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Neuro-immune interactions of neural stem cell transplants: From animal disease models to human trials

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Abstract

Stem cell technology is a promising branch of regenerative medicine that is aimed at developing new approaches for the treatment of severely debilitating human diseases, including those affecting the central nervous system (CNS).

Despite the increasing understanding of the mechanisms governing their biology, the application of stem cell therapeutics remains challenging. The initial idea that stem cell transplants work in vivo via the replacement of endogenous cells lost or damaged owing to disease has been challenged by accumulating evidence of their therapeutic plasticity. This new concept covers the remarkable immune regulatory and tissue trophic effects that transplanted stem cells exert at the level of the neural microenvironment to promote tissue healing via combination of immune modulatory and tissue protective actions, while retaining predominantly undifferentiated features.

Among a number of promising candidate stem cell sources, neural stem/precursor cells (NPCs) are under extensive investigation with regard to their therapeutic plasticity after transplantation. The significant impact in vivo of experimental NPC therapies in animal models of inflammatory CNS diseases has raised great expectations that these stem cells, or the manipulation of the mechanisms behind their therapeutic impact, could soon be translated to human studies.

This review aims to provide an update on the most recent evidence of therapeutically-relevant neuroimmune interactions following NPC transplants in animal models of multiple sclerosis, cerebral stroke and traumas of the spinal cord, and consideration of the forthcoming challenges related to the early translation of some of these exciting experimental outcomes into clinical medicines.

Keywords

Neural stem cells; Stem cell therapy; Immune modulation; Neuro-immune interactions; Clinical trials

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Introduction

The discovery of adult neurogenesis and the development of protocols that allow in vitro growth and significantly large scale-up of stem and precursor cells of the brain (Reynolds and Weiss, 1992) have fostered the development of innovative therapies aimed at stem cell transplantation for acute and chronic disorders of the nervous system (Cossetti et al., 2012). Motivated by the expectation of achieving CNS repair and/or regeneration via functional neural cell replacement, these studies have demonstrated a potential benefit of neural stem/precursor cell (NPC)-based experimental treatments in animal models of several neurological diseases (Martino et al., 2011). However, mounting evidence suggests that the effects orchestrated by transplanted NPCs are not only associated with the generation of new neurons or glial cells but also that the pathological setting in which these cells are transplanted critically determines the outcome (Cossetti et al., 2012). Cell replacement is therefore only one of the multiple ways in which transplanted NPCs promote tissue repair, and a much more complex therapeutic scenario should be foreseen. The concept of stem cell therapeutic plasticity (Martino and Pluchino, 2006) (or functional multipotency) (Teng et al., 2011) has therefore emerged, as it describes the multiple way(s) grafted NPCs which mediate systemic homeostasis, e.g. by the secretion of tissue trophic factors, as well as interaction with tissue-resident vs. -infiltrating immune cells, at the level of the inflammatory tissue context in which they are either transplanted or to which they migrate after transplantation.

The newest picture is therefore that stem cell therapies, contrary to single-molecule-based pharmaceutical interventions, hold the potential to deliver a complex series of information to a multitude of targets in the diseased microenvironment (Cossetti et al., 2012). While no final mechanisms (or direct evidence) of stem cell-to-host immune system interaction is yet available, a number of studies are now focussing on the cellular signalling that exists between grafted stem cells and endogenous target cells, with the aim of clarifying its physiological or circumstantial nature, and elucidating its molecular signature and therapeutic potential.

Here we will review the most recent evidence of immune modulation following syngeneic NPC transplants in animal models of multiple sclerosis, spinal cord injury and stroke, and discuss the next challenges related to the translation of some of these exciting experimental outcomes into clinical medicines.

Multiple sclerosis

Multiple sclerosis (MS) is a complex, highly debilitating CNS autoimmune disease that constitutes the most common cause of neurological disability in young adults (Compston and Coles, 2002). The main pathological hallmark of MS is the presence of highly heterogeneous, chronic inflammatory and demyelinating perivascular lesions within the CNS (Compston and Coles, 2002; Dyment and Ebers, 2002; Flugel et al., 2001; Lucchinetti et al., 2000; Noseworthy et al., 2000; Wingerchuk et al., 2001). Most of the demyelinated regions undergo partial remyelination and show structural repair and recovery of function (Barkhof et al., 2003; Chang et al., 2002; Compston, 1996, 1997; Prineas et al., 1993; Raine

and Wu, 1993). However, remyelination in MS is typically patchy and incomplete, and ultimately fails (Blakemore et al., 2002; Franklin, 2002; Franklin and Ffrench-Constant, 2008). The failure of remyelination in MS has multiple causes:

- **i.** Inadequate provision of OPCs (recruitment failure) (Chari et al., 2003) or a failure of recruited OPCs to differentiate into remyelinating oligodendrocytes (differentiation failure) (Jepson et al., 2012; Syed et al., 2011);
- **ii.** Ageing of the perilesional microenvironment where recruited OPCs show impaired differentiation into oligodendrocytes (Ruckh et al., 2012);
- **iii.** Inhospitable environment generated by pro-inflammatory Th1/Th17 cells and cytokines (Steinman, 2007); and
- **iv.** Anatomical barriers around chronic lesions impeding the recruitment of OPCs (Franklin, 2002).

Furthermore, recurring inflammation may have profound consequences on the health of anatomically intact axons, resulting in progressive and irreversible damage/dysfunction that accounts for the degenerative nature of MS (Franklin and Ffrench-Constant, 2008; Patrikios et al., 2006). The sequential involvement of most of the above processes underlies the clinical course of MS, which is characterised by recurrent episodes of relapses that eventually leave temporary or persistent deficits, to finally deteriorate into a secondary chronic progressive phase (Compston and Coles, 2002).

The issue of (stem) cell therapies for MS has therefore gained in complexity as its success relies on the capacity of transplanted (stem) cells to target the specific sites of disease, integrate into the host tissue and eventually differentiate into neural functional cells (neurons and glia), while surviving in the chronically inflamed CNS environment. This adds crucial concerns of identification of cell source, its constitutive vs. reactive immunogenicity, window of opportunity, route of cell delivery, as well as ways in which to help the integration and long-term survival of grafted cells in the ‘inhospitable’ inflammatory CNS environment. All these are critical aspects of stem cell therapies that must be critically considered when envisaging therapeutic cell transplants for MS (Martino et al., 2011).

The route of cell administration has always been a major constraint for stem cell transplantation in CNS diseases and appeared to be very much dependent on the presence of focal vs. multifocal lesions to target. With MS being a multifocal disease, it is unrealistic to propose lesion-targeted injection of cells (Pluchino and Martino, 2008). This is also complicated by the fact that it is generally difficult to determine which of the multiple lesions identified by magnetic resonance imaging (MRI) would underscore clinical significance, and whether they would eventually be amenable to effective (therapeutically relevant) remyelination upon (stem) cell therapy (Chen et al., 2007).

Following a number of successful proof-of-concept in vivo remyelination studies with focally-transplanted glial progenitor cell types, including oligodendrocytes (Blakemore and Crang, 1988), OPCs (Groves et al., 1993; Windrem et al., 2004, 2008) and Schwann cells (Blakemore, 1977; Zujovic et al., 2012), NPCs were the very first candidate stem cells for systemic cell treatment of experimental autoimmune encephalomyelitis (EAE), as an animal

model of MS [reviewed in Pluchino et al. (2004) and Goldman et al. (2012)]. During the last decade, rodents and non-human primates with acute, relapsing and chronic EAE (Martino and Pluchino, 2006) have been treated with NPCs injected intracerebroventricularly (icv), intrathecally (it) or intravenously (iv). These studies have shown that systemically injected NPCs enter the CNS where they survive for months in perivascular inflammatory areas while retaining mostly undifferentiated features, and remarkably reduce the clinical and pathological burden of the disease (Martino and Pluchino, 2006). Interestingly, the majority of these reports have substantially failed to show convincing differentiation and integration of transplanted NPCs in vivo, but rather contributed to the provocative idea that NPC transplants would work through mechanisms other than direct cell differentiation that imply the interaction between NPCs and immune cells (Cossetti et al., 2012; Martino and Pluchino, 2007).

The first evidence that NPCs possess immune-like features came from the observation that systemically injected NPCs use functional leukocyte-specific cell adhesion molecules (such as CD44 and very late antigen [VLA]-4) and inflammatory chemokine receptors (e.g. CCR2, CCR5 and CXCR4) to interact with activated ependymal and endothelial cells and ultimately enter the brain (Ben-Hur et al., 2003; Einstein et al., 2003; Pluchino et al., 2003). Once into the CNS, NPCs are found around inflamed blood vessels, in close contact with endogenous neural cells (e.g. astrocytes and neurons) and CNS-infiltrating blood-borne $CD45^+$ immune cells, while creating niche-like areas that are ultrastructurally and molecularly reminiscent of the prototypical stem cell niches from which NPCs were derived (Pluchino et al., 2005). NPC transplants are also associated with significantly reduced glial scar formation (Pluchino et al., 2003) and local inflammatory response (Ben-Hur et al., 2003; Einstein et al., 2006; Pluchino et al., 2005, 2009a, 2009b), which in turn lead to the increased survival and recruitment of endogenous neural cells (e.g. oligodendroglial progenitor cells) participating in the brain's intrinsic reparative response upon myelin damage (Einstein et al., 2009; Pluchino et al., 2005). The underlying molecular mechanisms by which transplanted NPCs confer this broad tissue protection were first indirectly linked to the increased in vivo bioavailability of major neurotrophins (Chu et al., 2004a, 2004b; Einstein et al., 2006; Lu et al., 2003; Pluchino et al., 2003; Teng et al., 2002) and to the modulation of the host environment into one that is more permissive for regeneration. Several neurotrophins that are found increased after NPC transplants have also been shown to inhibit EAE, and neurotrophins like insulin-like growth factor (IGF)-1 and glial growth factor (GGF)-2 promote the survival and proliferation of oligodendrocyte lineage cells (Barres et al., 1992; Canoll et al., 1996, 1999; Mason et al., 2000).

However, the observation that (icv)-injected NPCs primarily attenuate brain inflammation, in correlation with a reduction of $CD3^+$ T cells and an increase in $CD25^+$ and $CD25^+/CD62L^+$ regulatory T cells (Einstein et al., 2003), suggested a completely novel mechanism of action that deserved further investigation. The iv injection of NPCs also protects against chronic neural tissue loss as well as disease-related disability in EAE, via induction of the apoptosis of blood-borne CNS-infiltrating encephalitogenic T cells (Pluchino et al., 2005). In vitro, NPCs increase the apoptosis of antigen-specific Th1 pro-inflammatory (but not Th2 anti-inflammatory) cells selectively through the engagement of death receptors, including FasL, TRAIL and APO3L, on the surface of NPCs (Pluchino et al., 2005). Mouse and rat

NPCs also inhibit T cell activation and proliferation in response to T cell receptor (TCR)-mediated stimuli (Einstein et al., 2003; Fainstein et al., 2008). NPC-T lymphocyte co-cultures suggest that part of the anti-proliferative effect of NPCs might depend on the inhibition of IL-2 and IL-6 signalling on T lymphocytes (Fainstein et al., 2008). Knight et al. show that NPCs have a selective pro-apoptotic effect on Th17 cells in vitro via a FasL-dependent mechanism, identifying the axis Fas-Birc3 as an additional survival pathway for NPCs (Knight et al., 2010). NPCs also suppress T cell proliferation by the reactive production of nitric oxide (NO) and prostaglandin E2 (PGE2). Interleukin (IL)-10-transduced NPCs show enhanced ability to induce remyelination, neuronal repair and immune suppression after systemic NPC injection in EAE mice (Yang et al., 2009). Human NPCs suppress the proliferation of non-human primate activated T cells through both direct cell-to-cell contacts and via the release of soluble mediators into the culture supernatant (Kim et al., 2009; Pluchino et al., 2009a).

Other studies have shown that systemically injected NPCs also inhibit EAE by a peripheral immune modulation in lymph nodes (Einstein et al., 2007; Pluchino et al., 2009b). Einstein et al. first showed that EAE-derived lymph node cells were strongly inhibited by NPCs in the production of pro-inflammatory cytokines in response to MOG35-55. Furthermore, primed T cells from mice treated with NPCs were also deficient in their ability to adoptively transfer EAE, thus demonstrating a long-lasting inhibition of the encephalitogenicity of the T cells that are transferred to a naïve host, rather than an effect specific to the in vivo environment (Einstein et al., 2007).

We have reported a specific and almost exclusive targeting of the peripheral immune system in SJL mice with PLP-induced EAE in which NPCs were injected subcutaneously (sc) at 3 and 10 days post-immunisation (dpi) (Pluchino et al., 2009b). After sc injection in EAE, NPCs consistently accumulate and persist in draining lymph nodes, where they increase the availability of major stem cell regulators (including bone morphogenetic protein [BMP]-4) and interact with lymphoid dendritic cells (DCs) that are hindered in their maturation to professional antigen-presenting cells (APCs). In vitro, NPCs specifically impair the maturation of immature DCs (iDCs) via a BMP-4-dependent mechanism (Pluchino et al., 2009b). A recent study has shown that the preventive intravenous administration of NPCs ameliorates EAE by selectively inhibiting the differentiation of encephalitogenic T helper 17 (Th17) through secreted factors. Recently, Cao et al. have identified leukaemia inhibitory factor (LIF) as the first key factor responsible for the observed inhibition of Th17 cell differentiation by transplanted NPCs, and elucidated the signalling pathway behind this novel mechanism of action, where LIF antagonises interleukin (IL)-6-induced Th17 cell differentiation through the ERK-dependent inhibition of STAT3 phosphorylation (Cao et al., 2011).

Whether most of the immune regulatory effects of systemically injected NPCs in EAE act directly in the CNS or in the periphery is still an unanswered question and further studies are needed to establish the absolute relevance of these pre-clinical data in EAE – where peripheral lymphoid organs play an important role in the regulation of the immune responses to self myelin antigens – and indeed the possibility that both sites of action may become interrelated and pathophysiologically relevant to the future applications of NPCs in MS

(Kokaia et al., 2012; Martino et al., 2011). Evidence of the neuro-immune interactions observed following NPC transplantation in EAE is shown in Table 1 and summarized in Fig. 1.

Spinal cord injuries

Spinal cord injuries (SCIs) are devastating and debilitating conditions affecting all regions of the world – predominantly in young adults – which are associated with severe physical, psychological, social and economic burdens on patients and their families [reviewed in Ho et al. (2007) and van den Berg et al. (2010)]. An important premise for the development of effective treatments for SCIs is the precise understanding of the main pathophysiological events following the acute injury and how these interact in the development of established anatomical and functional deficits (Rowland et al., 2008).

The most common mechanisms of SCI developed into two exclusive broad chronological phases that are sustained by the primary and secondary mechanisms of injury. Primary injuries include shearing, laceration, and acute stretching vs. acceleration–deceleration events, which very rarely lead to complete transection or disruption of the anatomical continuity of the spinal cord (Rowland et al., 2008). The severity of neurological injury, the level of the injury and the presence of a zone of partial cord preservation are accepted predictors of recovery and survival after SCI. Indeed, the presence of spared axons crossing the injury site holds great therapeutic potential as a substrate of a number of emerging therapeutic strategies (Wilson et al., 2012).

A number of processes are triggered by primary injuries and lead to much more prolonged secondary injury phases that start within a few hours and remain active up to weeks after SCIs; thus, progressively exacerbating the consequences of the mechanical injury to the cord. Immediate, acute, intermediate and chronic secondary injury phases have been identified, characterised and staged (Rowland et al., 2008). Some of these phases include the early traumatic severing of the axons, the death of neurons and glia and the instantaneous loss of function at and below the injury level (spinal shock) (Ditunno et al., 2004; Kakulas, 2004). The spinal cord undergoes diffuse swelling and the central grey matter shows signs of petechial haemorrhage (Tator and Koyanagi, 1997). Cell necrosis and the activation of local astrocytes and microglia are also observed immediately after the injury (Watanabe et al., 1999). Subsequent secondary events include free radical production, ionic (e.g. Ca^{++}) deregulation (Tymianski et al., 1993) and glutamate excitotoxicity (Li and Stys, 2000) (immediate — hours); changes in the blood–brain barrier (BBB) permeability (Noble and Wrathall, 1989), activation of a multifaceted inflammatory response that involves soluble inflammatory mediators and a variety of cells such as astrocytes, microglia, T cells, neutrophils and cord-infiltrating monocytes (Donnelly and Popovich, 2008; Fleming et al., 2006), oligodendrocyte apoptotic cell death and demyelination (Crowe et al., 1997) (acute — days); astroglial proliferation and formation of a gliotic scar (Silver and Miller, 2004) that interferes with axonal sprouting (intermediate — weeks to months); and retrograde Wallerian axonal degeneration (Coleman and Perry, 2002), dynamic maturation of the lesion up to the development of cysts and/or syrinxes, and delayed neuronal dysfunction and neuropathic pain (Stoodley, 2000) (chronic — several months).

Despite the important advances in the understanding of SCI pathophysiology, to date, virtually all therapies that have shown promise at the preclinical stage of study have failed to translate into clinically effective treatments. For this reason, the potential of NPC transplantation to drive spinal cord repair has been widely investigated, as NPCs may provide an effective treatment by either directly replacing those cells lost owing to the injury (e.g. oligodendrocytes), influencing/modifying the environment in a way that supports axonal regeneration, providing neuroprotection, or re-setting the inflammatory response to a mode that heals the damaged tissue (Sahni and Kessler, 2010).

Adult mammalian NPCs from different sources have been transplanted into a wide range of SCI models with significant clinical improvement. Most of the studies have delivered NPCs focally to increase their viability at the injury site. While evidence exists in support of an astroglial differentiation default mode shown by transplanted embryonic NPCs in both the developing and the adult cord (Lepore et al., 2006), some pioneering work suggests that NPCs survive, migrate and generate functional remyelinating oligodendrocytes, which promote functional recovery when transplanted subacutely (namely 2 weeks) – but not chronically (namely 8 weeks) – after SCI (Karimi-Abdolrezaee et al., 2006).

However, other approaches have more convincingly established that the transplantation of somatic NPCs in experimental SCI yields a generally low degree of survival – as the injury creates a highly toxic environment – and differentiation (Bottai et al., 2008; Cao et al., 2002), most of which was biassed towards a glial fate (Cao et al., 2001; Pfeifer et al., 2004; Vroemen et al., 2003), thus challenging the initial expectation of achieving predominant neuronal/oligodendroglial cell replacement (Cao et al., 2001; Vroemen et al., 2003) in vivo.

There is also a growing belief that the severity (and type) of the injury, as well as the time after injury at which cells are transplanted, are two major key factors influencing the capability of grafted NPCs to affect the healing of the damaged spinal cord tissue. As such, rat spinal cord-derived somatic NPCs failed to induce any detectable functional recovery when transplanted hyperacutely at the level of injury in a severe (35-g) clip-induced SCI model (Parr et al., 2007), but were indeed significantly efficacious when transplanted as early as 9 days after injury in a milder (27-g) model of SCI (Parr et al., 2008). Furthermore, the homogeneity vs. heterogeneity of the neural stem/progenitor cell preparation is a key point. As such, prominent neuronal differentiation is reliably achieved with primary foetal tissue and when glially and neuronally restricted progenitors are combined, but not when only neuronally restricted progenitors are grafted to the injured spinal cord [reviewed in (Fischer, 2000)].

Strategies to overcome the observed poor survival and differentiation potential of transplanted NPCs have included combination with valproic acid (Abematsu et al., 2010) or neurotrophic growth factors (Bonner et al., 2010, 2011), which promoted neuronal differentiation and established functional synapses between host axons and graft neurons at the injury site.

The use of engineered NPCs transduced with transcription factors or survival genes (Hwang et al., 2009; Lee et al., 2009), as well as the co-transplantation with ‘scaffold’ cells such as

mesenchymal/stromal stem cells or olfactory ensheathing cells (Oh et al., 2010; Wang et al., 2010), has increased the survival, migration and differentiation of transplanted NPCs and the final functional recovery observed.

Furthermore, the combined transplantation of adult NPCs with minocycline treatment and growth factor delivery promoted cell survival and remyelination of spared axons into a rat model of compressive SCI (Karimi-Abdolrezaee et al., 2006). Transplanted NPCs were mainly incorporated into the white matter of the dorsal or lateral column and substantially differentiated along the oligodendroglial lineage. Exogenous (NPC-derived) myelin basic protein (MBP)-positive cells were found in close association with endogenous neuronal processes, finally correlating with the recovery of locomotor function (Karimi-Abdolrezaee et al., 2006). Interestingly, the net functional significance of exogenous remyelination in promoting behavioural recovery has been further confirmed by the recent observation that mice receiving NPC-derived oligodendrocytes isolated from myelin-deficient mice failed to acquire locomotor functionality after SCI (Yasuda et al., 2011). The concomitant use of chondroitinase ABC (ChABC) to reduce the presence of chondroitin sulphate proteoglycans (CSPGs) together with NPC transplantation in a chronic model of compressive SCI greatly increased the long-term survival, migration and integration of grafted cells (Karimi-Abdolrezaee et al., 2010).

In the majority of cases, the transplantation of NPCs has resulted in the significant recovery of functions that were highly specific to the treatment applied, as they were completely abolished in human NPC-transplanted SCI mice treated with diphtheria toxin (DT; as human cells are about 100,000 times more sensitive to DT than mouse cells) (Cummings et al., 2005). Interestingly, when more lineage-restricted neural precursors have been transplanted, a much higher rate of neuronal differentiation has been achieved, presumably because these latter cells are less sensitive than NPCs to inhibitory signals coming from the environment (Han et al., 2002; Yan et al., 2007).

Based on some of these assumptions, several strategies have been developed to promote regeneration and functional recovery by delivering biomaterial scaffolds engineered with cells and/or bioactive molecules (e.g. hydrogels, sponges, single- and multi-channelled guidance tubes, and nanofibre scaffolds (Bamber et al., 2001; Chen et al., 2010; Hurtado et al., 2006; Johnson et al., 2010; Taylor et al., 2006; Tobias et al., 2003; Wang et al., 2011; Xiong et al., 2012; Zeng et al., 2011). Interestingly, the implantation of a scaffold–neural stem cell unit into an adult rat hemisection model of SCI led to overall tissue preservation with limited atrophy and scar formation that finally resulted in significant functional improvement up to 1 year post-engraftment (Teng et al., 2002).

Alternative routes of administration (e.g. systemic) have also been investigated to avoid the damage to the spared cord tissue at the time of focal cell injection, as well as other procedure-related complications, with the final aim of improving the chances of translation into clinical practise. After injection into the tail vein of nude SCI mice, human NPCs were able to reach the injury site (Takeuchi et al., 2007), leading to significantly better behavioural recovery compared with SCI mice transplanted intraspinally with NPCs (Bottai et al., 2008).

Importantly, the shaping of new intraspinal circuits should be tightly controlled to avoid serious side effects such as aberrant host fibre sprouting associated with allodynia-like hypersensitivity (Hofstetter et al., 2005; Macias et al., 2006). These latter effects of transplanted NPCs may derive from their ability to release biological molecules able to protect damaged cells and/or promote endogenous CNS repair/regeneration. Indeed, in vitro (Hawryluk et al., 2012; Kamei et al., 2007) and in vivo (Lu et al., 2003) studies have shown how NPCs can release a milieu of biological factors able to attract injured axons and promote the growth of host spinal axons after injury.

As such, strong parallel evidence suggests that undifferentiated NPCs can also influence the injury environment at the spinal cord through the establishment of functional interactions with endogenous neural and immune cells. On the one hand, the transplantation of mouse NPCs both provided a cellular substrate and secreted several neurotrophic factors (e.g. nerve growth factor [NGF], brain-derived neurotrophic growth factor [BDNF] and GDNF), leading to substantial host axonal re-growth (Lu et al., 2003). Also, adult NPCs themselves were shown to induce the up-regulation of neurotrophic factors and chemokines such as BDNF, NGF and LIF, and inflammatory molecules such as TNF-α (Bottai et al., 2008). In line with this, NPCs, engineered to express a human NT-3 capable of binding to both trkB and trkC, showed increased survival and enhanced myelin formation, although they led only to a modest improvement in locomotor function, when transplanted into a chronic SCI model (Kusano et al., 2010). Furthermore, when combined with a self-antigen (e.g. myelin)-specific T cell vaccination that is expected to promote recovery from CNS insults provided that their activity (in terms of onset, intensity and duration) is well controlled (Butovsky et al., 2001, 2006), NPCs transplanted in mice with SCI migrated to the injury site and synergistically promoted functional recovery via modulation of the nature and intensity of the local T cell and microglial response, expression of BDNF and Noggin, and appearance of newly formed neurons from endogenous precursor-cell pools (Ziv et al., 2006). This immune regulatory effect was also present when NPCs were transplanted locally (at the lesion borders) into an extremely severe (e.g. 200 kdynes) model of contusion SCI. Sub-acutely (but not chronically) transplanted NPCs remained undifferentiated and altered the inflammatory infiltrate of the injured spinal cord by reducing the proportion of ‘classically-activated’ (M1) inflammatory macrophages, and increasing that of regulatory T cells, in turn promoting the healing of the injured cord (Cusimano et al., 2012).

Thus, NPC transplantation in SCIs contributes to anatomical and behavioural recovery in SCI models through cell replacement and integration of grafted cells into local circuits, as well as tissue trophic effects. Recent evidence has identified an additional novel protective mechanism that grafted undifferentiated NPCs exert on innate immune responses to remodel the perilesional inflammatory environment towards a tissue-healing mode (Kokaia et al., 2012; Martino et al., 2011).

Evidence of the main outcomes following syngeneic NPC transplantation in experimental SCIs is shown in Table 1 and summarized in Fig. 1.

Stroke

Clinical recovery after stroke remains very poor despite advances in therapy, and stem cell treatment is considered a promising alternative (Lindvall and Kokaia, 2011). Transplantation of NPCs with different delivery strategies, intraparenchymal (ipc) or icv injection, as well as systemic administration, has been shown to improve clinical signs in experimental stroke models (Liu et al., 2009; Pluchino et al., 2010).

Irrespective of the route of administration, transplanted NPCs migrate towards the infarct site in models of transient middle cerebral artery occlusion (MCAo) and intracerebral haemorrhage (ICH) (Chu et al., 2003, 2004b; Darsalia et al., 2007; Jeong et al., 2003; Kelly et al., 2004; Modo et al., 2002; Roitberg et al., 2006; Zhang et al., 2003), where gradients of pro-inflammatory cytokines and chemokines are released (Bacigaluppi et al., 2008), and CCL2/CCR2 and CXCL12/CXCR4 play a crucial role in transendothelial recruitment and intraparenchymal migration, respectively (Andres et al., 2011a; Darsalia et al., 2007; Imitola et al., 2004). Once they reach the ischaemic boundary zone (IBZ), grafted NPCs interact with the inflammatory environment, as suggested by the increase in the gene expression levels of VEGF, CXCL12/SDF1-α and TGF-β in the NPC-transplanted mouse brain with middle cerebral artery (MCA) occlusion 4 h after the insult (Capone et al., 2007). Similarly, the up-regulation of the cell adhesion molecule VCAM-1 onto the surface of endothelial cells facilitates the targeting and the subsequent extravasation of CD49d expressing NPCs to the site of injury (Guzman et al., 2008).

Intracerebrally transplanted NPCs survive up to 1 month, migrate towards the peri-infarct area and partially differentiate into mature neurons, finally resulting in a reduced lesion volume and functional recovery. Their beneficial effects were first attributed mainly to a combined action between trophic support and the ability of newly generated neurons to establish new synapses (Ishibashi et al., 2004). NPC-derived neurons have been described to produce neurotransmitters, form dendrites and show electrophysiological properties characteristic of integrated functional neurons (Buhnemann et al., 2006; Englund et al., 2002; Park et al., 2002). In most of the cases, it has been observed that transplanted NPCs remained confined to the penumbra (Jin et al., 2011), leading to a decrease in white matter atrophy and a reduction in endogenous apoptotic cells (Shen et al., 2010). Remarkably, NPCs grafted close to the lesion edges showed less prominent survival, most likely because of a gradient of factors and molecules released from the core of the injury that might have influenced NPC migration and maturation (Darsalia et al., 2007). Histopathological and MRI analyses showed that NPCs may also have profound effects on the white matter reorganisation within the IBZ (Jiang et al., 2006). More importantly, the number of cells surviving the transplantation does not strictly correlate with the observed clinical restoration. Indeed, a very low percentage of surviving cells (0.28%) could result in a robust behavioural recovery (Bacigaluppi et al., 2009).

On the other hand, NPCs injected systemically into MCAo mice mostly maintain an undifferentiated phenotype, while accumulating at the boundaries of the lesioned area (Bacigaluppi et al., 2009; Sun et al., 2010). Therefore, besides the (limited) cell replacement, NPCs are believed to also exert tissue trophic and immune modulatory effects (Bacigaluppi

et al., 2008) in stroke models [reviewed by (Kokaia et al., 2012; Martino et al., 2011)]. In line with this, the sub-acute (delayed) NPC injection after MCAo has been shown to significantly down-regulate multiple RNA species involved in inflammation, including IFN-γ, TNF-α, IL-1β, IL-6 and leptin receptor (Bacigaluppi et al., 2009). NPCs may exert an immune modulatory action, while in an undifferentiated state, causing a profound down-regulation of inflammatory *lymphoid* (T cells) and *myeloid* cells (macrophages) within inflamed brain areas. While the inhibition of the T cell responses by NPCs is quite an established concept (Ben-Hur, 2008), the effects on microglia/macrophages at the ischaemic injury site remain controversial, as professional phagocytes can exert both protective and deleterious effects after brain injuries, including stroke (Iadecola and Anrather, 2011). In addition to having a beneficial effect on axonal sprouting (Daadi et al., 2010), NPC transplantation promotes the infiltration of $CD11b^+$ myeloid cells in the brain of MCAo mice (Capone et al., 2007; Daadi et al., 2010), thus suggesting that some myeloid cell activation might be required for transplanted NPCs to exert part of their neuroprotective action (Capone et al., 2007). Mice with MCAo, selectively ablated of CD11b-positive microglia or mineralocorticoid receptor (MR)-expressing macrophages, show exacerbation or reduction of the ischaemia-dependent brain injury, respectively (Frieler et al., 2011; Lalancette-Hebert et al., 2007). However, other studies show a significant reduction in microglia/macrophages in the brain of mice with either ischaemic or haemorrhagic stroke after NPC transplantation, with improved neuronal survival and locomotor functions (Bacigaluppi et al., 2009; Lee et al., 2008). Interestingly, when injected systemically into mice with collagenase-induced intracerebral haemorrhage (ICH), only very few transplanted NPCs migrated into the brain, with the majority of them accumulating predominantly at the level of the spleen. In ICH mice, only the hyperacute (e.g. 2-h) NPC injection resulted in decreased brain oedema, inflammatory infiltration and neurological deterioration. Consistently, splenectomy prior to ICH induction eliminated the positive effect on oedema and the inflammation of transplanted NPCs (Lee et al., 2008).

Thus, preclinical research in animal models of stroke shows remarkable behavioural and pathological recovery through a number of bystander mechanisms that grafted NPCs employ to neutralize free radicals, inflammatory cytokines, excitotoxins, lipases peroxidases and other toxic metabolites released following an ischaemic event (Bacigaluppi et al., 2009; Ourednik et al., 2002). Once again, NPC transplants exert different therapeutic effects (e.g. cell replacement, neurotrophic support, central vs. peripheral immunomodulation, etc.) in response to the (inflammatory) signature of the tissue in which they are transplanted, or migrate to after systemic cell injection (Kokaia et al., 2012; Martino et al., 2011). Evidence of the main outcomes following syngeneic NPC transplantation in experimental stroke is shown in Table 1 and summarized in Fig. 1.

Towards clinical trials

Based on the encouraging results collected pre-clinically during the last 5–7 years (Table 1), phase I clinical trials have started to be conducted, both in fatal and non-fatal incurable neurological diseases where the risk/benefit ratio is in theory favourable (Aboody et al., 2011). Besides the unquestionable care regarding the characterisation and manufacture of the medicinal product (Rayment and Williams, 2010), one of the other important hurdles in

the design of clinical study for (stem) cell therapy trials is defining end-points, as these will be the measure of the trial's failure or success. This is particularly challenging given the inflammatory and degenerative nature of some of the target neurological disorders under consideration and the complexity posed by the rate of progression and lack of validated surrogate disease markers. The overall goal of these phase I NPC human studies is therefore to determine whether the transplantation of NPCs is feasible and safe – before checking for efficacy – with the primary aim of determining the maximum tolerated dose and potential dose-limiting toxicities. Secondary end-points are usually correlative studies (e.g. imaging the biodistribution of transplanted cells, assessing their immunogenicity, tracking their survival and fate in vivo) that would expand the knowledge gained from conducting these pilot studies. Long-term follow-up for the assessment of late toxicity is also important, particularly in patients with non-fatal conditions, who might live for many years after transplant.

Following the experimental evidence that human NPCs engraft robustly, migrate extensively and produce sufficient levels of palmitoyl protein thioesterase-1 (PPT1) to alter the behaviour and neuropathology of immunodeficient *Ppt1*$^{-/-}$ mice, as a model of infantile neuronal ceroid lipofuscinosis (NCL) (Tamaki et al., 2009), the first open-label dose-escalating phase I human study involving the transplantation of allogeneic NPCs was started in May 2006 at the Oregon Health and Science University (OHSU, Portland, OR, USA), investigating infantile and late-infantile NCL Batten disease. The rationale was that early intervention with stem cell transplants would supply an exogenous source of the missing enzyme sufficient for the uptake and cross-correction of host cells. A total of 6 subjects underwent transplantation in a single-stage procedure with direct delivery of StemCell, Inc. proprietary single-donor allogeneic free-floating cultured, foetally derived brain human NPCs (HuCNS-SC®) to the cerebral hemispheres and lateral ventricles. Immune suppression was administered for 12 months after transplantation. This study has now been completed and only 1 out of 6 patients has been reported to die from disease progression 11 months after treatment. The cell transplantation and combination with prolonged immune suppression were both well tolerated (Steiner et al., 2010).

Neural stem/precursor cell transplants also have the potential to replace lost or injured cell types, including neurons, astrocytes and oligodendrocytes (Lepore and Maragakis, 2007). While NPC-derived neurons may reconstruct circuits or create novel connections; graft-derived glial cells may be able to replace dysfunctional endogenous dead cells, thus restoring some level of normal (synaptic) communication and preventing further excitotoxic motor neuron cell death, as described for neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and leukodystrophies (Allaman et al., 2011; Lobsiger and Cleveland, 2007).

In September 2009, NeuralStem, Inc. initiated a phase I trial in amyotrophic lateral sclerosis (ALS) at the Emory University School of Medicine (Atlanta, GA, USA), using proprietary single-donor allogeneic adherent cultured, foetally derived spinal NPCs (NSI-566RSC). NSI-566 cells were surgically implanted in a total of 12 patients via multiple injections directly into the thoracic spinal cord (either unilateral or bilateral). Combined immune suppression with early methylprednisolone and prednisone, and late maintenance tacrolimus

and mycophenolate mofetil was administered until the end of the study. The clinical assessments demonstrated no evidence of acceleration of disease progression with the planned 18 month post-transplantation follow-up (Glass et al., 2012; Riley et al., 2012). In June 2012, the Azienda Ospedaliera Santa Maria (Terni, Italy) enrolled the first of a total of 18 ALS patients (subdivided into three independent groups, according to the disability at enrolment) and treated them with intraspinally implanted allogeneic, free-floating, cultured, foetally derived brain NPCs. This study has to date completed the enrolment of the fourth and final patient of treatment group A [main inclusion criteria: inability to walk and forced vital capacity (FVC) < 60%], who underwent transplantation in November 2012 [source: http://www.neurothon.com/trial.htm]. The patients in this study will be followed for 36 months post-transplantation (clinicaltrials.gov identifier no. NCT01640067).

StemCell, Inc. has also sponsored another two phase I trials with HuCNS-SC® in the X chromosome-linked co-natal leukodystrophy Pelizaeus–Merzbacher disease (PMD) and age-related macular degeneration (AMD). With the PMD trial at the University of California, San Francisco (UCSF, San Francisco, CA, USA; clinicaltrials.gov identifier no. NCT01005004), HuCNS-SC® was directly delivered through multiple injections into the brain of a total of 4 male patients, and immune suppression was administered for 9 months after transplantation. Multiple assessments were conducted to evaluate safety and to detect evidence of myelin formation after HuCNS-SC® transplantation, as suggested by the transplantation of xenogeneic human NPCs into the brains of neonatal or juvenile immunodeficient *shiverer* × *RAG2*$^{-/-}$ (*Shi-id*) mice, a model of PMD (Uchida et al., 2012). After a 1-year follow-up no clinical or radiological adverse effects were directly attributed to the donor cells. Furthermore, serial neurological evaluations, developmental assessments, as well as MRI and MR spectroscopy, including high-angular resolution diffusion tensor imaging (DTI), suggest durable cell engraftment and donor-derived myelin (starting at 9 months post-transplant) in the white matter of at least 3 of the 4 transplanted patients (Gupta et al., 2012).

The direct endogenous oligodendrocyte cell replacement operated by human NPC transplants in early chronic SCI in immunodeficient NonObese Diabetic/severe combined immunodeficiency (NOD-scid) mice (Salazar et al., 2010) inspired a further StemCell, Inc.-sponsored phase I/II clinical trial started in March 2011 at the University Hospital Balgrist (Zurich, Switzerland). A single dose (20×10^6 cells) of HuCNS-SC® was directly implanted through multiple injections into the thoracic spinal cord of the first patient (of a total of 12) with chronic thoracic (T2–T11) SCI, and immune suppression administered for 9 months after transplantation. This trial is enrolling patients 3–12 months after complete and incomplete cord injuries. The estimated completion date of this study is March 2016 (clinicaltrials.gov identifier no. NCT01321333).

A rescue effect on endogenous retinal ganglion cells (RGCs) (McGill et al., 2012) is the idea behind the StemCell, Inc.-sponsored AMD trial at the Retina Foundation of the Southwest (Dallas, TX, USA), where HuCNS-SC® is being delivered directly into the subretinal space of one eye in a single transplant procedure in a total of 16 patients. Immune suppression is not administered. The patient enrolment started in June 2012, and the estimated completion date is March 2014 (clinicaltrials.gov identifier no. NCT01632527).

In June 2012, the Glasgow Southern General Hospital (Scotland) enrolled the first patient (of a total of 12) into the dose-escalating *Pilot Investigation of Stem Cells in Stroke* (PISCES) phase I trial to be transplanted in a single-stage procedure with direct cerebral (intraparenchymal) delivery of Reneuron, Ltd. proprietary single-donor allogeneic adherent cultured, *c-myc* immortalised foetally derived brain human NPCs (CTX0E03). Immune suppression is not administered (clinicaltrials.gov identifier no. NCT01151124). Two recent studies in rats have provided some evidence in support of the multiple ways in which the CTX NPCs promote repair in the stroke-damaged brain, both through the up-regulation of (neo)angiogenesis (Hicks et al., in press), and through the recruitment of pro-neurogenic $CD11b^{+}$ macrophages/microglial cells in the ischaemic brain (Hassani et al., 2012).

Finally, there are not as yet any clinical trials using NPCs in MS, despite the solid pre-clinical data with somatic NPC transplants in EAE (Payne et al., 2011). However, there is general sense that they will start soon; and that the approach to MS with non-hematopoietic (neuroprotective) stem cell strategies will significantly benefit from the collection and interpretation of the data from those phase I trials that are now being done on diseases that are only apparently not strictly linked to MS (Martino et al., 2010). A consensus paper has been produced by a group of experts to define the uniform guidelines on the development of hematopoietic and non-hematopoietic stem cell therapies for MS. The main topics of this document include the identification of the ideal route of stem cell injection, the adequate dosage and type of cells, the appropriate protocols of in vitro expansion, the disease level of patients and the statistical power of trials (Martino et al., 2010). Furthermore, new expectations are arising from the use of induced pluripotent stem (iPS) cell-derived NPCs, which would eventually be available as autologous (e.g., if derived from the somatic cells of the same patient) cell sources.

These explorative trials play a key role in stem cell medicine for inflammatory CNS disorders, as they hold a great potential that goes far beyond the straightforward interpretation of disease-specific outcomes.

Both phase II dose-escalation studies and the inclusion of non-fatal diseases with larger population bases will finally be facilitated once human safety is established. The focus of phase II studies will also include certain clinical outcomes (e.g., transition to a different grade of disability) that can be measured and result in a benefit for the patient. The active clinical trials with NPCs are shown in Table 2.

Conclusions and future directions

Stem cell-based therapies hold great promise in regenerative neuroscience (Park et al., 2010). The huge advances made over the last few years have enormously deepened our knowledge about the biology of stem cells, leading to global reconsideration of their therapeutic potential and mechanisms of action, as well as their intrinsic limitations (Martino et al., 2011). Importantly, the use of animal models that closely mimic different aspects of human pathologies has also contributed to increasing our perception of the technical and biological challenges that still need to be faced before translating into clinical practise.

Neural stem/precursor cells not only give rise to neural cells for functional replacement, but also exert immune modulatory and tissue trophic effects, both in vitro and in vivo, after transplantation (Martino et al., 2011). As such, stem cells can be thought of as dynamic microsystems able to engage in active communications with surrounding neighbours and adapt accordingly. We also know that transplanted cells present the peculiar ability to sense the environment, specifically homing to the sites of damage (Martino et al., 2011). This is of extreme importance when considering multifocal diseases, where the in situ injection of stem cells is far from being achievable.

These new stem cell therapies may indeed help to explain the evidence that somatic stem cell sources of diverse embryonic origin, even with low capabilities of neural differentiation, efficiently promote CNS repair (Martino and Pluchino, 2006; Uccelli et al., 2008). Accordingly, stem cell therapeutic plasticity (or functional multipotency) should be viewed as the intrinsic capacity of stem cells to adapt their fate and function(s) to specific environmental needs occurring as a result of different pathological conditions (Martino and Pluchino, 2006; Teng et al., 2011).

Nevertheless, before introducing stem cell treatment wholesale into clinics, it is crucial to confront several inevitable issues. First, the biological and functional differences occurring between the rodent and the human host, which may in part explain the numerous failures encountered so far, where exciting pre-clinical data did not necessarily translate into promising clinical outcomes. Thus, an accurate choice of the model and the transition to non-human primates is still of vital importance. Further, the establishment of common guidance to precisely define the treatment strategies, the maximum tolerated cell dose, the standard conditions for scale-up and manipulation (prior to injection), the window of opportunity to treat and the most appropriate route for cell delivery are all high priority tasks to guarantee the uniformity of measures (Martino et al., 2010).

The next big challenge will be to understand the dynamics and the layers of host-to-graft-to-host interactions and to develop reliable surrogate markers that may enable us to assess some of the key outcome measures of stem cell therapies, including (but not only) stem cell survival, distribution and differentiation/integration. We also envisage that the in-depth understanding of stem cell-mediated immune modulatory actions has a real chance of resulting in (or contributing to) the development of more efficacious therapies for neurological disorders.

Unfortunately though, many of these efforts risk being corrupted by so-called stem cell tourism. Patients are attracted by false promises made by dubious scientists all over the world. In most cases, what is being sold as a miracle is in fact a fallacy that may be seriously harmful to patients' safety. An example of this is a young patient suffering from ataxia telangiectasia who developed a donor-derived brain tumour following neural stem cell transplantation (Amariglio et al., 2009).

Such experiences should once more encourage scientists, clinicians and regulators to work together to ensure that the huge efforts made so far and the credibility of the scientific community are not obfuscated by the development of deregulated cell-based therapies.

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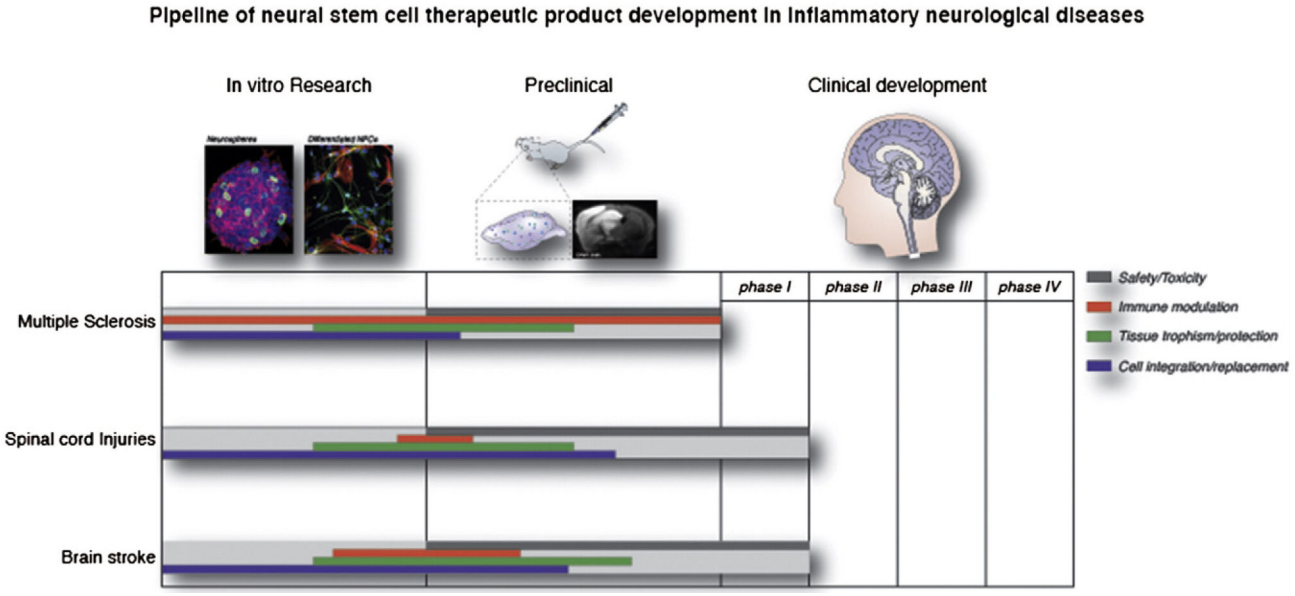

Fig. 1.

Overview diagram of the novel evidence of stem cell mediated effects – including neuro-immune interactions – that have inspired the present pipeline of neural stem cell therapeutic product development in multiple sclerosis, spinal cord injuries and brain stroke. In neurospheres, magenta is for Vimentin and green is for Phospho-Histone; in differentiated NPCs, red is for glial fibrillary acidic protein (GFAP) and green is for microtubule-associated protein 2 (MAP-2). Nuclei are stained with 4′,6-diamidino-2-phenylindole (DAPI). The magnetic resonance image (MRI) is a representative diffusion weighted image (DWI) coronal scan from the brain of a mouse 24 h after transient (45′) middle cerebral artery occlusion (MCAo).

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Table 1

Neuro-immune interaction following neural stem cell transplants in animal disease models.

Disease model	Species	Transplant features				Observed effect(s)	Proposed mechanism(s)	References
		Cell type	Cell no./animal	Route	Time			
Experimental Autoimmune Encephalomyelitis (EAE)								
Acute EAE	Rat	Rat neurospheres	$1.5–2 \times 10^4$	icv or it	Disease peak	Cell differentiation (neuronal and glial) and tissue trophism	None	(Ben-Hur et al., 2003)
Acute EAE	Rat	Rat neurospheres	2×10^4	icv	0 dpi	Immune regulation (central)	None	(Einstein et al., 2003)
Chronic EAE	Mouse	Mouse NPCs	1×10^6	iv or it	22 dpi	(Low) cell differentiation and tissue trophism	Inhibition of reactive gliosis	(Pluchino et al., 2003)
Chronic EAE	Mouse	Mouse neurospheres	2.5×10^3	icv	6 dpi	Immune regulation (local)	Reduction of CNS inflammatory infiltrates, increase in regulatory T cells	(Einstein et al., 2006)
Chronic EAE Passive EAE	Mouse	Mouse neurospheres	1×10^6	iv	8 dpi	Immune regulation (peripheral)	Suppression of encephalitogenic T cells	(Einstein et al., 2007)
Chronic EAE	Mouse	Human ES cell-derived NPCs	5×10^5	icv	7 dpi	Immune regulation (local)	Suppression of encephalitogenic T cells	(Aharonowiz et al., 2008)
Chronic EAE	Mouse	IL-10-transduced mouse NPCs	1.5×10^6	Iv or icv	10, 22 or 30 dpi	Immune regulation (local, peripheral) and cell differentiation	Induction of T cell apoptosis, promotion of myelin debris clearance	(Yang et al., 2009)
Chronic EAE	Mouse	Mouse and human ES cell-derived NPCs	2×10^6	iv	0 or 10 dpi	Immune regulation (peripheral)	LIF-mediated inhibition of Th17 cell differentiation	(Cao et al., 2011)
Chronic EAE	Mouse	Mouse MSC-derived NPCs	3.5×10^4-6.1×10^6	it	21, 28 and 35 dpi	Tissue trophism	None	(Harris et al., 2012)
Chronic EAE Passive EAE	Mouse	CCR5-transduced mouse BM-derived NPCs	1.5×10^6	iv	22 dpi (peak)	Immune regulation	None	(Yang et al., 2012)
Chronic EAE	Common Marmoset	Human NPCs	$2–6 \times 10^6$	it or iv	Disease onset	Immune regulation (central)	Suppression of encephalitogenic T cells, impairment of dendritic cell maturation	(Pluchino et al., 2009a)
Relapsing EAE	Mouse	Mouse NPCs	1×10^6	iv	Disease onset or first relapse	Immune regulation (central)	Induction of T cell apoptosis	(Pluchino et al., 2005)
Relapsing EAE	Mouse	Mouse NPCs	0.5×10^6	sc	3 and 10 dpi, or 10 dpi only	Immune regulation (peripheral)	BMP-4-dependent hindrance of dendritic cell maturation	(Pluchino et al., 2009b)
Relapsing EAE	Mouse	Mouse NPCs and olig2-transduced NPCs	1.5×10^5	icv	Disease onset or first relapse	Immune regulation (central) and tissue trophism	None	(Sher et al., 2012)
Spinal Cord Injuries (SCI)								
Compression (T7)	Rat	Mouse NPCs	$3–4 \times 10^5$	ipc	14 or 56 dpi	Cell differentiation (oligodendroglial)	Remyelination	(Karimi-Abdolrezaee et al., 2006)
Compression (T7)	Rat	Mouse NPCs	4×10^5	ipc	49 dpi	Cell differentiation (oligodendroglial)	Remyelination	(Karimi-Abdolrezaee et al., 2010)
Compression (T8–T9)	Rat	Human NPCs	2.5×10^5	ipc	7 dpi	Cell differentiation (neuronal and glial)	None	(Mothe et al., 2011)

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Disease model	Species	Transplant features				Observed effect(s)	Proposed mechanism(s)	References
		Cell type	Cell no./animal	Route	Time			
Contusion (C5)	Common marmoset	Human NPCs	1×10^6	il	9 dpi	Cell differentiation (neuronal and glial)	Integration in endogenous neuronal circuitries	(Iwanami et al., 2005)
Contusion (T8)	Rat	Rat NPCs	$3–5 \times 10^5$	il or ipc	10 dpi	Cell differentiation (glial)	None	(Cao et al., 2001; Karimi-Abdolrezaee et al., 2006)
Contusion (T8)	Mouse	Mouse NPCs	1×10^6 or 1×10^5	Iv or ipc	Acute	Tissue trophism	Reduction of apoptosis	(Bottai et al., 2008)
Contusion (T8–T9)	Rat	Rat NPCs	1×10^5	ipc	7 dpi	Cell differentiation (neuronal and glial)	None	(Hofstetter et al., 2005)
Contusion (T9)	Mouse	Human NPCs	7.5×10^4	ipc	9 dpi	Cell differentiation (neuronal and glial)	Integration into endogenous neuronal circuitry	(Cummings et al., 2005)
Contusion (T9–T10)	Rat	Rat G-NPCs	5×10^5	il	Acute	Axonal (re)growth	Reduction of astrogliosis and promotion of axon sprouting	(Hill et al., 2004)
Contusion (T9–T10)	Mouse	Mouse NPCs + VPA and HDAC inhibitor	5×10^5	il	7 dpi	Cell differentiation (neuronal and glial)	Integration into endogenous neuronal circuitries	(Abematsu et al., 2010)
Contusion (T9–T10)	Mouse	Human iPS cell-derived NPCs	1×10^6	il	7 dpi	Cell differentiation (neuronal and glial) and tissue trophism	Integration into endogenous neuronal circuitries	(Fujimoto et al., 2012)
Contusion (T10)	Mouse	Mouse NPCs	5×10^5	il	9 dpi	Cell differentiation (oligodendroglial)	Remyelination	(Yasuda et al., 2011)
Contusion (T10)	Mouse	Human iPS cell-derived NPCs	5×10^5	ipc	9 dpi	Cell differentiation (neuronal and glial), tissue trophism	Integration into endogenous neuronal circuitries, remyelination and neuroprotection	(Nori et al., 2011)
Contusion (T12)	Mouse	Mouse NPCs	5×10^5	it	7 dpi	Immune regulation (local) and tissue trophism	T cell mediated activation of microglia with a protective phenotype	(Ziv et al., 2006)
Contusion (T12)	Mouse	Mouse NPCs	$0.75–1.5 \times 10^5$	ipc	7 and 21 dpi	Immune regulation (local)	Reduction of the M1-like macrophage population	(Cusimano et al., 2012)
Hemisection (C1)	Rat	Rat NPCs	5×10^5	il	Acute	Cell differentiation (neuronal)	Integration into endogenous neuronal circuitries	(Bonner et al., 2011)
Hemisection (C3)	Rat	NT-3-transduced rat NPCs Rat NPCs	1×10^5	il	Acute	Tissue trophism	Neuroprotection	(Lu et al., 2003)
Hemisection (C3)	Rat	Rat NPCs	$4.8–5.4 \times 10^5$	il	Acute	Cell differentiation (astro and oligodendroglial)	None	(Vroemen et al., 2003)
Hemisection (C4)	Rat	Rat G-NPCs	1×10^5	il or ipc	7–10 dpi	Cell differentiation (astro and oligodendroglial)	None	(Han et al., 2004)
Hemisection (C4)	Rat	Rat NPCs	4×10^5	il	Acute	Cell differentiation (neuronal and glial)	None	(Lepore and Fischer, 2005)
Transection (T10)	Rat	Mouse NPCs + gelfoam	3×10^5	il	Acute	Tissue trophism	Neuroprotection	(Gu et al., 2012)
Hemisection (T9–T10)	Rat	Mouse NPCs + PLGA scaffold	5×10^5	il	Acute	Tissue trophism	Reduction of astrogliosis	(Teng et al., 2002)
Stroke								
MCAo	Rat	Rat NPCs	1×10^5	it	2 dpi	Cell differentiation (neuronal)	None	(Zhang et al., 2003)
MCAo (10′ or 90′)	Rat	Human NPCs	5×10^6	iv	1 dpi	Cell differentiation (neuronal, glial)	None	(Chu et al., 2003, 2004b)
MCAo (30′ or 60′)	Rat	Human NPCs	3×10^5	ipc	7 or 14 dpi	Cell differentiation (neuronal and glial)	None	(Darsalia et al., 2007; Kelly et al., 2004)

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Disease model	Species	Transplant features				Observed effect(s)	Proposed mechanism(s)	References
		Cell type	Cell no./animal	Route	Time			
MCAo (ED-1 or 120′)	Rat	Mouse ES cell-derived NPCs	1×10^5	ipc	7 dpi	Cell differentiation (neuronal and glial)	None	(Buhnemann et al., 2006; Wei et al., 2005)
MCAo (70′)	Rat	Human NPCs	8×10^5	ipc	21–28 dpi	Tissue trophism	None	(Pollock et al., 2006)
MCAo (180′)	Rat	Human NPCs	1×10^6	iv	2 dpi	Tissue trophism	None	(Jiang et al., 2006)
MCAo (NA)	Cynomolgus monkey	Human neurospheres	$2–4 \times 10^5$	ipc	7 dpi	Cell differentiation (neuronal)	None	(Roitberg et al., 2006)
MCAo (NA)	Mouse	Mouse ES cell derived NPCs	5×10^4	ipc	7 dbi	Tissue trophism	None	(Pignataro et al., 2007)
MCAo (90′)	Rat	Rat adult and ES cell-derived NPCs	1×10^5	ipc	Acute	Cell differentiation, tissue trophism	None	(Takahashi et al., 2008)
MCAo (20′)	Rat	Human ES cell-derived NPCs	5×10^4	ipc	1 dpi	Tissue trophism	Reduction of endogenous apoptosis	(Zhang et al., 2009)
MCAo (60′)	Rat	Human NPCs	4.5×10^3-4.5×10^5	ipc	28 dpi	Tissue trophism	Restoration of endogenous neurogenesis	(Stroemer et al., 2009)
MCAo (120′)	Rat	GDNF-transduced rat NPCs	5×10^5	icv	3 dpi	Tissue trophism, cell differentiation (neuronal)	None	(Chen et al., 2009)
MCAo (45′)	Mouse	Mouse NPCs	1×10^6	iv	3 dpi	Immune regulation (local) and tissue trophism	Reduction of microglial activation and neuronal death	(Bacigaluppi et al., 2009)
MCAo (90′)	Rat	HIF-1 *α*-transduced rat NPCs	1×10^6	icv	1 dpi	Tissue trophism	Promotion of angiogenesis	(Wu et al., 2010)
MCAo (permanent)	Rat	Human ES cell-derived NPCs	1.2×10^5	ipc	14 dpi	Tissue trophism	Enhancement of endogenous neurogenesis	(Jin et al., 2011)
MCAo (permanent)	Rat	Human ES cell-derived NPCs	5×10^4	ipc	1 dpi	Tissue trophism	Enhancement of endogenous neurogenesis	(Daadi et al., 2010)
MCAo (permanent)	Rat	Human ES cell-derived NPCs + Matrigel	1.2×10^5	ipc	21 dpi	Tissue trophism, cell differentiation (neuronal)	Integration into endogenous neuronal circuitries	(Jin et al., 2010)
MCAo (permanent)	Rat	Human ES cell-derived NPCs	5×10^4	ipc	1 dpi	Tissue trophism	Enhancement of endogenous neurogenesis and angiogenesis	(Zhang et al., 2011)
MCAo (permanent)	Rat	Human NPCs	1×10^5	ipc	7 dpi	Tissue trophism	Neuroprotection	(Andres et al., 2011b)
CCAo (10′ + 10′)	Mongolian gerbils	Human NPCs	5×10^5	ipc	4 dpi	Tissue trophism and cell differentiation (neuronal)	Integration into endogenous neuronal circuitries and neuroprotection	(Ishibashi et al., 2004)
ICH	Rat	Human NPCs	5×10^6	iv	1 dpi	Tissue trophism, cell differentiation (glial and neuronal)	Neuroprotection and integration into endogenous neuronal circuitries	(Jeong et al., 2003)
ICH	Mouse	VEGF-transduced human NPCs	2×10^5	ipc	7 dpi	Tissue trophism	Promotion of angiogenesis	(Lee et al., 2007)

Abbreviations: BM: bone marrow; BMP-4: bone morphogenetic protein 4; CCAo: common carotid artery occlusion; dpi: days post immunisation/injury; dbi: days before immunisation/injury; ED-1: endothelin-1; ES cells: embryonic stem cells; GDNF: glial-derived neurotrophic factor; G-NPCs: glial-restricted NPCs; HDAC: histone deacetylase inhibitor; HIF-1 *α*: hypoxia-inducible factor 1 *α*; ICH: intracerebral haemorrhage; icv: intracerebroventricular; il: intralesional; ipc: intraparenchymal (perilesional); iPS cells: induced pluripotent stem cells; it: intrathecal; iv: intravenous; LIF: leukaemia inhibitory factor; MCAo: middle cerebral artery occlusion; MSC: mesenchymal stem cells; NT-3: neurotrophin 3; sc: subcutaneous; VEGF: vascular endothelial growth factor; VPA: valproic acid.

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Table 2

Active clinical trials with neural stem/precursor cells.

Sponsor and place	Disease	Trial phase	Patients (no)	Age at enrolment (y)	Follow-up (months)	Transplant features					Status	Principal investigator	Trial identifier	Outcome and notes
						Cell type	Cell no./patient	Route	Time after disease/injury	Immune suppression				
StemCells, Inc. at University Hospital Balgrist-Uniklinik Zurich, (Switzerland)	Thoracic spinal cord injuries (SCI)	I/II	12	18–60	12	HuCNS-SC® (foetal, brain-derived, allogeneic, single donor)	2×10^7	Multiple injections, single dose, intramedullar	≥3 months	Y (9 months pt)	AR	Armin Curt, MD	NCT01321333	NA
ReNeuron, Ltd. at Glasgow Southern General Hospital, Glasgow (UK)	Stable ischaemic stroke (PISCES)	I	12	60–85	24	CTX0E303 (foetal, brain-derived, c-myc immortalised, allogeneic, single donor)	$2–20 \times 10^6$	Single injection, four ascending doses, intracerebral (putamen)	0.5–5 years	NA	AR	Keith Muir, MD	NCT01151124	NA
Neuralstem, Inc. at Emory University, Atlanta (USA)	Amyotrophic lateral sclerosis (ALS)	I	18	>18	48	NSI-566RSC (foetal, spinal cord-derived, allogeneic, single donor)	$0.5–1 \times 10^6$	Multiple injections, intraspinal	≥ 1.5 years	Y(≥4 months pt)	AnR	Eva Feldman, MD, PhD	NCT01348451	(Glass et al., 2012; Riley et al., 2012)
Azienda Ospedaliera Santa Maria, Terni (Italy)	ALS	I	18	20–75	36	Foetal, brain-derived, allogeneic, single donor	NA	Multiple injections, single dose, intraspinal	> 6 months	NA	AR	Angelo Vescovi, PhD	NCT01640067	NA
StemCells, Inc. at University of California, San Francisco (USA)	Pelizaeus–Merzbacher disease (PMD)	I	4	0.5–5	12	HuCNS-SC®	3×10^8	Multiple injections, single dose, intracerebral	NA	Y (9 months pt)	AnR	Stephen Huhn, MD	NCT01005004	(Gupta et al., 2012)
StemCells, Inc. at Oregon Health and Science University, Portland (USA)	Neuronal ceroid lipofuscinosis (NCL)	I	6	1.5–12	13	HuCNS-SC®	$0.5–1 \times 10^9$	Multiple injections, single dose, intracerebral	NA	Y (12 months pt)	C	Robert Steiner, MD	NCT00337636	(Steiner et al., 2010)
StemCells, Inc. Retina Foundation of the Southwest, Dallas (USA)	Age-related macular degeneration (AMD)	I/II	16	>50	12	HuCNS-SC®	$0.2–1 \times 10^6$	Single injection, single dose, subretinal	NA	Y (3 months pt)	AR	David Birch, PhD	NCT01632527	NA

Abbreviations: pt: post-transplant; NA: information not available. AR: actively recruiting; AnR: actively not recruiting; C: completed.