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Review

The neural stem cell secretome and its role in brain repair

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HIGHLIGHTS

- Transplantation of human NSCs has shown beneficial effects in rodent models of neurodegenerative diseases.
- The secretome of human NSCs plays a pivotal role in promoting neuroprotection and regeneration.
- Human NSC-derived extracellular vesicles as a promising candidate therapy.
- Clinical trials have found human NSC transplantation to be safe and well-tolerated.

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ABSTRACT

Compelling evidence from experimental animal disease models and early-phase clinical trials identifies the transplantation of neural progenitor/stem cells (NSCs) as a viable path towards the development of clinically applicable exogenous stem cell therapies.

Building from current advances in the field of NSC biology and following the positive outcomes of NSC transplantation studies, the contemporary view is that transplanted NSCs act as local '*factories*' capable of producing and secreting a wide array of immune and neurotrophic factors. This has launched a '*stem cell race*' to identify the mechanisms behind stem-cell mediated repair in what has been labeled the *paracrine hypothesis*. This hypothesis proposes that NSC grafts act as a natural source of potent biologics capable of modulating and promoting the restoration of several key functions in the central nervous system (CNS) tissue following acute or chronic tissue damage.

Investigators have been inspired to examine novel ways to harness and utilize the pro-regenerative properties of NSC therapies as an alternative approach to a more classical (small molecule based) treatment of CNS diseases.

In this review, we will discuss the most recent findings of human NSC (hNSCs) transplants in experimental animal models of CNS diseases that identify of hNSC-secreted factors, including those trafficked within extracellular membrane vesicles (EVs), and the outcomes of recent clinical trials utilizing hNSC therapeutics in CNS diseases.

1. Introduction

A growing body of literature into understanding and dissecting the biology of NSCs has elucidated a wide array of beneficial properties of these cells. This has led to major strides in the field in harnessing their restorative, regenerative, and protective potential for the development of pre-clinical therapies in the treatment of many common and devastating neurodegenerative diseases, such as: spinal cord injury (SCI), Parkinson's disease (PD) (Redmond et al., 2007; Han et al., 2015; L'Episcopo et al., 2018), Alzheimer's disease (AD) (Duncan and Valenzuela, 2017), multiple sclerosis (MS) (Pluchino et al., 2003; Pluchino and Martino, 2008; Pluchino et al., 2009; Peruzzotti-Jametti et al., 2018; Volpe et al., 2019), amyotrophic lateral sclerosis (ALS) (Glass et al., 2012), and stroke (Bacigaluppi et al., 2008; Lee et al., 2008; Hermann et al., 2014; Bacigaluppi et al., 2016; Bernstock et al., 2019). Despite historical attempts to utilize stem cell transplants as therapies in diseases of the CNS, recent literature has suggested that the effects orchestrated by NSC transplants is not limited to the de novo generation of graft-derived neurons and glial cells (Martino and Pluchino, 2006; Martino et al., 2011; Boese et al., 2018). Data has in fact led investigators to re-think the use of whole-cell transplants to derive benefit. Rather, the introduction of factors produced by neural stem cells has been found to provide a supportive milieu that allows injured cells to resist further degeneration, promote repair, drive

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Table 1
Main growth factors detected in the hNSC-derived secretome.

Genes					
Gene ID	Gene Name	Disorder	Technique	Condition	Reference
VEGF	Vascular endothelial growth factor	Ischemic Stroke	qPCR	*in vitro*	(Hicks et al., 2013)
VEGF, Vegf		ALS	qPCR	*in vivo*	(Kondo et al., 2014)
GDNF	Glial cell line-derived neurotrophic factor	SCI	qPCR	*in vivo*	(Romanyuk et al., 2015)
Gdnf		ALS	qPCR	*in vivo*	(Kondo et al., 2014)
Nt3	Neurotrophin-3	ALS	qPCR	*in vivo*	(Kondo et al., 2014)
BFGF	Basic fibroblast growth factor	Ischemic stroke	qPCR	*in vitro*	(Hicks et al., 2013)
EGF	Epidermal growth factor	Ischemic stroke	qPCR	*in vitro*	(Hicks et al., 2013)
NGF	Nerve growth factor	SCI	qPCR	*in vivo*	(Romanyuk et al., 2015)
FGF8	Fibroblast growth factor 8	SCI	qPCR	*in vivo*	(Romanyuk et al., 2015)
Proteins					
Protein ID	Protein Name	Disorder	Technique	Condition	Reference
BDNF	Brain-derived neurotrophic factor	Ischemic stroke	IF	*in vivo*	(Huang et al., 2014)
		Alzheimer's disease	WB	*in vivo*	Park et al., 2013; (Lee et al., 2015)
		Parkinson's disease	LC-MS/MS	*in vitro*	(Mendes-Pinheiro et al., 2018)
VEGF	Vascular endothelial growth factor	Ischemic stroke	WB, ELISA	*in vitro*	(Hicks et al., 2013)
		Alzheimer's disease	WB	*in vivo*	(Lee et al., 2015)
GDNF	Glial cell line-derived neurotrophic factor	Parkinson's disease	LC-MS/MS	*in vitro*	(Mendes-Pinheiro et al., 2018)
NGF	Nerve growth factor	Alzheimer's disease	WB	*in vivo*	Park et al., 2013; (Lee et al., 2015)
BFGF	Basic fibroblast growth factor	Ischemic stroke	WB, ELISA	*in vitro*	(Hicks et al., 2013)
EGF	Epidermal growth factor	Ischemic stroke	WB, ELISA	*in vitro*	(Hicks et al., 2013)
IGF-1	Insulin-like growth factor	Alzheimer's disease	WB, ELISA, IF	*in vitro*	(McGinley et al., 2016)

Abbreviations: qPCR, quantitative polymerase chain reaction; ALS, amyotrophic lateral sclerosis; SCI, spinal cord injury; IF, immunofluorescence; WB, western blot; ELISA, enzyme-linked immunosorbent assay; LC-MS/MS, liquid chromatography with tandem mass spectrometry.

regeneration of injured tissue, and decrease inflammation (Table 1) (Drago et al., 2013; Doeppner et al., 2017; Yang et al., 2018).

The classical view that NSC grafts only function through structural cell replacement has been recalibrated over the past decade with research demonstrating their capabilities to sense diverse signals, migrate to specific biological niches, and execute complex behaviors (Pluchino et al., 2010). NSCs are being extensively investigated for their capacity to signal to the endogenous host cells upon transplantation, reduce chronic inflammation and promote regeneration in experimental CNS diseases (Martino and Pluchino, 2006). Following NSC transplantation, sustained graft-to-host exchanges of signals has led to trophic effects on endogenous brain cells and beneficial modulatory actions on innate and adaptive immune responses that have ultimately promoted the healing of the injured CNS (Fig. 1) (Pluchino and Martino, 2008; Pluchino et al., 2010; Pluchino et al., 2005; Serra et al., 2008; Pluchino et al., 2009; Cossetti et al., 2012; Pluchino and Cossetti, 2013; Cossetti et al., 2014).

Considerable efforts are currently underway to develop, standardize, and assess the safety of autologous hNSC transplantation as a therapy for many chronic CNS diseases, with a small group of clinical trials at differing stages of progression (Table 2). As an alternative approach, the use of stem cell secreted factors for the development of treatments is receiving significant attention from investigators. Active exploration is underway way to refine technologies for use in exploiting stem cell secreted factors in the development of treatments using these naturally produced biologics. This has required the extensive application of analytical techniques such as targeted/untargeted proteomics and metabolomics to identify potential novel therapeutic factors from the NSC secretome (Table 1) (Drago et al., 2016; Shoemaker and Kornblum, 2016; Iraci et al., 2017). Analytical technologies coupled with the use of gene expression approaches to develop modified NSCs capable of actively releasing discrete levels of specific beneficial factors is an area of intensive investigation. For example, this review will highlight papers that use genetically-engineered hNSCs to secrete an increase of beneficial factors, such as IGF-1 (insulin-like growth factor 1), in rodent models of neurodegenerative disease (Park et al., 2013; McGinley et al., 2016). Finally, the development of cell-free (or *acellular*) therapeutics that use hNSC-derived biologics, instead of whole, allogeneic NSCs, has emerged as a concept in regenerative medicine to provide beneficial effects via modulation of the function of tissue-resident CNS cells.

In this review, we will outline the most current literature regarding human NSC transplantation in animal models of neurodegenerative diseases and how their secretome promotes regeneration, neuroprotection, and dampens inflammation. We will also discuss the outcomes of completed early clinical trials of hNSC transplants in human patients and discuss the growing field of *acellular* therapeutics utilizing the NSC secretome.

2. NSC transplantation in neurodegenerative disorders

NSCs are self-renewing, multi-potent cells characterized by their capacity to differentiate into multiple cell types, including astrocytes, oligodendrocytes, and neurons, making them an ideal therapeutic candidate for neurodegenerative disorders. In mammalian embryonic development these cells give rise to neurons and glia, and during adulthood have been found to persist as small populations within the subventricular zone (SVZ) near the lateral ventricles and in the subgranular zone (SGZ) of the hippocampus (Ma et al., 2009). Rodent NSCs (rNSCs) can be extracted directly from neural tissue, such as the neuroectoderm in the developing fetus, and from the SVZ or SGZ in postnatal and adult tissue (Lee et al., 2008). These NSCs can then be propagated *in vitro* for eventual transplantation in rodent disease models.

The initial success of transplantation studies in laboratory research is largely attributed to the immunological compatibility between the donor cells and the host (Tullis et al., 2014). Allografts, where the transplanted cells and the host are not genetically identical, but members of the same species is most commonly performed in the lab, which would include rodent NSCs into rodents.

This review will outline primarily xenografts, where donor tissue will be from a different species compared to the host (ie. human cells into rodents). On the other hand, autographs are transplants where the transplanted, autologous, tissue comes from the host, which is the most ideal in human transplants. Differences in major histocompatibility complex (MHC) antigens, in humans known as the human leukocyte

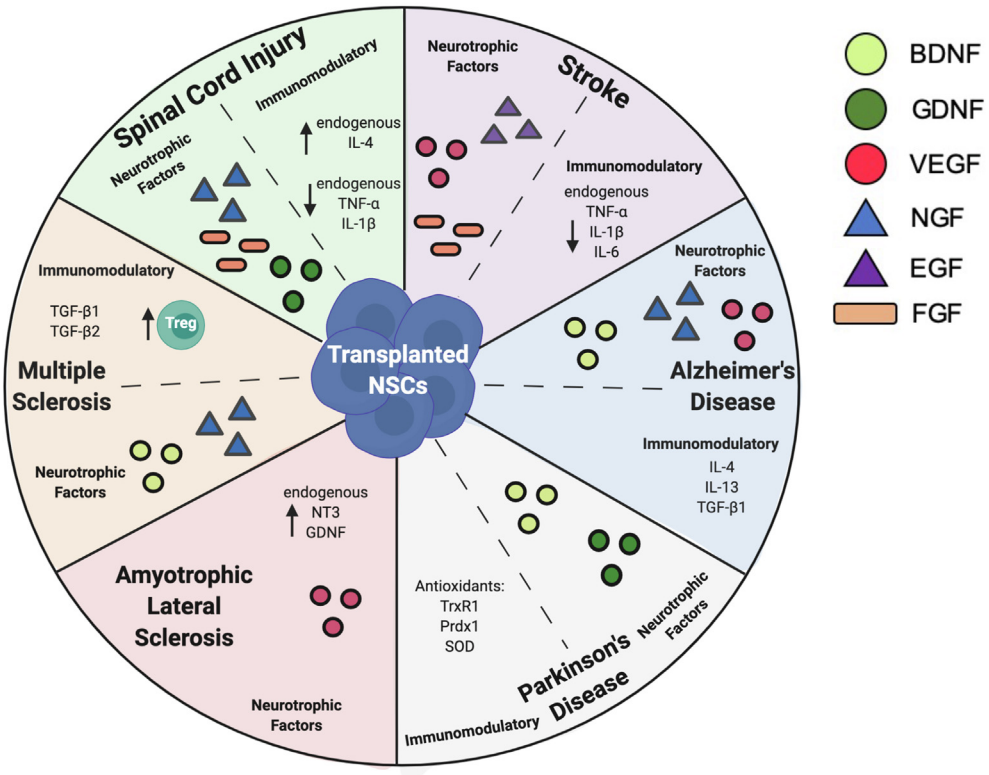

Fig. 1. The observed exogenous hNSC secretome in rodent models of neurodegenerative disease. Up arrow (↑) indicates an increase; down arrow (↓) indicates a decrease.

antigen (HLA), determines the successful integration of the transplant. If the donor and the recipient are sufficiently matched in the MHC region and immune suppression is used transplants are generally successful (Tullis et al., 2014). When not matched, and no immunosuppressants are given, the transplant will be deemed foreign by the host and rejected by the adaptive human system, which then initiates an immune response to remove foreign pathogens. In the case of CNS transplants this reaction would be extremely debilitating if not fatal, therefore human clinical trials have proceeded with caution.

Many initial studies of transplantation in neurodegenerative disease models have been performed using rodent cells into rodent hosts, but this review will focus on human NSC transplants into rodent models of disease. This is important to test how grafted human cells react *in vivo* in a disease model. Furthermore, there are a wealth of animal models, including transgenic mice, which have been generated that closely mimic the pathological and behavioral deficits that are associated with neurodegenerative diseases, and therefore are ideal models to test human NSCs.

Human NSCs can be derived in the same ways as rodent NSCs, including from fetal/embryonic tissue, postmortem adult tissue, and more recently from the derivation of induced pluripotent stem cells (iPSC) and directed stem cell technology (Thier et al., 2012). Many of the studies reviewed utilize hNSCs generated from fetal tissue, which raises ethical concerns about tissue source and genetic stability if these cells have been maintained long term in culture.

The advent of iPSC technology has enabled differentiated NSCs to bypass potential immunogenicity issues associated with transplantation, and circumvent the ethical issues associated with the use of fetal tissue. A limitation of fetal cell transplantation is the small amount of tissue available, which does not meet the current needs of patients with neurodegenerative diseases. NSCs derived from fetal tissue can be expanded, but research suggests that their expandability is limited, which raises concern on their genetic stability over multiple passages (Signer and Morrison, 2013). Nevertheless, NSCs derived from iPSCs still raise concerns due to their potential tumorigenic nature. Several groups have reported teratoma formation following the transplantation of iPSC-derived cells, and even de novo mutations in mitochondrial DNA which produce immunogenic neoepitopes (Duinsbergen et al., 2009; Seminatore et al., 2010; Nori et al., 2015; Deuse et al., 2019). Even if attempts to remove undifferentiated iPSCs from differentiated NSCs are successful, the risk of tumor formation is not completely eliminated and caution needs to be taken (Itakura et al., 2017). Furthermore, graft rejection is still a major unresolved issue in the field (Simonson et al., 2015). Despite major advances in stem cell biology, the field has not yet overcome this hurdle. Findings from pre-clinical animal models have revealed a critical need to utilize genetically immunodeficient mice and/or a strict regimen of immunosuppressive therapy in parallel to stem cell transplants to ensure graft survival (Swijnenburg et al., 2008; Pomeshchik et al., 2015). Therefore, extensive pre-clinical studies must be performed to ensure good manufacturing practices (GMP) are established and upheld to ensure long-lasting transplant survival and limited graft rejection in the clinical setting. Lastly, it is important that transplanted cells possess an intact and stable genome, however it is currently unknown how iPSC reprogramming affects genomic and epigenomic stability. Some research has suggested that long-term expansion of cells and cellular reprogramming can alter the genetic stability and introduce dangerous genetic aberrations in the cells (Zhang et al., 2018). It is therefore important to continue research on optimizing cellular reprogramming and establishing standards to check for genomic integrity before clinical applications. New technology within the past couple of years has been developed which generates directly induced NSCs from fibroblasts, bypassing the iPSC stage, named induced NSCs (iNSCs) (Thier et al., 2012). These cells therefore avoid the ethical implications associated with using fetal tissue and eliminate the pluripotent tumorigenic state. Current work has deemed rodent iNSCs to be safe when transplanted into a mouse model of MS, and promote anti-inflammatory activity (Peruzzotti-Jametti et al., 2018). Future work is being carried out on human iNSCs to determine their safety and

Table 2
Human NSC clinical trials for neurodegenerative diseases.

Start Year	Sponsor	Trial Location	Disease/Injury	Clinical Phase	Cell Therapy	Identifier	Results	Reference
2013	Neuralstem Inc.	US	Spinal cord injury	I	NSCs	NCT01772810	Spinal grafting of cells was found to be safe	(Curtis et al., 2018)
2014	StemCells, Inc	US and Canada	cervical spinal cord injury	II	HuCNS-SC (fetal, brain-derived NSCs from single donor); transplanted into spinal cord	NCT02163876	Safe and well-tolerated Some motor function increase Study terminated based on business decision	(Levi et al., 2019)
2016	Chinese Academy of Sciences	China	thoracic and cervical spinal cord injury	I/II	NSCs with NeuroRegen Scaffold	NCT02688049	NA, estimated completion December 2019	
2017	University of Zurich	Switzerland	thoracic spinal cord injury	I/II	HuCNS-SC	NCT03069404	NA, estimated completion April 2021	
2010	ReNeuron Limited	UK	Stroke	I	NSCs	NCT01151124	Implanted cells induced no adverse events	(Kalladka et al., 2016)
2017	Suzhou Neuralstem Biopharmaceuticals	China	Ischemic stroke	I	NSCs transplanted into peri-infarct area	NCT03296618	Transplantation was well tolerated Preliminary clinical benefits	(Zhang et al., 2019)
2018	ReNeuron Limited	US	Stroke	II	NSCs	NCT03629275	NA, estimated completion May 2020	
2013	Tisch Multiple Sclerosis Research Center of New York	US	Progressive multiple sclerosis	I	Autologous MSC-derived NSCs	NCT01933802	Intrathecal dosing of cells was well tolerated, improvement in neurological disability noted in some patients	(Harris et al., 2018)
2017	Tisch Multiple Sclerosis Research Center of New York	US	Progressive multiple sclerosis	II	Autologous MSC-derived NSCs	NCT03355365	NA, estimated completion November 2023	
2017	IRCCS San Raffaele	Italy	Progressive multiple sclerosis	I	NSCs	NCT03269071	NA, estimated completion August 2020	
2017	Casa Sollievo della Sofferenza IRCCS	Italy and Switzerland	Secondary-progressive multiple sclerosis	I	NSCs	NCT03282760	NA, estimated completion February 2021	
2015	Cyto Therapeutics Pty Limited	Australia	Parkinson's disease	I	ISC-hpNSC injected into striatum and substantia nigra	NCT02452723	NA, estimated completion June 2020	
2017	Chinese Academy of Sciences	China	Parkinson's disease	I	ESC-derived NSCs implanted in striatum	NCT03119636	NA, estimated completion December 2020	
2012	Neuralstem Inc.	US	ALS	II	NSCs	NCT01730716	Safe grafting of cells, no indication of improved survival	(Goutman et al., 2018)
2012	Azienda Ospedaliera Santa Maria	Italy	ALS	I	NSCs	NCT01640067	Safe grafting of cells with no adverse effects	(Mazzini et al., 2019)

Abbreviations: NA, not available; ISC, International Stemcell Corporation; hpNSC (human pathogenic NSC); iPS, induced pluripotent stem; MSC, mesenchymal stem cells.

tolerability in mice.

2.1. Spinal cord injury

Decades of spinal cord injury (SCI) research has made it abundantly clear that the ability to design treatments that induce long-distance regeneration of injured axons to improve function has remained elusive. This development is hampered on two fronts: by the limited regenerative capabilities of nervous tissue and the inability to replace lost and injured neurons. However, accumulated evidence from explorative studies of rodent NSC transplants into rodent SCI models have shown tremendous therapeutic benefit via neurotrophic growth factor production and secretion promoting axonal regeneration, modulating the immune response, and preserving host tissue (Ogawa et al., 2002; Teng et al., 2002; Lu et al., 2003; Ziv et al., 2006; Cusimano et al., 2012; Hawryluk et al., 2012). This has led to the investigation of whether human NSC xenografts are also capable of beneficial growth factor production and secretion in pre-clinical SCI animal models as a promising next step in the development of NSC based therapies for human benefit (Iwanami et al., 2005; Yamane et al., 2010).

Recent studies have investigated the beneficial effect of xenotransplantation of hNSCs in a rat compression injury model of SCI, which causes inflammation and some neuronal death (Amemori et al., 2015; Romanyuk et al., 2015; Ruzicka et al., 2017; Karova et al., 2019). In one study, human induced pluripotent stem cell-derived NSCs (hiPSC-NSCs) (5×10^5 cells) were transplanted into the injury epicenter one week following SCI and animals were observed for up to 17 weeks. Remarkably, animals displayed a rapid and significant motor improvement as early as two weeks after hiPSC-NSC transplantation, reaching a plateau at eight weeks post-transplantation with sustained benefit until the end of the observation period. In contrast, rats receiving saline injections exhibited minimal functional recovery beyond the spontaneous recovery typically observed in this SCI model. Morphometric evaluation of the spinal cord grey and white matter revealed a robust sparing of tissue in hiPSC-NSCs transplanted rats, with a significant increase in tissue volume observed two months after SCI (Romanyuk et al., 2015). In wanting to determine the source of neurotrophin production (i.e., endogenous vs. exogenous), gene expression analysis of rat and human brain derived neurotrophic factor (*Bdnf*), vascular endothelial growth factor (*Vegf*), nerve growth factor (*Ngf*), neurotrophin-3 (*Nt3*), fibroblast growth factor 8 (*Fgf8*), and glial cell line-derived neurotrophic factor (*Gdnf*) were assessed at eight weeks post-transplantation in injured host tissue. These specific neurotrophins were profiled due to their known roles in promoting neuronal and glial maturation (Habtemariam, 2018). Surprisingly, gene expression of rat neurotrophins did not change following hiPSC-NSC transplantation, rather the human genes *NGF*, *FGF8*, and *GDNF* were significantly upregulated in the host tissue compared to mRNA levels of pre-differentiated hiPSCs (Fig. 1, Table 1) (Romanyuk et al., 2015). The results from this study suggest that transplantation of hNSCs does not influence the expression of host/endogenous neurotrophins in the injured rat spinal cord. Rather, the evidence points towards exogenous hNSCs as capable of long-term graft survival and maintaining an active gene expression profile for neurotrophin production to provide a permissive environment for both exogenous and endogenous axonal sprouting and functional recovery following SCI.

In a follow-up study by Amemori *et al.*, rats with a compression induced SCI in the thoracic region were given an intrathecal injection of hiPSC-NSCs (5×10^5 cells) at a site distal (caudal) to the lesion epicenter one week following the injury (Amemori et al., 2015). Moderate functional improvements were observed in rats receiving intrathecal xenografts. However intrathecal hiPSC-NSCs had poor long-term survival being undetectable at the site of administration or in the spinal cord tissue. Further, while improvements of white matter sparing and increased axonal sprouting were observed in these animals, endogenous and exogenous expression of neurotrophic growth factors was absent (Amemori et al., 2015). Thus, despite moderate therapeutic benefit of intrathecally transplanted hiPSC-NSC, the absence of long-term engraftment and survival has revealed a critical need to identify the optimal route and site of administration.

Transplanted rNSCs have been shown to possess potent immune regulatory properties *in vivo* after transplantation into an animal model of SCI (Cusimano et al., 2012). To determine if hNSCs have similar immunomodulatory capabilities, Ruzicka *et al.* compared NSCs derived from hiPSCs or an immortalized spinal fetal line (SPC-01) in a pre-clinical compression induced SCI model in rats (Ruzicka et al., 2017). One week following SCI, hiPSC-NSCs (5×10^5 cells) or SPC-01 (5×10^5 cells) were transplanted intralesionally. Both cell types showed high survival at two months post-engraftment and resulted in improved functional recovery, while only the hiPSC-NSC treated animals had significant white matter sparing. Further, both hiPSC-NSC and SPC-01 transplants resulted in reduced astrogliosis surrounding the central lesion area as evidenced by a smaller GFAP $^{\pm}$ volume. On the other hand, only hiPSC-NSC treated spinal cords showed a down-regulation of CD86 and CD163, markers of M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages, respectively, highlighting the potential of transplanted hNSCs to modulate reactive astrocytes and microglia within the lesioned microenvironment. Analysis of cytokine and chemokine expression from lesioned spinal cord tissue following transplanted hiPSC-NSCs and SPC-01 s revealed a decrease in pro-inflammatory cytokines TNF-α (Ousman and David, 2001), IL-1β (Eskan et al., 2008), and IL-2 (de Rham et al., 2007) and chemokines MIP-1α (Ousman and David, 2001) and CCL5 (i.e. RANTES) (Lin et al., 2011) and an increase in anti-inflammatory factors IL-4 (Fenn et al., 2014) and IL-12p70 (Yaguchi et al., 2008) (Fig. 1). This suggests the immunomodulatory potential of hiPSC-NSC and SPC-01 xenografts in shaping the lesion microenvironment towards a more permissive state for regeneration of damaged tissue.

Further investigation into the immunomodulatory effect of transplanted NSCs focused on the NF-κB pathway (Karova et al., 2019). NF-κB is an inducible transcription factor that mediates immune and inflammatory responses (Pizzi et al., 2002) and inhibition of this pathway has been shown to improve functional recovery after SCI (Brambilla et al., 2005). Rats were subjected to a compression-induced SCI and one week after injury SPC-01 s (5×10^5 cells) were transplanted intralesionally. A significant reduction in both TNF-α production and nuclear translocation of NF-κB was observed in the rats receiving transplants (Karova et al., 2019). This resulted in increased tissue sparing and reductions in astrogliosis, a cell type known to have increased NF-κB activation in SCI (Brambilla et al., 2005). This was attributed to a paracrine effect of transplanted SPC-01 s in modulating the immune response, however no further profiling of the transplanted cells secretome was performed to provide conclusive evidence.

All of these studies point to potentially important interactions between transplanted hNSCs and the lesion environment to reconfigure the deleterious inflammatory state to protect and promote tissue regeneration (Fig. 1). However, there is a severe lack of mechanistic findings of immune modulation following human xenografts in rodent models of disease, in part due to the heavy use of immunodeficient animals to assess functional and molecular outcomes. In fact, studies rely almost exclusively on superficial, indirect correlative cause and effect relationships to establish an immunomodulatory role for transplanted cells. Another caveat that is poorly addressed in the field is the potential for opportunistic infections directed at the human xenografts. As current pre-clinical models rely on immunodeficient or immunosuppressed (immune competent) animals living in ultra-clean environments, the risk of infection is rare. Therefore, future studies are warranted to assess these questions to provide a more While the described findings of transplanted hNSCs in SCI are promising and hold great potential for their use as an adjunctive therapy, a more intense and deeper investigation into the mechanisms driving these cells is needed to make this potential become a reality.

2.2. Ischemic stroke

The global burden of stroke is immense, being the second leading cause of death and major disability worldwide (Mozaffarian et al., 2016), with rising incidence rates due to an ever-increasing aged population (Sander et al., 2015). Despite intensive efforts to develop treatments for improved post-stroke care, effective treatments are limited and do little to address post-stroke injury, which leads to extensive CNS damage, including inflammation, erosion of blood–brain-barrier (BBB) integrity, neuronal death, and substantial loss of tissue volume (Lipton, 1999; White et al., 2000; Mena et al., 2004; Besancon et al., 2008; Kulik et al., 2008; Iadecola and Anrather, 2011; Petrovic-Djergovic et al., 2016). Thus, to develop fully applicable and effective therapeutic strategies for the regeneration of post-stroke ischemic tissue, therapies must be designed to interact with and modulate multiple mechanisms of action to target multiple pathways. Previous attention has focused on the identification of targets to promote neurogenesis from endogenous NSCs, however it is now known the endogenous pool of NSCs is not capable of supplying enough cells to meet the demands required for the repair of neurological damage (Arvidsson et al., 2002; Kokaia and Lindvall, 2003; Thored et al., 2006; Pluchino et al., 2008). Therefore, the supplemental engraftment of exogenous hNSCs into the post-stroke brain is an attractive option to provide immunomodulatory, neurotrophic, and reparative support (Boese et al., 2018; Hicks et al., 2013; Huang et al., 2014; Eckert et al., 2015; Ryu et al., 2016; Bernstock et al., 2017; Lee et al., 2017).

The middle cerebral artery occlusion model (MCAO) is a thoroughly validated pre-clinical model of ischemic stroke that is extensively used to investigate the post-stroke brain (Canazza et al., 2014). Utilizing this model, Huang *et al.* transplanted human fetal NSCs (1×10^5 cells) into the mouse hippocampus 24 h following MCAO to determine the potential benefit on post-stroke outcomes (Huang et al., 2014). Mice receiving hNSC transplants showed rapid, significant improvements in behavioral outcomes, most notably within the first week post-transplantation. Improved behavioral outcomes were concomitant with reductions in infarct volume and BBB damage (Huang et al., 2014). A significant increase in the production of BDNF, a major neurotrophin known to promote functional recovery and neuroprotection after stroke (Emanueli et al., 2003), was also observed in the lesioned cortex in hNSC transplanted animals compared to non-transplanted, suggesting that the tissue preservation effects of transplanted hNSCs works through stimulating BDNF secretion. Tissue preservation was also attributed, in part, to significant reductions in the expression of pro-inflammatory cytokines *Tnf-α*, *Il-6*, and *Il-1β*, cell adhesion molecules ICAM-1 and VCAM-1, and chemokines *Mcp-1* and *Mip-1α*, as well as dampened inflammation and microglia/macrophage activation, suggestive of an immunomodulatory effect of hNSC transplants in post-stroke recovery (Fig. 1). A follow-up study from the same team using hiPSC-NSC transplants (1×10^5 cells) 24 h following MCAO in mice identified a similar immunomodulatory and neurotrophic profile (Huang et al., 2014; Eckert et al., 2015). As well, decreased levels of IgG and matrix metalloproteinase (MMP) activity was observed in rodents receiving hNSCs (Eckert et al., 2015). The presence of parenchymal IgG and increased MMP activity is associated with the dysfunction of tight junctions between endothelial cells that comprise the BBB, which suggests improved BBB integrity following hNSC transplantation (Yang et al., 2007; Chen et al., 2009).

Additional studies using the MCAO model in rats has provided further evidence of a potent paracrine effect of transplanted hNSCs. For example, Lee *et al.* found that delayed transplantation (7 days post-stroke) of hiPSC-NSCs (1×10^6 cells) into post-stroke rats resulted in significantly lower amounts of Iba-1 $^{\pm}$ and ED1 $^{+}$ microglia cells, reduced expression of glial fibrillary acidic protein (GFAP) positive astrocytes, and increased angiogenesis (Lee et al., 2017). Enhanced angiogenesis seems to be a common effect of transplanted hNSCs (Hicks et al., 2013; Ryu et al., 2016; Lee et al., 2017). A similar study also identified increased angiogenesis in a rat MCAO model following transplantation of hNSCs (4.5×10^5 cells), which was attributed to the increased production and secretion of pro-angiogenic factors VEGF, basic fibroblast growth factor (bFGF), and epidermal growth factor (EGF) as evidenced by *in vitro* biochemical analysis (Fig. 1, Table 1) (Hicks et al., 2013). Transplanted hNSCs have also been suggested to influence the proliferation and differentiation of endogenous NSCs. Ryu *et al.* found that hNSCs (6×10^5 cells) transplanted 24 h post-stroke into rats were capable of stimulating the proliferation of endogenous rNSCs in the subventricular zone and dentate gyrus of the ischemic hemisphere. Further, enhanced differentiation of endogenous rNSCs into neurons and GFAP $^{+}$ astrocytes within these regions was also observed (Ryu et al., 2016). Surprisingly, there was a complete absence of hNSCs observed within these regions using human nuclear antigen staining, indicating that transplanted hNSCs did not migrate to these brain regions. This suggests that hNSCs acted through a paracrine fashion to promote proliferation.

2.3. Alzheimer's disease

There are approximately 50 million people living with dementia resulting in an estimated global cost of care upwards of US$818 billion. However, due to national demographics identifying a rapidly aging global population, the number of people with dementia is anticipated to rise to 132 million by 2050 (Alzheimer's disease facts and figures, 2015; Alzheimer's disease facts and figures, 2016). Alzheimer's disease (AD) is the most commonly associated pathology with dementia and has been the focus of intense investigative efforts to develop clinically useful therapies to combat the significant social, economic, and medical impacts of this disease. As mentioned previously, the use of exogenous hNSC-based therapies to restore degenerated neuronal networks has seen success in SCI and stroke, with similar studies occurring in rodent models of aging and AD (Duncan and Valenzuela, 2017). For example, hNSCs genetically engineered to over-express choline acetyltransferase (ChAT) and transplanted into aged mice improved their cognitive function and physical activity (Park et al., 2013). Park and colleagues used an immortalized NSC line that were previously developed from a 15-week old human fetus and infected the cells with a retrovirus to induce over-expression of the human ChAT gene. In AD patients decreased activity of ChAT is observed, which is responsible in acetylcholine (ACh) synthesis (Terry and Buccafusco, 2003). This is associated with the dysfunction of the presynaptic cholinergic system, therefore causing cognitive deficits (Coyle et al., 1983). Enhancing ACh concentration in AD is a goal of many treatments (Terry and Buccafusco, 2003), as a result modifying hNSCs to over-express ChAT and transplanting these cells may be a viable therapeutic.

Aged (18 months) wild type outbred ICR mice received 4×10^5 hNSCs-ChAT via intracerebroventricular injection without immunosuppression. Aged animals (18 months) typically show severe impairment in learning and memory function, but 4 weeks after transplant demonstrated significant improvement in behavioral and cognitive functions compared to their aged counterparts. 5 weeks after transplantation there was a significant increase in ACh concentration and the transplanted cells were found to differentiate into neurons and astrocytes. This was associated with higher levels of BDNF and NGF in the aged animals, along with increases in cholinergic system genes, which are normally down regulated in aged animals. This study demonstrates how genetically engineering the secretome of hNSCs can aid in the restoration of age-related changes, but also provide an increase in neuroprotective factors such as BDNF and NGF. However, the long-term effects, including if the increase in ACh is long term, of the transplanted genetically engineered hNSCs are unknown.

Another example is a study which used human cortical NSC lines, derived from fetal tissue, and transfected the cells with a lentiviral vector to induce expression of insulin-like growth factor-1 (IGF-1) (McGinley et al., 2016). IGF-1 is a trophic factor that is essential for

neural development and function and exerts neuroprotective effects on neurons (Russo et al., 2005). Reduced levels have been associated with cognitive decline (Miltiadous et al., 2011). *In vitro* experiments assaying amyloid-β toxicity in neurons co-cultured with the IGF-1 hNSCs using *trans*-well inserts revealed a neuroprotective paracrine mechanism compared to non-modified hNSCs co-culture and no co-culture (McGinley et al., 2016).

The double transgenic AD mouse model, APP/PS1, which expresses a chimeric mouse/human amyloid precursor protein (APP) and a mutant human presenilin (PS1) protein was used. These mutations are associated with early-onset AD and cause AD-like pathology in mice. At 11 weeks of age mice were given bilateral injections into the hippocampus at 3 sites, injecting a total of 180,000 cells, accompanied with immunosuppressive treatment using tacrolimus. 10 weeks post-transplantation the grafted cells were found in the hippocampal region of the AD mice and were differentiated into neurons (McGinley et al., 2016). The study did not further investigate if the transplantation improved behavior or AD pathology in the mice but provided feasibility that the genetically modified human cells are safe when transplanted into rodents and can integrate and differentiate after 10 weeks. Further work is needed to understand how the transplanted hNSC-IGF-1 integrate within the AD-like environment and how their elevated secretion of IGF-1 can potentially provide neuroprotection.

Lastly, Lee et al. transplanted human NSCs derived from fetal tissue into the transgenic NSE/APPsw AD mouse model, which carries the mutated human APP gene (Lee et al., 2015). hNSCs were injected ($1x10^5$) bilaterally into the lateral ventricles, and mice received immunosuppression (cyclosporine A). 5 weeks after transplantation the mice had improved spatial memory and demonstrated a decrease in tau phosphorylation, Aβ42 levels, and gliosis, which are all highly upregulated in this AD mouse model (Lee et al., 2015). 7 weeks after transplantation the hNSCs engrafted, migrated widely from the transplantation site, and differentiated mostly towards immature neurons. *In vitro* work demonstrated that the hNSCs secreted trophic factors, including BDNF, NGF, and VEGF, and in parallel these factors were found increased in the transplanted mice and believed to be associated with the decrease in tau phosphorylation (Fig. 1). In addition, human neuroblastoma cells expressing the mutated APP gene treated with hNSC CM decreased the production of Aβ. Moreover, co-culturing hNSCs with inflammatory microglia in a transwell system decreased inflammation through the secretion of anti-inflammatory factors (TGFβ1, IL4, IL13). Nevertheless, exactly what factors are involved in attenuating the glial responses in the AD mice with transplants is unknown. Overall, this study implicates the regenerative and protective effect of the hNSC secretome *in vitro* as well as *in vivo* and provides proof of principle that these cells can be transplanted safely in rodents and migrate and integrate within the CNS (Lee et al., 2015).

The complete molecular mechanisms that mediate the positive effects described in aged as well as AD-like mice with hNSC transplants have not yet been fully realized, but the likely cause is the paracrine release of neurotrophic factors, such as BDNF, NGF, and VEGF (Table 1) (McGinley et al., 2016; Lee et al., 2015), which further supports a beneficial effect of transplanted hNSCs in AD (Fig. 1). Additionally, more work to understand the factors within the hNSC secretome that provide the greatest benefit for dementia and AD pathology can be harnessed through genetic-engineering to exploit the natural processes.

2.4. Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease affecting adults after AD (de Rijk et al., 2000). PD is associated with both motor and non-motor dysfunction and involves the substantial loss of dopaminergic neurons (DA) in the substantia nigra throughout the course of the disease (Cheng et al., 2010). Despite PD patients having access to established therapeutic approaches, such as drug treatment (L-DOPA) (Fahn et al., 2004) and deep-brain stimulation (Limousin et al., 1995), PD is an incurable disease and these treatment strategies only temporarily alleviate the associated clinical symptoms. Theoretically, replacing DA neurons in the striatum using fetal grafts could offer a cure for these patients, but these trials have faced difficulties. Tissue from human fetal ventral mesencephalon (FVM) has been used a source of DA cells grafted into patients. Remarkably these cells were shown to promote clinical benefits in these patients, and at postmortem were shown to integrate and survive for decades (Wenning et al., 1997; Hallett et al., 2014; Li et al., 2016). However, there was a high incidence of graft-induced dyskinesia reported, attributed to the presence of serotoninergic neurons within the transplant (Politis et al., 2011). In addition, in both a rat PD model (fetal rat tissue transplanted into rats with PD injury) and in patients grafted with fetal tissue it was shown that a host-to-graft transfer of disease can occur. In rats it was found that the grafted cells can take up host-derived α-synuclein, express the protein which can then aggregate in the nucleus to form Lewy bodies (Kordower et al., 2011). Interestingly, this study infected the rats with green fluorescent protein (GFP) as a control for the α-synuclein and found no transplanted cells expressing GFP; only α -synuclein transferred to the grafted cells (Kordower et al., 2011). Fetal cells transplanted in PD patients were found to last up to 16 years, but were found to develop α-synuclein-positive Lewy bodies (Li et al., 2008). Grafting of fetal neuronal tissue may therefore be limited and lead to failure in long-term transplantations.

It was also believed that these trials, using FVM, used a sub-optimal amount of fetal tissue (Freed et al., 2001; Olanow et al., 2003). Although these results are promising, they still pose concerns, including the ethical and logistical challenges that are associated with grafting FVM. There is a limited amount of fetal tissue to supply the amount of patients with PD. In addition, these grafts did not contain a pure population of DA cells, inducing dyskinesia. Therefore, re-focusing efforts on the utilization of NSCs, which offer scalability and ease ethical concerns (fibroblast-derived), may hold more promise (Monni et al., 2014; Choi et al., 2017;18.).

One paper from 2018 has investigated the secretome of human NSCs in the 6-hydroxidopamine (6-OHDA) model of PD (Mendes-Pinheiro et al., 2018). 6-OHDA, when injected, selectively destroys DA and noradrenergic neurons and is therefore used as a model of PD. Human NSCs were derived from the cortex of a 13-week old fetus that were then serially subcultured (Baghbaderani et al., 2010). 6-OHDA was unilaterally injected directly into the medial forebrain bundle of rats and 5 weeks later the animals received hNSC transplants or hNSC secretome (conditioned media) injected directly in the substantia nigra pars compacta (SNpc) and into 4 striatum coordinates. 200,000 cells were injected into the SNpc and 50,000 cells into each coordinate of the striatum. hNSC conditioned medium was collected after 24 h on the cells. Interestingly, motor performance of the animals, evaluated at 1, 4, and 7 weeks after treatment, was significantly improved in the group treated with the hNSC conditioned media (CM) when compared with both the untreated 6-OHDA group and hNSC-transplanted group (Mendes-Pinheiro et al., 2018). Histological analyses of tyrosine hydroxylase positive neurons (DA neurons) 13 weeks after the lesion, in both the SNpc and striatum, revealed an increase in cell survival in only the hNSC CM group (Mendes-Pinheiro et al., 2018). Whether the hNSC CM provided support to help promote neuronal survival or provide regeneration after injury remains unanswered. Very little to no recovery in motor performance and TH neuron survival was seen in the animals injected with hNSCs due to a very low rate of survival of cells upon transplantation, which could be caused by a lack of immunosuppressive treatment. Other work using iPS-derived human NSCs injected into the same rat PD model with immunosuppression showed successful transplantation along with improvement in motor disability, but did not investigate the secretome (Han et al., 2015).

In order to identify factors in the hNSC secretome the researchers used a non-targeted proteomic analysis based on a combined mass

spectrometry (MS) approach. They identified the well-known and expected neurotrophic factors GDNF and BDNF, which both support the growth and survival of mature neurons in PD models (Fig. 1, Table 1) (Baquet et al., 2005; Falk et al., 2010). Analyzing their proteomic data they identified the majority of secreted proteins to be involved in the cellular process and metabolic process. This includes the secretion of antioxidant agents, such as TrxR1, Prdx1, and SOD enzymes, which have been implicated in DA neuronal survival in PD models (Lee et al., 2008; Arodin et al., 2014; Filograna et al., 2016). Overall, this is one of the first papers to directly demonstrate the neuroprotective and regenerative qualities of the human NSC secretome in a rodent PD model (Mendes-Pinheiro et al., 2018). Further investigation of the hNSC secretome, and how it interacts with the environment, is imperative to understand the complicated molecular pathways towards the clinical utility of these cells.

2.5. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal, adult onset neurodegenerative disease and the most common motor neuron disease in adults (Cleveland and Rothstein, 2001). It is characterized by the selective degeneration of both upper and lower motor neurons of the primary motor cortex and brainstem and spinal cord, respectively (Comley et al., 2015). 90–95% of ALS cases are sporadic, while the remaining 20%, familial, are linked to differing point mutations in the Cu/Zn superoxide dismutase 1 gene (SOD1). Currently, only one drug has been approved by the United States Food and Drug Administration (FDA) for the treatment of ALS (Gurney et al., 1998), however it does not stop the progression of the disease. Therefore, a critical gap in available treatment options for ALS patients exists. Based upon the pathology of disease involving the degeneration of motor neurons, transplanted hNSCs ideally offer the potential to provide trophic support as well as a cell replacement strategy.

Two recent studies have explored the therapeutic potential of transplanted hNSCs in rodent models of ALS (Popescu et al., 2013; Kondo et al., 2014). This first study generated hNSCs from fibroblast-derived iPSCs. SOD1 rats, which harbor a mutation in the gene which causes ALS-like pathology and symptoms, were injected with 10^5 hNSCs in the ventral horns of the lumbar spinal cord (L4-L5) before the onset of disease (3 months old) and maintained on immunosuppression (Popescu et al., 2013). Mutations in the *SOD1* gene account for 20% of familial, genetic ALS and some sporadic ALS cases (Rosen et al., 1993). Rodents with SOD1 mutations develop severe degeneration of motor neurons, which leads to the progressive paralysis of the hindlimbs and forelimbs, and eventually death (Browne and Abbott, 2016). 60 days after transplantation the cells were found to differentiate into mature neurons. This study provided evidence that iPSC-derived hNSCs can be safely transplanted into rodents with ALS-like disease but is limited as the transplants were performed before the onset of symptoms, and the study did not analyze behavior or disease-induced pathology.

A similar study by Kondo *et al.* generated hNSCs from iPSCs and differentiated these cells using leukemia inhibitory factory (LIF)/bone morphogenic protein (MBP) signaling into what they termed hiPSC-GRNPs (glial-rich neural progenitors). This protocol generated an enriched population of NESTIN positive neural stem cells (68.4% of the population) and the other half positive for GFAP, which they remarked to be immature astrocytes. Previous work demonstrated that rodent astrocyte transplant into a mutant SOD1 rat promoted neural protection so this study wanted to determine if an enriched glial population from human cells could do the same (Xu et al., 2011). Along with immunosuppressive treatment they transplanted 40,000 hiPSC-GRNPs bilaterally into the lumbar section of spinal cords of 90 day old mutant SOD1 mice (Kondo et al., 2014). At this age the mice have already started demonstrating disease progression (Kondo et al., 2014). 10–40 days after transplantation mice showed improvement in their clinical motor score and lifespan was extended by 7.8% compared to the non-transplanted SOD1 mutants. At the end stage of disease progression (140–170 days; 50–80 days post-transplant) the transplanted hiPSC-GRNPs were found to survive in the lumbar spinal cord. Interestingly compared to the first paper discussed above (Popescu et al., 2013) the majority of the transplanted cells differentiated into astrocytes, while differentiation into neurons or oligodendrocytes was low. In order to investigate the factors secreted by the transplanted hiPSC-GRNPs, human-specific primers were generated to assay mRNA in the lumbar spinal cord tissue (Kondo et al., 2014). Only expression of human *VEGF* was found increased in the host tissue, while injection of the human cells increased endogenous expression of *Vegf*, *Nt3*, and *Gdnf* (Fig. 1, Table 1). Furthermore, transplantation of hNSCs increased phosphorylated AKT in the tissue, downstream of VEGF signaling, which has previously been found to be important for cell survival in ALS (Lunn et al., 2009). Although this study only showed modest benefits after human cell transplantation in mice with ALS-like disease, it demonstrated that iPSC-derived GRNPs were safe and tolerated in these mice.

These two studies offer important insights in how hNSCs could be used to treat ALS. Further work needs to be carried out to establish how the secretome from transplanted hNSCs can promote neuroprotection, and hopefully regeneration in this devastating disease. In addition, it is critical to study in these rodent models how long-term transplantation of these cells can affect disease progression and the ideal time to transplant these cells, whether it be before disease onset or during disease progression.

2.6. Multiple sclerosis

Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS and is the most common inflammatory neurological disease leading to non-traumatic disability in young adults worldwide (Global, regional, and national burden of multiple sclerosis, 2016; Wallin et al., 2019). MS is characterized by the formation of lesions (demyelination of neurons) within the white matter of the brain and spinal cord and the grey matter of the cortex, which leads to a gradual accumulation of neurological disabilities (Bo et al., 2006; Calabrese et al., 2013; Lublin et al., 2014). MS patients are broadly sub-divided into two clinically distinct disease phenotypes: the relapsing-remitting (RR) course, which is followed by a progressive phase (secondary progressive), and the primary-progressive course, when progression proceeds in the absence of preceding relapses or remissions (Lublin et al., 2014). Demyelination in MS is in part caused by immune cells, T cells, that gain entry into the CNS, recognize myelin as foreign and destroy it. It is unknown how MS is caused, but the autoimmune component of the disease has been hypothesized to be due to genetics and environmental factors (ie. exposure to certain viruses, geographic location) (Compston and Coles, 2008). A wide-range of disease-modifying therapies (DMTs) exist to slow the progression of the disease to improve the quality of life for patients, but the disease still progresses with no cure (Thompson et al., 2018). Despite the chronic demyelination that occurs throughout the natural history of the disease, the neural tissue, seemingly, remains remarkably responsive to neurotrophic and neurorestorative cues to initiate regeneration of the damaged tissue (Franklin and Ffrench-Constant, 2008; Franklin and Ffrench-Constant, 2017). Therefore, transplantation of human NSCs in these patients may offer a restorative therapy through two potential mechanisms: altering immune cell functioning and promoting neuroprotection and regeneration.

The establishment of NSCs as a viable therapeutic option in the treatment of MS has been well-established using rNSCs (Pluchino et al., 2003; Peruzzotti-Jametti et al., 2018). For example, previous work has indicated that transplanted rodent NSCs can secrete trophic factors such as LIF, which can promote endogenous regeneration in rodent models of MS, and can help buffer accumulated metabolites (Peruzzotti-Jametti et al., 2018; Laterza et al., 2013). Given this foundation, recent

evidence from hNSC transplants into rodent models of MS have further strengthened this line of investigation (Peruzzotti-Jametti et al., 2018; De Feo et al., 2017). Two recent studies have investigated the ability of transplanted hNSCs to dampen the immune response via the secretion of anti-inflammatory factors and induce a pro-regenerative environment in a viral model of MS (Chen et al., 2014; Plaisted et al., 2016).

Chen et al., generated hNSCs from a human embryonic stem cell line and injected these cells into mice with a viral model of MS (Chen et al., 2014). These mice were injected with a mouse strain of hepatitis virus (JHMV) which results in an immune-mediated demyelination with clinical and histologic similarities to MS. Destruction of myelin in this model occurs due to the activation of T cells within the CNS caused by the virus. Mice were infected intracranially and were transplanted with 2.5×10^5 hNSCs at T9 of the spinal cord 14 days after infection without immunosuppression (Chen et al., 2014). Injection of the hNSCs resulted in a reduction in the severity of the clinical disease that was sustained out to 6 months after transplant. Interestingly, the hNSCs were found to survive only for 1 week after transplantation, but were found to dampen the presence of T cells along with activated macrophage/microglia and reduce severity of demyelination within the spinal cord 21 days after transplantation (Chen et al., 2014). Pathological and clinical improvement was associated with an increase of regulatory T cells (Tregs), which suppress the immune system, in the hNSC transplanted mice. The hNSC secretome was attributed to causing a long-lasting response on the activated T cells, even though the transplanted cells were found to be transient within the tissue (lasting only a week). Conditioned media from the hNSCs revealed an increase in TGF-β1 and TGF-β2, both anti-inflammatory cytokines (Fig. 1). Neutralizing antibodies specific for TGF-β1 and TGF-β2 blocked the ability of hNSC conditioned media to inhibit proliferation of activated T cells. This study demonstrates how the secretome from hNSCs can have a long-lasting effect on the microenvironment by modulating activated immune cells (Chen et al., 2014). Further understanding the impact of the hNSC secretome in this model can help in the development of future therapeutics for this disease.

In the same rodent model of MS, Plaisted and colleagues transplanted 250,000 hNSCs, derived from a human iPSC line, in the spinal cord at T8-T9 14 days post injection without immunosuppressive treatment (Plaisted et al., 2016). Just as the aforementioned study, hNSCs were undetectable 8 days after transplantation, but at 21 days post transplantation the animals displayed reduced demyelination. In addition, they found restriction of inflammatory T cell migration into the spinal cord with a concomitant increase in regulatory anti-inflammatory T cells, associated with increased secretion of TGF-β1 and TGF-β2 (Fig. 1) (Plaisted et al., 2016). These 2 studies provide a proof of concept that hNSCs, derived from either embryonic stem cells for iPSCs, are able to be transplanted safely and provide beneficial effects via secretion of anti-inflammatory cytokines in this viral rodent model of MS (Fig. 1) (Chen et al., 2014; Plaisted et al., 2016).

Despite these promising pre-clinical findings, caution must be used regarding the source of hNSCs. Two recent studies have identified inherent defects and an altered secretome in hNSCs derived from iPSCs generated from patients with primary progressive multiple sclerosis arising from the development of a premature aging phenotype (Nicaise et al., 2017; Nicaise et al., 2019). Therefore, extensive health screenings must be carried out when developing human cells lines for the stable, expandable use of hNSCs in the clinical setting. This work suggests that autologous hNSCs may need to be genetically engineered to secrete factors that are inflammatory as well as pro-regenerative before use in the clinic or banks of healthy human cells could be generated for clinical use. There also is some discrepancy in the pathology of the relapsing form of MS compared to the progressive form, where pro-inflammatory reactive T cells are much more prominent in the relapsing form, while DMTs which target the infiltration of immune cells into the CNS do not provide benefit to progressive MS patients (Compston and Coles, 2008). Therefore, hNSCs could be engineered to secrete factors that benefit pathology in relapsing MS (ie. TGF-β) as well as factors that would benefit regeneration (ie. LIF), which is stalled in progressive MS.

3. Therapeutic efficacy of human NSC-secreted biologics for the treatment of neurodegenerative diseases

The material presented thus far has shown the progress being made in determining the viability of hNSC transplants as a therapeutic strategy. One recurring theme from the recent data is the effect of the secretome from the transplanted hNSCs, which exerts a beneficial and therapeutic effect through the secretion of potent immunomodulatory and/or neurotrophic factors (Drago et al., 2013). This has led to an alternate, but parallel, approach wherein significant repair of the diseased and damaged brain may be achieved via the non-invasive injection of the biologics secreted by hNSCs. Functional and biochemical analysis of the rNSC secretome has revealed diverse and complex properties of rNSC-secreted factors (Cossetti et al., 2014; Shoemaker and Kornblum, 2016; Iraci et al., 2017; Stevanato et al., 2016; Ma et al., 2019); some with therapeutic potential (Rong et al., 2019). This has spurred an interest amongst a growing number of investigators to identify similar properties of the hNSC secretome. Thus, we will focus on recent literature identifying the beneficial properties of hNSC-derived extracellular vesicles (EVs) and conditioned medium (CM) as treatments in pre-clinical models of neurodegenerative diseases.

3.1. Therapeutic potential of human NSC-extracellular vesicles and conditioned media

Broadly, EVs are nano-sized independent signaling vehicles that transport a defined cargo, such as proteins, mRNA, and miRNA, to target cells at local and distant sites (Tkach and Thery, 2016). Despite intensive research into the biology of EVs, their complex biogenesis and physiological role are only now being partially understood (Colombo et al., 2014). An interesting facet of stem-cell EVs is the bidirectional exchange of information between stem cells and target cells, which has been shown to lead to the activation of regenerative programs (Camussi et al., 2013). This remarkable feature of stem-cell EVs, their ability to transfer functional material to other cells, is of particular importance for hNSC-derived EVs in identifying and characterizing the relevant mechanisms with the highest potential for treating complex neurodegenerative diseases (Vogel et al., 2018). Despite the glaring lack of detailed characterization, growing interest into their potential as mediators of cell–cell communication has generated a significant increase in attention from the scientific community. However, to date, only a handful of studies have been conducted highlighting the promise these small vesicles hold.

Two recent studies by Webb *et al.* using a rodent and porcine model of stroke have highlighted beneficial effects of hNSC-EVs (Webb et al., 2018; Webb et al., 2018). Using the MCAO animal model of stroke, labeled hNSC-EVs were intravenously injected during the acute phase of stroke to establish the targeting preference of hNSC-EVs. Imaging at 1 and 24 h after injection revealed efficient targeting of hNSC-EVs to the infarcted region of the brain (Webb et al., 2018). Additional *ex vivo* imaging analysis found a significant decrease in tissue loss in the hNSC-EV treated animals with a concomitant improvement in motor function and episodic memory. Analysis of blood using quantitative flow cytometry during the acute post-stroke phase revealed that hNSC-EVs promoted the polarization of circulating macrophages from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype, as well as increased the induction of Tregs leading to a down-regulation of pro-inflammatory effector Th17 cells (Webb et al., 2018). The findings from these studies revealed the efficacy of hNSC-EVs in providing neuroprotecton, ameliorating the motor and memory impairments, and dampening the inflammation common to this pre-clinical model. After the exciting results generated from the rodent model, the same group set out to investigate the potency of hNSC-EVs in a porcine model of

stroke. Following permanent MCAO, hNSC-EVs were administered at three time points during the acute phase. Consistent with the results from the rodent model, stroked pigs had decreased lesion volume and brain swelling, as well as increased fluid movement through the brain (Webb et al., 2018). hNSC-EV treatment was also found to preserve white matter integrity in the brain when examined three months after stroke. Furthermore, normal exploratory behavior, motor activity and faster recovery was observed in pigs receiving the hNSC-EV injections (Webb et al., 2018). Identification of enhanced recovery in a larger mammalian species is hugely exciting for the potential promise hNSC-EVs hold in the development of clinically relevant therapies. Additionally, results from the both the rodent and porcine studies had similar outcomes when compared to the results generated from the hNSCs transplantation studies.

Progress has also been made in the use of cell-free, conditioned media (CM) from hNSCs for treatment in neurodegenerative diseases. A study by Mendes-Pinheiro *et al.* found that injection of CM from hNSCs into the 6-OHDA rodent model of PD had a neuroprotective effect as evidenced by a significant increase in DA neuron survival, which was outlined above (Mendes-Pinheiro et al., 2018). Despite the study being limited in scope mechanistically, the results indicate that the hNSC secretome in either isolation or conjunction with parallel treatments (I.e. hNSC-EVs or hNSC transplants) could provide immediate and long-term benefit in patients with chronic neurodegenerative diseases.

Currently, it is unknown if hNSC-EVs/CM from different or even identical hNSC lines result in the same therapeutic profile in treating all neurodegenerative diseases. Further, characterization of EVs and CM from the available clinical-grade hNSC lines must be carried out to determine the precise proteomic map and assess functional outcomes in other pre-clinical animal models of neurodegenerative diseases. Expectations must be tempered as more investigative work is required before these future therapies become a reality.

4. Human NSC transplantation in clinical trials

As described above, the pre-clinical animal models of disease have established that exogenous hNSC transplants are a promising tool to promote tissue regeneration in neurodegenerative diseases. Current treatments options for these diseases are either non-existent or limited and only effective at ameliorating symptoms or delaying the progression. All known neurodegenerative diseases are currently incurable. Thus, stem cells are an exciting field that needs to be approached with caution, with correctly designed clinical trials to determine the long-term safety and efficacy. The current pre-clinical animal models using human NSCs are encouraging enough to justify Phase I and II clinical trials in small patient cohorts.

Current clinical trials involving hNSCs are interested in establishing the safety of transplantation, methodology of transplantation, the optimal number of cells to be transplanted, source of the cells, and the collection of initial results detailing changes in disease pathology following transplantation (Table 2). Analyzing the efficacy of stem cell therapies is a challenging determination due to a variety of issues. First, heterogeneity of cohorts is hard to avoid, especially the (i) type and severity of the injury or disease, (ii) when transplantation will occur, and (iii) the current DMTs of individual patients, among many other variables. Secondly, the metrics assessed during follow-up need to be designed to assess if some therapeutic benefit has been reached, which will vary from patient to patient depending on age, type of injury or disease, and when intervention was initiated. Therefore, initial phase I and II clinical trials for hNSC transplantation are critical to the future use of these cells as therapeutics.

4.1. Spinal cord injury clinical trials

Current treatment options for spinal cord injury (SCI) include surgical interventions and rehabilitative care, which does not provide a cure for these patients. Phase I trials using hNSCs in SCI have found spinal grafting of these cells to be safe and well-tolerated (Table 2). These trials have been performed in patients with thoracic as well as cervical injuries and have been done using allogenic (donor tissue) hNSCs, with some lines approved by the FDA (Curtis et al., 2018). These hNSCs have been derived from fetal spinal cord and expanded serially in culture before transplantation. The initial phase I trials have demonstrated that injection in patients with chronic spinal cord injury can be safely performed, with no identified adverse effects 18–27 months post-procedure (Curtis et al., 2018). A prominent phase II trial, that was terminated because it did not reach required clinical efficacy threshold, reiterated the initial findings in patients with cervical spinal cord injury. They found hNSC transplantation to be safe and well-tolerated after a year in patients with chronic cervical SCI, and showed a trend in increased motor performance in the patients, but did not achieve the efficacy needed (Levi et al., 2019). This phase II trial used a proprietary stem cell line termed HuCNS-SCs that have been derived under GMP conditions and are an allogenic line obtained from fetal brain tissue (Tsukamoto et al., 2013). These initial studies in SCI have fallen short in therapeutic expectations but have laid the groundwork to demonstrate the safety of allogenic hNSC transplantation in the spinal cord. Further studies are needed to confirm potential benefits, the proper dosage, the best time to administer cells, the patient population that would benefit the most, and the best source to obtain the hNSCs. In addition, advances in genetic engineering may provide a way to genetically alter hNSCs to secrete specific factors known to support SCI regeneration.

4.2. Ischemic stroke clinical trials

A large number of studies in pre-clinical animal models has shown an improvement in stroke recovery after hNSC transplantation (Huang et al., 2014; Eckert et al., 2015). Most of these studies have shown that these transplanted cells not only replace damaged cells, but also provide trophic support that improves the damaged environment creating a path towards regeneration. The company ReNeuron Limited has initiated both phase I and II clinical trials using allogenic hNSCs derived from fetal tissue to transplant in the putamen region of stroke patients. Their phase I clinical trial reported no cell-related adverse effects a year after transplantation with claims of mild improvements observed in neurological function (Kalladka et al., 2016). Currently, a phase II trial is underway to evaluate the clinical benefit of NSC transplants in patients after stroke (Table 2). A second phase I trial out of China has demonstrated safe transplantation of NSCs in the *peri*-infarct area of ischemic stroke patients, using allogenic hNSCs from fetal tissue. Reported findings are similar to those from the ReNeuron Limited trial, transplants were well-tolerated with no adverse immune reactions. Even though this clinical trial was not designed to evaluate efficacy, the authors claim that preliminary clinical benefits were identified by observing new tissue at the site of injection 24 months after using MRI, but no conclusions can be made on the efficacy of this treatment (Zhang et al., 2019). Extensive work is still required before the implementation of hNSCs into the clinic for stroke patients, and the results of the current phase II trial may provide an understanding on their efficacy in treating patients.

4.3. Alzheimer's disease clinical trials

Based on growing interest, and the continuation of safe and well-tolerated phase I clinical trials using hNSCs, stem cell therapies may be a new hope for Alzheimer's disease patients. In animal models, transplanted hNSCs provide a functional graft, but it is still unknown whether they would be well tolerated and integrated in the host AD brain (Kwak et al., 2018). Preclinical studies using AD animal models have provided promising results, but human clinical trials are still in their infancy. There are still many unanswered questions associated with this disease, and further research will provide a better understanding for

how targeted hNSC therapy could provide regeneration and neuroprotection. Thus far there have been no reported clinical trials investigating the safety and tolerability of hNSCs in AD patients.

4.4. Parkinson's disease clinical trials

In the first phase I clinical trial of its kind, the International Stem Cell Corporation has received approval to inject a new human parthenogenetic derived NSC line (ISC-hpNSC) in patients with Parkinson's disease, which have been cleared by the FDA for clinical use. Parthenogenetic cells are derived from the chemical activation of unfertilized oocytes which generates ES-like cells that can be differentiated in culture (Revazova et al., 2007). This bypasses the ethical concerns associated with fetal tissue. These cells will be grafted next to the caudate nucleus, putamen, and substantia nigra and are primed to be used for providing trophic support and cellular replacement, in contrast to just injecting dopaminergic neurons. Phase I trials using hNSCs in PD have yet to generate any safety data, but these cells were found to engraft safely in a nonhuman primate model of PD and based on previous clinical trials with hNSCs they are expected to be safe and well-tolerated (Table 2) (Gonzalez et al., 2016).

4.5. Amyotrophic lateral sclerosis clinical trials

ALS is currently incurable and only two drugs have been approved, having only modest benefits (Mazzini et al., 2019). Initial phase I clinical trials using fetal hNSCs, expanded *in vitro*, and transplanted into the lumbar spinal cord were found to be safe and well-tolerated (Goutman et al., 2018). Recently, results from a follow up phase I trial have been published (Table 2). This study had a larger patient cohort and used the same line of fetal hNSCs transplanted into the lumbar and cervical spinal cord. After surgery these patients were followed up monthly for a year, and then every 3 months until death. This study determined that the concentration of hNSCs transplanted was safe and well-tolerated, with no immunological reactions. Even though this study was not designed to determine the efficacy of the treatment, it was still a secondary endpoint. Overall the transplantation was not found to extend life, however 50% of the patients reported a transient functional improvement after surgery, but these results are difficult to interpret due to a lack of a placebo group within the experimental design (Mazzini et al., 2019). These preliminary results have provided sufficient promise to continue towards phase II trials, which are currently in preparation by the same group (Mazzini et al., 2019).

4.6. Multiple sclerosis clinical trials

hNSC transplantation for MS patients is a promising way to provide neuroprotection through remyelination and dampening of the immune response. Patients with the progressive form of MS have very limited therapeutic opportunities, therefore hNSC therapy has been primarily targeted towards this population as it may offer the opportunity to slow down disease progression. As reviewed above, in rodent models hNSCs can have peripheral immunomodulatory effects and indirectly influence remyelination. Currently hNSC clinical trials are limited, but a recent interest of their efficacy in progressive MS has started. A phase I clinical trial, which used autologous mesenchymal stem cell (MSC)-NSCs administered intrathecally in progressive MS patients established their safety and tolerability after 24 months (Harris et al., 2018). Being a phase I study, it was not designed to assay efficacy, but authors found a positive improvement of disability in 40% of the patients treated, which they attributed to the multiple injections of human MSC-NSCs. These claims should be taken with caution, as the study did not have a placebo group, but warrants a larger phase II study in the future. This work has initiated a phase II trial (Table 2) using autologous human MSC-NSCs in a larger progressive MS cohort with the addition of a placebo group in order to determine their efficacy in promoting neuroprotection. In addition, two other phase I clinical trials using allogenic fetal-derived hNSCs have recently started in order to determine the safety profile in both intrathecal and intracerebroventricular transplantation. Current work suggests a dysfunction in the endogenous oligodendrocyte progenitor population, which facilitates the need for exogenous cell therapy to provide protective immunomodulatory properties and stimulate regeneration (Jakel et al., 2019; Schirmer et al., 2019). Despite the progress made, further work is required to understand disease processes and to verify safety and efficacy before the optimal hNSC therapy can be generated and targeted for the clinic.

5. Conclusions

There are still several questions that need to be addressed for the clinical translation of hNSC therapies, such as the optimal cell source and the overall long-term safety. The current influx of phase I clinical trials using NSCs will eventually give an answer on the long-term safety, especially on the concern of tumor formation and immuno-rejection. The generation of induced pluripotent stem cells (iPSCs) from patient cells has portended the use of autologous transplants, which would then circumvent immunological incompatibility as well as the ethics associated with fetal tissue. The differentiation of hiPSCs into hNSCs has become a well-known method, but the possibility of having a few remaining pluripotent cells becomes an issue when transplanting into patients. The direct conversion of human somatic cells into hNSCs bypasses the pluripotent state, making them an ideal candidate for transplantation (Thier et al., 2012). This methodology can be used to overcome histocompatibility through the use of autologous cells with negligible teratogenic potential but has yet to make it to clinical trial. The appropriate source of NSCs for transplantation may be different depending on disease. For example, autologous-derived hNSCs from certain diseases may harbor genetic and epigenetic mutations, therefore an allogenic source would provide greater benefit, but may cause histocompatibility issues. Overall, the continuation in basic research and phase I and II clinical trials using hNSCs will provide a more thorough understanding of the biological mechanisms underpinning their beneficial impact in neurodegenerative diseases.

Knowing how hNSCs repair and promote regeneration, whether it be through trophic modulation, cellular replacement, or both, will allow for a more targeted cellular therapy in the future.

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Competing financial interests

S.P. is co-founder and CSO at CITC Ltd. and iSTEM Therapeutics, and co-founder and Non-executive Director at Asitia Therapeutics. L.P.J. is Head of Research at iSTEM Therapeutics.

References

Redmond Jr., D.E., Bjugstad, K.B., Teng, Y.D., et al., 2007. Behavioral improvement in a primate Parkinson's model is associated with multiple homeostatic effects of human neural stem cells. PNAS 104, 12175–12180.

Han, F., Baremberg, D., Gao, J., et al., 2015. Development of stem cell-based therapy for Parkinson's disease. Transl. Neurodegener. 4, 16.

L'Episcopo, F., Tirolo, C., Peruzzotti-Jametti, L., et al., 2018. Neural stem cell grafts promote astroglia-driven neurorestoration in the aged parkinsonian brain via wnt/beta-catenin signaling. Stem cells (Dayton, Ohio) 36, 1179–1197.

Duncan, T., Valenzuela, M., 2017. Alzheimer's disease, dementia, and stem cell therapy. Stem Cell Res. Ther. 8, 111.

Pluchino, S., Quattrini, A., Brambilla, E., et al., 2003. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. Nature 422, 688–694.

Pluchino, S., Martino, G., 2008. The therapeutic plasticity of neural stem/precursor cells in multiple sclerosis. J. Neurol. Sci. 265, 105–110.

Pluchino, S., Zanotti, L., Brini, E., Ferrari, S., Martino, G., 2009. Regeneration and repair in multiple sclerosis: the role of cell transplantation. Neurosci. Lett. 456, 101–106.

Peruzzotti-Jametti, L., Bernstock, J.D., Vicario, N., et al., 2018. Macrophage-Derived Extracellular Succinate Licenses Neural Stem Cells to Suppress Chronic Neuroinflammation. Cell Stem Cell 22 (355–368), e313.

Volpe, G., Bernstock, J.D., Peruzzotti-Jametti, L., Pluchino, S., 2019. Modulation of host immune responses following non-hematopoietic stem cell transplantation: Translational implications in progressive multiple sclerosis. J. Neuroimmunol. 331, 11–27.

Glass, J.D., Boulis, N.M., Johe, K., et al., 2012. Lumbar intraspinal injection of neural stem cells in patients with amyotrophic lateral sclerosis: results of a phase I trial in 12 patients. Stem cells (Dayton, Ohio) 30, 1144–1151.

Bacigaluppi, M., Pluchino, S., Martino, G., Kilic, E., Hermann, D.M., 2008. Neural stem/precursor cells for the treatment of ischemic stroke. J. Neurol. Sci. 265, 73–77.

Lee, S.T., Chu, K., Jung, K.H., et al., 2008. Anti-inflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. Brain 131, 616–629.

Hermann, D.M., Peruzzotti-Jametti, L., Schlechter, J., Bernstock, J.D., Doeppner, T.R., Pluchino, S., 2014. Neural precursor cells in the ischemic brain - integration, cellular crosstalk, and consequences for stroke recovery. Front. Cell. Neurosci. 8, 291.

Bacigaluppi, M., Russo, G.L., Peruzzotti-Jametti, L., et al., 2016. Neural Stem Cell Transplantation Induces Stroke Recovery by Upregulating Glutamate Transporter GLT-1 in Astrocytes. J. Neurosci. 36, 10529–10544.

Bernstock, J.D., Peruzzotti-Jametti, L., Leonardi, T., et al., 2019. SUMOylation promotes survival and integration of neural stem cell grafts in ischemic stroke. EBioMed. 42, 214–224.

Martino, G., Pluchino, S., 2006. The therapeutic potential of neural stem cells. Nat. Rev. Neurosci. 7, 395–406.

Martino, G., Pluchino, S., Bonfanti, L., Schwartz, M., 2011. Brain regeneration in physiology and pathology: the immune signature driving therapeutic plasticity of neural stem cells. Physiol. Rev. 91, 1281–1304.

Boese, A.C., Le, Q.E., Pham, D., Hamblin, M.H., Lee, J.P., 2018. Neural stem cell therapy for subacute and chronic ischemic stroke. Stem Cell Res. Ther. 9, 154.

Drago, D., Cossetti, C., Iraci, N., et al., 2013. The stem cell secretome and its role in brain repair. Biochimie 95, 2271–2285.

Doeppner, T.R., Traut, V., Heidenreich, A., et al., 2017. Conditioned medium derived from neural progenitor cells induces long-term post-ischemic neuroprotection, sustained neurological recovery, neurogenesis, and angiogenesis. Mol. Neurobiol. 54, 1531–1540.

Yang, H., Wang, C., Chen, H., et al., 2018. Neural stem cell-conditioned medium ameliorated cerebral ischemia-reperfusion injury in rats. Stem Cells Int. 2018, 4659159.

Pluchino, S., Cusimano, M., Bacigaluppi, M., Martino, G., 2010. Remodelling the injured CNS through the establishment of atypical ectopic perivascular neural stem cell niches. Arch. Ital. Biol. 148, 173–183.

Pluchino, S., Zanotti, L., Deleidi, M., Martino, G., 2005. Neural stem cells and their use as therapeutic tool in neurological disorders. Brain Res. Brain Res. Rev. 48, 211–219.

Serra, P.A., Pluchino, S., Marchetti, B., Desole, M.S., Miele, E., 2008. The MPTP mouse model: cues on DA release and neural stem cell restorative role. Parkinsonism Related Disord. 14 (Suppl 2), S189–193.

Pluchino, S., Zanotti, L., Brambilla, E., et al., 2009. Immune regulatory neural stem/precursor cells protect from central nervous system autoimmunity by restraining dendritic cell function. PLoS ONE 4, e5959.

Cossetti, C., Alfaro-Cervello, C., Donega, M., Tyzack, G., Pluchino, S., 2012. New perspectives of tissue remodelling with neural stem and progenitor cell-based therapies. Cell Tissue Res. 349, 321–329.

Pluchino, S., Cossetti, C., 2013. How stem cells speak with host immune cells in inflammatory brain diseases. Glia 61, 1379–1401.

Cossetti, C., Iraci, N., Mercer, T.R., et al., 2014. Extracellular vesicles from neural stem cells transfer IFN-gamma via Ifngr1 to activate Stat1 signaling in target cells. Mol. Cell 56, 193–204.

Drago, D., Basso, V., Gaude, E., et al., 2016. Metabolic determinants of the immune modulatory function of neural stem cells. J. Neuroinflamm. 13, 232.

Shoemaker, L.D., Kornblum, H.I., 2016. Neural Stem Cells (NSCs) and Proteomics. Mol. Cellular Proteomics: MCP 15, 344–354.

Iraci, N., Gaude, E., Leonardi, T., et al., 2017. Extracellular vesicles are independent metabolic units with asparaginase activity. Nat. Chem. Biol. 13, 951–955.

Park, D., Yang, Y.H., Bae, D.K., et al., 2013. Improvement of cognitive function and physical activity of aging mice by human neural stem cells over-expressing choline acetyltransferase. Neurobiol. Aging 34, 2639–2646.

McGinley, L.M., Sims, E., Lunn, J.S., et al., 2016. Human cortical neural stem cells expressing insulin-like growth factor-I: A novel cellular therapy for Alzheimer's disease. Stem Cells Transl Med 5, 379–391.

Ma, D.K., Bonaguidi, M.A., Ming, G.L., Song, H., 2009. Adult neural stem cells in the mammalian central nervous system. Cell Res. 19, 672–682.

Lee JP, McKercher S, Muller FJ, Snyder EY. Neural stem cell transplantation in mouse brain. Curr Protoc Neurosci 2008;Chapter 3:Unit 3 10.

Tullis, G.E., Spears, K., Kirk, M.D., 2014. Immunological barriers to stem cell therapy in the central nervous system. Stem Cells Int 2014, 507905.

Thier, M., Worsdorfer, P., Lakes, Y.B., et al., 2012. Direct conversion of fibroblasts into stably expandable neural stem cells. Cell Stem Cell 10, 473–479.

Signer, R.A., Morrison, S.J., 2013. Mechanisms that regulate stem cell aging and life span. Cell Stem Cell 12, 152–165.

Duinsbergen, D., Salvatori, D., Eriksson, M., Mikkers, H., 2009. Tumors originating from induced pluripotent stem cells and methods for their prevention. Ann. N. Y. Acad. Sci. 1176, 197–204.

Seminatore, C., Polentes, J., Ellman, D., et al., 2010. The postischemic environment differentially impacts teratoma or tumor formation after transplantation of human embryonic stem cell-derived neural progenitors. Stroke; a journal of cerebral circulation 41, 153–159.

Nori, S., Okada, Y., Nishimura, S., et al., 2015. Long-term safety issues of iPSC-based cell therapy in a spinal cord injury model: oncogenic transformation with epithelial-mesenchymal transition. Stem Cell Rep. 4, 360–373.

Deuse, T., Hu, X., Agbor-Enoh, S., et al., 2019. De novo mutations in mitochondrial DNA of iPSCs produce immunogenic neoepitopes in mice and humans. Nat. Biotechnol.

Itakura, G., Kawabata, S., Ando, M., et al., 2017. Fail-safe system against potential tumorigenicity after transplantation of iPSC derivatives. Stem Cell Rep. 8, 673–684.

Simonson, O.E., Domogatskaya, A., Volchkov, P., Rodin, S., 2015. The safety of human pluripotent stem cells in clinical treatment. Ann. Med. 47, 370–380.

Swijnenburg, R.J., Schrepfer, S., Govaert, J.A., et al., 2008. Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. PNAS 105, 12991–12996.

Pomeshchik, Y., Puttonen, K.A., Kidin, I., et al., 2015. Transplanted human induced pluripotent stem cell-derived neural progenitor cells do not promote functional recovery of pharmacologically immunosuppressed mice with contusion spinal cord injury. Cell Transplant. 24, 1799–1812.

Zhang, M., Wang, L., An, K., et al., 2018. Lower genomic stability of induced pluripotent stem cells reflects increased non-homologous end joining. Cancer Commun (Lond) 38, 49.

Ogawa, Y., Sawamoto, K., Miyata, T., et al., 2002. Transplantation of in vitro-expanded fetal neural progenitor cells results in neurogenesis and functional recovery after spinal cord contusion injury in adult rats. J. Neurosci. Res. 69, 925–933.

Teng, Y.D., Lavik, E.B., Qu, X., et al., 2002. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. PNAS 99, 3024–3029.

Lu, P., Jones, L.L., Snyder, E.Y., Tuszynski, M.H., 2003. Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. Exp. Neurol. 181, 115–129.

Ziv, Y., Avidan, H., Pluchino, S., Martino, G., Schwartz, M., 2006. Synergy between immune cells and adult neural stem/progenitor cells promotes functional recovery from spinal cord injury. PNAS 103, 13174–13179.

Cusimano, M., Biziato, D., Brambilla, E., et al., 2012. Transplanted neural stem/precursor cells instruct phagocytes and reduce secondary tissue damage in the injured spinal cord. Brain 135, 447–460.

Hawryluk, G.W., Mothe, A., Wang, J., Wang, S., Tator, C., Fehlings, M.G., 2012. An in vivo characterization of trophic factor production following neural precursor cell or bone marrow stromal cell transplantation for spinal cord injury. Stem Cells Dev. 21, 2222–2238.

Iwanami, A., Kaneko, S., Nakamura, M., et al., 2005. Transplantation of human neural stem cells for spinal cord injury in primates. J. Neurosci. Res. 80, 182–190.

Yamane, J., Nakamura, M., Iwanami, A., et al., 2010. Transplantation of galectin-1-expressing human neural stem cells into the injured spinal cord of adult common marmosets. J. Neurosci. Res. 88, 1394–1405.

Amemori, T., Ruzicka, J., Romanyuk, N., Jhanwar-Uniyal, M., Sykova, E., Jendelova, P., 2015. Comparison of intraspinal and intrathecal implantation of induced pluripotent stem cell-derived neural precursors for the treatment of spinal cord injury in rats. Stem Cell Res. Ther. 6, 257.

Romanyuk, N., Amemori, T., Turnovcova, K., et al., 2015. Beneficial effect of human induced pluripotent stem cell-derived neural precursors in spinal cord injury repair. Cell Transplant. 24, 1781–1797.

Ruzicka, J., Machova-Urdzikova, L., Gillick, J., et al., 2017. A comparative study of three different types of stem cells for treatment of rat spinal cord injury. Cell Transplant. 26, 585–603.

Karova, K., Wainwright, J.V., Machova-Urdzikova, L., et al., 2019. Transplantation of neural precursors generated from spinal progenitor cells reduces inflammation in spinal cord injury via NF-kappaB pathway inhibition. J. Neuroinflamm. 16, 12.

Habtemariam, S., 2018. The brain-derived neurotrophic factor in neuronal plasticity and neuroregeneration: new pharmacological concepts for old and new drugs. Neural Regen. Res. 13, 983–984.

Ousman, S.S., David, S., 2001. MIP-1alpha, MCP-1, GM-CSF, and TNF-alpha control the immune cell response that mediates rapid phagocytosis of myelin from the adult mouse spinal cord. J. Neurosci. 21, 4649–4656.

Eskan, M.A., Benakanakere, M.R., Rose, B.G., et al., 2008. Interleukin-1beta modulates proinflammatory cytokine production in human epithelial cells. Infect. Immun. 76, 2080–2089.

de Rham, C., Ferrari-Lacraz, S., Jendly, S., Schneiter, G., Dayer, J.M., Villard, J., 2007. The proinflammatory cytokines IL-2, IL-15 and IL-21 modulate the repertoire of

mature human natural killer cell receptors. Arthritis Res. Therapy 9, R125.
Lin, M.S., Sun, Y.Y., Chiu, W.T., et al., 2011. Curcumin attenuates the expression and secretion of RANTES after spinal cord injury in vivo and lipopolysaccharide-induced astrocyte reactivation in vitro. J. Neurotrauma 28, 1259–1269.
Fenn, A.M., Hall, J.C., Gensel, J.C., Popovich, P.G., Godbout, J.P., 2014. IL-4 signaling drives a unique arginase+/IL-1beta+ microglia phenotype and recruits macrophages to the inflammatory CNS: consequences of age-related deficits in IL-4Ralpha after traumatic spinal cord injury. J. Neurosci. 34, 8904–8917.
Yaguchi, M., Ohta, S., Toyama, Y., Kawakami, Y., Toda, M., 2008. Functional recovery after spinal cord injury in mice through activation of microglia and dendritic cells after IL-12 administration. J. Neurosci. Res. 86, 1972–1980.
Pizzi, M., Goffi, F., Boroni, F., et al., 2002. Opposing roles for NF-kappa B/Rel factors p65 and c-Rel in the modulation of neuron survival elicited by glutamate and interleukin-1beta. J. Biol. Chem. 277, 20717–20723.
Brambilla, R., Bracchi-Ricard, V., Hu, W.H., et al., 2005. Inhibition of astroglial nuclear factor kappaB reduces inflammation and improves functional recovery after spinal cord injury. J. Exp. Med. 202, 145–156.
Mozaffarian, D., Benjamin, E.J., Go, A.S., et al., 2016. Heart disease and stroke statistics-2016 update: a report from the American Heart Association. Circulation 133, e38–360.
Sander, M., Oxlund, B., Jespersen, A., et al., 2015. The challenges of human population ageing. Age Ageing 44, 185–187.
Lipton, P., 1999. Ischemic cell death in brain neurons. Physiol. Rev. 79, 1431–1568.
White, B.C., Sullivan, J.M., DeGracia, D.J., et al., 2000. Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. J. Neurol. Sci. 179, 1–33.
Mena, H., Cadavid, D., Rushing, E.J., 2004. Human cerebral infarct: a proposed histopathologic classification based on 137 cases. Acta Neuropathol. 108, 524–530.
Besancon, E., Guo, S., Lok, J., Tymianski, M., Lo, E.H., 2008. Beyond NMDA and AMPA glutamate receptors: emerging mechanisms for ionic imbalance and cell death in stroke. Trends Pharmacol. Sci. 29, 268–275.
Kulik, T., Kusano, Y., Aronhime, S., Sandler, A.L., Winn, H.R., 2008. Regulation of cerebral vasculature in normal and ischemic brain. Neuropharmacology 55, 281–288.
Iadecola, C., Anrather, J., 2011. The immunology of stroke: from mechanisms to translation. Nat. Med. 17, 796–808.
Petrovic-Djergovic, D., Goonewardena, S.N., Pinsky, D.J., 2016. Inflammatory disequilibrium in stroke. Circ. Res. 119, 142–158.
Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z., Lindvall, O., 2002. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat. Med. 8, 963–970.
Kokaia, Z., Lindvall, O., 2003. Neurogenesis after ischaemic brain insults. Curr. Opin. Neurobiol. 13, 127–132.
Thored, P., Arvidsson, A., Cacci, E., et al., 2006. Persistent production of neurons from adult brain stem cells during recovery after stroke. Stem Cells (Dayton, Ohio) 24, 739–747.
Pluchino, S., Muzio, L., Imitola, J., et al., 2008. Persistent inflammation alters the function of the endogenous brain stem cell compartment. Brain 131, 2564–2578.
Hicks, C., Stevanato, L., Stroemer, R.P., Tang, E., Richardson, S., Sinden, J.D., 2013. In vivo and in vitro characterization of the angiogenic effect of CTX0E03 human neural stem cells. Cell Transplant. 22, 1541–1552.
Huang, L., Wong, S., Snyder, E.Y., Hamblin, M.H., Lee, J.P., 2014. Human neural stem cells rapidly ameliorate symptomatic inflammation in early-stage ischemic-reperfusion cerebral injury. Stem Cell Res. Ther. 5, 129.
Eckert, A., Huang, L., Gonzalez, R., Kim, H.S., Hamblin, M.H., Lee, J.P., 2015. Bystander effect fuels human induced pluripotent stem cell-derived neural stem cells to quickly attenuate early stage neurological deficits after stroke. Stem Cells Transl. Med. 4, 841–851.
Ryu, S., Lee, S.H., Kim, S.U., Yoon, B.W., 2016. Human neural stem cells promote proliferation of endogenous neural stem cells and enhance angiogenesis in ischemic rat brain. Neural Regener. Res. 11, 298–304.
Bernstock, J.D., Peruzzotti-Jametti, L., Ye, D., et al., 2017. Neural stem cell transplantation in ischemic stroke: A role for preconditioning and cellular engineering. J. Cerebral Blood Flow Metab. 37, 2314–2319.
Lee, I.H., Huang, S.S., Chuang, C.Y., et al., 2017. Delayed epidural transplantation of human induced pluripotent stem cell-derived neural progenitors enhances functional recovery after stroke. Sci. Rep. 7, 1943.
Canazza, A., Minati, L., Boffano, C., Parati, E., Binks, S., 2014. Experimental models of brain ischemia: a review of techniques, magnetic resonance imaging, and investigational cell-based therapies. Front. Neurol. 5, 19.
Emanueli, C., Schratzberger, P., Kirchmair, R., Madeddu, P., 2003. Paracrine control of vascularization and neurogenesis by neurotrophins. Br. J. Pharmacol. 140, 614–619.
Yang, Y., Estrada, E.Y., Thompson, J.F., Liu, W., Rosenberg, G.A., 2007. Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat. J. Cerebral Blood Flow Metab. 27, 697–709.
Chen, B., Friedman, B., Cheng, Q., et al., 2009. Severe blood-brain barrier disruption and surrounding tissue injury. Stroke 40, e666–674.
2015 Alzheimer's disease facts and figures. Alzheimer's & dementia : the journal of the Alzheimer's Association 2015;11:332-384.
Alzheimer's disease facts and figures Alzheimer's Dementia 12 2016 459 509.
Terry Jr., A.V., Buccafusco, J.J., 2003. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. J. Pharmacol. Experiment. Therap. 306, 821–827.
Coyle, J.T., Price, D.L., DeLong, M.R., 1983. Alzheimer's disease: a disorder of cortical cholinergic innervation. Science 219, 1184–1190.
Russo, V.C., Gluckman, P.D., Feldman, E.L., Werther, G.A., 2005. The insulin-like growth factor system and its pleiotropic functions in brain. Endocr. Rev. 26, 916–943.
Miltiadous, P., Stamatakis, A., Koutsoudaki, P.N., Tiniakos, D.G., Stylianopoulou, F., 2011. IGF-I ameliorates hippocampal neurodegeneration and protects against cognitive deficits in an animal model of temporal lobe epilepsy. Exp. Neurol. 231, 223–235.
Lee, I.S., Jung, K., Kim, I.S., et al., 2015. Human neural stem cells alleviate Alzheimer-like pathology in a mouse model. Mol. Neurodegener. 10, 38.
de Rijk, M.C., Launer, L.J., Berger, K., et al., 2000. Prevalence of Parkinson's disease in Europe: A collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. Neurology 54, S21–23.
Cheng, H.C., Ulane, C.M., Burke, R.E., 2010. Clinical progression in Parkinson disease and the neurobiology of axons. Ann. Neurol. 67, 715–725.
Fahn, S., Oakes, D., Shoulson, I., et al., 2004. Levodopa and the progression of Parkinson's disease. New Engl. J.f Med. 351, 2498–2508.
Limousin, P., Pollak, P., Benazzouz, A., et al., 1995. Effect of parkinsonian signs and symptoms of bilateral subthalamic nucleus stimulation. Lancet (London, England) 345, 91–95.
Wenning, G.K., Odin, P., Morrish, P., et al., 1997. Short- and long-term survival and function of unilateral intrastriatal dopaminergic grafts in Parkinson's disease. Ann. Neurol. 42, 95–107.
Hallett, P.J., Cooper, O., Sadi, D., Robertson, H., Mendez, I., Isacson, O., 2014. Long-term health of dopaminergic neuron transplants in Parkinson's disease patients. Cell Rep. 7, 1755–1761.
Li, W., Englund, E., Widner, H., et al., 2016. Extensive graft-derived dopaminergic innervation is maintained 24 years after transplantation in the degenerating parkinsonian brain. PNAS 113, 6544–6549.
Politis, M., Oertel, W.H., Wu, K., et al., 2011. Graft-induced dyskinesias in Parkinson's disease: High striatal serotonin/dopamine transporter ratio. Mov. Disord. 26, 1997–2003.
Kordower, J.H., Dodiya, H.B., Kordower, A.M., et al., 2011. Transfer of host-derived alpha synuclein to grafted dopaminergic neurons in rat. Neurobiol. Dis. 43, 552–557.
Li, J.Y., Englund, E., Holton, J.L., et al., 2008. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat. Med. 14, 501–503.
Freed, C.R., Greene, P.E., Breeze, R.E., et al., 2001. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. New Engl. J. Med. 344, 710–719.
Olanow, C.W., Goetz, C.G., Kordower, J.H., et al., 2003. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. Ann. Neurol. 54, 403–414.
Monni, E., Cusulin, C., Cavallaro, M., Lindvall, O., Kokaia, Z., 2014. Human fetal striatum-derived neural stem (NS) cells differentiate to mature neurons in vitro and in vivo. Curr. Stem Cell Res. Ther. 9, 338–346.
Choi, D.H., Kim, J.H., Kim, S.M., Kang, K., Han, D.W., Lee, J., 2017.. Therapeutic potential of induced neural stem cells for Parkinson's disease. Int. J. Mol. Sci. 18.
Mendes-Pinheiro, B., Teixeira, F.G., Anjo, S.I., Manadas, B., Behie, L.A., Salgado, A.J., 2018. Secretome of undifferentiated neural progenitor cells induces histological and motor improvements in a rat model of Parkinson's disease. Stem Cells Transl. Med. 7, 829–838.
Baghbaderani, B.A., Mukhida, K., Sen, A., et al., 2010. Bioreactor expansion of human neural precursor cells in serum-free media retains neurogenic potential. Biotechnol. Bioeng. 105, 823–833.
Han, F., Wang, W., Chen, B., et al., 2015. Human induced pluripotent stem cell-derived neurons improve motor asymmetry in a 6-hydroxydopamine-induced rat model of Parkinson's disease. Cytotherapy 17, 665–679.
Baquet, Z.C., Bickford, P.C., Jones, K.R., 2005. Brain-derived neurotrophic factor is required for the establishment of the proper number of dopaminergic neurons in the substantia nigra pars compacta. J. Neurosci. 25, 6251–6259.
Falk, T., Gonzalez, R.T., Sherman, S.J., 2010. The yin and yang of VEGF and PEDF: multifaceted neurotrophic factors and their potential in the treatment of Parkinson's Disease. Int. J. Mol. Sci. 11, 2875–2900.
Lee, Y.M., Park, S.H., Shin, D.I., et al., 2008. Oxidative modification of peroxiredoxin is associated with drug-induced apoptotic signaling in experimental models of Parkinson disease. J. Biol. Chem. 283, 9986–9998.
Arodin, L., Miranda-Vizuete, A., Swoboda, P., Fernandes, A.P., 2014. Protective effects of the thioredoxin and glutaredoxin systems in dopamine-induced cell death. Free Radical Biol. Med. 73, 328–336.
Filograna, R., Godena, V.K., Sanchez-Martinez, A., et al., 2016. Superoxide Dismutase (SOD)-mimetic M40403 Is Protective in Cell and Fly Models of Paraquat Toxicity: IMPLICATIONS FOR PARKINSON DISEASE. J. Biol. Chem. 291, 9257–9267.
Cleveland, D.W., Rothstein, J.D., 2001. From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. Nat. Rev. Neurosci. 2, 806–819.
Comley, L., Allodi, I., Nichterwitz, S., et al., 2015. Motor neurons with differential vulnerability to degeneration show distinct protein signatures in health and ALS. Neuroscience 291, 216–229.
Gurney, M.E., Fleck, T.J., Himes, C.S., Hall, E.D., 1998. Riluzole preserves motor function in a transgenic model of familial amyotrophic lateral sclerosis. Neurology 50, 62–66.
Popescu, I.R., Nicaise, C., Liu, S., et al., 2013. Neural progenitors derived from human induced pluripotent stem cells survive and differentiate upon transplantation into a rat model of amyotrophic lateral sclerosis. Stem Cells Transl. Med. 2, 167–174.
Kondo, T., Funayama, M., Tsukita, K., et al., 2014. Focal transplantation of human iPSC-derived glial-rich neural progenitors improves lifespan of ALS mice. Stem Cell Rep. 3, 242–249.
Rosen, D.R., Siddique, T., Patterson, D., et al., 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362, 59–62.
Browne, E.C., Abbott, B.M., 2016. Recent progress towards an effective treatment of amyotrophic lateral sclerosis using the SOD1 mouse model in a preclinical setting. Eur. J. Med. Chem. 121, 918–925.

Xu, L., Shen, P., Hazel, T., Johe, K., Koliatsos, V.E., 2011. Dual transplantation of human neural stem cells into cervical and lumbar cord ameliorates motor neuron disease in SOD1 transgenic rats. Neurosci. Lett. 494, 222–226.
Lunn, J.S., Sakowski, S.A., Kim, B., Rosenberg, A.A., Feldman, E.L., 2009. Vascular endothelial growth factor prevents G93A-SOD1-induced motor neuron degeneration. Dev Neurobiol 69, 871–884.
Global, regional, and national burden of multiple sclerosis 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet Neurology 2019;18:269-285.
Wallin, M.T., Culpepper, W.J., Campbell, J.D., et al., 2019. The prevalence of MS in the United States: A population-based estimate using health claims data. Neurology 92, e1029–e1040.
Bo, L., Geurts, J.J., Mork, S.J., van der Valk, P., 2006. Grey matter pathology in multiple sclerosis. Acta Neurol. Scand. Suppl. 183, 48–50.
Calabrese, M., Favaretto, A., Martini, V., Gallo, P., 2013. Grey matter lesions in MS: from histology to clinical implications. Prion 7, 20–27.
Lublin, F.D., Reingold, S.C., Cohen, J.A., et al., 2014. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology 83, 278–286.
Compston, A., Coles, A., 2008. Multiple sclerosis. Lancet 372, 1502–1517.
Thompson, A.J., Baranzini, S.E., Geurts, J., Hemmer, B., Ciccarelli, O., 2018. Multiple sclerosis. Lancet (London, England) 391, 1622–1636.
Franklin, R.J., Ffrench-Constant, C., 2008. Remyelination in the CNS: from biology to therapy. Nat. Rev. Neurosci. 9, 839–855.
Franklin, R.J.M., Ffrench-Constant, C., 2017. Regenerating CNS myelin - from mechanisms to experimental medicines. Nat. Rev. Neurosci. 18, 753–769.
Laterza, C., Merlini, A., De Feo, D., et al., 2013. iPSC-derived neural precursors exert a neuroprotective role in immune-mediated demyelination via the secretion of LIF. Nat. Commun. 4, 2597.
De Feo, D., Merlini, A., Brambilla, E., et al., 2017. Neural precursor cell-secreted TGF-beta2 redirects inflammatory monocyte-derived cells in CNS autoimmunity. J. Clin. Investig. 127, 3937–3953.
Chen, L., Coleman, R., Leang, R., et al., 2014. Human neural precursor cells promote neurologic recovery in a viral model of multiple sclerosis. Stem Cell Rep. 2, 825–837.
Plaisted, W.C., Zavala, A., Hingco, E., et al., 2016. remyelination is correlated with regulatory T cell induction following human embryoid body-derived neural precursor cell transplantation in a viral model of multiple sclerosis. PLoS One 11, e0157620.
Nicaise, A.M., Banda, E., Guzzo, R.M., et al., 2017. iPS-derived neural progenitor cells from PPMS patients reveal defect in myelin injury response. Exp. Neurol. 288, 114–121.
Nicaise, A.M., Wagstaff, L.J., Willis, C.M., et al., 2019. Cellular senescence in progenitor cells contributes to diminished remyelination potential in progressive multiple sclerosis. PNAS.
Stevanato, L., Thanabalasundaram, L., Vysokov, N., Sinden, J.D., 2016. Investigation of Content, Stoichiometry and Transfer of miRNA from Human Neural Stem Cell Line Derived Exosomes. PLoS One 11, e0146353.
Ma, Y., Wang, K., Pan, J., et al., 2019. Induced neural progenitor cells abundantly secrete extracellular vesicles and promote the proliferation of neural progenitors via extracellular signal-regulated kinase pathways. Neurobiol. Disease 124, 322–334.
Rong, Y., Liu, W., Wang, J., et al., 2019. Neural stem cell-derived small extracellular vesicles attenuate apoptosis and neuroinflammation after traumatic spinal cord injury by activating autophagy. Cell Death Dis. 10, 340.
Tkach, M., Thery, C., 2016. Communication by extracellular vesicles: where we are and where we need to go. Cell 164, 1226–1232.
Colombo, M., Raposo, G., Thery, C., 2014. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu. Rev. Cell Dev. Biol. 30, 255–289.
Camussi, G., Deregibus, M.C., Cantaluppi, V., 2013. Role of stem-cell-derived microvesicles in the paracrine action of stem cells. Biochem. Soc. Trans. 41, 283–287.
Vogel, A., Upadhya, R., Shetty, A.K., 2018. Neural stem cell derived extracellular vesicles: Attributes and prospects for treating neurodegenerative disorders. EBioMedicine 38, 273–282.
Webb, R.L., Kaiser, E.E., Jurgielewicz, B.J., et al., 2018. Human neural stem cell extracellular vesicles improve recovery in a porcine model of ischemic stroke. Stroke 49, 1248–1256.
Webb, R.L., Kaiser, E.E., Scoville, S.L., et al., 2018. Human neural stem cell extracellular vesicles improve tissue and functional recovery in the murine thromboembolic stroke model. Transl. Stroke Res. 9, 530–539.
Curtis, E., Martin, J.R., Gabel, B., et al., 2018. A first-in-human, phase I study of neural stem cell transplantation for chronic spinal cord injury. Cell Stem Cell 22 (941–950), e946.
Levi, A.D., Anderson, K.D., Okonkwo, D.O., et al., 2019. Clinical outcomes from a multicenter study of human neural stem cell transplantation in chronic cervical spinal cord injury. J. Neurotrauma 36, 891–902.
Tsukamoto, A., Uchida, N., Capela, A., Gorba, T., Huhn, S., 2013. Clinical translation of human neural stem cells. Stem Cell Res. Ther. 4, 102.
Kalladka, D., Sinden, J., Pollock, K., et al., 2016. Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. Lancet 388, 787–796.
Zhang, G., Li, Y., Reuss, J.L., et al., 2019. Stable intracerebral transplantation of neural stem cells for the treatment of paralysis due to ischemic stroke. Stem Cells Transl. Med.
Kwak, K.A., Lee, S.P., Yang, J.Y., Park, Y.S., 2018. Current perspectives regarding stem cell-based therapy for Alzheimer's disease. Stem Cells Int. 2018, 6392986.
Revazova, E.S., Turovets, N.A., Kochetkova, O.D., et al., 2007. Patient-specific stem cell lines derived from human parthenogenetic blastocysts. Cloning Stem Cells 9, 432–449.
Gonzalez, R., Garitaonandia, I., Poustovoitov, M., et al., 2016. Neural stem cells derived from human parthenogenetic stem cells engraft and promote recovery in a nonhuman primate model of Parkinson's disease. Cell Transplant. 25, 1945–1966.
Mazzini, L., Gelati, M., Profico, D.C., et al., 2019. Results from Phase I clinical trial with intraspinal injection of neural stem cells in amyotrophic lateral sclerosis: a long-term outcome. Stem Cells Transl. Med.
Goutman, S.A., Brown, M.B., Glass, J.D., et al., 2018. Long-term Phase 1/2 intraspinal stem cell transplantation outcomes in ALS. Ann. Clin. Transl. Neurol. 5, 730–740.
Harris, V.K., Stark, J., Vyshkina, T., et al., 2018. Phase I trial of intrathecal mesenchymal stem cell-derived neural progenitors in progressive multiple sclerosis. EBioMedicine 29, 23–30.
Jakel, S., Agirre, E., Mendanha Falcao, A., et al., 2019. Altered human oligodendrocyte heterogeneity in multiple sclerosis. Nature 566, 543–547.
Schirmer, L., Velmeshev, D., Holmqvist, S., et al., 2019. Neuronal vulnerability and multilineage diversity in multiple sclerosis. Nature.