Trends in Molecular Medicine

CellPress REVIEWS

Opinion

Promises and Limitations of Neural Stem Cell Therapies for Progressive Multiple Sclerosis

Stefano Pluchino,[1,3,*,@] Jayden A. Smith,[2,3] and Luca Peruzzotti-Jametti[1,3]

Multiple disease-modifying medications with regulatory approval to treat multiple sclerosis (MS) are unable to prevent inflammatory tissue damage in the central nervous system (CNS), and none directly promote repair. Thus, there is an unmet clinical need for therapies that can arrest and reverse the persistent accumulation of disabilities associated with progressive forms of MS (P-MS). Preclinical research has revealed an unexpected ability of neural stem cell (NSC) therapies to provide neurotrophic support and inhibit detrimental host immune responses *in vivo* following transplantation into the chronically inflamed CNS. We discuss NSC transplantation as a promising therapy for P-MS, elaborate on the necessities of clinical trial validation and formalized usage guidelines, and caution about unscrupulous 'clinics' marketing unproven therapies to patients.

Highlights

Few drugs have been approved for the treatment of progressive multiple sclerosis (P-MS), and those that have are limited in their efficacy to active forms of the disease, and fail to halt degeneration or promote repair and regeneration.

State-of-the-art single-cell characterization of the diseased CNS is providing high-resolution insights into deficiencies of endogenous regenerative potential and the shortcomings of animal models of disease.

CNS stem cell transplantation has demonstrated encouraging therapeutic potential in preclinical studies of neurological diseases such as P-MS, and there is a growing understanding of the mechanisms of action through which they act on the injured CNS.

Comprehensive and well-designed clinical study of CNS stem cell therapies is essential to decisively establish their translational potential and safety, but the marketing of unproven treatments is flourishing in the interim.

The Unmet Clinical Needs in P-MS

MS is a chronic inflammatory disease of the CNS that affects >2 million people worldwide [1,2]. MS is a complex disease arising from a combination of genetic determinants and environmental risk factors. There is one main genetic determinant associated with MS susceptibility – a variant of the *HLA-DRB1* gene of the major histocompatibility complex – as well as many smaller genetic risk factors. Genome-wide association studies have elaborated this polygenic model of MS genetics and have been highly successful in uncovering genetic variants at specific loci associated with MS [3]. Nevertheless, these disease-associated genetic variants do not necessarily cause the disease, and could merely be disease markers.

The typical MS disease course includes sporadic attacks (relapses) of neurological dysfunction (sensory impairment, fatigue, and ataxia) that are partially or fully reversible (remission) over the course of days to weeks. Although inflammation and **demyelination** (see Glossary) are the main features of the characteristic MS lesions in the brain and spinal cord, neurological deficits in patients best correlate with axonal degeneration [4].

Eighty-five percent of patients first present with this relapsing-remitting form of MS (RR-MS) [5], for which several **immunomodulatory** disease-modifying therapies (DMTs) have been developed and demonstrate a striking effect on the frequency and severity of relapses [6]. However, within two decades of onset, 80% of untreated RR-MS patients evolve to a later phase of sustained disability termed secondary progressive MS (SP-MS) [7]. A lower proportion of MS patients (10–15%) present with constant accumulation of disabilities from the onset of symptoms, without early relapses or remissions (so-called primary progressive MS, PP-MS) [8]. Both PP- and SP-MS involve a sustained build-up of symptoms with an insidious increase in disability, and are referred to here collectively as progressive MS (P-MS).

Although substantial progress has been made in the development of DMTs for the treatment of RR-MS, most act via peripheral immunomodulation and have questionable effects on the

[1]Department of Clinical Neurosciences and National Institute for Health Research (NIHR) Biomedical Research Centre, University of Cambridge, Cambridge, UK
[2]Cambridge Innovation Technologies Consulting (CITC) Ltd, Cambridge, UK
[3]These authors contributed equally to this work

*Correspondence: spp24@cam.ac.uk (S. Pluchino).
@Twitter: @Pluchinolab

aggravation of disabilities and disease progression [9]. Indeed, approval of DMTs has thus far been made largely on the basis of a reduced number of clinical relapses and decreased magnetic resonance imaging (MRI) lesion activity. This basis effectively differentiates an active MS phenotype – defined by the presence of clinical relapses and/or the presence of disease-associated MRI activity – from non-active MS, a transition for which there are no appropriate biomarkers. Indeed, non-active MS may prove difficult to discern owing to the activity-dampening effects of current DMTs [10].

In addition, the few DMTs approved for the treatment of P-MS patients are typically repurposed (i.e., existing drugs repositioned for new therapeutic purposes) RR-MS therapies. As a consequence, their efficacy is largely restricted to patients with active disease, more typically those in the earlier stages of P-MS. The pathobiology underlying non-active P-MS is complex and distinct from that of RR-MS, involving multiple intricately entwined mechanisms and cell types [4,5] (summarized in Figure 1).

Combating the accumulation of disability associated with P-MS requires a shift of focus away from limiting early RR-MS-like inflammatory damage mediated largely by lymphocytes originating in the periphery. Instead, efforts should be made to interfere with those distinct mechanisms that dominate the chronic late non-active phases of MS, including persistent demyelination, glial reactivity, and axonal loss.

Understanding P-MS

A main goal of regenerative approaches to MS has long been to promote myelin repair after demyelination [11]. Studies in rodent models of MS have suggested the potential for the generation of new **oligodendrocytes** from oligodendrocyte precursor cells (OPCs) as a viable mechanism to enhance remyelination [12]. However, recent studies have highlighted possible limitations to the clinical translation of this strategy.

Yeung *et al.* applied a **^{14}C-based birth-dating** technique to assess the dynamics of oligodendrocyte generation in the **normal-appearing white matter** (NAWM) and in **shadow plaques** from post-mortem MS brains. By measuring the integration of ^{14}C derived from nuclear testing in genomic DNA and its washout properties, this seminal work describes that NAWM of individuals with severe MS bears a much larger fraction of recently generated OPCs and oligodendrocytes than does the white matter of controls. Furthermore, oligodendrocytes in shadow plaques are biologically as old as the patient, and therefore not being generated by locally recruited OPCs.

Innovations in **single-cell and single-nucleus RNA sequencing** (scRNA-seq/snRNA-seq) have also been particularly valuable in providing insights into the substantial transcriptional heterogeneity among the neural and immune cells that drive neurodegenerative diseases such as MS, but also highlight fundamental discrepancies between the human CNS and animal models. Yeung and colleagues have reported that remyelination of the human MS CNS is sustained by old mature oligodendrocytes, and not by local OPCs [13]. A second comprehensive snRNA-seq study of human oligodendrocytes in MS has further supported these findings, showing that mature oligodendrocytes have increased expression of myelin-related genes, suggesting that they play a direct role in remyelination [14]. *Ex vivo* nuclear transcriptome data also confirmed a striking depletion of OPCs and of an opalin-expressing oligodendrocyte population of intermediate maturity in the MS brain, where pathology arises from (i) loss of mature oligodendrocytes, and (ii) skewing of the differentiation program towards alternative immunomodulatory transcriptional signatures [14,15]. These data have been confirmed by Schirmer

Glossary

^{14}C-Based birth-dating: a technique utilizing the globally elevated content of ^{14}C in all biological material synthesized during the peak of atmospheric atomic bomb tests in the early 1960s, and its characteristic washout in the years thereafter, to determine the age of cellular components.
CD20: a cell-surface marker of B cells that is targeted by monoclonal antibody drugs such as ocrelizumab leading to antibody-dependent cell-mediated cytotoxicity and thus immunosuppression.
Demyelination: damage to and/or loss of the myelin sheath of neurons, leading to impaired neuronal function.
Embryonic stem cell (ESC): pluripotent cells derived from the inner cell mass of a blastocyst.
Hematopoietic stem cell (HSC): a class of multipotent stem cell, located primarily in the blood or bone marrow, that can differentiate into different types of blood cells (myeloid and lymphoid lines).
Immunomodulation: regulation of the function of the immune system, often in an effort to attain homeostasis, either through direct action on immune cells (e.g., using drugs) or via secreted factors (e.g., using cells).
Induced pluripotent stem cell (iPSC): a cell, generated by reprogramming of a differentiated cell type such as a skin cell, that is capable of differentiating into any other cell type.
Mesenchymal stromal cell (MSC): a broad class of multipotent cells derived from various sources, commonly bone marrow and adipose tissue.
Mononuclear phagocyte (MP): a class of immune cells, including central nervous system (CNS)-infiltrating macrophages and CNS-resident microglia, that are implicated in the neuroinflammatory pathobiology of MS.
Neural stem cell (NSC): a multipotent self-renewing cell that is capable of differentiating along neural lineages (e.g., neural stem/progenitor cells).
Neuroprotection: preservation of neuronal structure and/or function.
Normal-appearing white matter (NAWM): macroscopically normal brain tissue that is at least 1 cm from a visible plaque.
Oligodendrocyte: a glial cell that produces the myelin coating of axons in the CNS.
Purine analog: an antimetabolite such as cladribine that resembles adenosine

et al., who also found an immunomodulatory signature in stressed myelinating oligodendrocytes of chronically active subcortical lesions [16].

Overall, these discoveries suggest that remyelination in the human MS brain is carried out by old oligodendrocytes, and they therefore imply that early therapeutic intervention aimed at protecting the oligodendrocyte inventory of the CNS may be effective. However, the paucity of lesion-resident surviving oligodendrocytes and the limited role of OPCs in MS may not adequately support the idea of therapeutically enhanced remyelination in P-MS that was predicted by animal models [12].

Region- and lineage-based transcriptional heterogeneity is highly variable in the MS CNS [16]. For example, upper-layer excitatory projection neurons in cortical lesions exhibit a distinct transcriptional stress profile, including enrichment of mitochondrial dysfunction, oxidative stress, and axonal degeneration pathways. This finding is supported by major changes in neuronal mitochondrial function and structure in P-MS patients, including altered morphology and aberrant expression of mitochondrial complexes and dynamics [17,18].

As the disease progresses, a switch from the adaptive towards the innate immune response occurs, causing a state of CNS compartmentalized low-grade inflammatory response in P-MS patients. **Mononuclear phagocytes** (MPs) are the key players of this innate immune response, and are directly responsible for the main pathological changes that lead to the progression of disability in P-MS patients [19,20]. Specific populations of chronically activated MPs include both tissue-resident (i.e., microglia and non-parenchymal macrophages) and infiltrating MPs (i.e., macrophages entering the CNS via the bloodstream). In P-MS, MPs accumulate in different regions of the brain where they release proteases, proinflammatory cytokines, and reactive oxygen species (ROS) that ultimately contribute to neurodegeneration and cortical atrophy [5,21–23]. The snRNA-seq study of Schirmer and colleagues showed an increase in microglia in MS tissue, and reactive microglia mapped primarily to regions proximal to subcortical lesions [16]. Although activated MPs are often reductively considered in terms of pro- or anti-inflammatory MPs, single-cell techniques emphasize the transcriptional, phenotypic and ultimately functional diversity of MPs [24,25], underscoring the need for caution in extrapolating from animal models to humans [26].

As we gain a better insight into the diversity of the cell types and subtypes underlying P-MS pathobiology, the need for a multimodal therapeutic approach is becoming clear. Effective treatments for P-MS will likely be combinatorial, providing not only **neuroprotection** and immunomodulation but also fostering repair and regeneration. The intrinsic regenerative potential of the adult human brain strikingly decreases with time after birth, as well as in the context of disease and physiological aging [27]. This aging-related loss of function specifically affects **neural stem cells** (NSCs) and arises from increased oxidative damage, genetic and epigenetic stress, as well as telomere attrition [28]. All these triggers may be advanced and exacerbated in the aged P-MS brain, contributing to homeostatic dysfunction of tissue stem cells [29,30]. Thus, conventional therapeutics intended to promote the regeneration of the MS brain from endogenous cellular sources may suffer from a dearth of targets in P-MS

It is in this context the innate multifunctionality and regenerative potential of exogenous NSC therapies should best be exploited.

Principles of CNS Stem Cell Therapies

Stem cell therapy is a broad concept encompassing the transplantation of different stem/progenitor cell types sourced from various tissues into prospective patients for therapeutic effect. With regards

or guanine and inhibits DNA synthesis, preventing the clonal expansion of lymphocytes.

Shadow plaques: sharply demarcated areas with reduced myelin density and disproportionately thin myelin sheaths, and which reflect a late phase of remyelination.

Single-cell and single-nucleus RNA sequencing (scRNA-seq/snRNA-seq): a method for characterizing the transcriptome of individual cells rather than of bulk tissue. snRNA-seq is restricted to the expression profile of single nuclei rather than of whole cells.

Sphingosine-1 phosphate receptor (S1PR): a class of lipid-binding receptors with diverse functions. The drug siponimod binds to the S1PR1 subtype on lymphocytes, preventing them from entering the CNS, as well as to the S1PR5 subtype on oligodendrocytes and astrocytes in the CNS.

Stem cell tourism: when a patient travels abroad to receive a stem cell therapy otherwise unavailable to them in their home country owing to inhibitive costs or regulatory prohibition.

Transdifferentiation: reprogramming of a mature somatic cell into a somatic cell of different lineage, bypassing a pluripotent stage.

to clinically validated procedures, this is still largely restricted to **hematopoietic stem cell** (HSC) transplantation. This is a procedure routinely used for the treatment of hematologic malignancies such as multiple myeloma and leukemias, and is also offering remarkable benefits in active RR-MS that fails to respond to DMTs [31]. The rationale behind HSC transplantation in blood or bone marrow cancers involves replacement of the host lymphohematopoietic compartment by donor-derived cells. In MS, however, HSC transplantation is often categorized as an immune reconstitution therapy (IRT). IRTs are intended to deplete components of the immune system, paving the way to self-renewal, but likely involve expansion of cell populations that survive immunosuppression leading to the acquisition of new (ostensibly protective) phenotypes [32]. Although transplantation of allogeneic HSCs can result in long-term deleterious side-effects owing to chronic graft-versus-host complications [33], the main short/medium-term risk of autologous HSC therapies is the increased susceptibility to infection as a result of the accompanying chemotherapeutic immunosuppressive regimen. Additional long-term effects include the development of secondary autoimmune problems and/or fertility issues. However, more comprehensive follow-up data are required [34]. Several MS DMTs, including the monoclonal antibodies alemtuzumab, rituximab, and ocrelizumab, and the nucleoside analog cladribine, are also categorized as IRTs.

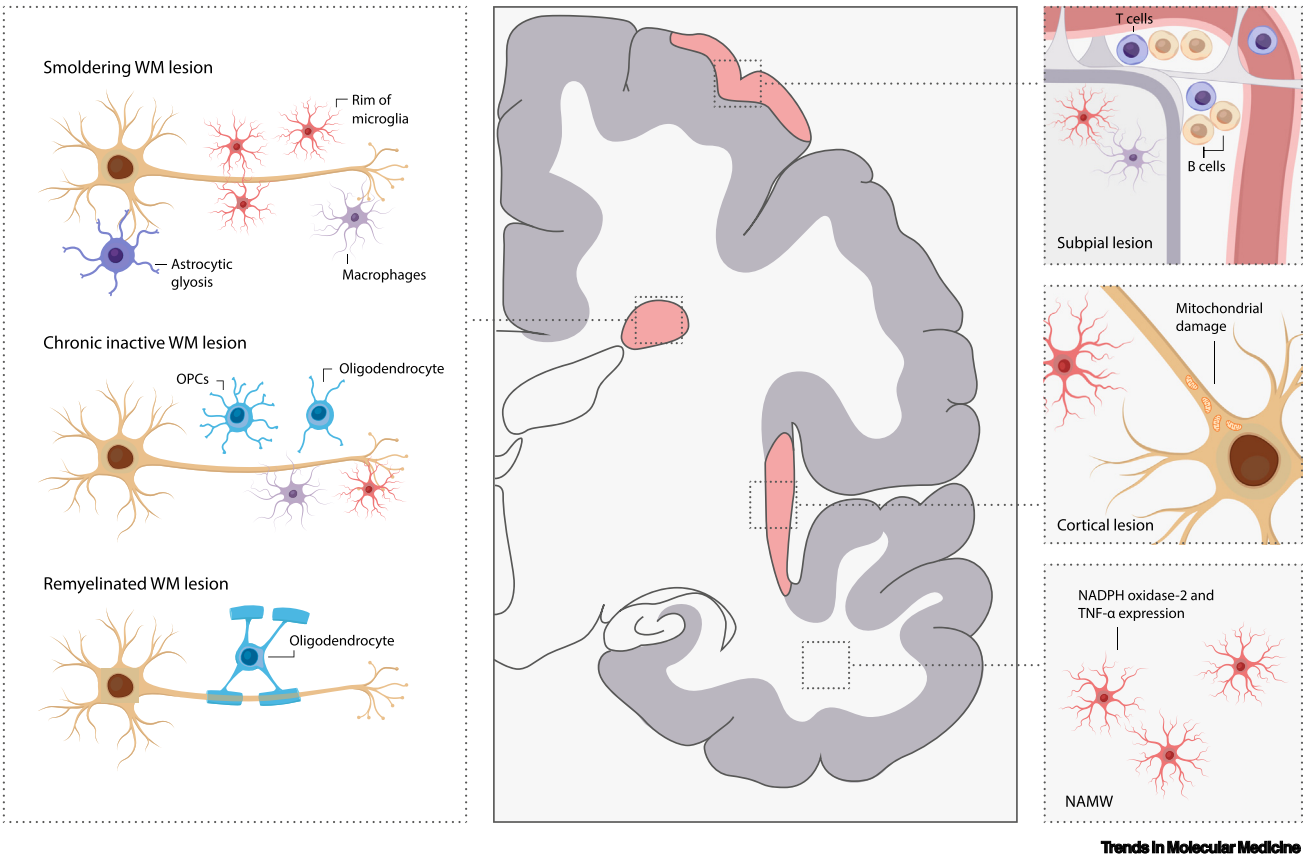

Figure 1. Pathobiology of Progressive Multiple Sclerosis (P-MS). Although early active white matter (WM) lesions fall into three major categories (patterns I–III), several factors determine the typical lesions found in P-MS. Lesion types typically found in P-MS include (i) smoldering lesions, characterized by astrocytic gliosis and a rim of activated mononuclear phagocytes (MPs) with slow myelin degeneration, (ii) chronic inactive lesions, where few oligodendrocytes and oligodendrocyte precursor cells (OPCs) are seen with no remyelination, and (iii) remyelinated lesions, where myelination is thin. Subpial lesions are also common in P-MS and are characterized by demyelination of the superficial cortex accompanied by activated MPs and lymphocyte activation in the overlying leptomeninges. In P-MS, changes are seen also in the cortex, with cortical lesions showing neurons with extensive mitochondrial damage, and in the normal-appearing white matter (NAWM), which shows a diffuse activation of microglia.

Mesenchymal stromal cells (MSCs) have garnered extensive interest in preclinical studies and clinical trials, but few MSC-based products have achieved marketing approval (e.g., lenzumestrocel for the treatment of amyotrophic lateral sclerosis, ALS). MSCs are multipotent *in vitro*, but their propensity for neural differentiation *in vivo* is limited [35]. Instead, MSCs are thought to act predominantly via an immunomodulatory mechanism *in vivo*, thus restricting their utility as a regenerative therapy [36]. Although relatively few randomized controlled clinical studies have studied the application of MSC therapies in MS [37], preliminary results from a large interventional (crossover) Phase II clinical trial on the use of mesenchymal stem cells for multiple sclerosis (MESEMS) suggests evidence of safety, some modest effects on relapses, but no effects on the primary outcome of the study – the number of contrast-enhancing lesions by MRI at 24 weeks in the MSC treatment group [38,39]. Nevertheless, uncertainties because of small trial sample sizes and a lack of uniformity with regards to MSC sources and routes of administration need to be addressed through more robust trials.

The ability to replace lost or damaged cells in the CNS demands cells that are capable of differentiating along neural lineages *in vivo*, such as NSCs [40]. Sources of NSCs include fetal or **embryonic stem cells** (ESCs), **induced pluripotent stem cells** (iPSCs), and **transdifferentiation** from somatic cells.

Although the clinical adoption of fetal and ESC-derived cell therapies has been hindered by ethical concerns, the development of iPSCs has provided an alternative source of pluripotent cells derived from abundant and convenient adult somatic cells [41]. However, despite some persuasive clinical outcomes, including the use of iPSC-derived retinal pigment epithelium for the treatment of age-related macular degeneration (AMD) [42], iPSC-derived NSCs have yet to fully surmount practical and safety challenges to clinical translation [43–45].

Perhaps the best chance to achieve regeneration of the CNS is through the use of NSCs directly induced from a patient's tissue (e.g., a skin or blood biopsy), so-called inducible (i)NSCs, which bypass the problematic pluripotency stage of iPSCs [46–48] and are able to recapitulate the properties and therapeutic potential of true NSCs [49]. Autologous iNSCs seemingly provide a means to circumvent the issue of immunogenicity, but the reality of the situation may be significantly more complex [50,51], and the possibility that genetic defects might adversely affect the therapeutic utility of autologously derived iNSCs in P-MS needs to be explored. Furthermore, considerations regarding practicality, cost of goods, and ease of regulatory compliance will ultimately have a significant influence on the choice of autologous versus allogeneic stem cell products [52].

Multiple prospective routes are available for the administration of stem cells for CNS applications, each of which comes with its own pros and cons. With regards to the treatment of P-MS, administration of NSCs into the cerebrospinal fluid by intrathecal or intracerebroventricular injection establishes a useful compromise between the invasiveness of the procedure and circumventing biological barriers [53]. Ultimately, the specific parameters of dose and timing for successful stem cell interventions are likely to be dictated by the cell type being administered and its mechanisms of action.

Mechanisms of Action

The multifunctionality of stem cells proves to be a double-edged sword: they provide a platform for combating disease and injury on multiple fronts (Figure 2), but often at the expense of well-defined therapeutic mechanisms and outcomes in clinical studies.

Early expectations for cell transplantation in the context of CNS disease were that exogenous cells would integrate into the damaged CNS tissue and subsequently differentiate into neural

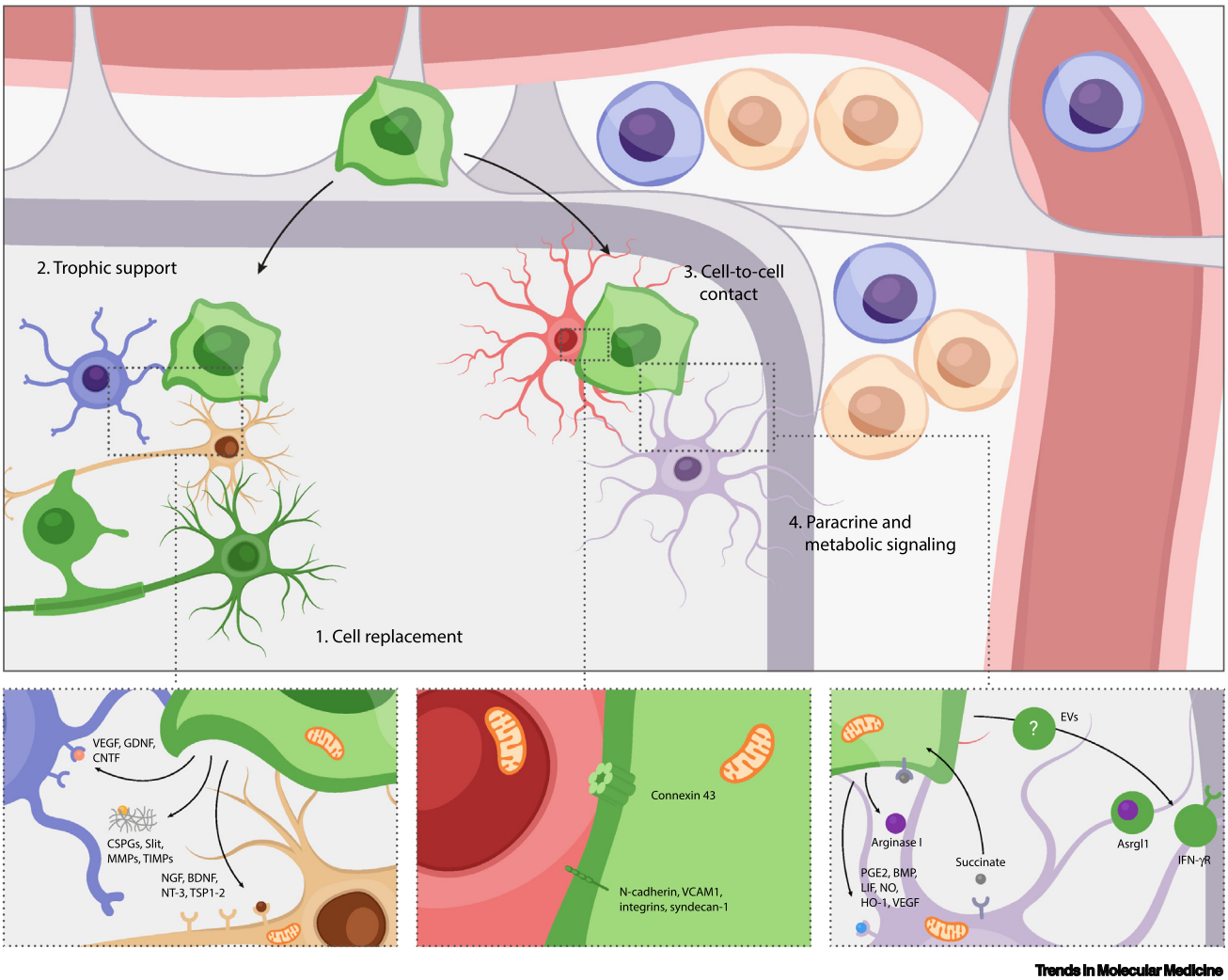

Figure 2. Mechanisms of Action of Central Nervous System (CNS) Stem Cell Therapies.

For a Figure360 author presentation of Figure 2, see https://doi.org/10.1016/j.molmed.2020.04.005.

These include (1) replacement of damaged cells, (2) (neuro)trophic support of injured tissue via paracrine factors, (3) immunomodulation of mononuclear phagocytes (MPs) via cell-to-cell contact, and (4) immunomodulatory effects via paracrine and metabolic signaling. Although CNS stem cells can replace neurons, oligodendrocytes, and astrocytes, they induce tissue regeneration by releasing glia-modulating factors (e.g., VEGF) and neurotrophic factors (e.g., NGF), as well as by remodeling the extracellular matrix. The immunomodulatory effect of CNS stem cells depends on their ability to traffic to sites of inflammation (pathotropism), where they contact MPs and release paracrine factors and enzymes (either naked or included in extracellular vesicles, EVs). Metabolic competition of the cellular graft for metabolites and substrates is also a key mechanism for immunomodulation of activated MPs.

cells. This mechanism is perhaps exemplified by the NSI-566 fetal NSC investigational product that is being studied study in ALS (clinicaltrials.gov registration NCT01348451[i] and NCT01730716[i]), spinal cord injury (SCI; NCT01772810[i]), and stroke (NCT03296618[i]). In preclinical and clinical studies, transplanted NSI-566 fetal NSCs were found to achieve synaptic integration and promote regeneration, with positive functional outcomes [54].

Nevertheless, most preclinical studies have found that cell replacement is secondary to other 'bystander' effects in which the transplanted cells modulate homeostasis favoring neuroprotection and immunomodulation via multiple mechanisms [53,55].

Table 1. Clinical Trials of CNS Stem Cells in Regenerative Neuroimmunology[a,b]

Condition	Sponsor/ coordinator	Cell type	Administration route (cell dose)	Study Design	Enrolment	Start	End	Notes	Registration[i]
ALS	Azienda Ospedaliera Santa Maria (Italy)	Fetal human NSCs	Intraspinal (unilateral or bilateral injections of 3 × 750 000 cells per side)	Phase I; open-label, single-arm	6	2011	2015	Completed No severe adverse effects related to procedure	NCT01640067
ALS	Neuralstem Inc. (USA)	Fetal human NSCs (NSI-566)	Intraspinal (unilateral or bilateral, lumbar and/or cervical; dose 0.5–16 × 10^6 cells)×	Phase I; open-label, single-arm	15	2009	2016	Completed Long-term (3 year) *post hoc* analysis of Phase I and II outcomes versus historical controls found significantly improved survival and function in NSC-treated cohorts [91]	NCT01348451
				Phase II; open-label, non-controlled, dose-escalating, multicenter	15	2013	2016		NCT01730716
ALS	Cedars-Sinai Medical Center (USA)	Fetal human NSCs expressing GDNF	Intraspinal (unilateral lumbar, dose unspecified)	Phase I/IIa; open-label, blinded, dose-escalating	18	2017	2019	Active, not recruiting Primary outcome measures: safety/ adverse events (12 months) Secondary outcome measures: functional, physiological, and biochemical assessments (up to 15 months)	NCT02943850
AMD	StemCells Inc. (USA)	Fetal human NSCs	Subretinal (unilateral, 0.2 × 10^6 or 1 × 10^6 cells)	Phase I/Phase II; open-label, dose-ranging	15	2012	2015	Completed, results unreported Primary outcome measures: adverse events (1 year) Secondary outcome measures: visual function (1 year)	NCT01632527
CP	The First Affiliated Hospital of Dalian Medical University (China)	NSCs	Unspecified	Phase unspecified; double-masked, parallel-controlled	20	2016	2019 (est.)	Recruiting Primary outcome measures: gross motor function changes (up to 1 year) Secondary outcome measures: fine motor function, spasticity, EEG, brain imaging	NCT03005249
HIE	Navy General Hospital (China)	NSCs	Intrathecal (three doses of 4 × 10^6 cells at 48–72 h, 5 days and 10 days after birth)	Phase unspecified; open-label, multiarm (NSC-treated, paracrine factor-treated, combination, no intervention)	120	2013	2017 (est.)	Status unknown Primary outcome measures: behavioral neurological assessment (up to 28 days) and adverse events Secondary outcome measures: motor function (up to 18 months) and CNS tumor imaging (up to 5 years)	NCT02854579
PD	Second Affiliated Hospital of Soochow University (China)	Fetal human NSCs	Intranasal (4 × 10^6 cells weekly for 4 weeks)	Phase II/Phase III; open-label, single-arm	12	2017	2018 (est.)	Status unknown Primary outcome measures: improvement in motor scores (up to 28 weeks) Secondary outcome measures: non-motor	NCT03128450

(continued on next page)

Table 1. (continued)

Condition	Sponsor/ coordinator	Cell type	Administration route (cell dose)	Study Design	Enrolment	Start	End	Notes	Registration[i]
								functional scores (cognition, sensory, fatigue, emotion, quality-of-life), brain imaging, immunology and biochemistry, safety (adverse events)	
PD	University of Jordan (Jordan)	Umbilical cord MSC-derived NSCs	Intrathecal and intravenous (dose unspecified)	Phase I/Phase II; open-label, single-arm	10	2018	2020	Recruiting Primary outcome measures: safety and tolerability (up to 6 months) Secondary outcome measures: gait, balance, biomarkers (up to 6 months)	NCT03684122
PD	Chinese Academy of Sciences (China)	ESC-derived NSCs	Intrastriatal (dose unspecified)	Phase I/Phase II; open-label, HLA-matched versus nonmatched	50	2017	2020	Active Primary outcome measures: adverse events (up to 6 months) Secondary outcome measures: functional, motor, quality-of-life disease scores; imaging (up to 12 months)	NCT03119636
PD	Allife Medical Science and Technology Co. Ltd (China)	iPSC-derived NSCs (autologous)	Unspecified	Phase I; open-label, single-arm	10	2019	2021	Not yet recruiting Primary outcome measures: adverse events (up to 1 year)	NCT03815071
PD	Cyto Therapeutics Pty Ltd (Australia)	Human parthenogenetic NSCs	Intracerebral (dose unspecified)	Phase I; open-label, single-arm, dose-ranging	12	2016	2020	Active, not recruiting Primary outcome measures: adverse events (up to 12 months). Secondary outcome measures: disease rating scores (up to 12 months)	NCT02452723
P-MS	IRCCS Ospedale San Raffaele (Italy)	Fetal human NSCs	Intrathecal (0.7–5.4 × 10^6 cells/kg body weight)	Phase I; open-label, non-controlled, dose-escalating	4	2017	2020 (est.)	Enrolling Primary outcome measures: feasibility, safety and tolerability up to 96 weeks, quality-of-life	NCT03269071
P-MS	Casa Sollievo della Sofferenza IRCCS (Italy)	Fetal human NSCs	Intracerebroventricular (dose unspecified)	Phase I; open-label, single-arm, dose-ranging, multicenter	24	2017	2021 (est.)	Active Primary outcome measures: feasibility, safety, and tolerability up to 1 year Secondary outcome measures: functional, cognitive, and neurophysiological changes, biomarkers, relapse rate	NCT03282760
P-MS	Tisch Multiple Sclerosis Research Center of New York (USA)	MSC-derived NSCs	Intrathecal (three doses of up to 1 × 10^7 cells, 3 month intervals)	Phase I; open-label, single-arm	20	2014	2017	Completed Safe and well-tolerated, signs of improvement in disability status, muscle strength, and bladder function at 9 months [92]	NCT01933802

Table 1. (continued)

Condition	Sponsor/ coordinator	Cell type	Administration route (cell dose)	Study Design	Enrolment	Start	End	Notes	Registration[i]
			Intrathecal (six treatments, dose unspecified, 2 month intervals)	Phase II; placebo-controlled crossover, quadruple-masked	50	2018	2023	Recruiting Primary outcome measures: disability status score (36 months) Secondary outcome measures: functional score and bladder function (36 months)	NCT03355365
SCI	Federal Research Clinical Center of Federal Medical and Biological Agency (Russia)	Autologous MSC-derived NSCs (in 3D biomatrix)	Intraspinal and intrathecal (dose unspecified)	Phase I/Phase II; open-label, factorial assignment (para- versus tetraplegic; acute, subchronic, chronic SCI)	30	2014	2018 (est.)	Status unknown Primary outcome measures: feasibility and safety (24 months) Secondary outcome measures: impairment score improvement, imaging (up to 3 years follow-up)	NCT02326662
SCI	Chinese Academy of Sciences (China)	NSCs (or MSCs) in scaffold	Intraspinal (10×10^6 cells in scaffold)	Phase I/Phase II; double-masked, parallel assignment	30	2016	2020	Enrolling by invitation Primary outcome measures: impairment scale and electrophysiology (24 months) Secondary outcome measures: functional independence, bowel and bladder function, and imaging (24 months); safety (6 months)	NCT02688049
SCI	Neuralstem Inc. (USA)	Fetal human NSCs (NSI-566)	Intraspinal (dose unspecified)	Phase I; open-label, single-arm	8	2014	2022	Recruiting Primary outcome measures: adverse events (6 months) Secondary outcome measures: graft survival assessed by imaging (60 months)	NCT01772810
SCI	StemCells, Inc. (USA)	Fetal human NSCs	Intraspinal	Phase I/Phase II; open-label, single-arm	12	2011	2015	Completed Safe and tolerable, hints of functional improvement [68]	NCT01321333
Stroke	Neuralstem Inc. (China, USA)	Fetal human NSCs (NSI-566)	Intracranial ($1.2–7.2 \times 10^7$ cells)	Phase I; open-label, non-controlled, escalating dose	18	2012	2018	Completed Transplant well-tolerated with evidence of therapeutic benefit by three different clinical outcome measures [54]	NCT03296618
Stroke	Allife Medical Science and Technology Co. Ltd (China)	iNSCs	Intracerebral (dose unspecified)	Phase I; open-label, single-arm	12	2019	2019	Not yet recruiting Primary outcome measures: treatment emergent adverse events (1 year)	NCT03725865
Stroke	ReNeuron Ltd (UK)	Fetal human NSCs (CTX)	Intracranial (20×10^6 cells)	Phase II; placebo-controlled (sham surgery), quadruple-masked	150	2018	2022	Recruiting Primary outcome measures: degree of disability or dependence in daily activities (6 months)	NCT03629275

(continued on next page)

Table 1. (continued)

Condition	Sponsor/ coordinator	Cell type	Administration route (cell dose)	Study Design	Enrolment	Start	End	Notes	Registration[i]
								Secondary outcome measures: various functional, cognitive, daily living, and quality-of-life scores (6 months); safety (12 months).	
Stroke	ReNeuron Ltd (UK)	Fetal human NSCs (CTX)	Intracranial (20×10^6 cells)	Phase II; multicenter, single-arm, open-label	23	2014	2017	Completed Primary outcome measures: functional test of upper limb function (3 months) Secondary outcome measures: functional recovery, independence, daily living, safety and tolerability (12 months)	NCT02117635
Stroke	ReNeuron Ltd (UK)	Fetal human NSCs (CTX)	Intracranial ($5–20 \times 10^6$ cells)	Phase I; open-label, dose-ranging	12	2010	2023	Active, not recruiting Primary outcome measures: adverse events (1 year) Secondary outcome measures: functional cognitive, quality-of-life scores (1 year)	NCT01151124

[a]CNS stem cell trials in neurological disease and injury (completed or ongoing) as registered at clinicaltrials.gov (1 February 2020).
[b]Abbreviations: ALS, amyotrophic lateral sclerosis; AMD, age-related macular degeneration; CP, cerebral palsy; ESC, embryonic stem cell; GDNF, glial-derived neurotrophic factor; HIE, hypoxic-ischemic encephalopathy; iPSC, induced pluripotent stem cell; MSC, mesenchymal stromal cell; NSC, neural stem cell; PD, Parkinson's disease; P-MS, progressive multiple sclerosis; SCI, spinal cord injury.

NSCs exert direct neuroprotective action through the secretion of neurotrophic factors [56]. For example, one of the most clinically advanced cell products, the CTX fetal NSC line, purportedly acts via a secreted cocktail of cytokines and growth factors that promote neurogenesis and axonal sprouting, and even angiogenesis [57]. The specific factors and mechanisms involved in these phenomena remain to be elucidated, although there is increasing interest in NSC lines engineered to overexpress specific neurotrophic factors [58].

Of relevance to MS, NSCs also inhibit the peripheral and perivascular activation of proinflammatory T cells and increase the numbers of anti-inflammatory T regulatory cells in animal models of the disease [59,60]. Transplanted NSCs ostensibly mediate T cell immunomodulation through juxtracrine interactions with immune cells and/or the release of paracrine/endocrine factors [61]. Examples of the latter include cytokines such as interleukin (IL)-10 [62], leukemia inhibitory factor (LIF) [63], and transforming growth factor (TGF)-β2 [64], as well as nitric oxide (NO) and prostaglandin E2 (PGE2) [65].

In the context of P-MS, the mechanisms of action of NSCs on MPs are of pivotal importance. Among these, the reprogramming of proinflammatory mononuclear phagocytes through sequestration of the immunomodulatory metabolite succinate and secretion of PGE2 is common to both NSCs and iNSCs [49]. Addressing the chronic activation of mononuclear phagocytes by means of modulating their mitochondrial metabolism is expected to be a key target of future molecular and cellular therapies for P-MS [20].

Overall, despite compelling preclinical evidence of the therapeutic effects of transplantation in animal models of MS, further well-designed clinical trials are warranted.

Human CNS Stem Cell Clinical Trials

NSCs have seen relatively few clinical trials in neurological disease so far, perhaps owing to practical and/or ethical issues surrounding the acquisition of NSCs [66].

Of those studies that have been conducted, most have been early-stage feasibility, safety, and tolerability assessments, leading to a general consensus of safety. The only clinical trials that have progressed through Phase II or Phase III efficacy studies have yielded inconsistent outcomes owing to variabilities in many factors, but have nevertheless unveiled some benefits in patients.

A broader list of clinical trials of NSCs in the treatment of neurological disease and injury is provided in Table 1, but clinical studies in this field were pioneered by the now-defunct StemCells Inc. (USA) [67]. StemCells Inc. obtained promising results in early-stage trials of intraparenchymal NSC transplantation in SCI (e.g., NCT01321333[i] [68]) but clinical efficacy was never observed in more robust follow-up studies [69]. The company was controversial a several levels, including its access to public funding, conflict of interest, and other issues.

Moreover, follow-up *in vivo* animal work comparing the clinical-grade NSC line and the research line from which it was derived revealed that the clinical line had no efficacy in a mouse model of SCI, even proving detrimental in some respects [70]. This discrepancy highlights the difficulty in translating preclinical results to a clinical setting, and demonstrates the importance of well-defined release criteria when manufacturing clinical-grade products.

Despite an abundance of encouraging preclinical data, clinical studies of NSCs in the context of P-MS are particularly limited, and there is a scarcity of human efficacy data. Current investigations include two ongoing small dose-ranging Phase I studies exploring the feasibility and safety of NSCs in the treatment of advanced P-MS: a monocentric study of intrathecal NSC delivery (Italy; NCT03269071[i]) and a multicentric study of intracerebroventricular delivery (Italy; NCT03282760[i]). The studies have follow-up lengths of 96 weeks and 1–5 years, respectively, with primary outcome measures focused on the occurrence of adverse events, and secondary outcomes pertaining to quality-of-life and functional improvements.

Although encouraging, larger rigorously controlled studies will be necessary to definitively establish the clinical utility of NSCs and to surmount regulatory barriers to translation.

False Promises and Poor Regulation of Stem Cell 'Clinics'

Notwithstanding insufficient evidence supporting their clinical use, unproven stem cell interventions are increasingly being marketed directly to consumers, providing false hope of therapies or even miraculous cures. Patients with chronic debilitating conditions such as P-MS are particularly susceptible to the lure of enticing but unproven interventions, understandably so given the slow progress in developing effective therapies for the disease [71]. Although often discussed in the context of **stem cell tourism**, where prospective patients will travel abroad to receive stem cell treatments in jurisdictions with less regulatory oversight [72], there is growing incidence of such clinics in affluent countries such as the USA [73–75], Canada [76], Australia [77], and the UK [78].

Although recent surveys by Frow *et al.* [74] and Turner and Knoepfler [73] found that the cells most commonly used in such treatments in the USA are classified as MSCs (or more broadly progenitor cells) derived from patient adipose tissue or bone-marrow, there remains a strong risk of premature exploitation of other, emerging cell therapies, including NSCs. Indeed, treatments for

Clinician's Corner

Recent decades have seen an evolution of therapies that can reduce the rate of new inflammatory lesions in RR-MS, but however have questionable effects on delaying secondary MS progression. Conflicting studies show either no effect [87] or limited effect [88] of injectable anti-inflammatory therapies (interferon-β or glatiramer acetate) on secondary progression. Instead, RR-MS patients treated with newer DMTs (natalizumab, alemtuzumab, or fingolimod) show a lower risk of conversion to SP-MS compared with untreated patients [9]. Nonetheless, this effect is highly dependent on disease duration, and 19% versus 38% of RR-MS patients convert to SP-MS after 5 years of natalizumab treatment, which plummets to 34% versus 48% at 6 years. Although early treatment is a key factor to be considered, these data suggest that the highest risk factor for progression is the time of disease duration, and ~60% of untreated RR-MS patients converting after 11 years [9].

Currently, drugs specifically indicated for P-MS include the anti-**CD20** B cell-depleting antibody ocrelizumab that is approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of PP-MS. This approval was based on clinical trial participants with a high proportion of active lesions. Indeed, the EMA indication is specific for 'early primary progressive MS in terms of disease duration and level of disability, and with imaging features characteristic of inflammatory activity'. The most common side-effects of ocrelizumab treatment are infusion-related reactions.

Similarly, both the **sphingosine-1 phosphate receptor** (S1PR) modulator siponimod (which inhibits lymphocyte infiltration into the CNS) and the **purine analog** cladribine (which selectively depletes lymphocytes) are approved by the FDA and EMA for the treatment of active SP-MS. Side-effects of siponimod can include bradycardia/bradyarrhythmia or macular edema, whereas cladribine therapy may result in lymphopenia or liver injury. All three of these lymphocyte-targeting therapies come with increased susceptibility to infection, including a small but serious risk of potentially fatal progressive multifocal leukoencephalopathy.

neurological/neurodegenerative conditions are among the top five most marketed applications of cell transplantation, and orthopedic issues, pain, and inflammation are the most commonly addressed ailments [73,74]. Such unproven 'therapies' are being offered despite limited (or absent) evidence for the efficacy of the clinical-grade lines and, in some cases, little scientific rationale for a given application [79,80]. Interventions are often provided under a false veneer of validity through registration as a clinical trial in databases such as clinicaltrials.gov, despite being at the patient's (often considerable) expense [81], and the qualifications of the providers frequently lie outside the scope of their expertise [82]. Most concerningly, incidences of adverse effects are on the rise and encompass a range of harm, from infection to blindness and even death (Box 1).

The regulatory intricacies of therapeutic stem cells differ substantially according to the national or regional authority that has jurisdiction, but stem cell 'clinics' are able to exploit loopholes and governance apathy to peddle unproven treatments. In the USA, stem cell interventions are under the regulatory authority of the US Food and Drug Administration (FDA) as set out under Title 21 of the Code of Federal Regulations Part 1271. This originally had no bearing on cells deemed to be minimally manipulated, and that were intended for homologous use or for the 'same surgical procedure' [83,84]. Many stem cell 'clinics' have evaded regulatory scrutiny by claiming that these exceptions apply to their unproven products (typically autologous MSCs); the FDA has subsequently clarified their guidance to close such loopholes and provide closer oversight, including serving a permanent injunction against one clinic [85]. In addition, growing pressure from scientific/clinical organizations such as the International Society for Stem Cell Research (ISSCR)[ii] to promote strengthened regulatory efforts, and action by companies such as Google to prohibit the advertising of unproven medical techniques[iii], are all important steps in the right direction.

Other putative PMS drugs currently undergoing late-stage clinical study include novel and emerging drugs such as the pleiotropic inhibitor ibudilast [89] and the HMG-CoA reductase inhibitor simvastatin [90], which have been found to yield reductions in brain atrophy in PMS patients.

There is a scarcity of pipeline DMTs that are able to effectively treat *bona fide* P-MS. The multifunctionality and regenerative potential of NSCs may position them as an ideal therapeutic alternative, but there must be a concerted effort to advance the technology through elegantly designed clinical studies to generate compelling evidence of safety and efficacy. Such efforts are currently underway in the context of RR-MS where autologous HSC transplantation is being directly compared with the best-available therapies in a multinational Phase III trial (NCT04047628[i]).

Box 1. Adverse Events Arising from Unproven Stem Cell Interventions

As recently reviewed by Bauer, Elsallab, and Abou-El-Enein [83], there have been at least 35 reported instances of adverse events arising from unproven stem cell interventions. Owing to lax follow-up and reporting by clinics, it is unclear to what extent these numbers reflect the true incidence of such harm [93]. Adverse events have been reported following treatments provided by clinics in 14 countries worldwide following the administration of a variety of different stem cell types (often ambiguous in nature) for the alleged treatment of a diversity of conditions.

Commonly reported complications included neoplastic growths and infections; the former condition reflects the poorly characterized interaction between specific cell sources and a human host, affirming the need for greater study of cell fate and patient susceptibility, whereas the latter highlights deficiencies in treatment protocols and manufacturing controls. Indeed, *Mycobacterium* is a common contaminant of laboratory cell cultures, and a recently reported case of *M. abscessus* infection appears to have arisen as the result of an unproven anticancer stem cell treatment [94]. Other adverse events attributed to such stem cell interventions include febrility, autoimmune reactions, cardiovascular events, and most alarmingly of all, several deaths [83]. Moreover, a rising trend of marketing stem cell treatments for the treatment of incurable CNS disorders has led to reports of neurological complications [95].

The use of unproven stem cell treatments as a putative MS therapy has been associated with at least one reported instance of an adverse event: a young 'stem cell tourist' is described as having suffered catastrophic demyelinating encephalomyelitis after receiving multiple intrathecal doses of MSCs [96]. Adverse events have also been associated with the administration of NSCs for other neurological conditions, and one of the earliest-reported complications was the occurrence of a brain tumor in a young ataxia telangiectasia patient who received fetal NSCs via the cerebellum and cerebrospinal fluid (CSF) [97]. Similarly, an ischemic stroke patient, who received a potpourri of different cell types (MSCs, ESCs, and fetal NSCs) intrathecally, is reported to have developed a glioproliferative lesion of the spinal cord [98].

More generally, unproven stem cell therapies have been associated with instances of vision loss following autologous adipose stromal cell treatments for AMD [99], infections and fever arising from the use of fetal olfactory ensheathing cells in treating SCI [100], a mucous-filled spinal mass following an olfactory mucosal cell-based SCI therapy [101], and cerebral hemorrhage (leading to death of an 18 month old baby in one case) following direct injection of bone marrow-derived stem cells into the brain to treat cerebral palsy[v].

Concluding Remarks

P-MS exemplifies the multifaceted pathological niche that is most likely to benefit from the putative therapeutic properties of CNS stem cells, but there is so far few clinical data to support such an application. Indeed, many issues remain to be addressed (see Outstanding Questions). Practical and ethical issues regarding the sourcing of appropriate cells have previously impeded the translation of preclinical results to clinical trials, but advances over the past 10–15 years in the generation of induced stem cells have provided significant opportunities in this regard. Nevertheless, even beyond the need for evidence of clinical efficacy, there remain considerable hurdles to the translation of CNS stem cells into an effective therapy, including considerations of manufacturing, quality control, and cost. For example, the cost of a single autologous iPSC treatment has been estimated at ~1 million US$ [86], but improvements in manufacturing scale and efficiency are likely to be met by a concomitant reduction in costs.

Despite the promise of CNS stem cells, there is a strong need for consensus statements from regulatory bodies and relevant international societies to mold the direction of clinical studies so as to obtain meaningful outcomes and maintain safety and ethical standards (e.g., the ISSCR *Practical Advice for Physicians and Ethics/Institutional Review Boards*[iv]). Ultimately, the best way forward is to promote good science and good practice in the best interests of the patient, thus providing a solid foundation upon which to demonstrate the value of CNS stem cell transplantation.

Outstanding Questions

What prompts the progression from RR-MS to SP-MS, and what biomarkers can be used to monitor this process?

Does the P-MS CNS have sufficient regenerative potential to be exploited by pharmaceutical interventions aimed at tissue repair?

To what extent do the different proposed mechanisms of action (innate vs adaptive immunomodulation, metabolic regulation of inflammation and glial reactivity, neurotrophic support, cell replacement, etc.) contribute to the therapeutic properties of NSC transplants?

Do preclinical results obtained in animal models accurately reflect the therapeutic potential of NSCs in the clinical setting?

What is the optimal dosage and administration route of NSCs, which cell source is the most practical (e.g., autologous vs. allogeneic), and what are the parameters of an ideal clinical study (timing, cohort size and identity, outcome measures, etc.)?

What is the half-life and/or long-term fate of NSCs once transplanted into a patient?

What actions can be taken by regulatory bodies and the stem cell community to better protect at-risk patients from 'clinics' providing unproven stem cell therapies?

Acknowledgments

We are grateful to Alexandra Nicaise, Giovanni Pluchino, and Cory Willis for critically reading the manuscript. The authors acknowledge the contribution of past and present members of the laboratory of S.P. who have contributed to (or inspired) this review. This work has received support from the National MS Society (USA, grant RG-1802-30200 to S.P.), the Italian Multiple Sclerosis Association (AISM, grant 2018/R/14 to S.P.), the US Department of Defense (DoD) Congressionally Directed Medical Research Programs (CDMRP) (grant MS-140019 to S.P.), and the Bascule Charitable Trust (RG 75149 and RG 98181 to S.P.). L.P.J. was supported by a research training fellowship from the Wellcome Trust (RG 79423). L.P.J. is currently the recipient of a senior research fellowship from the Fondazione Italiana Sclerosi Multipla (FISM; 2017/B/5) financed or cofinanced by '5 Per Mille' public funding.

Disclaimer Statement

S.P. is cofounder, CSO, and shareholder (>5%) of CITC Ltd and iSTEM Therapeutics, and cofounder and Non-Executive Director at Asitia Therapeutics. J.A.S. is a Project Manager and Senior Research Associate at CITC Ltd and CSO of Asitia Therapeutics. L.P.J. is Head of Research at iSTEM Therapeutics.

Resources

[i]This trial is registered at ClinicalTrials.gov

[ii]www.isscr.org/policy/positions-and-statements

[iii]www.statnews.com/2019/09/24/stem-cell-treatments-ads-banned-google/

[iv]www.isscr.org/docs/default-source/clinical-resources/isscr-stem-cell-based-clnical-trials-practical-advice_final_23jan2018.pdf?sfvrsn=2

[v]www.sciencemag.org/news/2011/05/authorities-shut-controversial-german-stem-cell-clinic

References

1. Reich, D.S. *et al.* (2018) Multiple sclerosis. *N. Engl. J. Med.* 378, 169–180
2. Stenager, E. (2019) A global perspective on the burden of multiple sclerosis. *Lancet Neurol.* 18, 227–228
3. Baranzini, S.E. and Oksenberg, J.R. (2017) The genetics of multiple sclerosis: from 0 to 200 in 50 years. *Trends Genet.* 33, 960–970
4. Mallucci, G. *et al.* (2015) The role of immune cells, glia and neurons in white and gray matter pathology in multiple sclerosis. *Prog. Neurobiol.* 127/128, 1–22
5. Dendrou, C.A. *et al.* (2015) Immunopathology of multiple sclerosis. *Nat. Rev. Immunol.* 15, 545–558
6. Derfuss, T. *et al.* (2020) Advances in oral immunomodulating therapies in relapsing multiple sclerosis. *Lancet Neurol.* 19, 336–347
7. Editorial (2012) Setting new standards in multiple sclerosis care and research. *Lancet Neurol.* 11, 835
8. Thompson, A.J. *et al.* (2018) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* 17, 162–173
9. Brown, J.W.L. *et al.* (2019) Association of initial disease-modifying therapy with later conversion to secondary progressive multiple sclerosis. *JAMA* 321, 175–187
10. Manouchehri, N. and Stüve, O. (2019) Trials and therapies in secondary progressive MS, simplified. *Nat. Rev. Neurol.* 15, 431–432

11. Cunniffe, N. and Coles, A. (2019) Promoting remyelination in multiple sclerosis. *J. Neurol.* Published online June 12, 2019. https://doi.org/10.1007/s00415-00019-09421-x
12. Franklin, R.J.M. and Ffrench-Constant, C. (2017) Regenerating CNS myelin – from mechanisms to experimental medicines. *Nat. Rev. Neurosci.* 18, 753–769
13. Yeung, M.S.Y. *et al.* (2019) Dynamics of oligodendrocyte generation in multiple sclerosis. *Nature* 566, 538–542
14. Jäkel, S. *et al.* (2019) Altered human oligodendrocyte heterogeneity in multiple sclerosis. *Nature* 566, 543–547
15. Falcão, A.M. *et al.* (2018) Disease-specific oligodendrocyte lineage cells arise in multiple sclerosis. *Nat. Med.* 24, 1837–1844
16. Schirmer, L. *et al.* (2019) Neuronal vulnerability and multilineage diversity in multiple sclerosis. *Nature* 573, 75–82
17. Mahad, D. *et al.* (2008) Review: Mitochondria and disease progression in multiple sclerosis. *Neuropathol. Appl. Neurobiol.* 34, 577–589
18. Mahad, D. *et al.* (2008) Mitochondrial defects in acute multiple sclerosis lesions. *Brain* 131, 1722–1735
19. Peruzzotti-Jametti, L. *et al.* (2014) The role of the immune system in central nervous system plasticity after acute injury. *Neuroscience* 283, 210–221
20. Peruzzotti-Jametti, L. and Pluchino, S. (2018) Targeting mitochondrial metabolism in neuroinflammation: towards a therapy for progressive multiple sclerosis. *Trends Mol. Med.* 24, 838–855
21. Voet, S. *et al.* (2019) Microglia in central nervous system inflammation and multiple sclerosis pathology. *Trends Mol. Med.* 25, 112–123
22. Wang, J. *et al.* (2019) Targeting microglia and macrophages: a potential treatment strategy for multiple sclerosis. *Front. Pharmacol.* 10, 286
23. Zrzavy, T. *et al.* (2017) Loss of 'homeostatic' microglia and patterns of their activation in active multiple sclerosis. *Brain* 140, 1900–1913
24. Hammond, T.R. *et al.* (2019) Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. *Immunity* 50, 253–271
25. Masuda, T. *et al.* (2019) Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. *Nature* 566, 388–392
26. Geirsdottir, L. *et al.* (2019) Cross-species single-cell analysis reveals divergence of the primate microglia program. *Cell* 179, 1609–1622
27. Kempermann, G. *et al.* (2018) Human adult neurogenesis: evidence and remaining questions. *Cell Stem Cell* 23, 25–30
28. van Deursen, J.M. (2014) The role of senescent cells in ageing. *Nature* 509, 439–446
29. Nicaise, A.M. *et al.* (2019) Cellular senescence in progenitor cells contributes to diminished remyelination potential in progressive multiple sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 116, 9030
30. Krysko, K.M. *et al.* (2019) Telomere length is associated with disability progression in multiple sclerosis. *Ann. Neurol.* 86, 671–682
31. Muraro, P.A. *et al.* (2017) Autologous haematopoietic stem cell transplantation for treatment of multiple sclerosis. *Nat. Rev. Neurol.* 13, 391–405
32. Lünemann, J.D. *et al.* (2020) Immune reconstitution therapies: concepts for durable remission in multiple sclerosis. *Nat. Rev. Neurol.* 16, 56–62
33. Mohty, B. and Mohty, M. (2011) Long-term complications and side effects after allogeneic hematopoietic stem cell transplantation: an update. *Blood Cancer J.* 1, e16
34. Sharrack, B. *et al.* (2020) Autologous haematopoietic stem cell transplantation and other cellular therapy in multiple sclerosis and immune-mediated neurological diseases: updated guidelines and recommendations from the EBMT Autoimmune Diseases Working Party (ADWP) and the Joint Accreditation Committee of EBMT and ISCT (JACIE). *Bone Marrow Transplant.* 55, 283–306
35. Nombela-Arrieta, C. *et al.* (2011) The elusive nature and function of mesenchymal stem cells. *Nat. Rev. Mol. Cell Biol.* 12, 126–131
36. Martin, I. *et al.* (2019) Challenges for mesenchymal stromal cell therapies. *Sci. Transl. Med.* 11, eaat2189
37. Oliveira, A.G. *et al.* (2019) Growing evidence supporting the use of mesenchymal stem cell therapies in multiple sclerosis: a systematic review. *Mult. Scler. Relat. Disord.* 38, 101860
38. Uccelli, A. *et al.* (2019) Mesenchymal stem cells for multiple sclerosis (MESEMS): a randomized, double blind, cross-over phase I/II clinical trial with autologous mesenchymal stem cells for the therapy of multiple sclerosis. *Trials* 20, 263
39. Uccelli, A. *et al.* (2019) Mesenchymal stem cells for multiple sclerosis (MESEMS): a multi-center, randomized, double blind, cross-over phase 2 clinical trial with autologous mesenchymal stem cells (MSC). In *ECTRIMS Online Library* (278579), pp. P1378
40. Tang, Y. *et al.* (2017) Current progress in the derivation and therapeutic application of neural stem cells. *Cell Death Dis.* 8, e3108
41. Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663–676
42. Mandai, M. *et al.* (2017) Autologous induced stem-cell-derived retinal cells for macular degeneration. *N. Engl. J. Med.* 376, 1038–1046
43. Lee, A.S. *et al.* (2013) Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. *Nat. Med.* 19, 998–1004
44. Zhao, T. *et al.* (2011) Immunogenicity of induced pluripotent stem cells. *Nature* 474, 212–215
45. Desgres, M. and Menasché, P. (2019) Clinical translation of pluripotent stem cell therapies: challenges and considerations. *Cell Stem Cell* 25, 594–606
46. Kim, J. *et al.* (2011) Direct reprogramming of mouse fibroblasts to neural progenitors. *Proc. Natl. Acad. Sci. U. S. A.* 108, 7838–7843
47. Thier, M. *et al.* (2012) Direct conversion of fibroblasts into stably expandable neural stem cells. *Cell Stem Cell* 10, 473–479
48. Han, D.W. *et al.* (2012) Direct reprogramming of fibroblasts into neural stem cells by defined factors. *Cell Stem Cell* 10, 465–472
49. Peruzzotti-Jametti, L. *et al.* (2018) Macrophage-derived extracellular succinate licenses neural stem cells to suppress chronic neuroinflammation. *Cell Stem Cell* 22, 355–368
50. Wood, K.J. *et al.* (2016) Understanding stem cell immunogenicity in therapeutic applications. *Trends Immunol.* 37, 5–16
51. Liu, X. *et al.* (2017) The immunogenicity and immune tolerance of pluripotent stem cell derivatives. *Front. Immunol.* 8, 645
52. Kurtz, A. *et al.* (2019) Linking scattered stem cell-based data to advance therapeutic development. *Trends Mol. Med.* 25, 8–19
53. Scolding, N.J. *et al.* (2017) Cell-based therapeutic strategies for multiple sclerosis. *Brain* 140, 2776–2796
54. Zhang, G. *et al.* (2019) Stable intracerebral transplantation of neural stem cells for the treatment of paralysis due to ischemic stroke. *Stem Cells Transl. Med.* 8, 999–1007
55. Martino, G. *et al.* (2011) Brain regeneration in physiology and pathology: the immune signature driving therapeutic plasticity of neural stem cells. *Physiol. Rev.* 91, 1281–1304
56. Willis, C.M. *et al.* (1729) The neural stem cell secretome and its role in brain repair. *Brain Res.* 2020, 146615
57. Sinden, J.D. *et al.* (2017) Human neural stem cell therapy for chronic ischemic stroke: charting progress from laboratory to patients. *Stem Cells Dev.* 26, 933–947
58. Marsh, S.E. and Blurton-Jones, M. (2017) Neural stem cell therapy for neurodegenerative disorders: the role of neurotrophic support. *Neurochem. Int.* 106, 94–100
59. Pluchino, S. *et al.* (2005) Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. *Nature* 436, 266–271
60. Einstein, O. *et al.* (2007) Neural precursors attenuate autoimmune encephalomyelitis by peripheral immunosuppression. *Ann. Neurol.* 61, 209–218
61. Pluchino, S. and Cossetti, C. (2013) How stem cells speak with host immune cells in inflammatory brain diseases. *Glia* 61, 1379–1401
62. Yang, J. *et al.* (2009) Adult neural stem cells expressing IL-10 confer potent immunomodulation and remyelination in experimental autoimmune encephalitis. *J. Clin. Invest.* 119, 3678–3691
63. Cao, W. *et al.* (2011) Leukemia inhibitory factor inhibits T helper 17 cell differentiation and confers treatment effects of neural progenitor cell therapy in autoimmune disease. *Immunity* 35, 273–284
64. De Feo, D. *et al.* (2017) Neural precursor cell-secreted TGF-β2 redirects inflammatory monocyte-derived cells in CNS autoimmunity. *J. Clin. Invest.* 127, 3937–3953

65. Wang, L. *et al.* (2009) Neural stem/progenitor cells modulate immune responses by suppressing T lymphocytes with nitric oxide and prostaglandin E2. *Exp. Neurol.* 216, 177–183
66. Gage, F.H. and Temple, S. (2013) Neural stem cells: generating and regenerating the brain. *Neuron* 80, 588–601
67. Smalley, E. (2016) Neural stem cell trailblazer StemCells folds. *Nat. Biotech.* 34, 677–678
68. Ghobrial, G.M. *et al.* (2017) Human neural stem cell transplantation in chronic cervical spinal cord injury: functional outcomes at 12 months in a phase II clinical trial. *Neurosurgery* 64, 87–91
69. Levi, A.D. *et al.* (2019) Clinical outcomes from a multi-center study of human neural stem cell transplantation in chronic cervical spinal cord injury. *J. Neurotrauma* 36, 891–902
70. Anderson, A.J. *et al.* (2017) Preclinical efficacy failure of human CNS-derived stem cells for use in the pathway study of cervical spinal cord injury. *Stem Cell Rep.* 8, 249–263
71. von Wunster, B. *et al.* (2018) Advising patients seeking stem cell interventions for multiple sclerosis. *Pract. Neurol.* 18, 472–476
72. Bowman, M. *et al.* (2015) Responsibilities of health care professionals in counseling and educating patients with incurable neurological diseases regarding 'stem cell tourism': caveat emptor. *JAMA Neurol.* 72, 1342–1345
73. Turner, L. and Knoepfler, P. (2016) Selling stem cells in the USA: assessing the direct-to-consumer industry. *Cell Stem Cell* 19, 154–157
74. Frow, E.K. *et al.* (2019) Characterizing direct-to-consumer stem cell businesses in the southwest United States. *Stem Cell Rep.* 13, 247–253
75. Knoepfler, P.S. and Turner, L.G. (2018) The FDA and the US direct-to-consumer marketplace for stem cell interventions: a temporal analysis. *Regen. Med.* 13, 19–27
76. Caulfield, T. and Murdoch, B. (2019) Regulatory and policy tools to address unproven stem cell interventions in Canada: the need for action. *BMC Med. Ethics* 20, 51
77. McLean, A.K. *et al.* (2015) Untested, unproven, and unethical: the promotion and provision of autologous stem cell therapies in Australia. *Stem Cell Res Ther* 6, 33–33
78. Erikainen, S. *et al.* (2019) Marketing experimental stem cell therapies in the UK: biomedical lifestyle products and the promise of regenerative medicine in the digital era. *Sci. Cult.* 28, 1–26
79. Sipp, D. *et al.* (2017) Marketing of unproven stem cell–based interventions: a call to action. *Sci. Transl. Med.* 9, eaag0426
80. Srivastava, A. *et al.* (2016) Part 1: defining unproven cellular therapies. *Cytotherapy* 18, 117–119
81. Snyder, J. *et al.* (2018) Crowdfunding for unproven stem cell–based interventions. *JAMA* 319, 1935–1936
82. Fu, W. *et al.* (2019) Characteristics and scope of training of clinicians participating in the US direct-to-consumer marketplace for unproven stem cell interventions. *JAMA* 321, 2463–2464
83. Bauer, G. *et al.* (2018) Concise review: a comprehensive analysis of reported adverse events in patients receiving unproven stem cell-based interventions. *Stem Cells Transl. Med.* 7, 676–685
84. Turner, L. (2018) The US direct-to-consumer marketplace for autologous stem cell interventions. *Perspect. Biol. Med.* 61, 7–24
85. US Food and Drug Administration (2019) *Statement on Stem Cell Clinic Permanent Injunction and FDA's Ongoing Efforts To Protect Patients from Risks of Unapproved Products*, FDA
86. Chakradhar, S. (2016) An eye to the future: researchers debate best path for stem cell–derived therapies. *Nat. Med.* 22, 116–119
87. Zhang, T. *et al.* (2015) Beta-interferon exposure and onset of secondary progressive multiple sclerosis. *Eur. J. Neurol.* 22, 990–1000
88. Signori, A. *et al.* (2016) Long-term impact of interferon or glatiramer acetate in multiple sclerosis: A systematic review and meta-analysis. *Mult. Scler. Relat. Disord.* 6, 57–63
89. Fox, R.J. *et al.* (2018) Phase 2 trial of ibudilast in progressive multiple sclerosis. *N. Engl. J. Med.* 379, 846–855
90. Chataway, J. *et al.* (2014) Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. *Lancet* 383, 2213–2221
91. Goutman, S.A. *et al.* (2018) Long-term phase 1/2 intraspinal stem cell transplantation outcomes in ALS. *Ann. Clin. Transl. Neurol.* 5, 730–740
92. Harris, V.K. *et al.* (2018) Phase I trial of intrathecal mesenchymal stem cell-derived neural progenitors in progressive multiple sclerosis. *EBioMedicine* 29, 23–30
93. Knoepfler, P.S. (2019) Rapid change of a cohort of 570 unproven stem cell clinics in the USA over 3 years. *Regen. Med.* 14, 735–740
94. Olmedo-Reneaum, A. *et al.* (2019) Rogue 'stem cell clinic' leads to *Mycobacterium abscessus* infection. *Lancet* 393, 918
95. Julian, K. *et al.* (2018) The 'growing' reality of the neurological complications of global 'stem cell tourism'. *Semin. Neurol.* 38, 176–181
96. Alderazi, Y.J. *et al.* (2012) Catastrophic demyelinating encephalomyelitis after intrathecal and intravenous stem cell transplantation in a patient with multiple sclerosis. *J. Child Neurol.* 27, 632–635
97. Amariglio, N. *et al.* (2009) Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. *PLoS Med.* 6, e1000029
98. Berkowitz, A.L. *et al.* (2016) Glioproliferative lesion of the spinal cord as a complication of 'stem-cell tourism'. *N. Engl. J. Med.* 375, 196–198
99. Kuriyan, A.E. *et al.* (2017) Vision loss after intravitreal injection of autologous 'stem cells' for AMD. *N. Engl. J. Med.* 376, 1047–1053
100. Dobkin, B.H. *et al.* (2006) Cellular transplants in China: observational study from the largest human experiment in chronic spinal cord injury. *Neurorehabil. Neural Repair* 20, 5–13
101. Dlouhy, B.J. *et al.* (2014) Autograft-derived spinal cord mass following olfactory mucosal cell transplantation in a spinal cord injury patient: case report. *J. Neurosurg. Spine* 21, 618–622