Cell-Based Therapeutic Strategies in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is an immune-mediated disease in which acute inflammatory demyelination leads to axonal injury and neurodegeneration, and is manifested clinically by relapsing–remitting neurological deficits superimposed on chronic accumulation of disability. MS treatments are largely immunomodulatory with little, if any, effect on neurodegeneration. Mesenchymal stem cells (MSCs) are pluripotent cells derived from adult tissues with intrinsic anti-inflammatory and repair-promoting properties. They cross the blood–brain barrier and target perivascular spaces, which are the sites of inflammatory cell infiltration in MS. In vitro, MSCs can be purified and expanded, labelled for post-transplant tracking and be manipulated to express surface receptors or neurotrophic factors for central nervous system (CNS) targeting or neuroprotection, respectively. Animal models of MS, traumatic CNS injury and neurodegenerative diseases demonstrate clinical and pathological benefits following MSC transplantation. Potentially, MSCs can be used to treat MS patients at various disease stages, which is the current focus of ongoing phase I/II clinical trials at multiple centres.

Keywords

Multiple sclerosis, mesenchymal stem cell, clinical trial, induced pluripotent stem cell, experimental autoimmune encephalitis

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Multiple sclerosis (MS) is a chronic immune-mediated neurodegenerative disorder characterised by central nervous system (CNS) demyelination, axonal injury, gliosis and eventual loss of both oligodendrocytes and neurons.¹ The aetiology of the disease is complex, and not entirely understood, though human and animal studies of immune-mediated demyelinating disease has elucidated some components of MS pathophysiology:²⁻⁴ Genetic susceptibility likely plays a role, with major histocompatibility complex (MHC) class I and II genes implicated in disease risk. A variety of other non-MHC genes may act as modest risk alleles individually, but together as a risk haplotype, may confer increased susceptibility.¹ Environmental factors have also been implicated in the pathogenesis and natural history of MS, including sun-exposure correlating to vitamin D synthesis, Epstein-Barr Virus (EBV) exposure and smoking, and these likely interact heterogeneously with genetic factors across the MS population.¹⁻² Thus, the natural history of MS varies between patients. The average age of onset is typically in the third decade and there is a peak prevalence in the fifth decade of life. Paediatric MS is rarer, particularly in patients aged younger than 9 years of age, though there is a growing recognition of early-onset MS and its implications for earlier disability than adult-onset MS.⁵⁻⁶ The prevalence of MS has been estimated at one to two per 1,000 in North America and northern Europe, but the incidence and prevalence vary geographically, seem to increase with latitude and appear to be increasing, particularly in women.¹ A systematic review of the financial burden of MS in the US found a range of $8,328–54,244 per patient per year, with approximately 77 % representing direct costs to the patient (i.e. prescription medications), making MS the second most costly disease in the US behind congestive heart failure.³⁻⁶

Clinically, MS most often begins with a relapsing–remitting course (RRMS) during which acute symptomatic episodes accompany active inflammation detected by gadolinium-enhanced magnetic resonance imaging (MRI), followed by a period of convalescence and recovery; on average relapses occur every 1–2 years. Many of these patients evolve to a secondary progressive stage characterised by neurodegeneration, brain atrophy and gradual accumulation of clinical disability despite a paucity of clinical relapses and MRI lesion activity. Approximately 20 % of patients present with a primary progressive course, in which disability accumulates gradually from onset without acute relapses.⁷

Current Treatments in Relapsing–Remitting Multiple Sclerosis

The mainstay of MS therapy has been anti-inflammatory disease-modifying drugs (DMDs) that target various immune system pathways to decrease or prevent the continuation of an immune-mediated destructive process.⁸ In the clinical context, the goal of therapy is twofold: 1) minimising accumulated disability associated with clinical relapses; and 2) preventing the MRI lesion activity, which can occur in the absence of clinical symptoms. The mechanisms of action, route of delivery, safety, tolerability and side-effect profiles differ between DMDs, though they all serve these two principles. Thus, DMD treatment should be considered early following an MS diagnosis, since the majority of...
Multiple Sclerosis

Table 1: A Comparison of Autologous and Allogenic Mesenchymal Stem Cell Properties as Potential Treatments for Multiple Sclerosis

<table>
<thead>
<tr>
<th>Source</th>
<th>Autologous MSCs</th>
<th>Allogenic MSCs</th>
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<tbody>
<tr>
<td>Myeloablation required</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Immediately available/commercially available</td>
<td>No</td>
<td>Potentially</td>
</tr>
<tr>
<td>Risk of host rejection</td>
<td>No</td>
<td>Potentially</td>
</tr>
<tr>
<td>Immunosenstisiation with repeated administration</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Potential for disease transmission</td>
<td>No</td>
<td>Potentially</td>
</tr>
<tr>
<td>Tumorigenic</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Potential for enzyme replacement or gene therapy</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Regulatory hurdles</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Potentially iPSC derived</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

iPSCs = induced pluripotent stem cells; MS = multiple sclerosis; MSC = mesenchymal stem cell.

Inflammatory destruction is thought to occur early in the disease.11 The first DMDs were the interferon-betas, shown to reduce MS relapses and MRI lesion activity in a series of double blind, placebo-controlled multicentre clinical trials in the early to mid-1990s.16–18 Glatiramer acetate was the next DMD to be introduced in the 1990s and, until recently, these medications were the only DMDs available to RRMS patients, and were ineffective for patients with secondary progressive MS. While used for acute MS relapses, steroids are not effective as long-term therapy in MS.5,16

A relative explosion of DMDs has been approved in the last decade, offering new oral, injectable and intravenous infusions, and targeting novel pathways in the immune system. However, these new therapeutic agents are expensive and burdened with their own unique side effects and, while moderately to highly effective in RRMS, they fail to prevent or treat the neurodegeneration and clinical deterioration of progressive MS. A recent mouse model of RRMS treated with DMDs demonstrated that neurodegeneration continues even after the complete cessation of autoimmune relapses.12 In this model, early cessation of inflammation was neuroprotective, but did not prevent gliosis and neuronal loss, while late cessation had no neuroprotective effects. This again demonstrates the importance of initiating DMD treatment early after diagnosis, but also highlights an unmet need in MS treatment: neuroprotection to prevent neurodegeneration. Thus, the search for new MS treatments continues, with the goal to not only suppress the underlying inflammatory process, but also to halt the disease entirely and potentially rebuild the injured CNS. Cell-based therapies have been proposed for the treatment of MS. As we will review, they offer many advantages over standard pharmaceutical DMDs in their availability, tolerability, safety and potential for manipulation as gene/enzyme delivery systems.

Stem Cells and their Clinical Application

Stem cells are immature, pluripotent progenitor cells that remain proliferative across multiple mitoses, and are found across development from embryogenesis, through foetal development, and, as we now recognise, are found in adult organs. Tissue-specific stem cells vary in their ability to differentiate into mature cell types, and typically respond to their environmental signals to fulfill the needs of that tissue. Thomas et al.3,4 were the first to demonstrate successful intravenous bone marrow infusion from unrelated human donors into six patients, with several demonstrating clinical evidence of graft ‘take’, and none demonstrating untoward reactions.8 Prior to this publication, studies in rodent and primate models demonstrated the complexity of obtaining bone marrow-derived pluripotent cells and the myriad clinical challenges in their use as therapeutic agents. In the intervening half century, animal and human experiments using a variety of pluripotent stem cells, but predominantly haematopoietic stem cells (HSCs) from bone marrow, have yielded feasible, tolerable and clinically relevant therapies for a variety of conditions including haematological malignancies,10–21 primary immunodeficiencies,22 thalassaemia23 and sickle cell disease,24 neurodegenerative diseases,25–27 inborn errors of metabolism28–30 and autoimmune diseases.31 However, these are not without their clinical and ethical pitfalls, including significant morbidity and mortality in some settings, expense, unexpected consequences such as graft-versus-host disease and donor-derived leukaemias and myeloproliferative disorders following umbilical cord stem cell (UCSC) transplants.32

Autologous HSC transplants have been studied in MS, though to date only small, uncontrolled trials have been reported. The rationale for autologous HSC in MS is to ‘reset’ the immune system using various chemotherapeutic agents and/or total body irradiation to ablate myeloproliferative cells, then to reconstitute the immune system with self-tolerant lymphocytes, thereby halting the autoimmune inflammatory process. There remains debate regarding the intensity of immunoablation required prior to autologous HSC transplant, as drug toxicity, side effects and infection risk are responsible for much of the transplant-related morbidity and mortality. In aggregate, these studies demonstrated mild to moderate progression-free survival rates and reduction in gadolinium-enhancing lesions on MRI, and relatively high mortality rates (2–8 %).33–35 Burt et al.36 reported 100 % progression-free survival at 3 years for RRMS and 81 % for progressive MS using a non-myeloablative conditioning protocol, with no treatment-related deaths.36 Recently, 48 patients in Sweden were treated with autologous HSC transplantation and an intermediate-intensity ablation, with no treatment-related mortality, and demonstrated 87 % relapse-free survival and MRI event-free survival at 5 years.37 Together, these reports suggest in the appropriate population, using less-intensive or non-ablative protocols, these treatments may be safe and effective. That said, it is difficult to separate the role of pre-transplantation immunoablation from the function of HSCs as immunomodulators in the reported success of these treatments.38

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are non-HSCs found in bone marrow, which can be harvested, cultured, propagated in vitro and purified, and then induced both in vitro and in vivo to differentiate into mesodermal cells. MSC-like cells have been found in perivascular spaces in multiple tissues, suggesting this may be their native niche in vivo, where they aid in tissue repair and immunological homeostasis.39 They have been well-characterised in animal and human models, and as research and therapeutic agents they avoid the ethical maelstroms associated with embryonic or foetal stem cells.40 MSCs are characterised by their lack of haematopoietic surface markers (CD14, CD11b, CD19, CD34, CD45 and HLA-DR) and presence of CD73, CD90 and CD105 surface markers, their ability to adhere to plastic dishes in vitro and their ability to differentiate in vitro into adipocytes, osteoblasts and chondrocytes.41 MSCs from a variety of tissues can be induced in vitro to neural differentiation, and transplanted neurally differentiated...
MSCs have demonstrated neuronal phenotypes in vivo, though others have demonstrated the failure of neurally differentiated MSCs to function as neurons and integrate into neural circuitry. Thus, the role of MSCs in treatment of neuropathology is less likely repletion of lost neurons, but rather immunomodulatory and supportive (reviewed in reference 46). Safety and tolerability studies in animals and humans demonstrate that MSCs are relatively easy to harvest from marrow, propagate in a small sample of bone marrow, propagate and administer in vivo, 50,51 and in at least one human patient with transfusion-dependent beta-thalassaemia, though clinical trials remain underway. MSCs provide an analogous delivery system for gene therapy replacement and gene therapies. For example, haemoglobinopathies are curable by one-time haematopoietic stem cell transplant, but the paucity of donors and morbidity and mortality associated have limited this practice. Recent trials using lentiviral transformation of autologous HSCs with beta- and gamma-globin genes and their regulatory elements have been successful in vitro and in at least one human patient with transfusion-dependent beta-thalassaemia, though clinical trials remain underway. MSCs provide an analogous delivery system for gene therapy where haematopoiesis is not the goal of the therapy. In mouse models of neonatal hypoxic ischaemic encephalopathy, MSCs transformed with growth factor genes including brain-derived neurotrophic factor, epidermal growth factor-like 7, persephin and sonic hedgehog, increased neural stem cell proliferation and improved motor function compared with empty vector MSCs. In humans, umbilical cord-derived MSC transplant in infantile Krabbe restored blood galactocerebroside activity, prevented pre-symptomatic patients from becoming fully symptomatic and halted MS = multiple sclerosis; MSC = mesenchymal stem cell; N/V = nausea and vomiting; PPMS = primary progressive MS; RRMS = relapsing–remitting MS; SPMS = secondary progressive MS; VEP = visually evoked potential.

Table 2: A Summary of the Four Published Phase I/II Clinical Trials Investigating the Use of Mesenchymal Stem Cells to Treat Multiple Sclerosis

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year</th>
<th>Number of Patients</th>
<th>MS Type</th>
<th>MSC Source</th>
<th>Route and Dose</th>
<th>Side Effects</th>
<th>Other Adverse Events</th>
<th>Follow Up (Months)</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonab</td>
<td>2010</td>
<td>22</td>
<td>SPMS</td>
<td>ABM</td>
<td>Intrathecal: 29.5 x 10^6 cells</td>
<td>Fever ’almost all’ N/V 2/22 Headache 3/22 LE weakness 2/22</td>
<td>None</td>
<td>12</td>
<td>1) Intrathecal MSC transplant is safe and tolerable in progressive MS 2) No group EDSS change at 12 mo 3) Halting of further T2 MRI lesion at 12 mo in 15/22 (62.2%), New T2 or Gd-enhancing MRI lesions at 12 months in 6/22 (27.3 %)</td>
</tr>
<tr>
<td>Connick</td>
<td>2012</td>
<td>10</td>
<td>SPMS</td>
<td>ABM</td>
<td>Intravenous: 1.6 x 10^6 cells/kg bodyweight</td>
<td>Pruritus</td>
<td>None</td>
<td>6</td>
<td>1) Intravenous MSC transplant is safe and tolerable in progressive MS 2) Improved minimal angle of resolution visual acuity (statistically significant group effect) 3) Improved low contrast visual acuity (statistically significant group effect) 4) VEP decreased latency and increased response amplitude (statistically significant group effect) 5) Increased optic nerve area (statistically significant group effect) 6) Reduced disability progression (EDSS) (statistically significant group effect)</td>
</tr>
<tr>
<td>Karussis</td>
<td>2010</td>
<td>15</td>
<td>RRMS</td>
<td>ABM</td>
<td>Intrathecal: 63.5 x 10^6 cells Intravenous: (5 patients only) 24.5 x 10^6 cells</td>
<td>Fever 10/15 Headache 10/15 Meningismus 1/15 Rigidity 2/15 Leg pain 1/15 Confusion 1/15 Neck pain 1/15 Walking difficulty 4/15</td>
<td>None</td>
<td>6</td>
<td>1) Intrathecal and intravenous MSC transplant is safe and tolerable in RRMS patients 2) Clinical improvement (decreased EDSS) at 1, 3 and 6 mo (statistically significant group effect) 3) Increased CD4+ CD25+ regulatory T cells, decreased proliferative lymphocyte responses, and CD40+, CD83+, CD86+ and HLA-DR on myeloid dendritic cells at 24 hour post-transplant</td>
</tr>
<tr>
<td>Yamout</td>
<td>2010</td>
<td>7</td>
<td>SPMS</td>
<td>ABM</td>
<td>Intra-cisternal and intrathecal 3–5 x 10^6 cells</td>
<td>Transient encephalopathy with seizures day 3 post-transplant</td>
<td>None</td>
<td>12</td>
<td>1) Intrathecal and intrathecal MSC transplant is safe and tolerable in progressive MS 2) Stability or improvement in EDSS in 6/7 patients at 6 and 12 months; note 1 patient worsened after improvement at 6 mo due to osteoporotic vertebral fractures 3) Improvement in visual contrast sensitivity by 1–3 lines in 5/6 patients at 12 mo; no change in OCT measures at 12 mo 4) New or enlarging T2 MRI lesions in 5/7 patients at 3 mo; New Gd-enhancing lesions in 3/7 at 3 mo, incomplete data at 12 mo</td>
</tr>
</tbody>
</table>

AABM = autologous bone marrow; EDSS = Expanded Disability Status Scale; Gd = gadolinium; LE = lower extremity; mo = months; MRI = magnetic resonance imaging; MS = multiple sclerosis; MSC = mesenchymal stem cell; N/V = nausea and vomiting; PPMS = primary progressive MS; RRMS = relapsing–remitting MS; SPMS = secondary progressive MS; VEP = visually evoked potential.
Multiple Sclerosis

Table 3: Unresolved Matters in Mesenchymal Stem Cell Transplantation for Multiple Sclerosis

| MSC Source                                    | initiation of ERT | Neuroprotection in chronic MS | Therapeutic efficiency in acute/remitting phase | Therapeutic potential for chronic progressive phase | Safety and tolerability
<table>
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<tbody>
<tr>
<td>Autologous versus allogeneic</td>
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<tr>
<td>Bone marrow, umbilical cord, adipose, iPSC</td>
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<tr>
<td>Universal donor versus directed donor</td>
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<tr>
<td><strong>MSC Culture Conditions and Processing</strong></td>
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<tr>
<td>Culture protocol and engineering</td>
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<tr>
<td>Foetal bovine serum versus serum-free media</td>
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<tr>
<td>Cryopreservation/thawing effects on MSC viability and function</td>
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<tr>
<td>Priming or engineering to produce neurotrophic factors or surface receptors for tissue-specific trafficking</td>
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<tr>
<td><strong>Ideal Patient Population</strong></td>
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<tr>
<td>Newly diagnosed versus prolonged disease course</td>
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<tr>
<td>RRMS, PPMS, SPMS</td>
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<tr>
<td>Paediatric MS</td>
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<tr>
<td>Pregnancy or child-bearing age</td>
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<tr>
<td><strong>Dosing</strong></td>
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<tr>
<td>Ideal delivery route and dosage</td>
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<tr>
<td>Number and interval of doses</td>
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<tr>
<td>Dosing based on duration of benefit</td>
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<tr>
<td>Fresh versus cryopreserved/thawed just before infusion</td>
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<tr>
<td><strong>Other</strong></td>
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<tr>
<td>Role of concomitant DMD treatment</td>
<td></td>
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<tr>
<td>Use as an anti-inflammatory or regenerative strategy or both</td>
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<tr>
<td>Labelling and in vivo localisation of MSCs</td>
<td></td>
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<tr>
<td>Potency markers</td>
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</table>

**Table 3**

DMD = disease-modifying drug; iPSC = induced pluripotent stem cell; MS = multiple sclerosis; MSC = mesenchymal stem cell; PPMS = primary progressive MS; RRMS = relapsing–remitting MS; SPMS = secondary progressive MS.

progression of demyelination in patients symptomatic at the time of transplant.56 Furthermore, in mucopolysaccharidosis (MPS), where enzyme replacement therapies (ERT) have helped alleviate or slow many of the systemic manifestations of the disease, neurological deterioration continues as the replaced enzyme molecules are too large to cross the blood–brain barrier. HSCs and, more recently, UCSCs, which contain MSCs, have been used to restore α-L-iduronidase (IDUA) production in Hunter’s disease patients, resulting in clearance of the neurotoxic glycosaminoglycans and improved neurological function.57 These modalities require HLA- and ethnicity-matched donors, pre-treatment with immunosuppressive and myeloablative processes and their success is incumbent upon achieving threshold donor-host chimerism for sufficient enzyme production. Potentially, allogeneic or autologous MSCs genetically transformed in vitro with the missing enzyme’s gene, could provide the same function as HSCs and UCSCs without requiring immunoaablation or antigenically matched donors. Cell-based therapy also potentially avoids immunoreactivity to patients to exogenous ERT products, as patients who achieve therapeutic circulating levels of enzyme following UCSC transplant have not been found to produce autoantibodies directed against these enzymes.58 However, recent preclinical data in mice transplanted with IDUA-transformed MSCs did produce significant antibody titres, raising questions about safety and efficacy in human trials.59

Preclinical studies of MSCs have shown promise in various neurological disease models including stroke, traumatic injury, amyotrophic lateral sclerosis and Parkinson’s disease.60–62 MSCs have been found to migrate to perivascular niches (the site of autoimmune inflammatory damage in MS) and towards sites of inflammation.63 Intravenous MSC administration has been effective in reducing clinical symptoms in the well-characterised animal model of MS, experimental autoimmune encephalitis (EAE). In this model, animals are sensitised with myelin-specific proteins to induce a relapsing–remitting or progressive inflammatory demyelinating disease.64 MSC infusion not only improved clinical symptoms in EAE mice, these animals had decreased inflammatory cell infiltration into the CNS, and decreased demyelination and axonal injury.65 Recently, Kassis et al.66 demonstrated similar clinical and histopathological benefits following transplantation of MSCs derived from animals with EAE, an animal model for autologous stem cells derived from human patients with MS.67 In a small study from humans, MSCs harvested from five MS patients with secondary progressive MS and five sex-matched (but not age-matched) controls were found to behave identically in vitro, further supporting the potential clinical utility for autologous MSC transplantation in MS.68 Together, the availability, ease of transplantation without immunoaablation and intrinsic properties of MSCs make them ideal for study as possible therapeutic agents in MS.

Mesenchymal Stem Cells and Multiple Sclerosis

The growing body of preclinical and clinical data supporting the use of MSCs and their intrinsic properties as immunomodulators were the impetus for proposed clinical trials in MS, as reviewed by the International MSCT Study Group.69 At the time of writing, a search for ‘mesenchymal stem cell’ and ‘multiple sclerosis’ on www.clinicaltrials.gov yielded 16 trials, one of which was terminated, and only one of which is listed as completed. The phase I/phase IIa trials reported in the literature thus far demonstrate overall good safety profiles and tolerability of autologous MSC transplantations (via intravenous, intrathecal or both routes), with mild transient fever and headache the most common reported side effects (see Table 2).70–72 Connick et al.73 report modest improvements in visual acuity, visual evoked potential latency and optic nerve area, which they suggest may be indicative of MSC-related neural protection over the year of the study.73 We have recently completed a phase I study confirming the safety and tolerability of autologous MSC transplantaion in MS (NCT00813969) at the Cleveland Clinic (Cleveland, Ohio, US). Detailed results are forthcoming.

Future Directions

Establishing safety and tolerability of autologous MSC transplantation in MS will allow further exploration of the efficacy of this treatment on multiple aspects of MS pathophysiology. The experience of centres, such as ours and the studies included above, should allow for larger, multicentre phase II and III clinical trials to emerge in the near future. Still to be defined is the optimal dose and dosing strategy for MSC transplantation (i.e. single versus repeated dosing; intravenous versus intrathecal administration, etc.), as well as interactions between DMD treatment (historic or concurrent) with MSC transplant (see Table 3). Phase II studies will explore the role of MSCs in immunomodulation and neuroprotection in early RRMS, looking at clinical, functional, structural and electrophysiological measures; potentially, cerebrospinal fluid and serum biomarkers will be identified that can be measured pre- and post-MSC transplant as outcome measures that correlate, or predict, clinical responses. Indeed, in a small study from Iran, seven patients were intrathecally injected with MSCs, and FOXP3 expression in serum was assessed by quantitative real-time polymerase chain reaction (RT-PCR) as a proxy measure of circulating regulatory T cells. Six of seven patients in this study were found to demonstrate increased FOXP3 expression in serum status-post transplant.74 The methods and implications of this small study are limited, but point to the potential for immunological biomarkers that may predict response to cell-based therapies; we
Currently lack clinically validated biomarkers of disease progression or response to DMDs in MS.\textsuperscript{70,71}

What role might MSCs play in later stages of MS where neurodegeneration and microglial activation predominate? The current lack of effective DMDs or other approved therapies for progressive MS make MSC transplantation a potentially attractive alternative, and preclinical studies suggest mechanisms through which MSCs may target neurodegeneration in progressive MS. For example, MSCs have been shown to downregulate transforming growth factor-\( \beta \) in microglia and astrocytes following stroke,\textsuperscript{72} suggesting MSCs may be able to alter gliosis if transplanted even after extensive damage has occurred. Furthermore, native MSCs as well as cells modified to express growth factors, may be able to rescue neurons from apoptosis and promote axonogenesis in injured axons, potentially restoring damaged circuits. Other potentially exciting avenues include transplantation of neural precursor cells to aid remyelination or potentially replace lost neurons.\textsuperscript{73} Previous studies in Shiverer-immunodeficient mice with developmental dysmyelination demonstrated that neuronal progenitor cells transplanted into hypomyelinated brain parenchyma differentiated into oligodendrocytes, forming structurally and functionally normal myelin.\textsuperscript{74} Improved techniques in MSC purification, clonal expansion and characterisation as neuronal progenitors\textsuperscript{75} may enhance MSC transplantation efficacy, or provide alternative therapies for both MS and other neurodegenerative diseases, including direct transplantation into injured brain parenchyma.

Direct transplantation into the brain may be feasible for some degenerative diseases such as Parkinson’s disease or stroke, but the multifocality of MS pathology makes this strategy less promising. One concern about intravascular or intrathecal administration of MSCs is knowing where in the body these cells accumulate, which has implications on their mechanisms of action either locally or systemically as immunomodulators. Thus, post-transplant localisation of MSCs using exogenous labels and clinical imaging is currently under investigation. Superparamagnetic iron oxide (SPIO)-labelled MSCs function in vivo similar to unlabelled MSCs, and in both rodent and canine stroke models, SPIO-labelled MSCs are visible using MRI following both intra-arterial and intravenous administration.\textsuperscript{76–78} A phase I trial in healthy human volunteers demonstrated that peripheral blood mononuclear cells could be SPIO-labelled, injected intramuscularly and tracked to an iatrogenic subcutaneous inflammatory site; furthermore, MRI demonstrated labelling of the reticuloendothelial system and the skin lesion up to 7 days following SPIO-mononuclear cell injection.\textsuperscript{79} This study demonstrated the feasibility and tolerability of SPIO-labelled cell administration and tracking in humans. However, SPIO-labelled MSCs were found to exacerbate EAE mouse symptoms, and upon pathological evaluation, iron-deposition was found in brain tissue.\textsuperscript{80} Another murine model, SPIO aggregates in the liver were found to be cytotoxic, and exposure to strong magnetic fields (such as MRI) exacerbated SPIO aggregation, reactive oxygen species formation and cytotoxicity.\textsuperscript{81} Thus, SPIO-labelling and tracking of MSCs post-transplantation is potentially feasible in humans, but may be limited in its application due to cytotoxic side effects in general, and specific consequences in inflammatory diseases such as MS.

Another labeling technique showing promise in preclinical studies is perfluorocarbon nanoparticle labelling of cells in vitro followed by in vivo imaging with \( ^{19} \text{F} \) MRI. This technique provides a very high signal to noise ratio due to the relative absence of intrinsic fluorine signal in body tissues compared with protons.\textsuperscript{82–84} Advantages of these techniques over SPIO-labelling include 1) the absence of cytotoxic cation or lipid-based transfection agents needed for cell labelling; 2) a relatively high level of perfluorocarbon uptake by progenitor/precursor cells such as MSCs; 3) the perfluorocarbons are inert, non-toxic compounds not found to cause cytotoxicity or reactive oxygen species that limit SPIO use in humans; 4) the ability to potentially quantify small numbers of labelled cells \textit{in situ} at clinical strength MRI (3T); and 5) potential labelling and tracking of more than one cell type using different perfluorocarbon preparations that give off unique MR spectra.\textsuperscript{85,86} Additionally, \textit{in vivo} perfluorocarbon emulsion infusion followed by \( ^{19} \text{F} \) MRI has been shown to preferentially detect areas of inflammation both in the CNS and systemically via preferential uptake by CD68+ macrophages, providing a non-invasive measure of disease activity in murine models of inflammatory disease.\textsuperscript{87–90} Thus, the advantage of these labelling modalities includes the ability to directly visualise (and potentially quantify) \textit{in situ} localisation of intravenously administered MSCs, or indirectly detect anti-inflammatory effects of MSCs by measuring the burden of inflammation \textit{pre-} and \textit{post}-transplant, respectively.

As we push the frontier of translational medicine, new techniques in disease detection and monitoring will allow researchers to ask new questions about MS pathology, and therefore the roles of therapies in mitigating these processes. The advent of clinical 7T MRI has illuminated the burden of cortical grey matter lesions in living patients with MS, where previously such evaluations were only feasible on post-mortem neuropathological studies. These cortical lesions identified \textit{in vivo} by 7T MRI potentially correlate with functional and neuropsychiatric deficits better than deep grey or white matter lesions.\textsuperscript{91,92} Utilising 7T MRI, cortical lesional analysis \textit{pre-} and \textit{post}-autologous MSC transplant may help correlate potential functional improvements with site-specific improvement in MS lesions. Targeting MSCs to the brain by genetic modification with brain-specific surface-receptors may be another potential tool for enhancing successful CNS engraftment of transplanted cells. This was demonstrated in an EAE mouse model in which human MSCs engineered to express a myelin oligodendrocyte glycoprotein-specific receptor were intranasally delivered and found to significantly improve EAE symptoms better than non-engineered human MSCs, and prevented further EAE induction.\textsuperscript{93}

Indeed, while MSCs are a promising therapeutic resource, other stem cell sources may supplant MSCs in the future. In particular, induced pluripotent stem cells (iPSC) are previously differentiated adult cells that have been reprogrammed by the introduction of four specific genes, and adopt embryonic stem cell morphology and behaviour.\textsuperscript{94} These cells can be induced to differentiate into neural precursors, and iPSC-derived neural precursors from mice have aided remyelination after transplantation into the spinal cord of EAE mice. This effect was secondary to secretion of leukaemia inhibitory factor (LIF), which promoted differentiation of endogenous oligodendrocyte precursors and survival of mature oligodendrocytes, rather than by repopulation and cell replacement.\textsuperscript{95} Alternatively, Najm et al.\textsuperscript{96} demonstrated direct reprogramming of adult mouse fibroblasts into induced oligodendrocyte precursor cells (iOPCs) that functioned \textit{in vitro} identically to endogenous mouse OPCs, and were able to enshroud axons and form compact myelination following transplantation into hypomyelinated mice.\textsuperscript{97} Thus, iPSCs may be alternative or adjunct cell sources to autologous MSCs in future therapies. Additionally, MSC-like cells have been generated from iPSCs \textit{in vitro} from several iPSC lines derived from different tissues, including gingiva, periodontal ligament and lung.\textsuperscript{98} These MSC-like cells behave \textit{in vitro} similar to adult MSCs and express identical surface markers, though their ability to differentiate \textit{in vivo} varied between cells derived from different tissues. At this
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time, there are significant limitations to the use of IPSCs including slow proliferation, concerns for incomplete reprogramming, immunogenicity and potential tumourigenicity. Currently, there is a proposed randomized trial of IPSCs in Japan in which six patients with exudative age-related macular degeneration will have autologous IPSC-generated retinal pigment epithelial sheet transplantation (please see http://www. riken-ibr.jp/AMD/english/research/index.html).

Conclusion

Cell-based therapies for MS are currently under exploration and potentially offer advantages over conventional DMDs. MSCs in particular are easily obtained from autologous sources, eliminating rejection risk and need for toxic immunomodulation therapies, and are well tolerated with minimal transplanted-side related effects. Preclinical data demonstrate intravenously administered MSCs target perivascular spaces and areas of active inflammation within and outside the CNS, can cross the blood–brain barrier and secrete anti-inflammatory cytokines to suppress local and systemic immune dysregulation. In animal models of MS, human MSCs are capable of decreasing clinical symptoms and histopathological evidence of immune-mediated demyelination. Furthermore, MSCs can be manipulated in vitro to produce neural precursors, express neuroprotective factors, brain-specific surface receptors to improve targeting of cells to the CNS and labelling for in vivo tracking post-transplantation. In addition to anti-inflammatory functions, MSCs may be able to alter neurodegenerative processes and potentially differentiate and integrate into neuronal circuitry, offering potential therapy to patients with progressive or later-stage MS. Other cell types, including IPSCs, IPSC-derived MSCs and induced OPCs may offer alternatives to MSCs, though at this time, autologous MSCs are an immediately available therapeutic source. Ongoing phase I and II trials will help elucidate their utility as MS treatment in the coming years.


31. Bianco Y, et al., Adult mesenchymal stem cells, adult neural crest stem cells and mesenchymal stem cells are not able to replace lost neurons in acute MPTP-lesioned mice, Proc Natl Acad Sci U S A, 2014;111:E583–90.


34. Bianco Y, et al., Adult mesenchymal stem cells, adult neural crest stem cells and mesenchymal stem cells are not able to replace lost neurons in acute MPTP-lesioned mice, Proc Natl Acad Sci U S A, 2014;111:E583–90.


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