doi:10.1093/brain/awx154 BRAIN 2017: 140; 2776–2796 | 2776

BRAIN
A JOURNAL OF NEUROLOGY

REVIEW ARTICLE

Cell-based therapeutic strategies for multiple sclerosis

Neil J. Scolding,[1] Marcelo Pasquini,[2] Stephen C. Reingold[3] and Jeffrey A. Cohen[4] on behalf of attendees at the International Conference on Cell-Based Therapies for Multiple Sclerosis

The availability of multiple disease-modifying medications with regulatory approval to treat multiple sclerosis illustrates the substantial progress made in therapy of the disease. However, all are only partially effective in preventing inflammatory tissue damage in the central nervous system and none directly promotes repair. Cell-based therapies, including immunoablation followed by autologous haematopoietic stem cell transplantation, mesenchymal and related stem cell transplantation, pharmacologic manipulation of endogenous stem cells to enhance their reparative capabilities, and transplantation of oligodendrocyte progenitor cells, have generated substantial interest as novel therapeutic strategies for immune modulation, neuroprotection, or repair of the damaged central nervous system in multiple sclerosis. Each approach has potential advantages but also safety concerns and unresolved questions. Moreover, clinical trials of cell-based therapies present several unique methodological and ethical issues. We summarize here the status of cell-based therapies to treat multiple sclerosis and make consensus recommendations for future research and clinical trials.

1 Department of Neurology, University of Bristol Southmead Hospital, Bristol BS10 5NB, UK
2 Center for International Blood and Marrow Transplant Research (CIBMTR), Medical College of Wisconsin, Milwaukee, WI 53226, USA
3 Scientific and Clinical Research Associates, LLC, Salisbury, CT 06068, USA
4 Neurological Institute, Cleveland Clinic, Cleveland, OH 44195, USA

Correspondence to: Jeffrey A. Cohen, M.D.,
Mellen Center for Multiple Sclerosis Treatment and Research,
Neurological Institute, Cleveland Clinic, 9500 Euclid Avenue,
Cleveland, OH 44195,
USA
E-mail: cohenj@ccf.org

Keywords: multiple sclerosis and neuroinflammation; stem cells; medical ethics; remyelination; clinical trials

Abbreviations: ATG = antithymocyte globulin; BEAM = carmustine, etoposide, cytarabine, melphalan; Cy = cyclophosphamide; DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; I/AHSCT = immunoablation followed by autologous haematopoietic stem cell transplantation; iPSC = induced pluripotent stem cell; MSC = mesenchymal stem cell; OPC = oligodendrocyte progenitor cell

Introduction

With multiple approved disease-modifying therapies (DMTs), there is a broad range of options to treat relapsing-remitting multiple sclerosis (Ingwerson *et al.*, 2016). However, less progress has been made in the treatment of progressive forms of the disease (Shirani *et al.*, 2016). While the positive impact of treatment on reducing the frequency of relapses and accrual of relapse-related disability has been demonstrated, none of the currently available agents halt disease progression or directly promote repair of pre-existing CNS damage. Moreover, all of the approved therapies have potential adverse events

Received December 30, 2016. Revised April 3, 2017. Accepted May 6, 2017. Advance Access publication July 21, 2017

that may compromise safety or adherence. All are expected to be ongoing life-long therapies as long as they remain safe and effective. Consequently, there is an imperative for new therapies that (i) are more effective in relapsing-remitting multiple sclerosis, particularly for patients with highly active disease who are at substantial risk for future disability; (ii) are effective in slowing or preventing progression; (iii) have the potential to reverse disability; and (iv) can be used safely with fewer delivery and adherence concerns.

Cell-based therapies have generated substantial interest as potential approaches to address these gaps by working through various mechanisms: regenerating the defective immune system that underlies multiple sclerosis by immunoablation followed by autologous haematopoietic stem cell transplantation (I/AHSCT); modifying both immune reactions and endogenous repair mechanisms using mesenchymal stem cells (MSCs) and other stem cells from bone marrow, adipose tissue, placenta, or other tissues; pharmacologic manipulation of endogenous stem cells to enhance their reparative capabilities; or replacing damaged or lost myelin-making oligodendrocytes by transplantation of oligodendrocyte progenitor cells (OPCs) or OPC-like inducible pluripotent stem cells (iPSCs) (Sarkar and Scolding, 2016).

In this review, we discuss the biology and potential utility of these cell-based therapeutic approaches in multiple sclerosis; summarize the progress made to date on testing in multiple sclerosis; discuss practical, scientific, clinical, regulatory, and ethical concerns; and make recommendations for future studies to move this therapeutic area forward. The review is based on an extensive literature search related to cell-based therapies for multiple sclerosis and on discussions at a consensus workshop, the International Conference on Cell-Based Therapies for Multiple Sclerosis, held 19–21 November 2015 in Lisbon Portugal under the auspices of the International Advisory Committee for Clinical Trials in Multiple Sclerosis (see Appendix 1 and Supplementary material for a list of conference participants).

Immunoablation followed by haematopoietic stem cell transplantation

Biological background and rationale

The rationale for I/AHSCT to treat multiple sclerosis is depletion of autoreactive effector cells with immunoablative agents (the conditioning regimen) followed by infusion of autologous haematopoietic stem cells to support immune system reconstitution with more normal immune function (Muraro *et al.*, 2005; Muraro and Abrahamsson, 2010). Analysis of circulating lymphocytes after I/AHSCT demonstrates reduction of circulating autoreactive effector T cells, predominantly Th17 rather than Th1, and emergence of recent thymic emigrants post-transplant, restoring a more regulatory milieu (Muraro *et al.*, 2005, 2014; Darlington *et al.*, 2013; Arruda *et al.*, 2015). The degree of reconstituted T cell repertoire variability is related to the intensity of the conditioning regimen (Muraro *et al.*, 2014). Muraro *et al.* (2014) reported the presence, pre-transplant, of circulating mucosal-associated invariant T cells (MAITs) characterized by a $CD8^+$, $CD161^{high}$ phenotype (Abrahamsson *et al.*, 2013). These MAITs exert a pro-inflammatory effect by promoting production of several cytokines thought to be associated with the pathogenesis of multiple sclerosis, including interferon-gamma and interleukin-17 (Lovett-Racke *et al.*, 2011). After transplantation, there was a significant reduction of this population in the peripheral blood in parallel with an increase of regulatory $CD4^+$, $CD25^{high}$, $CD127^-$, $FoxP3^+$ T cells. The initial benefit of I/AHSCT probably results from this and other comparable alterations in immune function. However, some studies have detected re-emergence of autoreactive effector cells despite a high intensity conditioning regimen and persistence of efficacy (Darlington *et al.*, 2013). Thus, the mechanisms responsible for sustained benefit of I/AHSCT are less well understood.

Practical/procedural background

Appropriate patients for I/AHSCT

Recognition of patients with multiple sclerosis most likely to benefit from I/AHSCT has evolved. Initial studies mainly enrolled patients with longstanding severe progressive multiple sclerosis, when inflammatory features are less prominent and neurodegeneration is the main underlying mechanism (Trapp and Nave, 2008). Benefit generally was modest, although some patients exhibited sustained slowing or stabilization of disability, but improvement in neurologic function was rarely seen (Burt *et al.*, 2015; Mancardi *et al.*, 2015). Also, patients with more severe neurologic disability had increased risk of adverse events (Mancardi and Saccardi, 2008). More recent studies (Table 1) focused on relapsing-remitting multiple sclerosis and demonstrated that patients with active inflammatory features appear to derive the most benefit from this approach (Burt *et al.*, 2012; Saccardi *et al.*, 2012; Muraro *et al.*, 2017). As a result, the current recommendation is for studies of I/AHSCT to enrol patients with highly active relapsing-remitting multiple sclerosis reflected by clinical relapses and MRI lesion activity, time from diagnosis within 5 years, and suboptimal response to available regulatory-approved DMTs (Burt *et al.*, 2012; Saccardi *et al.*, 2012). These criteria apply to only a limited subset of patients with multiple sclerosis but help define those at high risk for future disability despite available therapy. These recommendations have been somewhat controversial, as they suggest a relatively aggressive therapeutic approach for patients who may have little established disability (Soelberg Sorensen, 2016).

An important determinant of transplant success is the ability of patients to tolerate the conditioning regimen. Disease-related factors not only affect efficacy but also tolerability. In cancer patients, those with more advanced

disease, either with active cancer at time of transplant or refractory to prior therapy, have a higher failure rate. This is not only due to inability to control the disease with higher doses of chemotherapy, but also the increase in transplant-related morbidity or mortality from the cumulative effect of prior treatments. Similarly, multiple sclerosis patients with more severe disability or progressive disease also tend to have higher rates of transplant-related morbidity and mortality (Mancardi and Saccardi, 2008; Muraro *et al.*, 2017). The effects of prior multiple sclerosis DMTs on efficacy or safety of I/AHSCT are unknown.

In allogeneic haematopoietic stem cell transplantation for haematologic malignancies, the overall impact of the cancer on general health (the estimated the ability to work, perform activities of daily living and the need for hospitalization) is correlated with transplant outcome; lower performance scores are associated with higher post-transplant mortality. Similarly, the presence of key comorbid conditions also impacts transplant outcome. A high score on the Haematopoietic Cell Transplantation Comorbidity Index, which includes 17 items comprising past medical history (stroke, myocardial infarction, arrhythmia, autoimmune disease, prior solid tumours), end organ function (pulmonary, hepatic, renal and cardiac), and weight (obesity), is associated with increased post-transplant mortality (Sorror *et al.*, 2005, 2015; Elsawy and Sorror, 2016). Although comorbidities are less common in younger patients with multiple sclerosis, they have an important impact on multiple sclerosis disease outcomes (Marrie *et al.*, 2015). Their effects on the efficacy or safety of I/AHSCT to treat multiple sclerosis have not been explored.

Transplant procedure

I/AHSCT should be viewed as a multi-step process that leads to a combined therapeutic effect in multiple sclerosis. Adverse effects also can occur at each step. The typical sequence includes mobilization of peripheral blood haematopoietic stem cells, immunoablation via administration of a conditioning regimen, then infusion of haematopoietic stem cells to promote haematologic reconstitution.

Granulocyte colony stimulating factor (G-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF) are often used alone to mobilize haematopoietic stem cells from the bone marrow to the peripheral blood in healthy volunteer donors in an allogeneic donor transplant setting, but may cause worsening of neurologic manifestations in multiple sclerosis, either accentuation of pre-existing symptoms due to fever or *bona fide* relapses (Openshaw *et al.*, 2000). Therefore, in multiple sclerosis, the most common approach is administration of cyclophosphamide (Cy) plus G-CSF as a mobilizing agent, which helps deplete lymphocytes that will eventually be collected in the graft and lessens the chance of reinfusion of autoreactive T cells. Administration of Cy also may contribute to the therapeutic effect and adverse effects of I/AHSCT.

There are clear distinctions between the conditioning regimens used to treat malignancies and for non-malignant diseases such as multiple sclerosis. In the setting of AHSCT for malignancies, the conditioning regimen consists of high doses of chemotherapy to maximize disease control; the primary intent of subsequent haematopoietic stem cell infusion is to 'rescue' haematopoiesis. In the setting of allogeneic haematopoietic stem cell transplantation, the conditioning regimen can be classified within an intensity spectrum according to the type of chemotherapeutic agents and/or radiation selected, and their respective doses. High intensity regimens, also termed myeloablative, require haematopoietic stem cell infusion to prevent irreversible bone marrow damage. At the opposite end of the spectrum are lower intensity regimens, also termed non-myeloablative, which minimally affect haematopoiesis. Intermediate intensity regimens, also termed reduced intensity, fall in the middle of this spectrum. The indication, type of transplant, and population being treated determine the choice of regimen intensity. For example, in some instances for the same indication and transplant type, a high intensity regimen will be selected for patients younger than 65 years and a reduced intensity in patients older than 65 years. In general, high intensity regimens are selected to better control the cancer and minimize the risk of disease relapse, but are potentially associated with greater morbidity and mortality compared to lower intensity regimens.

In multiple sclerosis, like other autoimmune diseases, the optimal intensity of the conditioning regimen remains uncertain and is actively debated. The main objective is to balance lymphocyte depletion to eliminate pathologic autoimmunity with acceptable morbidity and mortality. The most commonly used conditioning regimen in multiple sclerosis has been BEAM (carmustine, etoposide, cytarabine and melphalan), which is considered an intermediate intensity regimen, combined with anti-thymocyte globulin (ATG) (Mancardi *et al.*, 2012, 2015; Muraro *et al.*, 2013; Burman *et al.*, 2014; Nash *et al.*, 2015; Shevchenko *et al.*, 2015). High intensity regimens, such as total body irradiation and busulfan, were initially used in multiple sclerosis but were either abandoned or modified because of toxicity. Atkins *et al.* (2016) reported the use of high dose busulfan and Cy (Bu/Cy) with infusion of a T cell depleted ($CD34^+$ cell selection) autologous graft (Atkins *et al.*, 2016). During the study, several modifications to the busulfan regimen were made to improve tolerability. The route of administration was switched from oral to intravenous, the dose was reduced (though still considered in the high dose range), and the target dose was adjusted based on the first busulfan dose pharmacokinetics. Conversely, Burt and colleagues (2009, 2015) have advocated a low intensity or non-myeloablative regimen—Cy or alemtuzumab followed by ATG—reporting good efficacy in relapsing-remitting multiple sclerosis with reduced toxicity and no mortality.

One potential concern with use of a less intense conditioning regimen is suboptimal multiple sclerosis disease control. One study demonstrated that recipients of non-myeloablative regimens had early reappearance of MRI

lesion activity post-transplant (Mancardi *et al.*, 2012). It is possible that the intensity of the conditioning regimen may need to be tailored to the clinical situation, although consensus on how to identify patients early with aggressive multiple sclerosis and poor prognosis is lacking. Another consideration is whether the specific drug combination within regimens considered the same intensity is associated with differential outcomes. Muraro *et al.* (2017) analysed data on 281 transplant recipients with multiple sclerosis worldwide in a retrospect registry-based study. Conditioning regimens varied greatly and when they were grouped according to intensity, there was no correlation with outcome. This observation may have been due to most patients having progressive multiple sclerosis and the proportion of patients with relapsing-remitting multiple sclerosis was not sufficient to demonstrate differential efficacy. Thus, the optimal regimen, Cy/ATG, BEAM-ATG, or Bu/Cy remains uncertain and, based on the available data, all remain acceptable options.

It remains uncertain whether haematopoietic stem cell transplantation should be considered merely bone marrow rescue or if it contributes to the therapeutic benefit of I/AHSCT. While less intensive conditioning regimens may not necessitate haematopoietic stem cell transplantation, the infusion of haematopoietic stem cells serves two purposes: (i) to reduce morbidity by shortening the duration of pancytopenia; and (ii) to increase benefit by promoting immune reconstitution with broader clonal diversity without auto-reactivity. Characteristics of the graft have received relatively little attention in multiple sclerosis. Some studies have administered a largely unmanipulated graft (Burman *et al.*, 2014; Burt *et al.*, 2015; Mancardi *et al.*, 2015). Other studies have positively selected $CD34^+$ cells *ex vivo* to remove any residual lymphocytes in the graft (Nash *et al.*, 2015; Atkins *et al.*, 2016). This step adds to the technical complexity of the transplant procedure but reduces the risk of reinfusion of potentially autoreactive lymphocytes. If haematopoietic stem cell transplantation is not merely rescue after immune depletion but, in fact, contributes to the efficacy of the procedure, further work is needed to optimize mobilization and graft processing to maximize potency.

Multiple sclerosis-related outcomes

Evaluation of therapeutic outcomes in multiple sclerosis is complex. Assessment of the success of a therapeutic intervention is more difficult than in I/AHSCT for malignant diseases, where transplant-related mortality, all-cause mortality, and/or malignant disease recurrence often are used as outcomes. Because multiple sclerosis is associated with only modest shortening of life-span (Goodin *et al.*, 2012), transplant-related mortality or all-cause mortality alone are not likely to be an informative efficacy outcome in studies of I/AHSCT in multiple sclerosis.

The outcomes most often used in multiple sclerosis clinical trials are relapses, confirmed worsening of disability measured by the Expanded Disability Status Scale (EDSS) or Multiple Sclerosis Functional Composite, MRI lesion activity and burden, and normalized whole brain volume (Cohen *et al.*, 2012). As summarized in Table 1, these endpoints have been used in trials of I/AHSCT in multiple sclerosis (Mancardi *et al.*, 2012, 2015; Muraro *et al.*, 2013; Burman *et al.*, 2014; Burt *et al.*, 2015; Nash *et al.*, 2015; Shevchenko *et al.*, 2015; Atkins *et al.*, 2016). Because of the potential risk associated with I/AHSCT and to distinguish its efficacy from that of available highly effective multiple sclerosis therapies, some workshop participants favoured a stringent outcome be utilized in future trials of I/AHSCT in multiple sclerosis, specifically event-free survival with a composite outcome comprising clinical relapses, MRI lesion activity (new/enlarged T_2-hyperintense lesions or gadolinium-enhancing lesions), confirmed disability worsening, and normalized whole brain volume. Whether the specific definitions of the outcome components should be those used for so-called 'no evidence of disease activity' (NEDA) in previous studies (Havrdova *et al.*, 2010; Giovannoni *et al.*, 2015; Kappos *et al.*, 2015; Rotstein *et al.*, 2015), or modified, e.g. to account for delayed efficacy in a highly active study population, was not decided at the workshop. Other workshop participants felt that early inhibition of inflammatory activity (clinical relapses and MRI lesion activity) had uncertain relation to long-term disease outcome (University of California San Francisco MS-EPIC Team *et al.*, 2016) and was not likely to distinguish I/AHSCT from available highly effective DMTs. They advocated focusing primarily on long-term disability accrual. Thus, an important issue, especially in relapsing-remitting multiple sclerosis, is how effectively I/AHSCT alters the long-term disease course, that is, delays or prevents development of progressive disease and disability accrual, compared to available therapies. This determination will require a randomized trial comparing I/AHSCT to DMTs with long-term follow-up. A related question is whether I/AHSCT affects the subsequent response to, or safety of, DMTs administered.

An additional efficacy outcome potentially relevant for clinical trials of I/AHSCT is confirmed improvement in disability, which has been demonstrated in trials of several multiple sclerosis DMTs (Jones *et al.*, 2010; Phillips *et al.*, 2011; Hauser *et al.*, 2015). Similarly, reversal of pre-existing disability also has been reported with I/AHSCT (Burt *et al.*, 2015; Atkins *et al.*, 2016). Although it is possible these interventions directly stimulate repair to some degree, it is more likely they unmask intrinsic repair mechanisms by effectively suppressing ongoing inflammatory damage (Chang *et al.*, 2002, 2008, 2012).

Financial cost

The annual costs of multiple sclerosis DMTs range from ~$50 000 to $70 000 (Hartung *et al.*, 2015) in the USA. The cost of I/AHSCT is ~$120 000 in the USA, which is incurred mainly in the first year with minimal direct costs subsequently. Thus, in contrast to DMTs for which cost accrues indefinitely, the financial cost of I/AHSCT is largely

Table 1 Contemporary clinical trials of I/AHSCT for multiple sclerosis

Study and study period	Design	*n*	Study population	EDSS median (range)	Mobilization	Conditioning regimen	Graft manipulation	Results
Italian (Mancardi *et al.*, 2012) 1996–2008	Multicentre case series	74	RRMS *n* = 33, SPMS *n* = 41	6.3 (3.5–9.0)	Cy/GF	BEAM/ATG	None	PFS[c] 66% at 5 years
CIBMTR/EBMT (Muraro *et al.*, 2013) 1995–2006	Multicentre case series	281	RRMS *n* = 45, progressive MS *n* = 236	6.5 (1.5–9.0)	chemo/GF 93%	Intensity: high 19%, intermediate 64%, low 27%	None 56%	PFS[h] 48% at 5 years
Swedish (Burman *et al.*, 2014) 2004–2013	Multicentre case series	48	RRMS *n* = 34, progressive MS *n* = 7	RRMS: 2.5 (0–6.5), progressive MS: 6.5 (5.0–7.5)	Cy/GF	BEAM/ATG *n* = 41, Cy/ATG *n* = 7	None	DFS[f] 68% at 5 years
Northwestern (Burt *et al.*, 2015) 2003–2014	Single centre case series	145	RRMS *n* = 118, SPMS *n* = 27	2.0 (3.0–5.5)[b]	Cy/GF	Cy/ATG, Cy/Alemtuzumab	None	64% improvement of EDSS at 4 years
ASTIMS (Mancardi *et al.*, 2015) 2004–2009	Multicentre, randomized, open-label, active comparator, phase 2	24	RRMS *n* = 9, SPMS *n* = 12	6.0 (5.5–6.5)	Cy/GF	BEAM/ATG	None	79% reduction in new T_2 MRI lesions
HALT-MS (Nash *et al.*, 2015) 2006–2009	Multicentre, single-arm, phase 2	21	RRMS *n* = 21	4.5 (3.0–5.5)	Steroids/GF	BEAM/ATG	$CD34^+$ selection	EFS[a] 78% at 3 years
Russian (Shevchenko *et al.*, 2015) 2005–2011	Single centre case series	99	RRMS *n* = 43, progressive MS *n* = 56	3.5 (1.5–8.5)	Steroids/GF	BEAM-like/ATG[d]	None	EFS[e] 88% at 3 years
Canadian (Atkins *et al.*, 2016) 2001–2009	Multicentre, single-arm, phase 2	24	RRMS *n* = 12, SPMS *n* = 12	4.3 (3.0–6.0)	Cy/GF	Busulfan, Cy, ATG	$CD34^+$ selection	EFS[i] 69.6% at 3 years

[a]EFS included: progression (increase of 0.5 in EDSS score compared to baseline, starting 6 months post-transplant and confirmed 3 months later), relapse (worsening or development of new neurologic sign and corresponding symptom lasting more than 48 h), MRI evidence of disease progression (two or more independent multiple sclerosis-related lesions gadolinium-enhancing or T_2-hyperintense lesions on brain MRI performed 1 year or more after transplant) or death.
[b]Represents interquartile range, the manuscript reported that 15 patients had the EDSS > 6.0.
[c]EFS: clinical and development of new brain lesions by MRI.
[d]BEAM-like: BEAM with dose reductions of etoposide, cytarabine and melphalan or the combination of carmustine and melphalan.
[e]EFS from the Russian study was freedom from progression (worsening of at least 0.5 points in EDSS for two consecutive assessments 3 months apart) or relapse (acute deterioration of neurologic function more than 24 h without other causes).
[f]DFS in the Swedish study classified events as relapse, new MRI manifestations, EDSS progression or death.
[g]Different progressive forms, the majority of participants had secondary progressive multiple sclerosis.
[h]PFS events included worsening of EDSS or initiation of new disease-modifying agent.
[i]EFS included clinical relapse, new or enhancing MRI lesion, or sustained EDSS worsening.
ASTIMS = autologous stem cell transplantation international multiple sclerosis; chemo = chemotherapy-based mobilization; CIBMTR = Center for International Blood and Marrow Transplant Research; DFS = disease-free survival; EBMT = European Blood and Marrow Transplant Group; EFS = event-free survival; GF = growth factors; HALT-MS = Hematopoietic cell autologous transplant for multiple sclerosis; MS = multiple sclerosis; PFS = progression-free survival; progressive MS = combined progressive forms of multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

front-loaded and may be less expensive overall. However, the procedure is not universally covered by health insurance in the USA, although there is variation across different centres and payors. Approaches in other transplant indications, e.g. myelodysplastic syndromes where Center for Medicare Services established Coverage with Evidence Development, allow coverage of transplant costs with the requirement for systematic and prospective data collection.

Clinical trials to date

I/AHSCT has been the most investigated cell-based therapeutic strategy for multiple sclerosis. Recent clinical studies are summarized in Table 1. Most studies were small or single centre case series with different patient populations, therapeutic protocols, and outcome measures. The published experience mostly comprises uncontrolled studies. The one randomized trial (Mancardi *et al.*, 2015) used mitoxantrone, an agent that is now largely less relevant as a comparator.

A recent retrospective analysis indicated that treatment with I/AHSCT achieved NEDA based on relapses, MRI lesion activity, and disability worsening in a higher proportion of multiple sclerosis patients (78–83% at 2 years) than reported in trials of the available DMTs (13–46%) (Sormani *et al.*, 2017). It should be noted that these studies had different patient populations and visit schedules, particularly the frequency of MRI scans, which can have a marked effect on NEDA rate. In addition to potent benefit on clinical measures and MRI lesion activity, I/AHSCT had potent efficacy on normalized whole brain volume loss. Following I/AHSCT there was initial acceleration of, followed by marked slowing after 2 years to levels approximating normal ageing (Roccatagliata *et al.*, 2007; Lee *et al.*, 2017).

Any evaluation of the utility of I/AHSCT needs to assess risk of mortality. In multiple sclerosis, mortality related to the disease may occur, but usually many decades after diagnosis. Thus, any therapy with significant risk of mortality will not readily be accepted. Mortality associated with I/AHSCT has decreased over the past two decades (Sormani *et al.*, 2016). With recent protocols, I/AHSCT is a safer procedure with mortality rates $<5\%$ (Muraro *et al.*, 2017), with some trials reporting no mortality (Burt *et al.*, 2015). Risks remain associated with the conditioning intensity, which necessitate careful patient selection (excluding participants with significant recent or chronic infection, liver disease, heart disease, etc.) and adequate supportive care during the 2–3-week aplastic phase. Optimal selection of patients, transplant procedure, timing of transplant, and post-transplant care help minimize the risk of transplant-related mortality. Some delayed adverse events occur late after I/AHSCT, but they are uncommon. The principal late adverse event is a 9% risk of a secondary autoimmune disorder within 5 years of I/AHSCT to treat autoimmune disease (Daikeler *et al.*, 2011). Thus, the front-loading of safety issues with I/AHSCT contrasts with multiple sclerosis DMTs, for which the risk of ongoing therapy accumulates over time related to chronic immune modulation or suppression.

Key questions/issues and recommendations

Workshop participants generally agreed on several consensus recommendations.

(i) In aggregate, the available evidence suggests I/AHSCT has substantial and sustained efficacy in suppressing inflammatory disease activity in multiple sclerosis. However, at present, it remains uncertain where the benefit-risk-cost profile of I/AHSCT places it in the treatment for relapsing-remitting multiple sclerosis relative to other available highly effective DMTs.

(ii) Patients most likely to benefit from I/AHSCT are relatively young e.g. 50 years of age or less, with relatively short disease duration e.g. 5 years or less, have active relapsing-remitting multiple sclerosis and accumulating disability but still are ambulatory, and have ongoing disease activity despite DMT. I/AHSCT is unlikely to benefit patients with longstanding progressive multiple sclerosis without recent inflammatory features (clinical relapses or MRI lesion activity).

(iii) We recommend a formal, multicentre, randomized phase 3 trial, comparing I/AHSCT head-to-head versus currently available highly effective therapy(ies) in a defined patient population. Issues concerning the trial design were discussed extensively, but further details still need to be determined (Box 1). Nevertheless, there was a substantial interest in the development of and participation in such a trial.

(iv) If I/AHSCT is performed to treat individual patients in clinical practice, comprehensive safety and efficacy data should be collected, the outcomes submitted to existing registries such as the Autoimmune Disease Working Party of the European Society for Blood and Marrow Transplant (EBMT) (Autoimmune Disease Working Party 2016) and the Autoimmune Diseases and Cellular Therapies Working Committee of the Center for International Blood and Marrow Transplant Research (CIBMTR) (Center for International Blood and Marrow Research 2016), and the results published. However, it is strongly encouraged that efforts be made to enrol such patients into formal clinical trials of I/AHSCT when available.

Enhanced endogenous cell therapy including mesenchymal stem cells

Biological background and rationale

Many stem cell types have potentially beneficial properties unrelated to trans-differentiation and cell replacement. These 'non-canonical' properties, some paracrine, may in some disorders play a greater therapeutic role than conventional cell replacement (Korbling and Estrov, 2003). In neurological disease, neural stem cells, MSCs from bone marrow or

Box 1 Key issues related to the proposed trial of I/AHSCT in multiple sclerosis

(1) Inadequacy of currently available evidence on which to base general treatment recommendations about I/AHSCT in multiple sclerosis. Thus, a phase 3 trial is needed.
(2) Characteristics of the optimal/appropriate study population
  (a) Age
  (b) Disease duration
  (c) Disease phenotype
  (d) Disability level
  (e) Required pre-study disease activity
  (f) Prior exposure to DMTs—Which are required? Which are allowed?
  (g) Key exclusion criteria relevant to safety
(3) Comparator group—best available therapy versus specific agent(s)
(4) Sample size and study duration to provide sufficient power to demonstrate clinically meaningful differences in efficacy and safety
(5) Primary outcome
  (a) Event-free survival using a composite outcome including clinical and imaging outcomes (NEDA or some modification) versus disability alone
  (b) Role of transplant-related or all-cause mortality and how to assess
(6) Treatment failure criteria, allowable/restricted rescue DMT, and how participants who change therapy are accounted for in the analysis
(7) Allowed use of symptomatic therapies
(8) Optimal conditioning regimen intensity
(9) Optimal graft manipulation (e.g. $CD34^+$ cell selection)
(10) Frequency and duration of follow-up for efficacy and safety

NEDA = no evidence of disease activity.

other sources including adipose tissue, and haematopoietic stem cells have all been shown to have therapeutic potential that depends on such non-canonical properties (Pluchino *et al.*, 2005; Uccelli *et al.*, 2008; Rice *et al.*, 2013). MSCs have attracted the most attention in this regard.

MSCs are present in most (possibly all) tissues (Da Silva Meirelles *et al.*, 2006; Phinney, 2012). Bone marrow contains various non-haematopoietic stem cells, including MSCs, and MSCs are themselves a heterogeneous population (Phinney, 2012). Within the bone marrow, they function to help maintain the haematopoietic stem cell developmental niche (Mendez-Ferrer *et al.*, 2010), but it is increasingly clear they also play a significant systemic role in repair in many tissues. In some diseased or damaged tissues, MSC differentiation into cells of the mesodermal lineage contributes to their putative benefit, for example, in liver and cardiac disease. Despite early reports of trans-differentiation into both neurons and oligodendrocytes (Woodbury *et al.*, 2000), this phenomenon probably does not play a significant role in potential repair-promoting effects of MSCs in the CNS. Rather, their multiple paracrine and other mechanisms of action are more relevant (Box 2), offering the prospect of ameliorating a number of the differing pathological processes contributing to tissue damage in multiple sclerosis through what might be termed 'enhanced endogenous cell therapy' (Korbling and Estrov, 2003; Rice and Scolding, 2004).

Some bone marrow cell subpopulations can reside for decades in the human brain after transplantation (Cogle *et al.*, 2004), though it is not yet clear which. There is no such evidence for MSCs specifically and, in fact, some evidence indicates that MSCs do not persist in tissues (von Bahr *et al.*, 2012). Thus, potential therapeutic benefit of MSC transplantation is likely to be self-limited, suggesting repeated administration will be necessary.

Practical/procedural background

Source and cell production

The question of whether MSCs from different tissue sources are identical in all properties is not wholly resolved (Strioga *et al.*, 2012; Li *et al.*, 2015). This uncertainty is partly a consequence of the continuing absence of any unique identifying marker of MSCs. They are consequently defined by a range of properties (Dominici *et al.*, 2006). So, the argument becomes almost circular: do cells with this same defining range of properties have identical additional, non-defining properties? There may be potentially important differences between MSCs derived from different sources, e.g. adipose tissue and bone marrow, and these differences may, in the future, influence the choice of tissue source.

The optimal dose of MSCs in any therapeutic use remains unknown, but a common target is 1–2 $\times$ 10^6 cells/

Box 2 Properties of MSCs and bone marrow-derived cells of potential therapeutic value in multiple sclerosis

- **Remyelination**
  Both MSCs and unseparated, non-expanded bone-marrow-derived cells promote myelin repair following intravenous injection (Sasaki *et al.*, 2001; Akiyama *et al.*, 2002). The mode of action is not clear. Intravenously-delivered bone marrow-derived cells successfully infiltrate the brain and spinal cord, inflamed or otherwise (Devine *et al.*, 2003; Gordon *et al.*, 2010); and they proliferate and migrate towards cytokines expressed in multiple sclerosis lesions (Rice and Scolding, 2010). Stimulation of CNS endogenous neural precursors (Munoz *et al.*, 2005; Bai *et al.*, 2009), and the release of trophic factors for oligodendrocytes might underlie this effect (Pisati *et al.*, 2007).
- **Reduced gliotic scar formation (Li *et al.*, 2005)**
  Gliosis is widely considered to inhibit spontaneous myelin repair.
- **Angiogenesis (Bronckaers *et al.*, 2014)**
  Angiogenesis would also likely enhance tissue repair.
- **Suppression of inflammation, immune modulation**
  Bone marrow-derived cells have pronounced immune-modulating properties (Prockop and Oh, 2012), affecting both innate and adaptive immune systems. Numerous studies have shown both MSCs and mixed populations of bone marrow-derived cells successfully to abrogate experimental autoimmune encephalomyelitis through increasingly well understood immunosuppressive actions (Bai *et al.*, 2009; Morando *et al.*, 2012). Many consider these immune effects sufficiently potent to justify clinical testing in relapsing-remitting multiple sclerosis [MEsenchymal StEm Cells for Multiple Sclerosis (MESEMS)] (Freedman *et al.*, 2010). However, it should be noted that animal and human MSC responses can be differentially modulated in both pro- and anti-inflammatory directions by environmental factors, such as pathogen-associated molecules and cytokines (Darlington *et al.*, 2010; Rozenberg *et al.*, 2016).
- **Neuroprotection**
  MSCs reduce axon loss in various immune-mediated experimental autoimmune encephalomyelitis models (Zhang *et al.*, 2006), but also in non-immune CNS injury, e.g. stroke models (Chen *et al.*, 2001). Neuroprotective mechanisms include the release of superoxide dismutase-3 (Kemp *et al.*, 2010) and of various neurotrophins (GDNF, BDNF, HGF) (Bai *et al.*, 2012). MSCs also promote CNS neuritis outgrowth, and remodelling (Shen *et al.*, 2011).
- **Cell fusion**
  Bone marrow-derived cells fuse with certain differentiated cell types, including neuronal subpopulations, a phenomenon which is increased in local or systemic inflammation or immune activation, and which likely represents a means of 'rescuing' damaged cells and restoring them to normal function (Johansson *et al.*, 2008; Kemp *et al.*, 2014). Transferring mitochondria from MSC to damaged cells can also protect tissue (Rice *et al.*, 2010; Prockop, 2012), membrane fusion (likely relating to nanotube formation or exosome transfer) representing the underlying mechanism common to both cell fusion and mitochondrial 'donation'. Fusion of infiltrating endogenous bone marrow-derived cells with Purkinje cells appears to occur spontaneously in multiple sclerosis (Rice *et al.*, 2010; Kemp *et al.*, 2012).

BDNF = brain-derived neurotrophic factor; GDNF = glial derived neurotrophic factor; HGF = hepatocyte growth factor.

kg body weight—a number that makes using primary MSCs near impossible for clinical use. Therefore, protocols for expanding cells are widely used (Mosna *et al.*, 2010), though it has become clear that cycles of expansion significantly attenuate many reparative and neuroprotective properties. In addition, the typical yield limits repeat dosing. New approaches to expansion therefore continue to be explored (Hoch and Leach, 2014).

Some studies used mixed/unseparated cells (Rice *et al.*, 2010); others administered purified and culture-expanded MSCs (Karussis *et al.*, 2010; Yamout *et al.*, 2010; Bonab *et al.*, 2012; Connick *et al.*, 2012; Cohen *et al.*, 2017). Some authors have studied modified MSCs adapted to express and secrete particular neurotrophins, though less so in multiple sclerosis models. Others have pre-differentiated MSCs—for example, using the classic neural stem cell mitogenic combination of epidermal growth factor and basic fibroblast growth factor, combined with 'neurosphere' culture techniques, to produce cells with MSC-derived neural stem cell properties (Harris *et al.*, 2012). Lack of comparative studies of different cell products and of *in vitro* markers that relate to therapeutic efficacy preclude recommendations on the optimal cell production protocol.

As in all clinical cell therapy endeavours, there is need for rigorous and stringent quality and safety control in cell production with, in the case of artificially expanded cells, assessment of phenotype and karyotype, mutagenesis testing, and microbiological analysis (Dominici *et al.*, 2006; Mosna *et al.*, 2010). This safety aspect represents one significant reason for caution in considering patient requests to purchase treatments from commercial clinics, particularly in countries where medical facilities are arguably less well regulated.

Route of delivery

Directly injecting cells into specific lesions would provide little benefit in the diffuse grey and white matter involvement

that characterizes multiple sclerosis. Cell therapy delivered systemically (as with any conventional drug) may have more rationale—and is safer. Most studies of MSCs have adopted intravenous injection (Rice *et al.*, 2010; Odinak *et al.*, 2011; Connick *et al.*, 2012; Li *et al.*, 2014; Llufriu *et al.*, 2014; Lublin *et al.*, 2014; Cohen *et al.*, 2017). Following intravenous injection, many cells are trapped in the lungs, but significant numbers still enter the CNS, become widely distributed, and can remain for decades, as shown in experimental models and in human subjects (Cogle *et al.*, 2004). Emerging evidence also suggests potential immune-modulating effects result from the interaction of MSCs and immune cells in the lung (Lee *et al.*, 2009; Odoardi *et al.*, 2012). Intra-arterial (carotid) delivery of bone marrow-derived MSCs has been explored in multiple system atrophy (Lee *et al.*, 2012), but not in multiple sclerosis to date. Concerns about micro-embolization have limited enthusiasm for this approach. Intrathecal delivery also has been tested in multiple sclerosis (Liang *et al.*, 2009; Riordan *et al.*, 2009; Karussis *et al.*, 2010; Yamout *et al.*, 2010). In the absence of a head-to-head comparison study, the optimal route of delivery remains uncertain.

Clinical results to date

Various groups have published small studies exploring feasibility and safety of MSC transplantation in multiple sclerosis (summarized in Table 2). These studies involved differing study populations, cell products, and routes of administration. The results generally supported the feasibility and safety of MSC transplantation in multiple sclerosis, as was expected based on studies in other conditions (Lalu *et al.*, 2012), including no evidence of ectopic tissue formation (von Bahr *et al.*, 2012). Transient aseptic meningitis was common with intrathecal delivery (Karussis *et al.*, 2010). Also, there have been case reports of acute disseminated encephalomyelitis after intrathecal MSC injection (Kishk *et al.*, 2013); a glioproliferative spinal cord tumour after intrathecal injection of a combination of mesenchymal, embryonic, and foetal neural stem cells (Berkowitz *et al.*, 2016); and severe visual loss in three patients with age-related macular degeneration after intravitreous injection of adipose tissue-derived stem cells (Kuriyan *et al.*, 2017). Some uncontrolled studies reported preliminary evidence of benefit on clinical, neurophysiological, or imaging outcomes (Rice *et al.*, 2010; Connick *et al.*, 2012; Cohen *et al.*, 2017).

The consensus among workshop participants was that further clinical trials were warranted. Larger, controlled phase 2 studies of both unseparated, non-expanded bone marrow-derived cells (Rice *et al.*, 2015*a*, *b*) and purified, culture-expanded MSCs [MEsenchymal StEm Cells for Multiple Sclerosis (MESEMS)] (Freedman *et al.*, 2010) are underway.

Key questions/issues and recommendations

Workshop participants identified several methodological issues concerning MSC transplantation in multiple sclerosis (Box 3).

Cell numbers and types

Cell dose currently is entirely empirical; there is little or no evidence indicating how many cells might be optimal. Similarly, there is no clear evidence whether multiple infusions would be needed, though this appears intuitively likely, and exploratory trials are underway (Rice *et al.*, 2015*b*). A further issue is the type of cell preparation to be used. Most studies used mixed mononuclear cell preparations or purified culture-expanded MSCs; some investigators studied MSC-derived neural progenitors (Harris *et al.*, 2012). MSCs are much studied experimentally, and there is an attractive rationale in using a purified homogenous cell population (Freedman *et al.*, 2010). However, bone marrow mononuclear preparations include many cell types, and there is good experimental evidence of benefit of such mixed preparations in repairing demyelination (Akiyama *et al.*, 2002) and suppressing inflammation, as well as clinical evidence in patients with stroke and other diseases (Savitz *et al.*, 2011). We do not know which cells among the bone marrow population are the most valuable therapeutically; there is no known benefit from excluding cell populations. Indeed, some evidence points to the superiority in certain experimental situations of using mixed mononuclear preparations over purified MSCs. The former simpler approach requires fewer technical resources and avoids the potential risks of genetic instability (Miura *et al.*, 2006), infection (Uhlin *et al.*, 2014), and altered phenotype that may accompany multiple cell cycling in culture for expansion. At present, it appears reasonable for both approaches to be pursued.

It is unclear whether the underlying biology of multiple sclerosis might affect MSC function. Some studies have demonstrated similar growth in culture, differentiation potential, surface antigen expression, and immunomodulatory properties of MSCs isolated from multiple sclerosis subjects versus non-multiple sclerosis controls (Papadaki *et al.*, 2005; Mazzanti *et al.*, 2008; Mallam *et al.*, 2010; Kassis *et al.*, 2013). Other studies reported notable functional differences (de Oliveira *et al.*, 2015; Redondo *et al.*, 2016; Sarkar *et al.*, 2016), suggesting autologous cell transplantation might not be appropriate. Commercial studies using pooled culture-expanded (heterologous) MSCs in non-multiple sclerosis conditions have, thus far, had disappointing efficacy results. Issues of donor variance, immunogenicity, culture expansion, epigenetic reprogramming, senescence, and (perhaps particularly) cryopreservation and thawing (Francois *et al.*, 2012; Chinnadurai *et al.*, 2014, 2016) may have contributed to these negative results.

Table 2 Published studies of MSC transplantation in multiple sclerosis

Reference	Study population[a] (*n*)	Post-treatment follow-up (months)	Cell product	Route of administration[b]	Efficacy outcomes	Adverse events (number of patients)
Bonab *et al.* (2007)	Progressive MS (10) unresponsive to treatment, EDSS 3.5–6.0	13–26 (mean 19)	Autologous, culture-expanded BM MSCs	IT	EDSS change (*n*): improved (1) stable (4), worse (5); MRI lesions (*n*): decrease (1), no change (7), increase (2)	No serious adverse events
Bonab *et al.* (2012)	SPMS (23), PRMS (2) unresponsive to treatment, mean EDSS 6.1	12	Autologous, culture-expanded BM MSCs	IT	Discontinued study ($n = 3$); EDSS change (*n*): improved (4) stable (12), worse (6); MRI lesion activity (n): none (15), present (6), no data (1)	Low-grade fever (all), nausea/vomiting (2), lower limb weakness (2), headache (3) No serious adverse events
Cohen *et al.* (2017)	RRMS (10), SPMS (14); clinical or MRI activity or worsening in the prior year; afferent visual pathway involvement; EDSS 3.5–6.5	6	Autologous, culture-expanded BM MSCs	IV	No benefit on MRI lesion activity; possible benefit on EDSS and whole brain MTR	No serious or severe adverse events
Connick *et al.* (2012)	SPMS (10), afferent visual pathway involvement, EDSS 5.5–6.5	5.8–10.2 (mean 7.0)	Autologous, culture-expanded BM MSCs	IV	Improvement in visual acuity and visual evoked response latency, increase in optic nerve area on MRI	Transient post-infusion rash (1), bacterial infection (2) No serious adverse events
Karussis *et al.* (2010)	RR or progressive MS (15 total) unresponsive to treatment, EDSS 4.0–8.0	6	Autologous, culture-expanded BM MSCs	IT (+IV in five)	Improved mean EDSS 6.7 to 5.9, no new enhancing MRI lesions at 6 months	Transient fever (10), headache (10), meningeal irritation and aseptic meningitis (1) No major adverse events
Li *et al.* (2014)	RR or SPMS (23), 13 treated versus 10 controls	12	Allogeneic human umbilical cord-derived MSCs	IV three times over 6 weeks, with corticosteroids	EDSS and relapses significantly improved compared to control group	None reported
Liang *et al.* (2009)	PPMS (1) unresponsive to treatment, EDSS 8.5	5	Allogeneic human umbilical cord-derived MSCs	IT + IV, with cyclophosphamide	Improved EDSS 8.5 to 5.5, decreased MRI lesion load	None reported
Llufriu *et al.* (2014)	RRMS (9) five treated versus four placebo, unresponsive to treatment, EDSS 3.0–6.0	12	Autologous, culture-expanded BM MSCs	IV	Non-significant decrease in cumulative number of enhancing MRI lesions	No serious adverse events
Lublin *et al.* (2014)	Treated (12): RRMS (7); SPMS (5) versus placebo (4): RRMS (3), SPMS (1); clinical or MRI activity or worsening in the prior year; EDSS 1.5–6.5	12	Allogeneic human placenta-derived MSCs	IV, one infusion (low dose) or two infusions (high dose)	No significant change in EDSS or enhancing MRI lesions	MS relapse (1), anaphylactoid reaction (1), superficial thrombophlebitis (1), headache (7), infusion site reaction (6)
Odinak *et al.* (2011)	MS, course not specified (8)	12	Autologous, culture-expanded BM MSCs	IV, three infusions	EDSS change (*n*): improved (6), stable (1), worse (1)	No significant adverse events reported
Rice *et al.* (2010)	Progressive MS with recent relapse (6); EDSS 4.5–6.5	12	Autologous Filtered, non-expanded whole BM aspirate	IV	Multi-modal evoked potential improvement	Transient increase in lower limb spasticity (2), urinary retention (1) No serious adverse events
Riordan *et al.* (2009)	RRMS (3), unresponsive to treatment	3–7	Autologous, non-expanded adipose SVF, allogeneic CD34 + cells, allogeneic MSCs	Two IV SVF infusions, multiple IV + IT infusions of CD34 + cells and MSCs	Clinical improvement	No side effects reported

(continued)

Table 2 Continued

Reference	Study population[a] (n)	Post-treatment follow-up (months)	Cell product	Route of administration[b]	Efficacy outcomes	Adverse events (number of patients)
Yamout *et al.* (2010)	SPMS (7); EDSS 4.5–7.5	12	Autologous, culture-expanded BM MSCs	IT (lumbar + cisternal)	EDSS change at 12 months (*n*): improved (5), stable (1), worse (1); MRI at 3 months (*n*): new/enlarging T_2 lesions (5/7), enhancing lesions (3/7)	Transient encephalopathy with seizures (1), cervical and back pain (1) No serious adverse events

[a]No control group unless otherwise noted.
[b]Single administration and without concomitant multiple sclerosis disease therapy unless otherwise noted.
BM = bone marrow; IT = intrathecal; IV = intravenous; MS = multiple sclerosis; MTR = magnetization transfer ratio; PPMS = primary progressive multiple sclerosis; PRMS = progressive relapsing multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; SVF = stromal vascular fraction.

Consensus recommendations

MSC and related cell therapy is an active area of research. Several phase 2 trials are already underway (Freedman *et al.*, 2010; Rice *et al.*, 2015*a*), which should clarify whether this approach is a potentially efficacious treatment for multiple sclerosis and in what phase of the disease. Workshop participants agreed it remains important to monitor carefully for long-term adverse effects, perhaps through international registries.

If the concept that underlies MSC therapy proves to be of benefit in diseases like multiple sclerosis, it may be that enhanced endogenous cell therapy will ultimately give way to molecular treatments. If the main beneficial effects of MSC therapy are paracrine, these might be more conveniently reproduced by directly using the principal effectors elaborated by infiltrating cells. The problem, however, is that not all the multiple therapeutic capacities of MSC-related populations are uniformly activated in all disease situations. Rather, infiltrating cells probably 'sense and react', with specific pathways triggered in response to the tissue and form of tissue damage (Murphy *et al.*, 2013). This process is likely dynamic, with the profile of administered cells and of those infiltrating tissues evolving with the progress of each individual disease or injury. It may be challenging to reproduce this by administering molecules rather than cells.

Pharmacological manipulation of endogenous repair mechanisms

Biological background and rationale

The traditional approach to develop remyelination-promoting pharmacologic therapies begins with basic studies of myelination and remyelination followed by development of agents that augment these processes. Such studies have identified a sizable number of candidate therapeutics (Kremer *et al.*, 2016). When the agents are novel, e.g. the anti-LINGO-1 monoclonal antibody opicinumab (Mi *et al.*, 2013), they then must go through the standard regulatory approval process of preclinical studies then phase 1–3 clinical trials (Mi *et al.*, 2013). However, a sizable number of already existing medications also may have the ability to promote remyelination (Kremer *et al.*, 2016); 'repurposing' these agents could expedite testing and regulatory approval. A complementary molecular approach involves using cultured OPCs or OPC-like iPSCs as the basis for high-throughput screening of libraries of already available drugs for their ability to stimulate remyelination (Deshmukh *et al.*, 2013; Mei *et al.*, 2014; Najm *et al.*, 2015). Molecules identified in the initial screens were further evaluated by increasingly stringent *in vitro* and *in vivo* testing, identifying the muscarinic antagonist benztropine, the antihistamine clemastine, the imidazole antifungal miconazole, and the topical steroid clobetasol as potential

Box 3 Key issues related to future trials of MSC transplantation in multiple sclerosis

(1) Cell product
  (a) Tissue source: bone marrow versus adipose tissue versus other tissues
  (b) Mixed/unseparated cells versus purified cultured-expanded cells
  (c) Cryopreserved and thawed versus unfrozen
  (d) Autologous versus heterologous
(2) Route of delivery: intravenous versus intrathecal versus intra-arterial versus a combination
(3) Dose and dosing
  (a) Cell number
  (b) Single versus multiple infusions
(4) Trial design
  (a) Appropriate study population
  (b) Primary trial outcome—should it focus on anti-inflammatory effects or repair?
  (c) Comparator
  (d) Allowed concomitant multiple sclerosis DMT(s): how might available multiple sclerosis DMTs affect success/viability of MSC transplantation?
  (e) Safety monitoring, short and long term
    (i) Infusion-related toxicity
    (ii) Acute and late infection
    (iii) Ectopic tissue formation
    (iv) Cancer
(5) How might underlying multiple sclerosis disease process affect success/viability of MSCs as a treatment approach?

candidates for further testing (Deshmukh *et al.*, 2013; Mei *et al.*, 2014; Najm *et al.*, 2015).

Practical/procedural background

Agents identified through the approaches described above are anticipated to have a wide range of mechanisms of action and pharmacological properties. Therefore, the design of proof-of-principle clinical trials in multiple sclerosis will vary according to the agent under study.

Clinical trials to date

A pilot study of clemastine fumarate to promote inherent remyelination showed improvement on visual evoked potentials in participants with multiple sclerosis-related chronic optic neuropathy (Green *et al.*, presented at the 2016 Annual Meeting of the American Academy of Neurology). The RENEW trial of opicinumab in acute optic neuritis demonstrated benefit on visual evoked potential latency recovery in the per-protocol analysis but not in the intention-to-treat analysis or on visual function or optical coherence tomography measures (Cadavid *et al.*, 2017). The SYNERGY trial of opicinumab in relapsing multiple sclerosis did not demonstrate benefit on the primary endpoint, percentage of participants with confirmed improvement on a composite outcome measure comprising EDSS, timed 25-foot walk, 9-hole peg test, and paced auditory serial addition test (Cadavid *et al.*, 2016).

Key questions/issues and recommendations

A number of studies of such agents are underway or planned. The key question is whether medications identified through basic studies of myelination/remyelination or high throughput screening, in fact, promote remyelination in patients with multiple sclerosis and how to demonstrate it. Some of the theoretical problems applying to the potential use of remyelinating cells, as outlined below, also apply—for example, the question of whether degenerated axons can support remyelination.

Oligodendrocyte progenitor cells and induced pluripotent cells

Biological background and rationale

Cell-based remyelinating strategies have long been of interest as a potential therapeutic approach in progressive multiple sclerosis. Glial progenitor cells expressing A2B5 but not polysialylated neural cell adhesion molecule can be isolated from foetal human brain (Windrem *et al.*, 2004), and when injected intracerebrally into hypomyelinating shiverer mice mediate widespread myelination and reversal of the clinical phenotype (Windrem *et al.*, 2008). These cells can be further purified by selecting for expression CD140a and

platelet-derived growth factor alpha receptor, which yields the more potent myelinogenic fraction (Sim *et al.*, 2011). Similarly, iPSCs might be used as a source of oligodendrocytes, with the potential for autologous cells to be used (Thiruvalluvan *et al.*, 2016). There are a number of potential therapeutic targets for such cells (Goldman *et al.*, 2012), including genetic dysmyelinating disorders, traumatic brain and spinal cord injury, and acquired inflammatory demyelinating disorders such as multiple sclerosis.

Practical/procedural background

Because OPCs are not expected to be capable of trafficking from blood or CSF into the CNS parenchyma, it is assumed that direct injection will be necessary for the cells to gain access to demyelinated lesions in multiple sclerosis, which introduces an additional level of technical and safety concerns. It appears that the cells have the capacity to migrate substantial distances within the CNS (Goldman *et al.*, 2012), so it may be possible to inject the cells into selected locations and still obtain widespread repair.

Another issue is that the current principal source of OPCs is foetal tissue, which provides a limited number of cells, given the finite proliferative capacity of OPCs in culture. Because such cells would be allogeneic, immunosuppression of the recipient is required to prevent rejection. It is reassuring that a previous study demonstrated that corticosteroids, interferon-beta, and azathioprine did not affect OPC proliferation, survival, or migratory capacity (Halfpenny and Scolding, 2003).

For the reasons outlined, generation of OPCs from iPSCs generated from the recipient is an attractive alternative approach. However, some studies of human iPSCs have detected frequent genetic modifications, including aberrant DNA methylation and mutations in genes implicated in cancer (Gore *et al.*, 2011; Hussein *et al.*, 2011; Lister *et al.*, 2011), raising the possibility of aberrant tissue formation or malignant transformation after transplantation. To design cell lines safe for human use, more research clearly is required concerning the mechanisms leading to genetic alterations in iPSCs. As a result, there will be substantial regulatory hurdles prior to human testing of such cells. These issues provide much of the impetus for identifying agents that stimulate remyelination by acting through intrinsic OPCs rather than relying on administration of exogenous cells.

Clinical trials to date

A phase 1 trial currently is planned by the New York State Consortium for Cell Therapy to evaluate the feasibility and safety of intracerebral injections of escalating doses of OPCs into multiple locations at a single time point in patients with secondary progressive multiple sclerosis (Goodman, 2016). To prevent transplant rejection, participants will receive tacrolimus and mycophenolate mofetil for 6 months then mycophenolate mofetil alone. Safety studies will include clinical assessment, laboratory studies, and brain MRI.

Key questions/issues and recommendations

The planned phase 1 study of OPC transplantation will focus on feasibility and safety as a prelude to proof-of-principle studies evaluating whether administration of exogenous OPCs augments remyelination in multiple sclerosis. Workshop participants supported further exploration of OPC transplantation in multiple sclerosis, but with some reservations (Box 4). Some studies have demonstrated sizable numbers of OPC in some chronically demyelinated lesions (Chang *et al.*, 2002), suggesting that lack of such cells is not the cause of inadequate remyelination in multiple sclerosis. Rather, the lack of factor(s) necessary to support and sustain remyelination, the presence of inhibitory factor(s) in the multiple sclerosis lesion environment, and inability of degenerated axons to support remyelination may be the main obstacles. Thus, administration of exogenous cells may not address the need. Even if the proof-of-principle with exogenous OPCs can be demonstrated with reasonable safety, there are several practical issues that need to be resolved, e.g. the appropriate cell dose and patient population most likely to benefit.

Ethical considerations

Cell-based therapies for multiple sclerosis are experimental, and strict adherence to ethical guidelines for human subject research (World Medical Association Declaration of Helsinki) helps preserve patient welfare and the integrity of the research process (Box 5) (Hyun *et al.*, 2008). Specific guidelines for human embryonic stem cell research broadly applicable to cell-based therapies for multiple sclerosis, including the most recent revision of international guidelines in 2016 (International Society for Stem Cell Research 2016; National Research Council and Institute of Medicine of the National Academies; Daley *et al.*, 2016), stress the ethics of procurement, derivation, banking, distribution, and use of cells and tissues, including somatic tissues and human totipotent or pluripotent stem cell lines.

Media attention has resulted in some cases of misrepresentation and exaggeration of therapeutic claims for cell-based therapies for multiple sclerosis and other diseases. In the consent process, patients need to be clearly informed that the cell-based therapy procedure is not 'standard of care'. Background information must include what is known about the procedures they are considering and what the goals are of the study in which they will participate. Close attention must be paid to known safety concerns and the potential for unanticipated adverse events.

The existence of many stem cell clinics around the world has resulted in 'medical tourism' by patients who believe they have exhausted other routes of treatment and are

willing sometimes to travel great distances to unregulated clinics for cell-based therapies (Lindvall and Hyun, 2009; Gunter *et al.*, 2010; New York Times, 2016). Patients are usually asked to pay for the care directly, without insurance reimbursement. Often such clinics are—for obvious reasons—based in jurisdictions with less stringent medical regulatory structures, and so there can often be little if any assurance of the expertise, quality of care (or even hygiene), or ethical standards of the provider centre, which is often unwilling or unable to seek more traditional financial support for their 'research'. Freestanding stem cell clinics, which are as yet largely unregulated, have also opened in Western Europe and the USA, so the issue is becoming more widespread (Turner and Knoepfler, 2016). In fact, there is a proposed change in the law in the USA, the so-called REGROW Act, which would remove the requirement for formal clinical trials for regulatory approval of cell-based therapies. Caution against this change has been urged (Nature Editorial, 2016).

Workshop participants agreed that, at present, all cell-based therapies lack definitive evidence for efficacy in multiple sclerosis and, thus, should be considered experimental and only pursued in rigorous clinical trials and observational studies with the expectation that the results will be published. Clinics offering such therapies should, at minimum, confirm that individuals with appropriate qualifications, training, and experience administer the treatment. There should be a written treatment plan, including how complications will be monitored and managed, that can be reviewed and approved by the treating physician. In the case of I/AHSCT, workshop participants acknowledged that there are rare patients with highly aggressive multiple sclerosis not adequately controlled by available DMTs for whom this approach can appropriately be considered as part of clinical practice. In this case, it should be performed at centres with experience both in the procedure and with managing multiple sclerosis, and the outcomes submitted to existing registries and ultimately published.

Workshop participants considered the complex issues of patient-funded research, in which study participants provide financial support for clinical trials. We acknowledged that this approach might be a potential option to allow progress in the field given the limited availability of grant funding. However, a number of concerns were emphasized. Having participants fund research, particularly pay-to-participate, can accentuate therapeutic misconception; may compromise the scientific merit and integrity of the trial; and should only be undertaken if the trial is reviewed, approved, and monitored by an independent review body, such as an institutional review board or data safety monitoring committee for the protection of human subjects in research (Wenner *et al.*, 2015).

Future directions

Cell-based therapies in multiple sclerosis have been pursued experimentally for at least four decades (Blakemore, 1977), and the past few years have witnessed considerable progress. Each of the cell-based approaches discussed above has begun to enter clinical trials. Much work remains, however. There is no clear consensus about their relative roles, especially in comparison with available DMTs, the specifics of the procedures, or the most appropriate patient population and study design to demonstrate short- and long-term safety and efficacy.

In spite of these uncertainties, there was agreement among workshop participants that I/AHSCT appears to have potent efficacy in relapsing-remitting multiple sclerosis though with significant safety concerns. The principal question is where I/AHSCT should be placed in the overall treatment for relapsing-remitting multiple sclerosis. Other cell-based therapeutic strategies—MSC or OPC/iPSC transplantation, and manipulation of endogenous stem cells—may be more helpful in patients with progressive forms of multiple sclerosis where degenerative mechanisms predominate, but this hypothesis remains to be confirmed. Thus, all forms of cell-based therapy for multiple sclerosis should be considered experimental at this time. There may be rare patients with highly aggressive relapsing-remitting multiple sclerosis who have failed available therapies for whom I/AHSCT may be justified. Other than these patients, cell-based therapy of multiple sclerosis should be pursued only in rigorous clinical trials. In all cases, comprehensive safety and efficacy data should be collected and submitted to existing registries, with the expectation that the results will be published.

It seems clear that the most efficient approach to cell-based therapeutic trials for a relatively uncommon disease with approved, available DMTs like multiple sclerosis, is not through the independent effort of many disconnected clinical centres, but through the development of stable, inclusive networks of investigators involved in a spectrum of cell-based therapies. Such networks can function beyond the organization of a single multicentre clinical trial and can establish protocols; undertake studies; and importantly set up registries to record transplantation protocols and outcomes. The Autoimmune Disease Working Party of the EBMT and the Autoimmune Diseases and Cellular Therapies Working Committee of the CIBMTR (Center for International Blood and Marrow Research, 2016) maintain longstanding registries of patients with autoimmune disorders, including multiple sclerosis, undergoing I/AHSCT. More recently, networks have been established for MSC transplantation trials (MESEMS) (Freedman *et al.*, 2010). Given the experimental status of cell-based therapy, we recommend all patients undergoing these procedures either in trials or in clinical practice are recorded in registries. In addition, important biological questions remain for all forms of cell-based therapy. Therefore, well designed mechanistic studies using validated methods for sample procurement and handling also should be included.

Undertaking clinical trials is a costly enterprise, even without the creation and maintenance of networks and collaborative working groups. It is unlikely that any single

Box 4 Key issues related to future trials of OPC transplantation in multiple sclerosis

(1) Cell product
  (a) Foetal versus derived from embryonic stem cells versus OPC-like iPSC lines derived from the recipient
    (i) Issue of genetic modifications in cultured autologous lines
    (ii) Potential for ectopic tissue formation
  (b) Need for immunosuppression for allogeneic cells
  (c) Cell manipulation to stimulate inherent remyelination capacity of exogenous OPCs
(2) Route of delivery
  (a) Is direct injection necessary? Can the cells be administered intrathecally?
  (b) What is the ability of injected cells to migrate to areas of need within the CNS
(3) Dose and dosing
  (a) Cell number
  (b) Injection into a single versus multiple locations
  (c) Single versus repeated administration
(4) Trial design
  (a) Appropriate study population
  (b) Allowed concomitant multiple sclerosis DMT(s). How do available multiple sclerosis DMTs affect the viability and function of transplanted OPCs?
  (c) Safety monitoring, short and long term
    (i) Adverse effects related to administration e.g. infection, haemorrhage
    (ii) Ectopic tissue formation
    (iii) Cancer
    (iv) Adverse effects related to immunosuppression
  (d) What is the effect of immunosuppression to prevent transplant rejection on the viability and/or function of transplanted cells?
  (e) How to monitor fate of injected cells
  (f) What information on efficacy can be obtained from a phase I study focusing on feasibility and safety?

source can meet all funding needs. Collaborative funding networks for such efforts have been created, for example, the International Progressive Multiple Sclerosis Alliance; the New York State Consortium for Stem Cell Therapy for Progressive Multiple Sclerosis; government agencies such as Immune Tolerance Network of the National Institute of Immunology Allergy and Infectious Disease and National Institute of Neurological Disorders and Stroke, the National Institute of Heart Lung and Blood, the National Cancer Institute, all of the United States National Institutes of Health; and others. Public-private funding consortia will likely be needed to raise sufficient funds to undertake studies and to create and maintain networks and registries. While there is documented interest in such support from private foundations, care needs to be taken to avoid confusion between the concepts of 'care' and 'research' in the absence of public support and independent oversight. The issues of private stem cell clinics, which 'sell' research that is supported by patients undergoing treatment, are fraught with ethical and practical concerns.

Given the interest in all forms of cell-based therapies for multiple sclerosis, and the increasing number of observational and randomized studies that are being done and proposed, there will likely be more opportunities than funds available. Prioritization among opportunities will be important for funders as well as investigators, and encouraging creation of research networks will encourage efficiencies for both scientific progress and expenditure of limited funds.

Concluding remarks

(i) Immunoablation followed by autologous haematopoietic stem cell transplantation appears to have potent and durable efficacy in relapsing-remitting multiple sclerosis though with significant safety concerns. The principal question is where this therapeutic approach should be placed in the overall treatment for relapsing-remitting multiple sclerosis.

(ii) There may be rare patients with highly aggressive relapsing-remitting multiple sclerosis who have failed available therapies for whom I/AHSCT may be justified as part of clinical practice. Other than these patients, cell-based therapy of multiple sclerosis should be pursued in clinical trials.

(iii) Cell-based therapy—transplantation, mobilization, and pharmacologic manipulation—may be helpful in patients with progressive forms of multiple sclerosis where

Box 5 Ethical issues related to cell-based therapies

(1) Studies must adhere to current general ethical standards for use of human subjects in research
   (a) Declaration of Helsinki accords
   (b) Local Institutional Review Board/Ethics committee requirements
(2) Studies must adhere to local guidelines for use of human embryonic and other stem cells related to procurement, cell production, banking, distribution, etc
(3) Issues related to the proliferation of stem cell clinics and medical tourism
   (a) Misconceptions created by media hype
   (b) Adequate informed consent
   (c) Confusion regarding the distinction between clinical care, innovation, and research
   (d) Legitimacy of patient funded research, particularly pay-for-participation
   (e) Need for independent oversight

degenerative mechanisms predominate, but this hypothesis remains to be confirmed.

(iv) All forms of cell-based therapy for multiple sclerosis should be considered experimental at this time. When it is pursued, comprehensive safety and efficacy data should be collected and submitted to existing registries, with the expectation that the results will be published. Because important biological questions remain for all forms of cell-based therapy, mechanistic studies should be included.

Acknowledgements

The International Advisory Committee on Clinical Trials in Multiple Sclerosis sponsored the International Conference on Cell-Based Therapies for Multiple Sclerosis. We are grateful for the active participation in the Conference by the participants (Appendix 1 and Supplementary material) who had the opportunity to review a draft of this manuscript prior to submission for publication.

Funding

The U.S. National Multiple Sclerosis Society and the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) funded the activities of the Committee and the Conference. The Americas Committee for Treatment and Research in Multiple Sclerosis (ACTRIMS) and the Multiple Sclerosis International Federation (MSIF) provided additional Conference support.

Conflicts of interest

J.A.C has received personal compensation for consulting for Merck, Novartis, and Receptos/Celgene and as a Co-Editor of Multiple Sclerosis Journal – Experimental, Translational and Clinical. M.P. has received consulting fees and/or travel grants from Atara and Baxalta. N.J.S. has received honoraria, research support, or educational support from Biogen, GSK, Merck Serono, Novartis, and Teva. S.C.R. has received personal consulting fees and/or travel support during the course of this work from the National Multiple Sclerosis Society, the European Committee for Treatment and Research in Multiple Sclerosis, F. Hoffmann-LaRoche, Ionis Pharmaceuticals, Medday Pharmaceuticals SA, MedImmune Inc., Merck Serono, Novartis, Observatoire Français de la Sclérose en Plaques, Opexa Therapeutics, Teva Pharmaceuticals Industries, and TG Therapeutics.

Supplementary material

Supplementary material is available at *Brain* online.

Appendix 1

Participants in the International Conference on Cell-Based Therapies for Multiple Sclerosis, 19–21 November 2015, Lisbon, Portugal: Harold Atkins; Brenda Banwell; Amit Bar-Or; Bruce Bebo; James Bowen; Richard Burt; Peter Calabresi; Jeffrey Cohen; Giancarlo Comi; Peter Connick; Anne Cross; Gary Cutter; Tobias Derfuss; Charles Ffrench-Constant; Mark Freedman; Jacques Galipeau; Myla Goldman; Steven Goldman; Andrew Goodman; Ari Green; Linda Griffith; Hans-Peter Hartung; Bernhard Hemmer; Insoo Hyun; Ellen Iacobaeus; Matilde Inglese; Burk Jubelt; Dimitrios Karussis; Patrick Küry; Douglas Landsman; Cornelia Laule; Roland Liblau; Giovanni Mancardi; Ruth Ann Marrie; Aaron Miller; Robert Miller; David Miller; Ellen Mowry; Paolo Muraro; Richard Nash; Daniel Ontaneda; Marcelo Pasquini; Daniel Pelletier; Luca Peruzzotti-Jametti; Stefano Pluchino; Michael Racke; Stephen Reingold; Claire Rice; Olle Ringdén; Alex Rovira; Riccardo Saccardi; Saud Sadiq; Stefanie Sarantopoulos; Sean Savitz; Neil Scolding; Per Soelberg Sorensen; Maria Pia Sormani; Olaf Stuve; Paul

Tesar; Alan Thompson; Maria Trojano; Antonio Uccelli; Bernard Uitdehaag; Ursula Utz; Sandra Vukusic; Emmanuelle Waubant; Alastair Wilkins.

References

Abrahamsson SV, Angelini DF, Dubinsky AN, Morel E, Oh U, Jones JL, et al. Non-myeloablative autologous haematopoietic stem cell transplantation expands regulatory cells and depletes IL-17 producing mucosal-associated invariant T cells in multiple sclerosis. Brain 2013; 136: 2888–903.

Akiyama Y, Radtke C, Honmou O, Kocsis JD. Remyelination of the spinal cord following intravenous delivery of bone marrow cells. Glia 2002; 39: 229–36.

Arruda LC, Lorenzi JC, Sousa AP, Zanette DL, Palma PV, Panepucci RA, et al. Autologous hematopoietic SCT normalizes miR-16, -155, 142-3p expression in multiple sclerosis patients. Bone Marrow Transplant 2015; 50: 380–9.

Atkins HL, Bowman M, Allan D, Anstee G, Arnold DL, Bar-Or A, et al. Immunoablation and autologous haematopoietic stem-cell transplanatation for agressive multiple sclerosis: a multicentre single-group phase 2 trial. Lancet 2016; 388: 576–85.

Autoimmune Disease Working Party. History of the Autoimmune Diseases Working Party, reason for its establishment and its context within the EBMT. Available from: https://www.ebmt.org/Contents/Research/TheWorkingParties/ADWP/Pages/Autoimmune-Diseases-Working-Party.aspx (22 August 2016, date last accessed).

Bai L, Lennon DP, Caplan AI, DeChant A, Hecker J, Kranso J, et al. Hepatocyte growth factor mediates mesenchymal stem cell-induced recovery in multiple sclerosis models. Nat Neurosci 2012; 15: 862–70.

Bai L, Lennon DP, Eaton V, Maier K, Caplan AI, Miller SD, et al. Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. Glia 2009; 57: 1192–203.

Berkowitz AL, Miller MB, Mir SA, Cagney D, Chavakula V, Guleria I, et al. Glioproliferative lesion on the spinal cord as a complication of "stem-cell tourism". N Engl J Med 2016; 375: 196–8.

Blakemore WF. Remyelination of CNS axons by Schwann cells transplanted from the sciatic nerve. Nature 1977; 266: 68–9.

Bonab MM, Sahraian MA, Aghsaie A, Karvigh SA, Hosseinian SM, Nikbin B, et al. Autologous mesenchymal stem cell therapy in progressive multiple sclerosis: an open label study. Curr Stem Cell Res 2012; 7: 407–14.

Bonab MM, Yazdanbakhsh S, Loft J, Alimoghaddom K, Talebian F, Hooshmand F, et al. Does mesenchymal stem cell therapy help multiple sclerosis patients? Iran J Immunol 2007; 4: 50–7.

Bronckaers A, Hilkens P, Martens W, Gervois P, Ratajczak J, Struys T, et al. Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis. Pharmacol Ther 2014; 143: 181–96.

Burman J, Iacobaeus E, Svenningsson A, Lycke J, Gunnarson M, Nilsson P, et al. Autologous haematopoietic stem cell transplantation for aggressive multiple sclerosis: the Swedish experience. J Neurol Neurosurg Psychiatry 2014; 85: 1116–21.

Burt RK, Balabanov R, Han X, Sharrack B, Morgan A, Quigley K, et al. Association of nonmyeloablative hematopoietic stem cell transplantation with neurologic disability in patients with relapsing-remitting multiple sclerosis. JAMA 2015; 313: 275–84.

Burt RK, Balabanov R, Voltarelli J, Barreira A, Burman J. Autologous hematopoietic stem cell transplantation for multiple sclerosis - if confused or hesitant, remember: treat with standard immune suppressive drugs and if no inflammation, no response. Mult Scler J 2012; 18: 772–5.

Burt RK, Loh Y, Cohen B, Stefosky D, Balabanov R, Katsamakis G, et al. Autologous non-myeloablative haemopoietic stem cell transplanatation in relapsing-remitting multiple sclerosis: a phase I/II study. Lancet 2009; 8: 244–53.

Cadavid D, Balcer L, Galetta S, Aktas O, Ziemssen T, Vanopdenbosch L, et al. Safety and efficacy of opicinumab in acute optic neuritis (RENEW): a randomised, placebo-controlled, phase 2 trial. Lancet Neurol 2017; 16: 189–99.

Cadavid D, Edwards KR, Hupperts R, Drulovic J, Montalban X, Hartung HP, et al. Efficacy analysis of opicinumab in relapsing multiple sclerosis: the phase 2b SYNERGY trial (abstract 192). Mult Scler J 2016; 22: 66.

Center for International Blood and Marrow Research. Available from: https://www.cibmtr.org/pages/index.aspx. (22 August 2016, date last accessed).

Chang A, Smith MC, Yin X, Fox RJ, Staugaitis SM, Trapp BD. Neurogenesis in the chronic lesions of multiple sclerosis. Brain 2008; 131: 2366–75.

Chang A, Staugaitis SM, Dutta R, Batt CE, Easley KE, Chomyk AM, et al. Cortical remyelination: a new target for repair strategies in multiple sclerosis. Ann Neurol 2012; 72: 918–26.

Chang A, Tourtellotte WW, Rudick RA, Trapp BD. Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. N Engl J Med 2002; 346: 165–73.

Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, et al. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke 2001; 32: 1005–11.

Chinnadurai R, Copland IB, Garcia MA, Petersen CT, Lewis CN, Waller EK, et al. Cryopreserved mesenchymal stromal cells are susceptible to T-cell mediated apoptosis which is partly rescued by IFNgamma licensing. Stem Cells 2016; 34: 2429–42.

Chinnadurai R, Garcia MA, Sakurai Y, Lam WA, Kirk AD, Galipeau J, et al. Actin cytoskeletal disruption following cryopreservation alters the biodistribution of human mesenchymal stromal cells *in vivo*. Stem Cell Reports 2014; 3: 60–72.

Cogle CR, Yachnis AT, Laywell ED, Zander DS, Wingard JR, Steindler DA, et al. Bone marrow transdifferentiation in brain after transplantation: a retrospective study. Lancet 2004; 363: 1432–7.

Cohen JA, Imrey PB, Planchon SM, Bermel RA, Fisher E, Fox RJ, et al. Pilot trial of intravenous autologous culture-expanded mesenchymal stem cell transplantation in multiple sclerosis. Mult Scler J 2017. Advance Access published on April 1, 2017, doi: 10.1177/1352458517703802.

Cohen JA, Reingold SC, Polman CH, Wolinsky JS, for the International Advisory Committee on Clinical Trials in Multiple Sclerosis. Disability outcome measures in multiple sclerosis trials: current status and future prospects. Lancet Neurol 2012; 11: 467–76.

Connick P, Kolappan M, Crawley C, Webber DJ, Patani R, Michell AW, et al. Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: an open-label phase 2a proof-of-concept study. Lancet Neurol 2012; 11: 150–6.

Da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 2006; 119: 2204–13.

Daikeler T, Labopin M, Di Gioia M, Abinun M, Alexander T, Miniati I, et al. Secondary autoimmune disease occurring after HSCT for an autoimmune disease: a retrospective study of the EBMT Autoimmune Disease Working Party. Blood 2011; 118: 1693–8.

Daley GQ, Hyun I, Apperley JF, Barker RA, Benvenisty N, Bredenoord AL, et al. Setting global standards for stem cell research and clinical translation: the 2016 ISSCR guidelines. Stem Cell Rep 2016; 6: 787–97.

Darlington PJ, Boivin M-N, Renoux C, Francois M, Galipeau J, Freedman MS, et al. Reciprocal Th1 and Th17 regulation by mesenchymal stem cells: implications for MS. Ann Neurol 2010; 68: 540–5.

Darlington PJ, Touil T, Doucet JS, Gaucher D, Zeidan J, Gauchat D, et al. Diminished Th17 (not Th1) responses underlie multiple sclerosis abrogation after hematopoietic stem cell transplantation. Ann Neurol 2013; 73: 341–54.

de Oliveira GLV, de Lima KWA, Colombini AM, Pinheiro DG, Panepucci RA, Palma PVB, et al. Bone marrow mesenchymal stromal cells isolated from multiple sclerosis patients have distinct gene expression profile and decreased suppressive function compared with healthy counterparts. Cell Transplant 2015; 24: 151–65.

Deshmukh VA, Tardif V, Lyssiotis CA, Green CC, Kerman B, Kim HJ, et al. A regenerative approach to the treatment of multiple sclerosis. Nature 2013; 502: 327–32.

Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. Blood 2003; 101: 2999–3001.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society For Cellular Therapy position statement. Cytotherapy 2006; 8: 315–17.

Elsawy M, Sorror ML. Up-to-date tools for risk assessment before allogeneic hematopoietic cell transplantation. Bone Marrow Transplant 2016; 51: 1283–300.

Francois M, Copland IB, Yuan S, Romieu-Mourez R, Waller EK, Galipeau J. Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-gamma licensing. Cytotherapy 2012; 14: 147–52.

Freedman MS, Bar-Or A, Atkins HL, Karussis D, Frassoni F, Lazarus HM, et al. The therapeutic potential of mesenchymal stem cell transplantation as a treatment for multiple sclerosis: consensus report of the International MSCT Study Group. Mult Scler 2010; 16: 503–10.

Giovannoni G, Turner B, Gnanapavan S, Offiah C, Schmierer K, Marta M. Is it time to target no evident disease activity (NEDA) in multiple sclerosis? Mult Scler Relat Disord 2015; 4: 329–33.

Goldman SA, Nedergaard M, Windrem MS. Glial progenitor cell-baed treatment and modeling of neurological disease. Science 2012; 338: 491–5.

Goodin DS, Reder AT, Ebers GC, Cutter G, Kremenchutsky M, Oger J, et al. Survival in MS: a randomized cohort study 21 years after the start of the pivotal IFNb-1b trial. Neurology 2012; 78: 1315–22.

Goodman AD. Stem cell therapy for MS. Mult Scler J 2016; 22: 8.

Gordon D, Pavlovska G, Uney JB, Wraith DC, Scolding NJ. Human mesenchymal stem cells infiltrate the spinal cord, reduce demyelination, and localize to white matter lesions in experimetnal autoimmune encephalomyelitis. J Neuropathol Exp Neurol 2010; 69: 1087–95.

Gore A, Li Z, Fung H-L, Young JE, Agarwal S, Antosiewicz-Bourget J, et al. Somatic coding mutations in human induced pluripotent stem cells. Nature 2011; 471: 63–7.

Green A, Gelfand J, Cree B, Bevan C, Boscardin WJ, Mei F, et al. Positive phase II double-blind randomized placebo-controlled crossover trial of clemastine fumarate for remyelination of chronic optic neuropathy in MS (ES1.008). Presented at the 2016 Annual Meeting of the American Academy of Neurology.

Gunter KC, Caplan AL, Mason C, Salzman R, Janssen WE, Nichols K, et al. Cell therapy medical tourism: time for action. Cytotherapy 2010; 12: 965–8.

Halfpenny CA, Scolding NJ. Immune-modifying agents do not impair the survival, migration or proliferation of oligodendrocyte progenitors (CG-4) *in vitro*. J Neuroimmunol 2003; 139: 9–16.

Harris VK, Faroqui R, Vyshkina T, Sadiq SA. Characterization of autologous mesenchymal stem cell-derived neural progenitors as a feasible source of stem cells for central nervous system applications in multiple sclerosis. Stem Cells Transl Med 2012; 1: 536–47.

Hartung DM, Bourdette DN, Ahmed SM, Whitham RH. The cost of multiple sclerosis drugs in the US and pharmaceutical industry: too big to fail? Neurology 2015; 84: 2185–92.

Hauser SL, Comi G, Hartung HP, Selmaj K, Trabousee A, Bar-Or A, et al. Efficacy and safety of ocrelizumab in relapsing multiple sclerosis - results of the interferon-beta-1a-controlled, double-blind, phase III OPERA I and II studies (190). Mult Scler J 2015; 21: 61–2.

Havrdova E, Galetta S, Stefoski D, Comi G. Freedom from disease activity. Neurology 2010; 74 (Suppl 3): S3–7.

Hoch AI, Leach JK. Concise review: optimizing expansion of bone marrow mesenchymal stem/stromnal cells for clinical applications. Stem Cells Transl Med 2014; 3: 643–52.

Hussein SM, Batada NN, Vuoristo S, Ching RW, Autio R, Narva E, et al. Copy number variation and selection during reprogramming to pluripotency. Nature 2011; 471: 58–62.

Hyun I, Lindvall O, Ahrlund-Richter L, Cattaneo E, Cavazzana-Calvo M, Cossu G, et al. New ISSCR guidelines underscore major principles for responsible translational stem cell research. Cell Stem Cell 2008; 3: 607–9.

Ingwerson J, Aktas O, Hartung HP. Advances in and algorithms for the treatment of relapsing-remitting multiple sclerosis. Neurotherapeutics 2016; 13: 47–57.

International Society for Stem Cell Research. Guidelines for stem cell research and clinical translation, 2016. Available from: http://www.isscr.org/docs/default-source/guidelines/isscr-guidelines-for-stem-cell-research-and-clinical-translation.pdf?sfvrsn=2. (22 August 2016, date last accessed).

Johansson CB, Youssef S, Koleckar K, Holbrook C, Doyonnas R, Corbel SY, et al. Extensive fusion of haematopoietic cells with Purkinje neurons in response to chronic inflammation. Nature Cell Biol 2008; 10: 575–83.

Jones JL, Anderson JM, Phuah CL, Fox EJ, Selmaj K, Margolin D, et al. Improvement in disability after alemtuzumab treatment of multiple sclerosis is associated with neuroprotective autoimmunity. Brain 2010; 133: 2232–47.

Kappos L, De Stefano N, Freedman MS, Cree BAC, Radue EW, Sprenger T, et al. Inclusion of brain volume loss in a revised meaure of "no evidence of disease activity" (NEDA-4) in relapsing-remitting multiple sclerosis. Mult Scler J 2015; 22: 1297–305.

Karussis D, Karageorgiou C, Vaknin-Dembinsky A, Gowda-Kurkalli B, Gomori JM, Kassis I, et al. Safety and immunologic effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. Arch Neurol 2010; 67: 1187–94.

Kassis I, Petrou P, Halimi M, Karussis D. Mesenchymal stem cells (MSC) derived from mice with experimental autoimmune encephalomyelitis (EAE) suppress EAE and have similar biological propoerties with MSC from healthy donors. Immunol Lett 2013; 154: 70–6.

Kemp K, Gray E, Wilkins A, Scolding N. Purkinje cell fusion and binucleate heterokaryon formation in multiple sclerosis cerebellum. Brain 2012; 135: 2962–72.

Kemp K, Hares K, Mallam E, Heesom KJ, Scolding N, Wilkins A. Mesenchymal stemm cell-secreted superoxide dismutase promotes cerebellar neuronal survival. J Neurochem 2010; 114: 1569–80.

Kemp K, Wilkins A, Scolding N. Cell fusion in the brain: two cells forward, one cell back. Acta Neuropathol 2014; 128: 629–38.

Kishk NA, Abokrysha NT, Gabr H. Possible induction of acute disseminated encephalomyelitis (ADEM)-like demyelinating illness by intrathecal mesenchymal stem cell injection. J Clin Neurosci 2013; 20: 310–12.

Korbling M, Estrov Z. Adult stem cells for tissue repair—a new therapeutic concept? N Engl J Med 2003; 349: 570–82.

Kremer D, Gottle P, Hartung H-P, Kury P. Pushing forward: remyelination as the new frontier in CNS diseases. Trends Neurosci 2016; 39: 246–63.

Kuriyan AE, Albini TA, Townsend JH, Rodriguez M, Pandya HK, Leonard RE, et al. Vision loss after intravitreal injection of autologous "stem cells" for AMD. N Engl J Med 2017; 376: 1047–53.

Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, et al. Safety of cell therapy with mesenchymal stromal

cells (SafeCell): a systematic review and meta-analysis of clinical trials. PLoS One 2012; 7: e47559.
Lee H, Narayanan S, Brown RA, Chen JT, Atkins HL, Freedman MS, et al. Brain atrophy after bone marrow transplantation for treatment of multiple sclerosis. Mult Scler J 2017; 23: 420–31.
Lee PH, Lee JE, Kim H-S, Song SK, Lee HS, Nam HS, et al. A randomized trial of mesenchymal stem cells in multiple system atrophy. Ann Neurol 2012; 72: 32–40.
Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 2009; 5: 54–63.
Li CY, Wu XY, Tong JB, Y9ng XX, Zhao JL, Zheng QF, et al. Comparative analysis of human mesenchyal stem cells from bone marrow and adipose tissue under xeno-free conditions for cell therapy. Stem Cell Res Ther 2015; 6: 55.
Li JF, Zhang DJ, Geng T, Chen L, Huang H, Yin HL, et al. The potential of human umbilical cord-derived mesenchymal stem cells as a novel cellular therapy for multiple sclerosis. Cell Transplant 2014; 23 (Suppl 1): S113–22.
Li Y, Chen J, Zhang CL, Wang L, Lu D, Katakowski M, et al. Gliosis and brain remodeling after treatment of stroke in rats with marrow stromal cells. Glia 2005; 49: 407–17.
Liang J, Zhang H, Hua B, Wang H, Wang J, Han ZC, et al. Allogeneic mesenchymal stem cells transplantation in treatment of multiple sclerosis. Mult Scler 2009; 15: 644–6.
Lindvall O, Hyun I. Medical innovation versus stem cell tourism. Sci Justice 2009; 324: 1664–65.
Lister R, Pelizzola M, Kida YS, Hawkins D, Nery JR, Hon G, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature 2011; 471: 68–73.
Llufriu S, Sepulveda M, Blanco Y, Marin P, Moreno B, Berenguer J, et al. Randomized placebo-controlled phase II trial of autologous mesenchymal stem cells in multiple sclerosis. PLoS One 2014; 9: e113936.
Lovett-Racke AE, Yang Y, Racke MK. TH1 versus Th17: are T cell cytokines relevant in multiple sclerosis? Biochim Biophys Acta 2011; 1812: 246–51.
Lublin FD, Bowen JD, Huddlestone J, Kremenchutzky M, Carpenter A, Corboy JR, et al. Human placenta-derived cells (PDA-001) for the treatment of adults with multiple sclerosis: a randomized, placebo-controlled, multiple-dose study. Mult Scler Relat Disord 2014; 3: 696–704.
Mallam E, Kemp K, Wilkins A, Rice C, Scolding N. Characterization of *in vitro* expanded bone marrow-derived mesenchymal stem cells from patients with multiple sclerosis. Mult Scler 2010; 16: 909–18.
Mancardi G, Saccardi R. Autologous haematopoietic stem-cell transplantation in multiple sclerosis. Lancet Neurol 2008; 7: 626–36.
Mancardi G, Sormani MP, Gualandi F, Saiz A, Carreras E, Merelli E, et al. Autologous hematopoietic stem cell transplantation in multiple sclerosis. A phase II trial. Neurology 2015; 84: 981–8.
Mancardi GL, Sormani MP, Di Gioia M, Vuolo L, Gualandi F, Amato MP, et al. Autologous haematopoietic stem cell transplantation with an intermediate intensity conditioning regimen in multiple sclerosis: the Italian multi-centre experience. Mult Scler 2012; 18: 835–42.
Marrie RA, Cohen J, Stuve O, Trojano M, Sorensen PS, Reingold S, et al. A systematic review of the incidence and prevalence of comorbidity in multiple sclerosis: overview. Mult Scler J 2015; 21: 263–81.
Mazzanti B, Aldinucci A, Biagioli T, Barilaro A, Urbani S, Dal Pozzo S, et al. Differences in mesenchymal stem cell cytokine profiles between MS patients and healthy donors: Implications for assessment of disease activity and treatment. J Neuroimmunol 2008; 199: 142–50.
Mei F, Fancy SPJ, Shen Y-AA, Niu J, Zhao C, Presley B, et al. Micropillar arrays as a high-throughput screening platform for therapeutics in multiple sclerosis. Nat Med 2014; 20: 954–60.
Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthus BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 2010; 466: 829–34.
MEsenchymal StEm Cells for Multiple Sclerosis (MESEMS). ClinicalTrials.gov Identifier: NCT01854957. Available from: https://clinicaltrials.gov/ct2/show/NCT01854957?term=01854957&rank=1, (22 August 2016, date last accessed).
Mi S, Pepinsky RB, Cadavid D. Blocking LINGO-1 as a therapy to promote CNS repair: from concept to the clinic. CNS Drugs 2013; 27: 493–503.
Miura M, Miura Y, Padilla-Nash HM, Molinolo AA, Fu B, Patel V, et al. Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation. Stem Cells 2006; 24: 1095–103.
Morando S, Vigo T, Esposito M, Casazza S, Novi G, Principato MC, et al. The therapeutic effect of mesenchymal stem cell trnasplantation in experimental autoimmune encephalomyelitis is mediated by peripheral and central mechanisms. Stem Cell ResTher 2012; 3: 3.
Mosna F, Sensebe L, Krampera M. Human bone marrow and adipose tissue mesenchymal stem cells: a user's guide. Stem Cells Dev 2010; 19: 1449–70.
Munoz JR, Stoutenger BR, Robinson AP, Spees JL, Prockop DJ. Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice. Proc Natl Acad Sci USA 2005; 102: 18171–6.
Muraro P, Pasquini M, Atkins H, Bowen JD, Farge D, Fassas A, et al. Long-term outcomes after autologous haematopoietic cell transplantation for multiple sclerosis: a joint study from the Center for International Blood and Marrow Research (CIBMTR) and the European Group for Blood and Marrow Transplantation (EBMT). Bone Marrow Transplant 2013; 48: S1.
Muraro PA, Abrahamsson SV. Resetting autoimmunity in the nervous system: the role of hematopoietic stem cell transplantation. Curr Op Investig Drugs 2010; 11: 1265–75.
Muraro PA, Douek DC, Packer A, Chung K, Guenaga FJ, Cassiani-Ingoni R, et al. Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. J Exp Med 2005; 201: 805–16.
Muraro PA, Pasquini M, Atkins HL, Bowen JD, Farge D, Fassas A, et al. Long-term outcomes after autologous hematopoietic stem cell transplantation for multiple sclerosis. JAMA Neurol 2017; 74: 459–69.
Muraro PA, Robins H, Malhotra S, Howell M, Phippard D, Desmarais C, et al. T cell repertoire following autologous stem cell transplantation for multiple sclerosis. J Clin Invest 2014; 124: 1168–72.
Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med 2013; 45: e54.
Najm FJ, Madhavan M, Zaremba A, Shick E, Karl RT, Factor DC, et al. Drug-based modulation of endogenous stem cells promotes functional remyelination *in vivo*. Nature 2015; 522: 216–20.
Nash RA, Hutton GJ, Racke MK, Popat U, Devine SM, Griffith LM, et al. High-dose immunosuppressive therpay and autologous hematopoietic cell transplantation for relapsing-remitting multiple sclerosis (HALT-MS). A 3-year interim report. JAMA Neurol 2015; 72: 159–69.
National Research Council and Institute of Medicine of the National Academies. Guidelines for Human Embryonic Stem Cell Research. Available from: http://www.nap.edu/read/11278/chapter/1. (22 August 2016, date last accessed).
Nature Editorial. FDA should stand firm on stem-cell treatments. US regulators must regain the upper hand in the approval system. Nature 2016; 535: 7–8.
New York Times. A cautionary tale of "stem cell tourism." Available from: http://www.nytimes.com/2016/06/23/health/a-cautionary-tale-of-stem-cell-tourism.html?_r=0. (22 August 2016, date last accessed).

Odinak MM, Bisaga GN, Novitskiĭ AV, Tyrenko VV, Fominykh MS, Bilibina AA, et al. Transplantation of mesenchymal stem cells in multiple sclerosis [in Russian]. Zh Nevrol Psikhiatr Im S S Korsakova 2011; 111: 72–6.

Odoardi F, Sie C, Streyl K, Ulaganathan VK, Schlager C, Lodygin D, et al. T cells become licensed in the lung to enter the central nervous system. Nature 2012; 488: 675–9.

Openshaw H, Stuve O, Antel JP, Nash R, Lund BT, Weiner LP, et al. Multiple sclerosis flares associated with recombinant granulocyte colony-stimulating factor. Neurology 2000; 54: 2147–50.

Papadaki HA, Tsagournisakis M, Mastorodemos V, Pontikoglou C, Damianaki A, Pyrovolaki K, et al. Normal bone marrow hematopoietic stem cell reserves and normal stromal cell function support the use of autologous stem cell transplantation in patients with multiple sclerosis. Bone Marrow Transplant 2005; 36: 1053–63.

Phillips JT, Giovannoni G, Lublin FD, O'Connor PW, Polman CH, Willoughby E, et al. Sustained improvement in expanded disability status scale as a new efficacy measure of neurologic change in multiple sclerosis: treatment effects with natalizumab in patients with relapsing multiple sclerosis. Mult Scler J 2011; 17: 970–9.

Phinney DG. Functional heterogeneity of mesenchymal stem cells: implications for cell therapy. J Cell Biol 2012; 113: 2806–12.

Pisati F, Bossolasco P, Meregalli M, Cova L, Belicchi M, Gavina M, et al. Induction of neurotrophin expression via human adult mesenchymal stem cells: implications for cell therapy in neurodegenerative diseases. Cell Transplant 2007; 16: 41–55.

Pluchino S, Zanotti L, Rossi B, Brambilla E, Ottoboni L, Salani G, et al. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. Nature 2005; 436: 266–71.

Prockop DJ. Mitochondria to the rescue. Nat Med 2012; 18: 653–4.

Prockop DJ, Oh JY. Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. Mol Ther 2012; 20: 14–20.

Redondo J, Sarkar P, Kemp K, Heesom KJ, Wilkins A, Scolding NJ, et al. Alterations in the secretome of MSCs isolated from patients with MS are in keeping with their reduced neuroprotective potential under conditins of oxidative stress (P669). Mult Scler J 2016; 22(S3): 321.

Rice CM, Kemp K, Wilkins A, Scolding NJ. Cell Therapy for multiple sclerosis: an evolving concept with implications for other neurodegenerative diseases. Lancet 2013; 382: 1204–13.

Rice CM, Mallam EA, Whone AL, Walsh P, Brooks DJ, Kane N, et al. Safety and feasibility of autologous bone marrow cellular therapy in relapsing-progressive multiple sclerosis. Clin Pharmacol Ther 2010; 87: 679–85.

Rice CM, Marks DI, Ben-Shlomo Y, Evangelou N, Morgan PS, Metcalfe C, et al. Assessment of bone marrow-derived Cellular Therapy in progressive Multiple Sclerosis (ACTiMuS): study protocol for a randomised controlled trial. Trials 2015a; 16: 463.

Rice CM, Marks DI, Walsh P, Kane NM, Guttridge MG, Redondo J, et al. Repeat infusion of autologous bone marrow cells in multiple sclerosis: protocol for a phase I extension study (SIAMMS-II). BMP Open 2015b; 5: e009090.

Rice CM, Scolding NJ. Adult stem cells - reprogramming neurological repair? Lancet 2004; 364: 193–9.

Rice CM, Scolding NJ. Adult human mesenchymal cells proliferate and migrate in response to chemokines expressed in demyelination. Cell Adh Migr 2010; 4: 235–40.

Riordan NH, Ichim TE, Min W-P, Wang H, Solano F, Lara F, et al. Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis. J Transl Med 2009; 7: 29.

Roccatagliata L, Rocca M, Valsasina P, Bonzano L, Sormani MP, Saccardi R, et al. The long-term effect of AHSCT on MRI measures of MS evolution: a five-year follow-up study. Mult Scler J 2007; 13: 1068–70.

Rotstein DL, Healy BC, Malik MT, Chitnis T, Weiner HL. Evaluation of no evidence of disease activity in a 7-year longitudinal multiple sclerosis cohort. JAMA Neurol 2015; 72: 152–8.

Rozenberg A, Rezk A, Boivin MN, Darlington PJ, Nyirenda M, Li R, et al. Human mesenchymal stem cells impact Th17 and Th1 responses through a prostaglandin E2 and myeloid-dependent mechanism. Stem Cells Transl Med 2016; 5: 1506–14.

Saccardi R, Freedman MS, Sormani MP, Atkins H, Farge D, Griffith LM, et al. A prospective, randomized, controlled trial of autologous haematopoietic stem cell transplantation for aggressive multiple sclerosis: a position paper. Mult Scler J 2012; 18: 825–34.

Sarkar P, Redondo J, Kemp K, Wilkins A, Scolding NJ, Rice CM. Reduced expression of mitochondrial fumarate hydratase contributes to impaired MSC-mediated neuroprotection in multiple sclerosis (139). Mult Scler J 2016; 22(S3): 38–9.

Sarkar P, Scolding N. Stem cells for multiple sclerosis. In: Tuszynski MH, editor. Translational Neuroscience. New York: Springer; 2016. p.259–73.

Sasaki M, Honmou O, Akiyama Y, Uede T, Hashi K, Kocsis JD. Transplantation of an acutely isolated bone marow fraction repairs demyelinated adult rat spinal cord axons. Glia 2001; 35: 26–34.

Savitz SI, Misra V, Kasam M, Juneja H, Cox CS, Alderman S, et al. Intravenous autologous bone marrow mononuclear cells for ischemic stroke. Ann Neurol 2011; 70: 59–69.

Shen LH, Xin H, Li Y, Zhang RL, Cui Y, Zhang L, et al. Endogenous tissue plasminogen activator mediates bone marrow strom cell-induced neurite remodeling after stroke in mice. Stroke 2011; 42: 459–64.

Shevchenko JL, Kuznetsov AN, Ionova TI, Melnichenko VY, Fedorenko DA, Kurbatova KA, et al. Long-term outcomes of autologous hematopoietic stem cell transplantation with reduced-intensity conditioning in multple sclerosis: physician's and patient's perspectives. Ann Hematol 2015; 94: 1149–57.

Shirani A, Okuda DT, Stuve O. Therapeutic advances and future prospects in progressive forms of multiple sclerosis. Neurotherapeutics 2016; 13: 58–69.

Sim FJ, McClain CR, Schanz SJ, Protack TL, Windrem MS, Goldman SA. CD140a identifies a population of highly myelinogenic, migration-competent and efficiently engrafting human oligodendrocyte progenitor cells. Nat Biotechnol 2011; 29: 934–41.

Soelberg Sorensen P. Haematopoietic stem cell transplants should be a second-line therapy for highly active MS - No. Mult Scler J 2016; 22: 1260–3.

Sormani MP, Muraro PA, Saccardi R, Mancardi G. NEDA status in highly active MS can be more easily obtained with autologous hematopoietic stem cell transplantation than other drugs. Mult Scler J 2017; 23: 201–4.

Sormani MP, Schiavetti I, Signori A, Muraro PA, Saccardi R, Mancardi G. Autologous haematopoietic stem cell transplantation in multiple sclerosis: a meta-analysis (P751). Mult Scler J 2016; 22: 374.

Sorror ML, Logan BR, Zhu X, Rizzo JD, Cooke KR, McCarthy PL, et al. Prospective validation of the predictive power of the Hematopoietic Cell Transplantation Comorbidity Index: a center for international blood and marrow transplant research study. Biol Blood Marrow Transplant 2015; 21: 1479–87.

Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood 2005; 106: 2912–19.

Strioga M, Viswanathan S, Darinskas A, Slaby O, Michalek J. Same or not the same? Comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. Stem Cells Dev 2012; 21: 2724–52.

Thiruvalluvan A, Czepiel M, Kap YA, Mantingh-Otter I, Vainchtein I, Kuipers J, et al. Survival and functionality of human induced pluripotent stem cell-derived oligodendrocytes in a nonhuman primate model for multiple sclerosis. Stem Cells Transl Med 2016; 5: 1550–61.

Trapp BD, Nave K-A. Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci 2008; 31: 247–69.

Turner L, Knoepfler P. Selling stem cells in the USA: assessing the direct-to-consumer industry. Cell Stem Cell 2016; 19: 154–7.

Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunology 2008; 8: 726–36.

Uhlin M, Wikell H, Sundin M, Blennow O, Maeurer M, Ringden O, et al. Risk factors for Epstein-Barr virus-related post-transplant lymphoproliferative disease after allogeneic hematopoietic stem cell transplantation. Haematologia 2014; 99: 346–52.

University of California San Francisco MS-EPIC Team, Cree BA, Gourraud PA, Oksenberg JR, Bevan C, Crabtree-Hartman E, et al. Long-term evolution of multiple sclerosis disability in the treatment era. Ann Neurol 2016; 80: 499–510.

von Bahr L, Batsis I, Moll G, Hagg M, Szakos A, Sundberg B, et al. Analysis of tissues following mesenchymal stromal cell therapy in humans indicate limited long-term engraftment and no ectopic tissue formation. Stem Cells 2012; 30: 557–64.

Wenner DM, Kimmelman J, London AJ. Patient-funded trials: opportunity or liability. Cell Stem Cell 2015; 17: 135–7.

Windrem MS, Nunes MC, Rashbaum WK, Schwartz TH, Goodman RA, McKhann G, et al. Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. Nat Med 2004; 10: 93–7.

Windrem MS, Schanz SJ, Guo M, Tian GF, Washco V, Stanwood N, et al. Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse. Cell Stem Cell 2008; 2: 553–65.

Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 2000; 61: 364–70.

World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Available from: http://www.wma.net/en/30publications/10policies/b3/index.html. (22 Aug 2016 date last accessed).

Yamout B, Hourani R, Salti H, Barada W, El-Hajj T, Al-Katoubi A, et al. Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: a pilot study. J Neuroimmunol 2010; 227: 185–9.

Zhang J, Li Y, Lu M, Cui Y, Chen J, Noffsinger L, et al. Bone marrow stromal cells reduce axonal loss in experimental autoimmune encephalomyelitis mice. J Neurosci Res 2006; 84: 587–95.