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Review article

Modulation of host immune responses following non-hematopoietic stem cell transplantation: Translational implications in progressive multiple sclerosis

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ABSTRACT

There exists an urgent need for effective treatments for those patients suffering from chronic/progressive multiple sclerosis (MS). Accordingly, it has become readily apparent that different classes of stem cell-based therapies must be explored at both the basic science and clinical levels. Herein, we provide an overview of the basic mechanisms underlying the pre-clinical benefits of exogenously delivered non-hematopoietic stem cells (nHSCs) in animal models of MS. Further, we highlight a number of early clinical trials in which nHSCs have been used to treat MS. Finally, we identify a series of challenges that must be met and ultimately overcome if such promising therapeutics are to be advanced from the bench to the bedside.

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1. Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by the dissemination of lesions throughout the brain and spinal cord, whose pathological hallmarks are variable degrees of inflammation, demyelination and axonal loss (Compston and Coles, 2002). In the majority of MS patients, the disease begins with a relapsing course (RRMS) followed by a progressive phase during which the gradual accumulation of a series of neurological disabilities becomes pronounced (secondary progressive, SPMS) (Lublin and Reingold, 1996; Lublin et al., 2014; Scalfari et al., 2014). When progression is observed from the onset without preceding relapses or remissions, the clinical course is defined as primary progressive (PPMS) (Ebers, 2004). Clinically, the transition from a RR to progressive phenotype marks the passage from a treatment responsive form of the disease to one that is refractory to currently available disease modifying treatments (DMTs) (Comi, 2013). Given the challenges associated with the treatment of patients who present with progressive MS, the importance of identifying the underlying pathobiological mechanisms that govern the transition from RR to progressive are at the center of current MS research (Lassmann et al., 2007). Accordingly, it has now become increasingly clear that many of the pathological events that characterize disease activity during acute inflammatory relapses can in fact be discerned from those that occur during progression, thereby highlighting the possibility of ultimately developing novel therapies capable of targeting chronic/progressive MS (Lassmann et al., 2012, Lublin et al., 2014).

Advances in histopathology, magnetic resonance imaging (MRI) and experimental animal models have recently contributed to the identification of novel aspects of MS pathophysiology. Such work has highlighted a complex role for immune responses in different MS phenotypes. The activation of the immune system has been recognized as a dynamic phenomenon, which not only drives tissue damage over the course of disease but may also influence brain repair/recovery via the modulation of functional and/or structural plasticity (Mallucci et al., 2015). In particular, the development of ultra-high-field scanners and the adoption of quantitative MRI techniques have contributed to identification of novel features of progressive MS pathology (Filippi and Rocca, 2005; Filippi et al., 2016; Rovaris et al., 2008). Quantitative MRI analysis has demonstrated the presence of diffuse neuronal tissue damage and concurrently highlighted the presence of cortical involvement in progressive forms of MS (Favaretto et al., 2015; Filippi and Rocca, 2005). Such findings have also been correlated with histological evidence, which collectively reveal the presence of diffuse normal appearing white matter (NAWM) inflammation driven by resident activated microglial cells (Rissanen et al., 2014).

Such results, when coupled to the reduced efficacy of DMTs (primarily targeting T and B cells) and to the limited radiological evidence of blood brain barrier (BBB) damage in progressive MS, have come to suggest that the pathophysiologic mechanisms occurring in late MS cases may reflect the compartmentalization of CNS neuroinflammation (Kutzelnigg et al., 2005). Further studies have provided additional emphasis by demonstrating that the cortical lesions in SPMS are associated with meningeal structures that are similar to the secondary B cell follicles contained within germinal centers (Serafini et al., 2004). This ectopic lymphoid tissue contains proliferating B cells, plasma cells, T cells and a network of follicular dendritic cells (FDC) and as such acts in similar manner to the aggregates witnessed in other organ-specific autoimmune diseases (*i.e.* Hashimoto's thyroiditis, myasthenia gravis and Sjogren's syndrome) (Aloisi and Pujol-Borrell, 2006), all of which have been related to the production of autoantibodies leading to the accumulation of tissue damage. Of note, these meningeal infiltrates in SPMS patients have been related to levels of cortical pathology (Magliozzi et al., 2007; Magliozzi et al., 2010), yet there is no evidence of similar structures in PPMS cases (Choi et al., 2012). The presence of B cell follicles supports the long standing clinical evidence of persistent oligoclonal bands (OCB) in the CSF of MS patients (Meinl et al., 2006), and also suggests that the CNS is capable of providing the required support needed for the long-term, *in situ* maintenance of B cells (Meinl et al., 2006). In summary CNS compartmentalized inflammation appears to dominate the pathobiology of progressive MS subtypes via the promotion of an environment favorable for progressive tissue degeneration (Lassmann, 2012; Meinl et al., 2008).

Understanding the spatial and temporal nature of inflammation throughout the clinical course of MS, it is reasonable to assume that the eradication of self-reactive immune cells via intense immunosuppression and/or immune reconstitution via the engraftment of autologous hematopoietic stem cells (HSC) would stabilize/improve patient outcomes in those suffering from severe forms of RRMS (Kimiskidis et al., 2008; Mancardi and Saccardi, 2008; Mancardi et al., 2005). Nonetheless, such approaches have not been effective for those patients with progressive forms of disease and/or high pre-transplantation disability scores. One such explanation for this failure may relate to the inability of such treatment paradigms to influence inflammation once it has been compartmentalized within the CNS (Bowen et al., 2012; Burt et al., 2003; Mancardi and Saccardi, 2008; Shevchenko et al., 2012). Advances in non-hematopoietic stem cell (nHSC) research are of particular interest in the context of chronic/progressive MS as per the recognition of their immune modulatory and neurotrophic actions in preclinical models of MS (Pluchino et al., 2009a; Pluchino et al., 2003; Pluchino et al., 2005). Contrary to single target based pharmaceutical interventions, nHSCs may be thought of as dynamic molecular medicines (Fischbach et al., 2013). nHSCs are capable of modulating the affected microenvironment present in progressive forms of MS via the secretion of paracrine factors, cell-to-cell interactions and/or exosome-mediated signalling, without the application of intensive pre-transplantation immune-ablation protocols (Cossetti et al., 2012).

In this review, we focus on the role of nHSCs in regulating both innate and adaptive immune responses in experimental models of MS. In particular, we explore the immune modulatory and tissue trophic proprieties of mesenchymal (MSCs) and neural stem cells (NSCs) that have been observed in both pre-clinical studies and early attempts to advance such therapies from the bench to the bedside. It is the authors' content that the successful translation of nHSC therapies will require the continued evaluation of both route(s) of transplantation and the underlying biology of the stem cell graft. Accordingly, we describe the benefits observed after either local or systemic nHSC transplantation and briefly highlight recent advances/clinical implications in stem cell reprogramming.

2. Evidence from disease/pathway models

2.1. Mesenchymal stem cells (MSCs)

MSCs are made up of a multipotent non-hematopoietic precursor cell population, which were first described within the bone marrow (BM) (Friedenstein et al., 1974) and then in the stroma of a wide range of additional tissues including adipose (AT) and those associated

with birth (*e.g.* decidua, umbilical cord, etc.) (Fraser et al., 2006; In 't Anker et al., 2004; Uccelli et al., 2008).

While the primary physiologic role of BM-MSCs is to support the processes underlying hematopoiesis (Mendez-Ferrer et al., 2010) recent studies have gone on to expand the biologic potential of MSCs and have come to suggest that MSCs may possess additional properties, including an ability to transdifferentiate into endodermal/ectodermal cells (Oswald et al., 2004; Woodbury et al., 2000) and to modulate immune responses (Uccelli et al., 2006). Such findings clearly suggest a role for the use of MSCs as a potential therapeutic tool for diseases in which tissue repair is needed and/or inflammation is extensive (Auletta et al., 2012; Wei et al., 2013).

BM and AT have been the most widely utilized source of MSCs due in part to the ease of harvest and the potential application of these cells as an autologous therapy (Hoogduijn and Dor, 2013). While BM aspiration and surgical harvest of adipose tissue (*i.e.* in case of liposuction, lipoplasty, or lipectomy) have provided robust materials for the study of MSCs, a lack of specific surface antigens has hindered the isolation of pure MSCs. Thus MSC cultures are typically composed of mixed, non-clonal populations of cells (Dominici et al., 2006; Galderisi and Giordano, 2014; McNiece, 2007; Samsonraj et al., 2015). Despite such limitations definitive MSC phenotypes have been discerned through a number of comparative studies (Baksh et al., 2007; Deuse et al., 2011; Hass et al., 2011; Hoogduijn and Dor, 2013). In line with the aforementioned, when MSCs derived from umbilical cord perivascular cells (UCPVCs) are compared with those isolated from the adult BM and AT, the neonatal MSCs display greater rates of proliferation (Baksh et al., 2007). Further, while BM-MSCs display contact-inhibited patterns of growth, UCPVC-MSCs continue to grow via multi-layering (Baksh et al., 2007). With regard to AT-MSCs, a certain range of heterogeneity has been demonstrated with cells isolated from different regions of the body, and evidence that *e.g.* subcutaneous-derived AT-MSCs grow slightly faster than omental-derived AT-MSCs provided (Van Harmelen et al., 2004). It is also interesting to note that MSCs from different sources are differentially influenced by the donor's age. As such, BM- and AT-MSCs decrease in therapeutic potential with age, whereas neonatal MSCs display reduced signs of senescence following multiple passages *in vitro* (Deuse et al., 2011). The optimal source of MSCs for cell-based therapies remains to be determined (Uccelli et al., 2008).

With regard to the pathobiology of progressive MS, *in vitro* findings have demonstrated that both mouse and human MSCs are capable of influencing the maturation and activity of innate immune cells/the associated response, whereas experimental settings evaluating the immunogenicity of MSCs have contribute to spread the idea that MSCs can be considered intrinsically immune privileged, thus overcoming the necessity for graft-host matching and considerably boosting the idea for allogenic MSCs transplantation in patients (Di Nicola et al., 2002; Le Blanc et al., 2003a; Tse et al., 2003). Adopting co-cultures involving cell subtypes of both innate or adaptive immunity, several studies reported that allogenic MSCs are capable of skewing the immune response toward an anti-inflammatory/tolerant phenotype through mechanisms that appear to be independent from the major histocompatibility complex (MHC). Despite human MSCs express intermediate levels of human leukocyte antigen (HLA) MHC class I, and can be induced to express MHC class II antigen by interferon γ (IFN-γ) treatment (Le Blanc et al., 2003b, Tse et al., 2003); MSCs do not eliciting a proliferative response from allogeneic lymphocytes, but to rather suppress their proliferation (Di Nicola et al., 2002, Le Blanc et al., 2003b, Tse et al., 2003).

Although it was initially suggested that the lack of co-stimulatory molecules like B7-1, B7-2, CD40, and CD40 ligand in MSCs would underpin the absence of T cell activation, following *in vitro* studies indicated a more complex scenario in which MSCs could actively influence the surrounding inflammatory environment in order to induce tolerance by means of mechanism which might not involve cell contact. In line with this concept, T-cell suppression was also observed in trans-well assays (Di Nicola et al., 2002) and a role for soluble factors (*i.e* prostaglandin E2, PGE_2) was suggested following the observation that MSCs were capable of down-regulate IFN-γ secretion from NK and Th1 cells and increase IL-4 secretion from Th2 cells, thus promoting a shift from a pro-inflammatory Th1-like phenotype to an anti-inflammatory Th2-like one (Aggarwal and Pittenger, 2005). Similarly, MSCs restrain DC maturation and functional properties through inhibition of TNF-α secretion and promotion of IL-10 secretion (Aggarwal and Pittenger, 2005). In line with this evidence, human MSCs inhibit the maturation of monocytes into macrophages, and cord-blood and $CD34^+$ hematopoietic progenitor cells into DCs (Jiang et al., 2005; Li et al., 2008; Nauta et al., 2006a; Ramasamy et al., 2007), as well as impair their antigen-presenting function (Chiesa et al., 2011, Jiang et al., 2005, Ramasamy et al., 2007). Analogously, MSCs hinder the cytotoxic activity of resting NK cells by down-regulating the expression of NKp30 and natural-killer group 2, member D (NKG2D) (Spaggiari et al., 2006), and diminish the respiratory burst of neutrophils, while inducing their spontaneous apoptosis through an IL-6-dependent mechanism (Raffaghello et al., 2008). Ensuing this evidence, the idea that MSCs could influence the inflammatory responses while maintaining a low/negligible immunogenic phenotype largely took place.

Nonetheless, in striking contrast to this notion murine allograft of MSCs has shown to be immune rejected by MHC class I- and class II-mismatched recipient (Eliopoulos et al., 2005) and be capable of inducing a memory T cell response after injection *in vivo* in immunocompetent hosts (Nauta et al., 2006b). Furthermore, recent studies have emphasized that whereas human MSCs limit Th1 responses, they concurrently induce Th17 responses. It appears that MSCs reciprocally regulate Th1 and Th17 effector T cell subsets, through a prostaglandin E2 (PGE2)-dependent and myeloid cell- mediated mechanism, which open novel questions regarding the potential pro-inflammatory effect induced by MSCs (Rozenberg et al., 2016). The substantial evidence of the immune modulatory capacity of MSCs has driven to the consideration that MSCs could induce anti-inflammatory responses sufficient to tame any pro-inflammatory T cell responses that they might concurrently induce (Rozenberg et al., 2016). It has also been suggested that MSCs may be considered immune-evasive rather than -privileged (Ankrum et al., 2014) and that such propriety is balanced between their expression of immunogenic and immunosuppressive factors, which is substantially affected by the nature of local inflammatory environment.

Herein we will focus on a number of pre-clinical studies in which MSC transplantation in rodents with EAE was employed in an effort to determine the mechanisms underlying the therapeutic efficacy of MSCs *in vivo*.

2.1.1. Systemic delivery of MSCs

The intravenous (i.v.) transplantation of syngeneic MSCs has led to beneficial effects in both RR (Marin-Banasco et al., 2014) and chronic EAE mouse models (Kurte et al., 2015). However, the beneficial effect of MSCs appears to be dependent on the time of delivery, especially in chronic EAE, with transplanted MSCs being most effective when administered either before disease onset, at disease onset or at the peak of disease (Kurte et al., 2015). The therapeutic effect of MSCs injected i.v. early in the course of disease appears to be related to their preferential accumulation within secondary lymphoid organs, rather than the CNS.

Since the activation of the adaptive immune system dominates the pathophysiology of EAE in its early phases, it is reasonable assume that MSCs may effectively induce an *in vivo* state of T cell unresponsiveness within the peripheral secondary lymphoid organs (Zappia et al., 2005). Specific immune actions occur - and as such may be interfered with - at the level of secondary lymphoid organs. In line with such findings evidence exists that i.v. injected MSCs suppress Th17 cells and increase the proportion of $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells in EAE (Luz-Crawford et al., 2013). While the effect of mouse MSCs on Th17 cells is now firmly established, some authors have come to suggest that this may in fact be true for other lymphocyte subtypes (*i.e.* Th1) (Glenn et al., 2015).

In the wake of the close dependence of B cell functions from T cell activation, it has also been proposed an influence of MSCs on B cell activation. To support this hypothesis, MSCs infusion in mice with proteolipid protein (PLP)-induced EAE lead to significant reduction of blood anti PLP139-151 antibodies, thereby suggesting that MSCs could block the pathogenic T and B cell response against PLP139-151. To further corroborate this data, MSCs have shown to alter the capacity of encephalitogenic lymph nodes cells (LNCs) to transfer full-blown EAE to healthy recipient mice. The transfer of encephalitogenic LNCs MSCs-treated has indeed demonstrated to produce a lower amount of PLP- specific IgG of all subclasses in transferred mice compared to healthy control (Gerdoni et al., 2007).

The i.v. delivery of MSCs also leads to beneficial effects within the CNS. These may be related to the antioxidant and neuroprotective activities exerted by MSCs (Lanza et al., 2009), as i.v. delivered MSCs also promote the regeneration of axons in the spinal cord of mice with myelin oligodendrocyte glycoprotein (MOG)-induced EAE (Mitra et al., 2015). As such it is tempting to speculate that part of these effects may be due to the release of neurotrophic growth factors by MSCs and/or to a bystander promotion of endogenous reparative responses (Constantin et al., 2009).

While *in vitro* studies have supported the idea that MSCs could transdifferentiate in cells of ectodermal lineage (Woodbury et al., 2000), the i.v. injection of MSCs in acute and chronic EAE mice has provided no evidence of transplanted MSCs expressing marker of neuroectodermal lineage within the CNS (Gerdoni et al., 2007, Zappia et al., 2005). Transplanted undifferentiated MSCs were found within the parenchyma (Gerdoni et al., 2007) and in the subarachnoid space of the spinal cord (Zappia et al., 2005), in close relationship with inflammatory infiltrates as early as one month upon injection, thus demonstrating a limited potential for direct cell replacement in the contest of a CNS exposed to chronic inflammation.

Pre-clinical studies have also investigated the intraperitoneal (i.p.) delivery of syngeneic MSCs in mouse models of EAE. When compared to i.v. delivered cells, the i.p. delivery of MSCs resulted in an increased number of splenic Treg cells and interleukin (IL)-4 levels, with a concordant decrease in the levels of both interferon (IFN)-γ and overall inflammatory cell infiltration in the brain (Yousefi et al., 2013). Other studies have confirmed the efficacy of i.p. delivered MSCs, showing that MSC-treated EAE mice display a significant reduction of $CD4^+$ T cell infiltration in the spinal cord, and a reduction in plasma levels of both IL-17 and tumor necrosis factor (TNF)-α (Rafei et al., 2009b).

Human MSCs have also been shown capable of ameliorating rodent EAE models, after either i.v. or i.p. cell delivery (Bai et al., 2009; Gordon et al., 2010; Guo et al., 2013). The i.v. injection of human MSCs induces functional recovery in both relapsing and chronic EAE in mice, ultimately reducing the extent of damage and increasing the number of endogenous oligodendrocyte lineage cells (Bai et al., 2009). Upon i.v. delivery, human MSCs infiltrate the CNS and preferentially accumulate at sites of myelin damage (Gordon et al., 2010). Host immune responses are also influenced by the i.v. delivery of human MSCs, with a reduction of T cell proliferation (Donders et al., 2015), IFN-γ producing Th1-like and IL-17 producing Th17-like inflammatory cells (Bai et al., 2009), and up-regulation of $CD1d^{high}CD5^+$ regulatory B cells (Guo et al., 2013). Interestingly, human MSCs are also intrinsically capable of fusing with endogenous Purkinje cells *in vitro*. This event increases in frequency upon the presence of TNF-α and/or IFN-γ (Kemp et al., 2011), and also occurs consistently *in vivo* in the EAE brain following the i.v. delivery of human MSCs, as suggested by the occurrence of heterokaryons, multi-nucleated cells containing genetic material from both the cellular graft and the host.

The i.p. delivery of human MSCs lead to relatively little CNS infiltration, yet the graft was still able to exert significant ameliorative and anti-inflammatory effects in EAE (Bravo et al., 2016; Gordon et al., 2008; Peron et al., 2012). Of note, some of these human MSC-dependent effects may be related to specific immune modulatory properties, which are lacking in rodent MSCs. For example, while tryptophan (trp) catabolism by indolamine-2,3-dioxygenase 1 (IDO1) activity contributes to the immunosuppressive phenotype of human MSCs, this does not seem to be the case for murine MSCs (Lanz et al., 2010). The systemic injection of both IDO1-proficient and phenotypically identical IDO1-deficient mouse MSCs both resulted in the amelioration of EAE (Lanz et al., 2010); and mouse MSCs were capable of inducing the expression of IDO1 in target $CD11c^+$ dendritic cells (DCs) (Matysiak et al., 2008). Similarly, a specific immune modulatory mechanism stimulated by i.v. injected human adipose tissue-derived MSCs may be related to their secretion of the human leukocyte antigen (HLA)-G, a non-classical HLA class I molecule, which has inhibitory effects in all immune cells (Shalaby et al., 2016). Human MSCs delivered i.p. have also shown direct effects on neurons via the recovery of the expression of glycogen synthase kinase (GSK)-3β (Tafreshi et al., 2014).

When envisioning the clinical translation of such cells, several additional factors must be considered. These include: (i) the source from which MSCs are derived (ii) their differentiation profile/potential and (iii) the vitality of the transplanted graft. Whether mouse MSCs are derived from healthy mice or from mice with EAE may also play an important role; yet human evidence exists to suggest that this may not be limiting (Mallam et al., 2010). The systemic injection of MSCs from EAE mice proved to be indistinguishable from effects induced by MSCs obtained from healthy syngeneic donors (Kassis et al., 2013). The i.p. delivery of adipose tissue-derived MSCs from EAE mice displayed no therapeutic effects (*vs* control MSCs) with regard to the progression of disease (Zhang et al., 2014). *In vitro* BM-derived MSCs from EAE mice, display higher rates of proliferation/apoptosis when compared to MSCs derived from healthy mice (Zacharaki et al., 2013). These findings are of particular interest as they highlight the role of the inflammatory microenvironments and suggest that deriving MSCs from active MS patients for autologous cell transplants might not be ideal. The importance of the inflammatory environment has also been shown in rodent experiments with allogeneic MSC transplants. Although allogeneic (Balb/c) MSC transplantation in C57Bl/6 MOG-induced EAE mice led to reduction of EAE scores (*vs* syngeneic MSC transplants), pre-treatment of allogeneic MSCs with IFN-γ increased the expression levels of major histocompatibility complex (MHC)-I and -II and completely inhibited their therapeutic effects (Rafei et al., 2009a).

The tissue from which MSCs are derived (*e.g.* bone marrow *vs* adipose tissue *vs* umbilical cord) may also have an effect in terms of migratory abilities and/or impact on the clinical and pathological disease outcomes of the stem cell graft (Payne et al., 2013). The MSC phenotype and pre-differentiation status is also important when considering its translational implications. While MSC exposed to neurogenic induction media (pre-neuralised) (Harris et al., 2012) may possess an enhanced potential for CNS integration and regeneration, evidence exists that efficacy in EAE is likely to be strictly dependent on the undifferentiated profile of MSCs that is coupled to secretion of immune modulatory factors (*i.e.* prostaglandin E2) (Matysiak et al., 2011). Finally, it still remains unclear as to whether the sustained vitality of the graft *in vivo* is indeed indispensable for the observed therapeutic post-MSC transplantation in animal models. As for murine MSCs, the source from which human MSCs are derived might play an important role in terms of the therapeutic potential of the graft. Mice with chronic EAE that were treated before disease onset with adipose tissue-derived human MSCs derived from young (<35 years) *vs* old (>60 years) donors displayed profoundly different outcomes. Human MSCs from old donors failed to ameliorate the neurodegeneration associated with chronic EAE, leading to increased CNS inflammation and demyelination *in vivo*, and increased splenocyte proliferation in co-cultures *in vitro* (Scruggs et al., 2013). Further work has shown that MSCs derived from human embryonic stem (hES) cells (hES-MSCs) significantly reduce clinical symptoms and prevent demyelination in EAE to significantly greater levels than that of human BM-derived MSCs. Such differences may also be related to the intrinsically greater ability of hES-MSCs to extravasate and enter the inflamed CNS

tissue (Wang et al., 2014). Such assets, when coupled to the fact that hES-MSCs may be expanded virtually indefinitely prior to transplantation, suggest that they may be ideally suited for large-scale clinical translation.

2.1.2. Local delivery of MSCs

Mouse MSCs have also shown preclinical efficacy in EAE models when delivered locally to the CNS, with the clinical course of chronic EAE having been ameliorated in treated animals following the intracerebroventricular (i.c.v.) delivery of MSCs (Kassis et al., 2008). The i.c.v. administration of MSCs induced a strong reduction in CNS inflammation in term of total number, size and cellularity of the lymphocytic infiltrates and presented a peculiar neuroprotective effect evidenced by a significant decrease in axonal loss (Kassis et al., 2008). Of note, similar axonal protection was not observed following i.v. MSC delivery (Kassis et al., 2008).

The i.c.v. transplantation of BM-derived MSCs has shown variable degrees of efficacy and adverse effects when performed in EAE mice displaying different disease severity. While effective in mild EAE phenotypes, i.c.v. delivery did not ameliorate the clinical deficits in mice with severe EAE scores (Grigoriadis et al., 2011). Moreover, local transplantation in severe EAE mice was associated with potentially adverse effects, including the formation of cellular masses, which were characterized by focal inflammation, demyelination, axonal loss and increased collagen-fibronectin deposition (Grigoriadis et al., 2011). Interestingly these masses were not observed post-MSC transplantation in mild EAEs, nor were they associated with the transplantation of NSCs (Grigoriadis et al., 2011). These findings are of considerable relevance when one considers clinical trials in MS as they have come to suggest that the i.c.v./intrathecal transplantation of MSCs may exhibit, under conditions of severe inflammation like that presented in mice with higher EAE score, a local pathology whose consequences remain to be elucidated (Grigoriadis et al., 2011).

In addition to this, the intravenous systemic injection of MSCs has shown comparable clinical and pathological amelioration to intratechal delivery in a study which compared both ways of cells administration (Morando et al., 2012), thus supporting the adoption of less invasive therapeutic approaches.

Human MSCs have also been shown to be efficacious in EAE, after i.c.v. transplantation. Human placental MSCs (PL-MSCs) transplanted i.c.v. in EAE mice decrease the disease severity and increase the animals' survival (Fisher-Shoval et al., 2012). PL-MSCs express anti-inflammatory mediators in response to pro-inflammatory cytokines (*e.g.* TNF-α, IL1-β) released by activated microglia and astrocyte in the CNS of EAE mice. Further reports have shown that following exposure to conditioned media from LPS-activated astrocytes, PL-MSCs secrete TNF-α-stimulated gene/protein 6 (TSG-6). Such results again suggest that human MSCs may influence activated microglia, astrocytes and/or inflammatory cells in EAE models (Fisher-Shoval et al., 2012; Getting et al., 2002; Milner et al., 2006).

Being that MSCs have an immune modulatory effect predominantly associated with the secretion of several bioactive molecules, one intriguing possibility for MS research/therapy would be the use of MSC-conditioned media (CM).

Interestingly, studies with CM from human MSCs have described a reduction in functional deficits and an increase in the number of oligodendrocytes and neurons in mice with chronic EAE (Bai et al., 2012). A recent study has described that a single i.v. injection of CM collected from MSCs derived from the stem cells of human exfoliated deciduous teeth (SHED) is capable of improving EAE scores, reducing demyelination/axonal injury, and promoting a switch in the microglia/macrophage phenotype from the M1-like (classically activated, pro-inflammatory) to the M2-like (alternatively activated, anti-inflammatory) state (Shimojima et al., 2016). Of note, treatment of EAE mice with the secreted ectodomain of sialic acid-binding Ig-like lectin-9, a major component of SHED-CM, recapitulated the effects of SHED-CM treatment, thereby demonstrating the possibility of deriving cell-free components that would mitigate some complications typically associated with cell-based therapeutics (Shimojima et al., 2016).

Hepatocyte growth factor (HGF) was identified as a critical factor in CM-MSC-stimulated recovery in EAE (Bai et al., 2012) with the administration of recombinant HGF at the peak of disease having been shown to promote both behavioral and pathological recovery and the functional blocking of cMet and the administration of anti-HGF antibodies having been shown to abolish MSC-CM mediated recovery (Bai et al., 2009; Sheth et al., 2008).

Finally, EAE mice treated with adipose tissue-derived MSC-CM or injected with MSCs themselves displayed a similar amelioration of clinical and pathological features. However, a greater reduction in lymphocytic proliferative responses and brain leukocyte infiltration was observed in CM-treated mice (Yousefi et al., 2016).

2.2. Neural stem/precursor cells (NSCs)

Neural stem/precursor cells (NSCs) are heterogeneous populations of self-renewing, multipotent cells present within both the developing and adult CNS that drive neurogenesis/gliogenesis (Bernstock et al., 2014; Martino and Pluchino, 2006). NSCs reside within specialized microenvironments located at level of the ganglionic eminence(s) within the embryo, and both the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) in the adult. NSCs obtained from either embryos or adults can be cultured/expanded *in vitro* if supplemented with multipotent growth factors (*i.e.* EGF and FGF) as free-floating spherical aggregates (*i.e.* neurospheres) and/or as adherent monolayers (provided the provision of an artificial matrix). Upon growth factor withdrawal, NSCs spontaneously differentiate into specific neuroectodermal lineages, ultimately becoming neurons, astrocytes and oligodendrocytes (Reynolds et al., 1992; Reynolds and Weiss, 1992).

The intrinsic ability of NSCs to differentiate into cells of the oligodendroglial lineage cultivated hope for their adoption as clinically relevant therapies in a number of demyelinating/dysmyelinating disorders. As such an extensive number of pre-clinical studies involving both rodent and non-human primate disease models have been employed to investigate the therapeutic potential of NSC transplantation in acute (Einstein et al., 2003), relapsing (Pluchino et al., 2009b, Pluchino et al., 2005) and chronic EAE (Einstein et al., 2006, Pluchino et al., 2003). It should be noted however that our current understanding of the prevailing mechanisms governing the beneficial effects displayed by transplanted NSCs revolves primarily around a number of graft-to-host interactions as opposed to the replacement of damaged tissue (Ben-Hur, 2008, Cossetti et al., 2012); the aforementioned has come to be known as *therapeutic plasticity* (Cossetti et al., 2012, Pluchino and Martino, 2008).

2.2.1. Systemic delivery of NSCs

The therapeutic value of NSC i.v. grafts in inflammatory neurodegenerative disorders has become readily apparent. Transplanted NSCs are able to home to the lesioned areas of the CNS where they establish atypical perivascular niches whose architecture is reminiscent of their native prototypical germinal niches (Martino and Pluchino, 2006, Pluchino et al., 2009a, Pluchino et al., 2009b, Pluchino et al., 2005). Such microenvironments allow undifferentiated stem cells to survive and in so doing provide neurotropic support, modulate inflammation, and/or migrate into the lesioned parenchyma and participate directly in cellular repair/replacement (Calabrese et al., 2007, Einstein et al., 2003, Pluchino et al., 2005). NSCs biological effects are exerted through a regulated cross talk with other components of the atypical niche, including endothelial cells, blood-born inflammatory cells, activated macrophages and microglia, and/or reactive astrocytes (Bacigaluppi et al., 2009, Cossetti et al., 2012, Cusimano et al., 2012, Pluchino and Cossetti, 2013, Sun et al., 2010). Within the inflamed CNS, bone morphogenetic protein (BMP)-4 and the BMP-4 antagonist Noggin promote the maintenance of an

undifferentiated phenotype in grafted NSCs (Pluchino et al., 2005). Moreover, the activation of microglia, astrocytes and/or endothelial cells within the injured CNS (Martino and Pluchino, 2006, Muller et al., 2006) produce chemokines such as stromal cell-derived factor (SDF)-1α/CXCL12, monocyte chemotactic protein (MCP)-1/CCL2, and vascular endothelial growth factor (VEGF) that collectively function as a homing beacons, not only for inflammatory cells, but also for implanted NSCs (Doetsch et al., 2002, Maslov et al., 2004, Mi et al., 2005, Muller et al., 2006, Pluchino and Cossetti, 2013, Soares and Sotelo, 2004). It should be noted that NSCs express adhesion molecules (*e.g.* [CD44], integrins [α4 β1]) and chemokine receptors (*e.g.* CCR1, CCR2, CCR5, CXCR3, CXCR4), thereby enabling them to follow concentration gradients of these chemokines and extravasate into the CNS via tethering, rolling and adhering to endothelial cells followed by trans-endothelial migration (Andres et al., 2011, Darsalia et al., 2007, Mueller et al., 2006, Pluchino et al., 2003, Pluchino et al., 2005). Factors such as basic fibroblast growth factor (bFGF) and insulin-like growth factor (IGF)-1 are also produced by activated astrocytes and support NSC proliferation, survival and/or differentiation (Lie et al., 2004, Muller et al., 2006, Soares and Sotelo, 2004). Conversely, hypertrophic glial fibrillary acidic protein (GFAP)-enriched astrocytes of the glial scar produce factors such as slit homologue (SLIT)-2, TNF-α and hyaluronan that repel NSCs and limit the regenerative potential of their progeny (Benedetti et al., 2000, Emsley et al., 2005, Muller et al., 2006, Soares and Sotelo, 2004).

Although glial cell replacement was initially thought to underlie the therapeutic effects exerted by NSC grafts, the remarkable functional recovery observed upon transplantation in different experimental models of CNS injury have shown minimal correlation with the number of grafted cells that ultimately undergo terminal differentiation (Ben-Hur, 2008, Cao et al., 2002, Cusimano et al., 2012). However NSCs can contribute to some cell replacement, mainly by differentiating into astrocytes (Cusimano et al., 2012), or progressing towards oligodendrocyte progenitor cells (Ben-Hur et al., 2003). Despite such potential it is the bystander effects of undifferentiated NSCs (*e.g.* the provision of trophic support and modulation of the immune response) that ultimately predominate (Martino and Pluchino, 2006). In EAE (murine and non-human primate) models of MS such bystander effects have been shown to modulate mediate myelin repair and axonal rescue (Einstein et al., 2009, Pluchino et al., 2003). These trophic and neuroprotective effects are exerted via the provision of neurotrophins, growth factors, developmental stem cell regulators, and immune modulators (all of which serve to modulate the microenvironment) (Cossetti et al., 2012, Martino and Pluchino, 2006, Pluchino and Cossetti, 2013). Interestingly, NSCs injected i.v. in EAE animal models stimulate the proliferation and differentiation of endogenous oligodendrocyte progenitor cells (OPCs), and consequently upregulate remyelination through the secretion of platelet-derived growth factor (PDGF)-α and bFGF (Einstein et al., 2009, Pluchino et al., 2003). As such NSCs culturing protocols which call for elevated amounts of epidermal growth factor (EGF) and fibroblast growth factor (FGF)-2) may serve to select growth factor-responsive cells that if transplanted into the inflamed CNS are more responsive to environmental signals and therefore release more neurotrophins and growth factors (Martino and Pluchino, 2006).

Transplantation of NSCs facilitates the switch to a more anti-inflammatory lesional environment (Martino et al., 2011, Pluchino and Cossetti, 2013). *In vitro* studies have shown that NSC 1) induce apoptosis of Th1 and Th17, but not Th2 lymphocytes through Fas ligand (FasL), TRAIL and Apo-3 ligand (APO3L) (Pluchino et al., 2005); 2) reduce T cell proliferation through nitric oxide and prostaglandin E (PGE)-2 (Wang et al., 2009), 3) reduce T cell receptor (TCR) dependent activation (Pluchino et al., 2009b); 4) inhibit IL-2 (T cell) and IL-6 (B cell) signaling (Fainstein et al., 2008, Kim et al., 2009); and 5) reduce local populations of monocytes and macrophages through cytotoxic TNF-α secretion (Ricci-Vitiani et al., 2007). Furthermore, NSC-induced increases in activated $CD11b^+$ microglia have been shown to lead to IGF-1, VEGF, transforming growth factor (TGF)-β, and brain-derived neurotrophic factor (BDNF) production, yielding better motor function and axonal sprouting; such findings again speak to the dynamic/context dependent nature of inflammation/inflammatory cells (Capone et al., 2007, Daadi et al., 2010, Lalancette-Hebert et al., 2007).

Beyond the CNS systemically delivered NSCs can also be found within secondary lymphoid organs where they serve to modulate inflammation (Einstein et al., 2007, Lee et al., 2008b, Pluchino et al., 2009a, Pluchino et al., 2009b). In lymph nodes, the increased bioavailability of BMP-4, BMP-7, sonic hedgehog (Shh) and Noggin, released by both transplanted NSCs and immune cells, promotes the survival and persistence of grafted NSCs outside the CNS, at the levels of ectopic perivascular germinal niche-like lymph node areas (Pluchino et al., 2009b). Therein, NSCs hinder the activation of myeloid DCs, limiting the expansion of antigen-specific encephalogenic T-cells (Pluchino et al., 2009b; Pluchino et al., 2005). Moreover, the presence of BMP-4 has also shown to contribute in delaying DC maturation (Pluchino et al., 2009b).

In an attempt to translate these immune modulatory proprieties the therapeutic potential of both human somatic (Pluchino et al., 2009a) and human ES-cell-derived NPCs (Aharonowiz et al., 2008, Pluchino et al., 2009a) has been investigated following the transplantation in non-human primates and mice with EAE, respectively. These studies have shown that human-derived cell functions are largely similar to those of animal-derived cells, thereby capable of promoting functional and pathological recovery. Nonetheless, the therapeutic use of these cells is limited by ethical constraints, genetic instability, and potential tumorigenicity (Tsuji et al., 2011, Yoo et al., 2013).

2.2.2. Local delivery of NSCs

Upon i.c.v. injection, NSC typically attenuate brain inflammation, reduce demyelination and axonal pathology and ultimately improve clinical endpoints in models of both acute and chronic EAE (Cossetti et al., 2012). Transplanted NSCs drive the reduction of perivascular infiltrates and $CD3^+$ T cells and the increase of $CD25^+$ or $CD25^+/CD62L^+$ Treg cells, accompanied by a down-regulation of inflammatory markers intercellular adhesion molecule 1 (ICAM-1), and lymphocyte function-associated antigen 1 (LFA-1) (Einstein et al., 2003, Pluchino et al., 2005). Transplanted NSCs also act via the inhibition of IL-2 and IL-6 signaling in T lymphocytes affecting both their activation and proliferation (Fainstein et al., 2008).

Immune regulation rather than neural differentiation has again been suggested as the putative mechanism by which i.c.v. injection of human NSCs (hNSCs) ameliorate EAE in non-human primates (Aharonowiz et al., 2008, Pluchino et al., 2009a). Non-human primates with EAE transplanted with hNSCs accumulated lower disability and displayed increased survival, as compared with sham-treated controls. In contrast to their mouse NSC counterparts, hNSCs display a limited cytotoxicity towards T cells *in vitro*. However, in inflammatory environments such as EAE, they exhibit a higher cytotoxic potential against mononuclear phagocytes (MPs) perhaps due to the elevated expression of TNF-α, TNF-like protein (TL) 1A and TRAIL (Ricci-Vitiani et al., 2007). Such effects on MPs support the potential adoption of hNSCs as a treatment for progressive MS, which shows CNS inflammatory infiltrates principally characterized by macrophages and microglia (Mallucci et al., 2015). Despite such promise, hNSCs require greater manipulation *in vitro* as compared to the rodent NSCs (Zhang et al., 2001). These features when coupled to the presence of human leukocyte antigens on their surface, have significantly limited the clinical translation of somatic hNSCs (Al Nimer et al., 2004, Odeberg et al., 2005).

The ability of NSCs to influence their microenvironment via the secretion of soluble factors has become further evident as NSC-CM has been shown capable of modulating microglial activation, proliferation, and phagocytosis *in vitro* (Mosher et al., 2012). Importantly, intrastriatal injection of NSC-CM induced a similar increase in the absolute number, activation and proliferation of microglia, which was comparable the effects obtained via the stereotaxic injection of NSCs (Mosher et al., 2012).

Transplanted mouse NSCs have demonstrated an ability to reduce the proportion of classically-activated (M1-like) macrophages, while promoting the healing of the injured CNS (Cusimano et al., 2012). In the case of NSC-CM injection then, VEGF was recognized as one of the principal mediators involved in the regulation of microglia, as the knock down of VEGF appeared to attenuate the effects of NSC-CM on microglia (Mosher et al., 2012). Of note NSC-CM has also shown to have striking effects on delayed neurodegeneration when injected i.v. (Doeppner et al., 2016). These results were again attributed to the high concentrations of VEGF within the CM, which reduces inflammation, whilst concurrently promoting angiogenesis (Doeppner et al., 2016).

2.3. Induced pluripotent stem cells (iPSC)

In 2006 Shinya Yamanaka and colleagues published a seminal study in which they described how mouse embryonic and adult fibroblasts were able to acquire properties similar to ES cells if transduced with four transcription factors (*i.e.* Oct3/4, Sox2, Klf4, and c-Myc) (Takahashi and Yamanaka, 2006). This first generation of iPSCs were similar to ES cells in morphology, proliferation and teratoma formation yet these iPSCs possessed a unique global gene expression pattern when compared to endogenous ES cells and as such failed to produce adult chimeric mice (Takahashi and Yamanaka, 2006). In 2007, germline transmission was achieved with mouse iPSCs (Okita et al., 2007), and soon afterwards iPS cells were being efficiently generated from human fibroblasts (Takahashi et al., 2007). Today, iPSC technology holds tremendous promise/potential for regenerative medicine and as such research groups have dedicated considerable time and effort to the efficient development of both mouse and human NSCs via the differentiation of somatic-derived iPSCs with the overarching aim of treating intractable neurological illness such as chronic/progressive MS.

In chronic EAE, mouse iPSC-derived NSCs (iPSC-NSCs) transplanted i.c.v. at disease onset induce stable amelioration of the disease. In this case, the neuroprotective effects are not dependent upon the differentiation of transplanted cells in functional oligodendrocytes, but instead are mainly mediated by the secretion of leukemia inhibiting factor (LIF) (Laterza et al., 2013). In turn, LIF secretion promotes endogenous OPC survival and functionality, and this is necessary to limit the BBB leakage and autoreactive lymphocytes extravasation, thereby reducing the inflammatory process (Laterza et al., 2013). When iPSC-NSCs are transplanted into the inflamed CNS, the majority of the environmental cues are pro-inflammatory signals that tend to repress the differentiation process while promoting bystander (*paracrine*) actions. In a similar study, Zhang and colleagues showed that murine iPSC-NSCs when transplanted i.c.v. in chronic EAE mice presenting severe hind limb weakness (usually 18 days post immunization) result in reduction T cell infiltration and amelioration white matter damage (Zhang et al., 2016).These effects were coupled by an improvement of EAE score, which was however less pronounced than that observed in mice being i.c.v. injected with syngeneic NSCs.

A schematic comparison of the main features and properties of MSC, NSC and iPSC therapeutics for EAE/MS is provided in Table 1.

3. Stem cell clinical trials in multiple sclerosis (MS)

The advent of DMTs has transformed treatment paradigms for patients in the early stages of MS. However, once inflammation becomes compartmentalized within the CNS, DMTs are no longer clinically effective. Accordingly, DMTs have questionable effects on the progressive accumulation of disabilities witnessed throughout the disease course in chronic/progressive MS patients (Hauser et al., 2013). While several novel DMTs have received regulatory approval for RRMS, they have had negligible effects on the persistent inflammation and the subsequent CNS degeneration that are the hallmarks of the progressive forms of the disease (Fox and Rhoades, 2012). Furthermore, early attempts to approach progressive MS via the application of putative neuroprotective therapies have failed (Kapoor et al., 2010). Given the clinical burden of progressive MS and the lack of treatment options, there is an urgent need for clinical trials utilizing novel therapeutics capable of (i) reducing inflammation once it has been compartmentalized within the CNS, (ii) providing trophic support to the brain, and (iii) increasing the plasticity and intrinsic repair capabilities of the CNS.

The transplantation of nHSCs fulfills all of these criteria and therefore represent both a promising and novel therapeutic approach for progressive MS patients. Of note, recent clinical investigations that have employed stem cell based products in regenerative neurology have been centered on addressing a wide spectrum of pathological conditions (*e.g.* Parkinson's disease, amyotrophic lateral sclerosis (ALS), spinal cord injury (SCI), stroke, MS) and as such have utilized a variety of stem cell types (Trounson and McDonald, 2015).

3.1. Current MS clinical trials - Search stratagem and criteria for inclusion

Our search strategy sought to identify all ongoing clinical trials utilizing stem cells in MS globally. As such, we have outlined a general search strategy along with individual key words and operators for each database utilized. Our analysis includes the results from searches of clincaltrials.gov (queried on 15/06/16) and the WHO's International Clinical Trials Registry Platform (ICTRP) (queried on 16/6/16). Search

Table 1
Summary of main features and properties of MSC, NSC and iPSC therapeutics for EAE/MS.

Features and properties	MSCs	NSCs	iPSC-NSC derivatives
Source(s)	Bone marrow, adipose tissue, dental pulp, birth associated tissues (*e.g.* decidua, umbilical cord)	Fetal brain and spinal cord	Somatic tissues (skin, hair follicles, peripheral blood monocytes)
Ethical concerns	None	Some	None
Immunogenicity	±	+	±
Graft options	Autologous and Allogeneic	Allogeneic	Autologous and Allogeneic
Immune suppression	N/Y	Y	N/Y
Stability *in vitro* (over passaging)	Limited	Good	Remarkable
Safety *in vivo* (systemic injection)	Good	Some	Not tested
Safety *in vivo* (CNS graft)	Some	Good	Good
Differentiation into neural cells *in vitro*	Some	Good	Good
Differentiation into neural cells *in vivo* (systemic injection)	Scarce	Some	Not tested
Differentiation into neural cells *in vivo* (CNS graft)	None	Some	Good
Immune modulation (adaptive)	Good	Good	Not tested
Immune modulation (innate)	Some	Some	Not tested

terms employed on clinicaltrails.gov were as follows: ("mesenchymal stem cell" OR "neural stem cell" OR "induced pluripotent stem cell" OR "induced neuronal stem cell") AND ("multiple sclerosis") yielding 19 trials. Advanced search terms employed on ICTRP were "Condition = sclerosis" "Intervention = stem", yielding 136 trials of which 16 involving progressive MS and non-hematopoietic cells sources.

3.1.1. Clinical trials with MSCs

MSCs have been at the forefront of reparative/regenerative medicine for several diseases, including MS (Mallucci et al., 2015). The immune modulatory, tissue protective and repair-promoting properties of MSCs demonstrated both *in vitro* and in relevant preclinical animal models make them an attractive potential therapy for MS and other conditions characterized by inflammation and/or tissue injury (Cohen, 2013).

Accordingly, in 2009 an international panel comprised of clinicians with expertise in the treatment of MS and basic scientists with know-how in stem cells/immunology formed a consortium entitled, the "International MSCT Study Group"; the aims were to derive a consensus on the utilization of MSCs for the treatment of MS, along with protocols for the culture of the cells and the treatment of patients. The consensus recognized that current evidence did support the i.v. administration of autologous MSCs as inhibitors of the autoimmune response in patients who continued to show inflammatory activity despite attempts to treat with immune modulatory agents (Freedman et al., 2010). Early clinical trials in MS that employed MSCs demonstrated that such procedures were not only clinically feasible and relatively safe but also emphasized an immediate induction of immune modulatory effects in patients (Cohen et al., 2010, Dulamea, 2015, Freedman et al., 2010, Yamout et al., 2010). Despite this it is prudent to acknowledge that the vast majority of these trials consisted of what were essentially unblinded clinical pilots and included very small cohorts of patients and should be considered anecdotal reports. As such results garnered regarding MSC therapy in MS should be carefully weighed.

Karussis et al. conducted a study in which 15 patients with MS (mean Expanded Disability Status Scale [EDSS] score, 6.7) and 19 with ALS (mean Amyotrophic Lateral Sclerosis Functional Rating Scale [ALSFRS] score, 20.8 [8.0]) were enrolled (Karussis et al., 2010). The mean ALSFRS score was reported as stable during the first 6 months of observation, whereas the mean EDSS score appeared to have slightly improved (Karussis et al., 2010). In line with preclinical animal studies, *ex vivo* immunological analyses revealed an increase in the proportion of $CD4^+CD25^+$ Treg cells, a decrease in the proliferative responses of lymphocytes, and the expression of $CD40^+$, $CD83^+$, $CD86^+$, and HLA-DR on myeloid DCs as early as 24 h after MSC transplantation (Karussis et al., 2010). No major adverse effects were reported during follow-up, thereby demonstrating that the transplantation of MSCs in both MS and ALS patients was feasible and relatively safe.

A recent randomized, double blind, placebo-controlled, crossover phase II study went on to investigate the safety/efficacy of MSCs RRMS patients unresponsive to conventional therapies. Efficacy was evaluated in terms of the cumulative number of gadolinium-enhancing lesions on MRI at 6 months and at the end of the study (*i.e.* 1 year) (Llufriu et al., 2014). Patients were randomized to receive IV $1–2 \times 10^6$ bone-marrow-derived-MSCs/kg body weight or placebo. After 6 months, the treatment was reversed and patients were followed for another 6 months. No serious adverse events were reported after treatment (Llufriu et al., 2014). At both 6 months and 1 year patients treated with MSCs trended toward lower mean cumulative numbers with regard to defined clinical endpoints (*e.g.* gadolinium-enhancing lesions), yet statistical significance was not achieved (Llufriu et al., 2014).

Further studies have examined the therapeutic utility of MSCs within cohorts of patients who suffer from progressive forms of MS. Connick and colleagues isolated, expanded, characterized, and administered MSCs in 10 patients with secondary progressive MS that involved the visual pathways (EDSS of 5.5–6.5) (Connick et al., 2012). The single autologous i.v. mean dose of MSC was ~1.6×10^6 cells/kg body weight (Connick et al., 2012). The autologous MSCs transplants were shown to induce an improvement in visual endpoints (*i.e.* visual evoked response latency). The neuroprotective effects of the MSCs again seemed to be independent of directed differentiation and/or cell replacement, but rather relied primarily on mechanisms related to immune modulation (Connick et al., 2012).

A second study looked at patients with progressive MS (EDSS 4.0–6.50) who were unresponsive to conventional treatments (Bonab et al., 2012). 25 patients were ultimately recruited and followed for 1 year after receiving a single intrathecal injection of *ex vivo* expanded MSCs (mean dose of ~2.95×10^7 cells). Again, no major adverse effects were reported post-infusion (Bonab et al., 2012). The clinical course of the disease, as measured via EDSS improved in 4/25 patients, deteriorated in 6/25 patients and remained consistent in 12/25 patients, whilst MRI evaluation revealed that 15/25 patients remained stable whereas 6/25 patients displayed new T2 and/or gadolinium enhanced lesions (Bonab et al., 2012). While promising, the authors themselves went on to acknowledge the need for an expansion of the study, booster injections and longer time of clinical follow-up (Bonab et al., 2012).

A recent open-label, phase I clinical trial designed to evaluate the safety, tolerability and preliminary efficacy of the intrathecal administration of autologous MSCs-derived NSCs has confirmed that such a treatment is safe with no major adverse outcomes having been reported (Harris et al., 2015). Critically, these MSCs-derived NSCs, have been shown to display a reduction in the expression of mesodermal markers and reduced capacity for adipogenic or osteogenic differentiation thereby retaining many of clinically relevant features of both MSCs and NSCs (Harris et al., 2012). In those patients that remain these cells are scheduled to be delivered in 3 doses (up to 10 million cells per injection), spaced 3 months apart after the pre-injection quality testing of the autologous MSC-NSCs that have been expanded from bone marrow (Harris et al., 2015, Harris et al., 2012). Secondary outcomes have been designed to observe trends in the clinically efficacy via neurological exams, MRIs, evoked potentials, and urodynamics testing. Result are pending (Harris et al., 2015).

A recent systematic review and meta-analysis of the clinical trials that made used i.v. delivery of MSCs in adult populations or mixed adult/pediatric populations included 36 studies with a total of >1000 participants with a numerous clinical conditions (*e.g.* ischemic stroke, Crohn's disease, cardiomyopathy, myocardial infarction, graft versus host disease [GVHD], and healthy volunteers) did not detect an association between acute infusion toxicity, organ system complications, infection, death or malignancy, yet did detect a significant association between MSCs and transient fever (Lalu et al., 2012). As such, while important advances have made with regard to the use of MSCs in MS, substantial challenges remain and include the nature, identity, function, mode of isolation and/or experimental handling of MSCs (Bianco et al., 2013). Allogeneic MSCs are both well tolerated and clinically effective in treating GVHD (Le Blanc et al., 2008) and autologous MSCs from MS individuals share almost identical functional properties in terms of their proliferation, phenotype/differentiation *in vivo* and immunosuppressive ability with those isolated from healthy individuals (Mallam et al., 2010, Mazzanti et al., 2008).

Further and structured study work will be required to identify the optimal dose(s), route(s) of delivery and/or duration of any observed therapeutic effects induced by the application of MSC-based therapies in MS (Cohen, 2013) (Table 2).

3.1.2. Clinical trials with NSCs

NSCs have been shown to exert remarkable trophic effects on endogenous brain cells and a litany of modulatory actions on both innate and adaptive immune responses in mouse (Pluchino et al., 2005) and non-human primates models of MS (Martino et al., 2011, Pluchino and

Table 2
Clinical trials with nHSCs in MS.

Sponsor/place	Form of MS	Trial phase	Patients (no)	Age (years)	Follow up (months)	Cell type	Cell no./patients (cells/kg)	Route	Immunosuppression	Disease duration (years)	Status	Trial number
American CryoStem Corporation (Cayman Islands)	Unspecified MS	I	100	18–65	60	Autologous adipose derived MSCs	–	Multiple intravenous infusion	No	>5	Recruiting	NCT02326935
Tisch Multiple Sclerosis Research Center of New York (US)	SPMS, PPMS	I	20	18–70	30	Neural Progenitor-derived MSCs	2-10 × 10^6	Multiple intratecal infusion	No	–	Active, not recruiting	NCT01933802
The Cleveland Clinic (US)	RRMS, SPMS, PPMS	I	24	18–55	6	Bone-marrow-derived mesenchimal stem cells (BM-MSCs)	2 × 10^6	Single intravenous infusion	No	–	Completed	NCT00813969
Royan Institute (Iran)	RRMS	I/II	22	18–55	12	BM-MSCs	–	Single intravenous infusion	No	2–10	Completed	NCT01377870
University of Cambridge, Medical Research Council (UK)	Unspecified MS	I/II	10	18–65	12	BM-MSCs	2 × 10^6	Single intravenous infusion	No	–	Completed (Connick et al., 2012)	NCT00395200
Karolinska Institutet (Sweden)	RRMS, SPMS, PPMS	I/II	15	18–50	4	BM-MSCs	–	Single intravenous infusion	No	2–10	Recruiting	NCT01730547
Antonio Uccelli (Italy)	RRMS, SPMS, PPMS	I/II	20	18–50	4	BM-MSCs	1-2 × 10^6	Single intravenous infusion	No	2–10	–	NCT01854957
University of Jordan (Jordan)	Unspecified MS	I/II	13	18–65	18	Primed BM-MSCs	–	intratecal infusion	No	–	Completed	NCT01895439
Shenzhen Beike Bio-Technology Co., Ltd. (China)	Progressive MS and NMO	I/II	20	18–60	12	Umbilical-cord-derived MSCs	–	Presumed intravenous	No	>2	–	NCT01364246
University Hospital, Toulouse (France)	RRMS, SPMS, PPMS	I/II	12	18–50	12	BM-MSCs	1-2 × 10^6	Multiple intravenous infusion	No	2–10	Recruiting	NCT02403947
Fundación Pública Andaluza Progreso y Salud (Spain)	SPMS	I/II	30	>18	12	Autologous adipose derived MSCs	1-4 × 10^6	Single intravenous infusion	No	–	Completed	NCT01056471
Germans Trias i Pujol Hospital (Spain)	RRMS, SPMS, PPMS	I/II	8	18–50	12	BM-MSCs	–	Single intravenous infusion	No	2–10	Active, not recruiting	NCT02035514
Translational Biosciences (Panama)	Unspecified MS	I/II	20	18–55	12	Umbilical-cord-derived MSCs	–	Multiple intravenous infusion	No	–	Completed	NCT02034188
Imperial College London (UK)	RRMS, SPMS, PPMS	I/II	19	18–50	36	BM-MSCs	1-2 × 10^6	Single intravenous infusion	No	–	Completed	NCT01606215
Fundación Pública Andaluza Progreso y Salud (Spain)	RRMS, SPMS	I/II	30	18–50	12	BM-MSCs	1-2 × 10^6	Multiple intravenous infusion	No	–	Recruiting	NCT01745783
Novo Cellular Medicine Institute LLP (Trinidad and Tobago)	RRMS	I/II	69	18–60	12	Umbilical-cord-derived MSCs	Total of ~100 × 10^6 (intratecal) and 50 × 10^6 (intravenous)	intravenous and intratecal infusion	No	>5	Recruiting	NCT02587715
Hadassah Medical Organization (Israel)	Unspecified MS	I/II	20	35–65	12	BM-MSCs	Total of ~60 × 10^6 (intratecal) and 20 × 10^6	intravenous and intratecal	No	>5	Completed (Karussis et al., 2010)	NCT00781872

(continued on next page)

Table 2 (*continued*)

Sponsor/place	Form of MS	Trial phase	Patients (no)	Age (years)	Follow up (months)	Cell type	Cell no./patients (cells/kg)	Route	Immunosuppression	Disease duration (years)	Status	Trial number
							(intravenous)	infusion				
Banc de Sang i Teixits (Spain)	RRMS, SPMS	I/II	8	18–60	36	BM-MSCs	–	Single intravenous infusion	No	–	Active, not recruiting	NCT02495766
Albert Saiz (Spain)	RRMS, SPMS, PPMS	II	9	18–50	12	BM-MSCs	1-2 × 10^6	Multiple intravenous infusion	No	2–10	Completed (Llufriu et al., 2014)	NCT01228266
Ottawa Hospital Research Institute (Canada)	RRMS, SPMS, PPMS	II	40	18–50	12	BM-MSCs	1-2 × 10^6	Multiple intravenous infusion	No	2–10	Recruiting	NCT02239393
Dimitrios Karussis (Israel)	Active and Progressive MS	II	36	25–65	12	BM-MSCs	–	intravenous and intratecal infusion	No	>3	Recruiting	NCT02166021
Danish Multiple Sclerosis Society (Denmark)	RRMS, SPMS	II	25	18–50	12	MSCs	–	intravenous infusion	No	2–10	On-going	EUCTR2012-000518-13-DK
SALK - Gemeinnützige Salzburger Landeskliniken Betriebsges. m.b.H. (Austria)	RRMS, SPMS, PPMS	II	5	18–50	12	MSCs	2 × 10^6	intravenous infusion	No	2–10	On-going	EUCTR2015-000137-78-AT
Australian Custom Pharmaceuticals Pty Ltd., Adult Stem Cell Foundation (Australia)	Unspecified MS	I/II	45	18–70	12	Adipose Derived Stem Cells (stromal vascular fraction, SVF)	10^6	intravenous infusion	No	–	Not yet recruiting	ACTRN12615000687594
Military Institute of Medicine, Central Clinical Hospital of the Ministry of National Defence in Warsaw (Poland)	RRMS, SPMS	I/II	20	27–58	18	Adipose Derived Stem Cells (stromal vascular fraction, SVF)	Total of 36 × 10^6	Multiple intratecal infusion	No	9,5 (RRMS),15,6 (SPMS)	Completed (Stepien et al., 2016)	–
Healeon Medical Inc. (US)	Unspecified MS	I/II	500	18–85	60	Adipose Derived Stem Cells (stromal vascular fraction, SVF)	–	Intravenous infusion	No	>6 months	Recruiting	NCT02939859
Ageless Regenerative Institute (US)	Unspecified MS	I/II	50	18–80	6	Adipose Derived Stem Cells (stromal vascular fraction, SVF)	–	intravenous infusion	No	>5	Recruiting	NCT01453764

Abbreviations: MSCs: mesenchymal stem cells; NMO: neuromyelitis optica; PP: primary progressive; RR: relapsing remitting; SP, secondary progressive.

Table 3
Clinical trials with nHSCs in other neurological diseases.

Sponsor/place	Disease	Trial phase	Patients (no)	Age (years)	Follow up (months)	Cell type	Cell no./patients (cells/kg)	Route	Immunosuppression	Disease duration (years)	Status	Trial number
Italian Ministry of Health (Italy)	ALS	I	10	20–60	24	Autologous BM-derived MSCs	11.4×10^6 to 120×10^6	Multiple intra-medullar injections	No	<3	Completed (Mazzini et al., 2010)	NCT01777646
Mayo Clinic (US)	ALS	I	27	>18	24	Autologous MSCs	5 treatment groups (from 10^7 to 10^8)	Single/multiple intratecal infusion	No	–	Active, not recruiting	NCT01609283
University of Warmia and Mazury (Poland)	ALS	I	30	18–65	18	Autologous BM-derived MSCs	–	Single/multiple intratecal infusion	No	>2	Enrolling by invitation	NCT02881489
University of Warmia and Mazury (Poland)	ALS	I	30	18–65	18	Allogeneic Wharton's jelly-derived MSCs	–	Single/multiple intratecal infusion	No	>2	Enrolling by invitation	NCT02881476
University of Sao Paulo General Hospital (Brazil)	ALS	I/II	28	18–70	10	Autologous BM-derived MSCs	–	Multiple intratecal infusion	No	–	Recruiting	NCT02917681
Fundación Pública Andaluza Progreso y Salud (Spain)	ALS	I/II	40	>18	6	Autologous Adipose Derived MSCs	4 treatment groups (from 1×10^6 to 4×10^6)	Intravenous infusion	No	6 months to 3 years	Recruiting	NCT02290886
Hadassah Medical Organization, Brainstorm Cell Therapeutics (Israel)	ALS	I/II	12	20–75	12	Autologous BM-derived MSCs	Total of ~60×10^6 (intratecal) and 24×10^6 (intravenous)	Multiple intramuscular or single intratecal infusion	No	<2	Completed (Petrou et al., 2016)	NCT01051882
Neuralstem Inc. (US)	ALS	I	18	>18	48	Allogenic fetal spinal cord-derived NSCs (NSI-566RSC)	0.5-1 $\times 10^6$	Multiple intraspinal injections	4 months	1.5	Active, not recruiting (Glass et al., 2012, Riley et al., 2012, Robberecht and Philips, 2013)	NCT01348451
Azienda Ospedaliera Santa Maria, Terni (Italy)	ALS	I	18	20–75	36	Allogenic fetal brain-derived NSCs	7.5×10^5	Multiple intraspinal injections	6 months	>6 months	Completed (Gelati et al., 2013, Mazzini et al., 2015, Robberecht and Philips, 2013)	NCT01640067
Shenzhen Hornetcorn Bio-technology Company, LTD (China)	Cerebral Hemorrhage	I	20	40–80	12	umbilical cord derived MSCs	2×10^7 (every week for four times)	Multiple intravenous infusion	–	>3 months	Active, not recruiting	NCT02283879
Instituto de Investigación Hospital Universitario La Paz (Spain)	Acute Ischemic Stroke	II	20	60–80	24	Allogenic adipose tissue derived MSCs	10^6	Single intravenous infusion	No	<12 h	Completed (Diez-Tejedor et al., 2014)	NCT01678534
University Hospital, Grenoble, European Commission H2020 program (France)	Acute Ischemic Stroke	II/III	400	>18	6	Adipose Derived MSCs	10^6	Single intravenous infusion	–	1–4 days	Recruiting	NCT02849613
Stemedica Cell Technologies, Inc. (US)	Chronic Ischemic Stroke	I/II	38	>18	12	Allogeneic adult BM derived MSCs	0.5–1.5 $\times 10^6$	Single intravenous infusion	–	>0.5	Active, not recruiting	NCT01297413
Sapporo Medical University (Japan)	Ischemic Stroke	I/II	12	20–75	12	Autologous BM derived MSCs	0.6 to 1.6 $\times 10^8$	Intravenous infusion	No	~1–3 months	Completed (Honmou et al., 2011)	–
Southern Medical University, Cellonis Biotechnology Co. Ltd. (China)	Ischemic Stroke	I/II	20	18–80	12	Autologous BM derived MSCs or Autologous endothelial progenitor cells (EPCs)	2.5×10^6	Multiple intravenous infusion	No	<7 days	Recruiting (Bang et al., 2005, Lee et al., 2010)	NCT01468064
Samsung Medical Center, Pharmicell	Ischemic Stroke	III	60	30–75	3	Autologous BM derived MSCs	1×10^6 to 1.2×10^8	Intravenous infusion	No	>3 months	Completed (Kim et al., 2013)	NCT01716481

(continued on next page)

Table 3 (*continued*)

Sponsor/place	Disease	Trial phase	Patients (no)	Age (years)	Follow up (months)	Cell type	Cell no./patients (cells/kg)	Route	Immunosuppression	Disease duration (years)	Status	Trial number
Co., Ltd. (Republic of Korea)												
ReNeuron Limited (UK)	Stable Ischemic Stroke	I	12	>60	24	Allogenic fetal-derived, c-myc immortalized NSCs (CTX0E303)	2-20 × 10^6	Single intra-cerebral injection	–	0.5–5	Active, not recruiting	NCT01151124
StemCells, Inc. (US)	Pelizaeus-Merzbacher Disease (PMD)	I	4 (4)	0.5–5	12 (48)	Allogeneic fetal brain-derived human Central Nervous System (CNS) Stem Cells (HuCNS-SC®)	3 × 10^8	Multiple intra-cerebral injections	9 months	–	Completed (Gupta et al., 2012) (Terminated)	NCT01005004 (NCT01391637)
StemCells, Inc. (US)	Neuronal Ceroid Lipofuscinosis (NCL)	I	6	1.5–12	13	Allogeneic fetal brain-derived HuCNS-SC®	0.5-1 × 10^9	Multiple intra-cerebral injections	12 months	–	Completed (Selden et al., 2013)	NCT00337636
Retina Associates of South Florida, MD Stem Cells (US)	Retinal Disease, Aged Macular Degeneration (AMD), Hereditary Retinal Dystrophy, Optic Nerve Disease, Glaucoma	I/II	300	>18	12	Autologous BM derived MSCs	–	Retro-bulbar, Subtenon, Intravenou, Intravitreal, Intraocular infusion	No	–	Recruiting (Weiss et al., 2015a, Weiss et al., 2015b)	NCT01920867
StemCells, Inc. (US)	AMD	I/II	15 (8)	>50	12 (48)	Allogeneic fetal brain-derived HuCNS-SC®	0.2-1 × 10^6	Single subretinal injection	3 months	–	Completed	NCT01632527 (NCT02137915)
StemCells, Inc. (US)	Geographic atrophy in AMD	II	3	50–90	12	Allogeneic fetal brain-derived HuCNS-SC®	0.2-1 × 10^6	Single subretinal injection	3 months	–	Completed	NCT02467634
Yonsei University (Republic of Korea)	Multiple System Atrophy (MSA)	II	27	<75	12	Autologous BM derived MSCs	4 × 10^7	Single intra-arterial, multiple intravenous infusion	No	–	Completed (Lee et al., 2008a, Lee et al., 2012, Sunwoo et al., 2014)	NCT00911365
Stemedica Cell Technologies, Inc. (US)	Alzheimer's Disease (AD)	II	40	55–80	18	Allogeneic MSCs	1.5 × 10^6	Single intravenous infusion	–	<3 months	Recruiting	NCT02833792
StemCells, Inc. (Canada, Switzerland)	Thoracic Spinal Cord injury	I/II	12	18–60	12 (48)	Allogeneic fetal brain-derived HuCNS-SC®	20 × 10^6	Multiple intra-medullar injections	9 months	3 months	Completed	NCT01321333 (NCT01725880)
StemCells, Inc. (Canada, US)	Cervical Spinal Cord injury	II	31	18–60	12	Allogeneic fetal brain-derived HuCNS-SC®	20 × 10^6	Multiple intra-medullar injections	–	3 months	Completed (Ahuja and Fehlings, 2016)	NCT02163876
University of Ulsan College of Medicine (Republic of Korea)	Cervical Spinal Cord Injury	III	16	16–65	16	Autologous BM derived MSCs	1.6 × 10^7 (intra-medullar) and 3.2 × 10^7 (subdural)	Multiple intra-medullar and single subdural injections	–	>1	Completed (Oh et al., 2016)	–
Cairo University|, (Egypt)	Chronic Spinal Cord Injury	I/II	80	10–36	18	Autologous BM derived MSCs	2 × 10^6	Intratecal infusion	–	3 months to 3 years	Completed (El-Kheir et al., 2014)	NCT00816803
Hospital Sao Rafael (Brazil)	Spinal Cord Injury	I	20	18–50	6	Autologous BM derived MSCs	5 × 10^6 cells/cm^3 (per lesion volume)	Multiple intra-medullar injections	–	–	Completed (Mendonca et al., 2014)	NCT01325103

Abbreviations: HuCNS-SC®: human Central Nervous System (CNS) Stem Cells, ALS: Amyotrophic Lateral Sclerosis, PMD: Pelizaeus-Merzbacher Disease, NCL: Neuronal Ceroid Lipofuscinosis, AMD: Age-Related Macular Degeneration, MSA: Multiple System Atrophy, AD: Alzheimer's Disease.

Cossetti, 2013, Pluchino et al., 2009a). Several phase I and II trials have been undertaken for the use of NSCs in several intractable CNS diseases (*e.g.* amyotrophic lateral sclerosis, stroke, Pelizaeus-Merzbacher disease, Batten's disease), but clinical trials in MS have yet to be undertaken (Chiu and Rao, 2011).

Serious practical limitations have been associated with the use of somatic NSCs therapies for human diseases. These include the ethics associated with the source from which somatic NSCs are derived (either fetal or embryonic), the immunogenicity of allogeneic grafts and the limited expandability/genotypic stability of NSCs over extensive passaging *in vitro* (Fox et al., 2012, Pearl et al., 2012, Ramos-Zuniga et al., 2012).

Further, recent reports that industry leaders, StemCells Inc. have terminated a "make-or-break" phase 2 clinical trial of its proprietary human CNS stem cell (HuCNS-SC) based therapy in spinal cord injuries is certain to hinder the translational progression of these HuCNS-SC via a diming of potential investment in NSC centered translational research (Smalley, 2016). This critical trial employed Stem Cells Inc.'s HuCNS-SC, which originated from a purified population of human NSC derived from human fetal (16–20 weeks) brain tissue that was sorted using the CD133 marker and expanded in culture; the resultant cell line retained both its capacity to self-renew and differentiate into oligodendrocytes, neurons and astrocytes (Tsukamoto et al., 2013). Despite such preclinical promise, StemCells Inc. is the second high-profile cellular therapy developer to fall short in the highly challenging neural therapies space (*i.e.* with Geron exiting in 2011–2012) in recent years (Frantz, 2012) (Table 3).

3.1.3. Clinical trials with iPSCs

The generation of induced pluripotent stem cells (iPSCs) from adult somatic cells (*e.g.* fibroblasts) has heralded the possibility of autologous transplants that would serve to circumvent histocompatibility barriers and the ethics associated with the source of the tissue. While iPSC technology displays tremendous promise with regard to regenerative medicine, the field of cellular reprogramming is one that is in its relative infancy. As such, the de-differentiation of somatic cells into pluripotent cells comes with an intrinsic set of limitations. These include major difficulties in obtaining differentiation into specific lineages and the required extensive *in vitro* passaging (Fong et al., 2010). Further one cannot exclude the possibility of remaining neoplastic pluripotent cells within the final medical product (Liang and Zhang, 2013).

The first trial to employ an individualized iPSC-based therapy (*i.e.* in age related macular degeneration [AMD]) was suspended due the identification of three single-nucleotide variations and three copy-number variants that were present in a second patients iPSCs which were not detectable in the patient's original fibroblasts (Garber, 2015). As of yet no trials utilizing iPSCs and/or iPSC derivatives have been attempted in MS.

4. Future directions of stem based clinical therapies

Advances in the direct conversion (*i.e.* transdifferentiation) of somatic cells into multipotent/stably expandable induced NSCs (iNSCs) is a massive step forward in the translational march needed to overcome histocompatibility barriers and avoid extensive *in vitro* manipulation of putative cell based therapies (Thier et al., 2012). iNSCs obtained from skin fibroblasts are stably expandable, tissue specific, display unlimited self-renewal and can be expanded *in vitro* giving rise to multiple subtypes of neurons, astrocytes and oligodendrocytes (Ruggieri et al., 2014, Thier et al., 2012). Compared with the circuitous two-step strategy used during the conversion of somatic cells to iPSCs (and subsequent differentiation into neural stem cells), the relatively straightforward reprogramming of iNSCs is highly efficient, direct and safe. The reprogramming method used to generate iNSCs requires only one step that is completed in <20 days *in vitro* and yields numerous colonies of mouse iNSCs (Thier et al., 2012). Moreover, iNSCs exhibit negligible teratogenic potential, while displaying attributes suggestive of potential for reparative/restorative therapies targeting neurological diseases. The aforesaid when coupled with the recent advances in non-integrating cellular reprogramming technology make these next generation stably expandable patient-specific stem cell therapeutics extremely attractive (Sancho-Martinez and Izpisua Belmonte, 2013). Despite such positive attributes clinical trials have yet to begin.

5. Challenges to the effective translational of stem cell therapeutics in MS

While it is critical to note that there have been few reports of safety issues arising from autologous or allogeneic transplants it is widely acknowledged that prior to the successful implementation of novel stem cell based therapeutics, a number of pertinent scientific, ethical, regulatory, translational and clinical challenges will need to be met/overcome in order to successfully capitalize on the transition from proof-of-concept studies in animal models to clinically relevant therapies in man. Major hurdles precluding the translation of stem-based therapies into the clinic are centered on the inevitable risks related to transplant rejection and fundamental ethical concerns that exist within our society (*i.e.* those relating to the source of the tissue). Further, many stem cell types are hindered by a limited expandability and/or genotypic stability over extensive passaging *in vitro* (Ramos-Zuniga et al., 2012, Rice et al., 2013). All of the aforementioned affect the commercial viability/potential of such stem-based therapeutics. Therefore the successful translational of such novel therapies may ultimately require public/private partnerships (Rao, 2013).

6. Conclusions

As per the abovementioned a vast array of preclinical and early clinical data have come to suggest the potential therapeutic utility of stem cell centered approaches in various neurological diseases and disorders including progressive MS. Despite such progress, further work will be required to categorically define the underlying immunological and neurobiological mechanisms causing progressive MS pathology and in so doing determine whether patients suffering from this disease will ultimately benefit from non-hematopoietic stem cell-based therapeutics. In accordance with such findings it is prudent to again note that nHSCs have been shown to possess a therapeutic profile that is distinct from that of small molecules and/or biologics. As such it is reasonable to envision that cellular therapies might radically change its aspect modifying the way on which immune suppressive "drugs" are designed and/or delivered and in so doing seek to exploit the unique ability of stem cells to migrate, integrate and ultimately modify the host's pathologic CNS microenvironment(s).

As such, it is the authors' contention that these distinct attributes may be ultimately be harnessed to treat the persistent CNS inflammation and/or tissue degeneration and/or demyelination that are hallmarks of chronic/progressive MS in the clinic.

Conflict of interest

No conflicts of interest to disclose.

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