

Stem Cell-based Therapies in Multiple Sclerosis

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Abstract

The method of intensive immunosuppressive therapy followed by autologous hematopoietic stem cell transplantation (HSCT) has been used in the last eighteen years for the management of severe forms of Multiple Sclerosis (MS) and has been claimed to yield superior results. However, it is still not an established method of MS treatment, because it has not demonstrated its superior efficacy in comparative trials, owing to methodological difficulties and lack of sufficient patient recruitment. The main criticism has been the transplant-associated toxicity and an approximately 3% risk of mortality. Based on the results of these studies, HSCT has a sustained effect in suppressing disease progression for long periods of time, while it may also bring about sustained clinical improvement, especially if patients are in the relapsing-remitting phase or have active Magnetic Resonance Imaging (MRI) lesions. Three particular points merit to be stressed: (a) the nearly 100% eradication of active Central Nervous System (CNS) lesions on MRI, sustained over time; (b) the dramatic effect on the so-called "malignant" MS forms; (c) the qualitative immunological changes post-HSCT resulting in reconstitution of the clonal diversity and in regeneration of regulatory cells. Whether the latter changes, can also result in immune tolerance is yet to be definitely shown. An alternative approach to HSCT involves the transplantation of Mesenchymal stem cells (MSCs). This interesting approach has been explored in a limited number of phase I/II studies with promising results that await confirmation in the context of larger scale, controlled trials. In conclusion, HSCT is not a therapy for the general population of MS patients; it is a powerful therapy with long-term benefits that need to be weighed against certain toxicity risks; and in critical situations, like the very aggressive, rapidly progressing and refractory "malignant" form, it may have a life-saving effect with a meaningful and long-lasting improvement of disability.

Keywords: Autologous hemopoietic stem cell transplantation; Mesenchymal stem cells; Multiple sclerosis; Transplant-related mortality

Abbreviations: HSCT: Hemopoietic Stem Cell Transplantation; MSC: Mesenchymal Stem Cells; PFS: Progression-Free Survival; TRM: Transplant-Related Mortality; MS: Multiple Sclerosis

Introduction

During the last two decades, accumulating experimental and clinical evidence suggested that hemopoietic stem cell transplantation (HSCT) might be used for the treatment of aggressive Multiple Sclerosis (MS), unresponsive to conventional immunomodulatory and immunosuppressive agents. In addition, there is emerging evidence that other types of stem cells, including mesenchymal (MSC) and neural cell precursors, olfactory ensheathing cells, oligodendrocyte progenitors and embryonic stem cells, might be used in Central Nervous System (CNS) demyelinating diseases with beneficial effects. These stem cell-based therapies have received much attention, both in the scientific and lay press, and those that have reached the stage of clinical trials (i.e. HSCT & MSC transplantation) will be briefly discussed in the following report.

I. Autologous hemopoietic stem cell transplantation for Multiple Sclerosis: rationale and clinical experience

In the early 1990's, a pivotal series of experiments [1-6] investigated, for the first time, the effects of Bone Marrow Transplantation (BMT) on the animal MS model experimental autoimmune encephalomyelitis (EAE). The impetus for designing these experiments was provided by the fact that nonspecific, conventional-dose immunosuppression, although effective in MS, is fraught with problems. Immunosuppressive agents act in a dose-dependent manner, and therefore, high doses can be more effective, but they are associated with morbidity risks. Low doses, on the other hand, are less effective and may even

induce relapses of EAE [7,8] possibly because of impairment of suppressor mechanisms. In addition, immunosuppressants need to be administered continuously, leading to long-term side effects, and upon discontinuation of the therapy, relapses commonly occur. For these reasons, it would be desirable, in a single therapeutic scheme, to administer high-dose immunosuppression, such as myeloablative chemotherapy, to maximize effectiveness, followed by hematopoietic stem cell rescue (transplant) to minimize morbidity and mortality. Indeed, in the transplant experiments, remissions of EAE could be attained in all animals after high-dose total body irradiation (TBI at 10 Gy) or high-dose chemotherapy followed by allogeneic, syngeneic, or pseudo-autologous BMT (transplant from syngeneic animals brought to an identical stage of disease) [1-6]. High-dose conditioning was required in order to achieve remission and also to prevent from relapses, which were caused either by residual host T-cells surviving the conditioning or by reinfused cells of the graft. These results established the efficacy of BMT in EAE and indicated that some form of T-cell depletion of the graft is necessary.

The exact mechanism by which BMT influenced the course of EAE was not entirely clear but was principally based on the profound and prolonged immunosuppression following high-dose chemo/radiotherapy and the deletion of autoreactive T-cell clones. In addition, the

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interesting possibility was raised that the replacement of the aberrant immune system by a pristine, expectantly non-autoreactive system, generated by the hemopoietic stem cell graft, may possibly restore self-tolerance [9].

At the clinical level, the first circumstantial evidence of the efficacy of BMT in MS was obtained from sporadic patients with concurrent demyelinating and malignant diseases who underwent BMT resulting in stabilization or even improvement in their neurological status [10,11]. On the basis of these encouraging experimental and clinical observations, phase I/II clinical trials were initiated in 1995 [12] in order to explore the feasibility of performing autologous HSCT in patients with aggressive MS. Over the last 15 years, more than 700 patients with refractory MS, the majority of which suffered from the secondary progressive subtype, have been treated with AHSCT with consistently good results. The conditioning regimens employed in these studies can be categorized as high-intensity (TBI plus cyclophosphamide (CY) or busulphan alone or in combination with CY), intermediate-intensity (i.e. the BEAM regimen containing etoposide, melphalan, carmustine and cytosine arabinoside) and low-intensity (CY or fludarabine-based protocols).

In the largest cohort (n=178), reported by the European Group for Blood and Marrow Transplantation (EBMT), improvement or stabilization of neurological state occurred in 63% of patients at a median follow-up of 41.7 months [13]. More recent studies provided similarly favorable results. Burt et al. [14] treated 21 patients with relapsing/remitting MS and frequent relapses despite interferon with a non-myeloablative HSCT and observed an improvement in EDSS score of at least 1 point compared to baseline in 81% of study participants and a progression-free survival (PFS) rate of 100% at a mean of 3 years follow-up. Shevchenko et al. [15] treated 50 MS patients with EDSS scores ranging from 1.5 to 8 and reported that HSCT was well tolerated and effective as 62% of study participants improved by at least 0.5 EDSS points and a PFS rate at 6 years was 72%. The same group of authors reported recently the outcome of a larger cohort (95 patients with various types of progressive and relapsing/remitting MS) treated with early or late HSCT and concluded that the PFS rate at 5 years was 92% after early vs. 73% after late HSCT emphasizing the need for timely intervention [16]. Atkins and Freedman [17] treated 17 patients with aggressive MS using a high-intensity protocol with *in vivo* and *ex vivo* purging of the graft and observed a progression-free survival rate of 75% at 3 years with absence of MRI activity or new relapses in the 5 years post-HSCT. Fassas et al. investigated the long-term outcome of HSCT for MS and reported that progression-free survival at 15 years was 44% for patients with active MRI lesions vs. 10% for those without [18]. It should be noted that a recent long-term follow-up study in 26 patients with advanced MS by Bowen et al. [19], did not confirm the impact of baseline activity on final outcome but, in line with previous reports, observed disease stabilization in a significant number of patients. Finally, numerous MRI studies have demonstrated that HSCT has an impressive and sustained effect in suppressing disease activity on MRI [18,20].

The mechanism by which autologous HSCT exerts its' beneficial effects in MS has not been fully resolved. It is well established that HSCT causes a profound and prolonged immunosuppression with low CD4+ cells lasting up to 2 years and more after transplantation. It is conceivable that the deletion of autoreactive T-cell clones, which are thought to play a pivotal role in the pathogenesis of MS, could explain the short-term beneficial, anti-inflammatory effect of AHSCT. In addition to the abrogation of CNS inflammation, HSCT is thought

to induce a number of qualitative immunologic changes including a decrease in memory T cells, expansion of thymic naïve CD4-cells, the creation of a new and diverse TCR repertoire and the generation of thymic CD4-25-FoxP3 regulatory cells [21]. A recent study investigating the functional capacity of the T-cell repertoire following HSCT to participate in new autoimmune disease activity reported the reemergence and *in vivo* expansion of functional autoreactive T cells. In addition, however, the authors observed significantly diminished Th17 and Th1/17 responses which correlated with the complete abrogation of clinical and radiological MS activity brought about by HSCT [22].

HSCT is a complex and intensive form of treatment that is inevitably associated with a small but significant mortality risk. Importantly, transplant-related mortality (TRM) has decreased significantly over time for a number of reasons. For instance in European centers, it fell from 7.3% in the period 1995–2000 [13] to 1.3% in 2001–2007 [23], probably due to improved patient selection, in accordance to available guidelines [24], and accumulating experience of Transplantation centers. In addition, the choice of the conditioning regimen appears to be of paramount importance. In the study by Shevchenko et al. [16] in 95 MS patients treated with a reduced intensity conditioning regimen, TRM was nil and the procedure was associated with significant improvement in the majority of the quality of life parameters. According to a recent position paper [25], intermediate-intensity protocols (such as BEAM) seem to offer advantages compared to high-intensity ones because the latter are associated with increased TRM [13] and lower progression-free survival, at least for SP MS [26].

The HSCT-associated toxicity and the transplant-related mortality in particular have caused much discussion and concern over the ethics of applying an aggressive form of treatment, such as HSCT, in MS. However, as already mentioned, with proper patient selection the transplant-related toxicity is possible to be lowered. Furthermore, other agents currently available for the same indications, i.e. mitoxantrone and natalizumab, are not entirely devoid of toxicity and can be used only for a restricted time period. In addition, the duration of the therapeutic effect after the discontinuation of these drugs is unknown. Overall, the clinical benefits and radiological improvement observed in the phase I/II clinical trials so far, seem to justify the further evaluation of the effectiveness of HSCT in the context of a larger- scale, randomized, controlled trial.

In conclusion, the above-cited data suggest that HSCT is not a therapy for patients with MS at large but should be offered, reasonably early, to rapidly deteriorating cases, still in the inflammatory phase of the disease, and to patients with the rare but clinically significant malignant form, in which it may be life-saving [27,28]. The next step is to find out whether HSCT is superior to other available forms of MS treatment. It is clear that only a randomized, comparative trial will be able to answer this question conclusively. Currently, there are a number of international collaborative efforts to this end, the results of which are eagerly awaited.

II. Mesenchymal stem cell transplantation for Multiple Sclerosis

The concept of Mesenchymal stem cell (MSC) transplantation for MS differs fundamentally from that of HSCT because it proposes the intravenous and/or intrathecal infusion of MSCs without preceding immunosuppression. These cells are then thought to migrate to areas of CNS inflammation and interact locally with paracrine and contact factors thereby exerting antiproliferative, immunomodulatory and prosurvival effects which in concert ameliorate the autoimmune process [29].

This concept was initially investigated in various animal models of Experimental Allergic Encephalomyelitis. These pioneering studies demonstrated that the i.v., i.p. or i.c.v. administration of MSCs resulted in potent immunomodulatory and, most importantly, neuroprotective effects, reflected in decreased axonal loss, improved neuronal survival, oligodendrocyte proliferation and remyelination [30-32].

Recently, three small-scale clinical studies investigated the feasibility of performing (MSC) transplantation for MS. Karussis et al. [33] administered MSCs in 15 MS patients (in 10 of them intrathecally and in 5 via combined intrathecal-intravenous routes) and concluded that the procedure was relatively safe with minor adverse effects (most commonly headache and transient fever). The mean EDSS score improved from 6.7 (1.0) to 5.9 (1.6) at 6 months post-transplant and a detailed immunologic analysis revealed a number of qualitative immunomodulatory effects. Interestingly, MRI tracking of MSCs labeled with superparamagnetic iron oxide displayed the presence of labeled cells in the occipital horns of the ventricles, indicating the possible migration of MSCs in the meninges, subarachnoid space, and spinal cord. Yamout et al. [34] administered intrathecally MSCs in 10 patients with advanced MS and observed transient encephalopathy with seizures (n=1) and transient cervical and low back pain (n=1). At 3-6 months post-transplant, EDSS scores were improved in 5/7, stabilized in 1/7 and worsened in 1/7 which was interpreted as an indication of clinical efficacy. On the other hand, MRI at 3 months revealed new or enlarging lesions in 5/7 and Gadolinium-enhancing lesions in 3/7 patients suggesting that MSC transplantation was not able to suppress disease-related radiological activity. Finally, Connick et al. [35] explored the safety and efficacy of an intravenous infusion of autologous bone-marrow-derived MSCs as a neuroprotective treatment in secondary progressive MS in the context of an open-label, phase 2a proof-of-concept study. Adverse events included a transient rash and self-limiting bacterial infections. For the efficacy assessment, the authors focused on anterior visual pathways as a model of the disease at large and observed statistically significant improvement in visual acuity, visual evoked potential latency and optic nerve area. These structural and functional changes were thought to be suggestive of a neuroprotective effect of MSCs.

One potential safety concern with the MSC transplantation is ectopic tissue formation in the CNS as observed after intraventricular administration of MSCs for the treatment of EAE particularly in animals with severe disease [36]. However, macroscopic and histological examination of autopsy material from 18 patients who had received HLA-mismatched MSCs intravenously for the treatment of complications of HSCT revealed no signs of ectopic tissue formation or malignant tumors of MSC-donor origin [37].

The safety aspects of MSC treatment are of critical importance and a number of relevant issues remain to be addressed including the optimal dose, culture regimen, route of administration and source of MSCs (i.e. autologous or allogeneic) [38]. Yet, despite these concerns, the preliminary results obtained so far are encouraging and a recent consensus from a panel of experts concluded that an international phase II study is warranted in order to better define the role of MSC transplantation in MS [39].

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