

Autologous Haematopoietic Stem Cell Transplantation for the Treatment of Multiple Sclerosis

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Abstract

Introduction: Autologous haematopoietic stem cell transplantation (auto-HSCT) has been performed for severe multiple sclerosis (MS) refractory to standard therapy with increasing frequency worldwide. However, experience in Asia employing this modality in MS has been limited. In this review, we explored the pathophysiology of autoimmunity and the underlying rationale for auto-HSCT in treating autoimmune diseases including MS, as well as existing published pre-clinical and clinical data. We aimed thereby to better understand the utility of treating MS with auto-HSCT and the feasibility of this procedure in Singapore. **Methods:** A Medline search was performed with the terms “haematopoietic stem cell transplantation”, “multiple sclerosis” and “autoimmune diseases” from 1996 to 2005. Both original papers and review articles were considered. **Main Findings:** The majority of publications were from Europe or the United States and most clinical series from single centres had relatively small numbers of patients. Worldwide, the number of patients reported has been less than 300 since 1997. Existing data support the feasibility and promise of this procedure and ongoing Phase III trials may serve to confirm this initial experience. **Conclusion:** Pre-clinical and early clinical data support the rationale for immunoablative therapy for autoimmune disorders. Auto-HSCT for severe MS is a feasible procedure and can be safely performed in centres with experience managing HSCT patients.

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Introduction

Multiple sclerosis (MS) is the most common cause of chronic neurological disability in young adults. It is characterised by a variable disease course with multiple sites of inflammation and demyelination within the central nervous system (CNS). Studies of its natural history suggest that patients may also have a reduced life expectancy¹ and about 50% of patients will require walking aids within at least 15 years of disease onset.²⁻⁴ As the disease is widely accepted to be of autoimmune aetiology, the mainstays of therapy have been immunosuppressive or immunomodulatory agents. These may alter the rate of progression of disease but none have so far been curative.⁵ In this review, we discuss the increasing use of haematopoietic stem cell transplantation (HSCT) over the last decade for the treatment of several autoimmune diseases, including MS.

Rationale of Autologous HSCT (Auto-HSCT) for MS

The Pathogenesis of Autoimmune Disease and Conceptual Basis of Auto-HSCT for Treatment of Autoimmune Disease

The development of clinical autoimmune disease is postulated to be due to an imbalance between factors favouring autoimmunity over self-tolerance. Underlying genetic susceptibility coupled with environmental factors result in a shift of the “immune rheostat” toward autoimmunity and loss of self-tolerance.^{6,7} An individual’s underlying susceptibility depends on genes which affect HLA expression, cytokine production, antigen/receptor signalling, costimulatory molecules and apoptosis pathways. For instance, many autoimmune diseases are associated with certain HLA alleles and there is a greater incidence of autoimmune disorders among syngeneic twins compared to the general population. The role of environmental factors

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however is supported by the fact that the concordance rate for syngeneic twins is one-third rather than the near-100% should genetic make-up be the only governing factor for the development of autoimmune disease. The prevalence of diseases like MS differs in various climates and retrospective studies of patients who have migrated from regions of high to low prevalence suggest the role of environmental factors such as infections or drugs. It is the combination of both underlying susceptibility coupled with multiple exposures at different time-points which is thought to alter the immunological milieu in the individual leading to the development of clinical autoimmune disease.⁸

MS is thought to be caused by activated T-cells specific for self antigens which migrate into the CNS when the blood-brain barrier is disrupted, possibly by an infection. These antigens include myelin basic protein and myelin oligodendrocyte glycoprotein. Antibodies may also gain access into the CNS and amplify the immune response further. The resultant axonal demyelination leads to a blockade in nerve conduction which, in the initial stages of the disease, may be repaired. This resolution of acute inflammation and repair accounts for the remission/resolution of acute flares of MS. However, repeated episodes of inflammation result in irreversible axonal injury, scarring and loss of oligodendrocyte precursors manifested in later-stage disease with secondary progression and permanent disability.⁵

Auto-HSCT is postulated to work by allowing the delivery of immunosuppressive or immunoablative therapy at high doses with the stem cell rescue hastening myeloid recovery. Full functional T-cell recovery, on the other hand, usually lags behind by up to 12 months⁹ and the practice of T-cell depletion either via the conditioning regimen or graft manipulation with CD34 selection delays immune recovery further. However, the limited T-cell repertoire alters the balance in favour of tolerance over autoimmunity. While the individual's genetic makeup remains susceptible to developing autoimmune disease, the combination of re-exposure to the putative "trigger" and a functional T-cell repertoire would be required to shift the balance toward autoimmunity again.

Pre-clinical Data

Extrinsic autoimmune encephalomyelitis (EAE) is a disorder induced in animal models by injecting an adjuvant with myelin basic protein or brain/spinal cord tissue. The disease manifests in Buffalo rats as a chronic relapsing/remitting syndrome resembling MS with characteristic inflammatory lesions throughout the CNS.¹⁰ Studies have been performed on EAE rats in which the animals first receive an allogeneic transplant from a resistant strain followed by immunisation with myelin basic protein.

Animals that achieved full donor chimerism were prevented from developing EAE.¹¹⁻¹³ This technique was then extended to animals which already had EAE to see if allogeneic transplant could treat established disease. When EAE animals with established disease were treated in the acute phase with allogeneic transplant, all animals went into a complete remission and did not suffer relapses, as opposed to control mice.^{10,12} Autologous/syngeneic transplants in EAE mice and rats have also resulted in complete remissions in these animals.^{14,15} However, with allogeneic transplants, there could be potential for more longlasting remissions, given the establishment of an entirely new immune system.

Anecdotal Evidence

Patients with co-existent autoimmune diseases have undergone both autologous and allogeneic HSCT for a haematological malignancy and there have been reports of improvement in their autoimmune disorder. Mandalfino et al¹⁶ reported on 3 cases of MS who achieved neurologic stability following HSCT (2 autologous, 1 allogeneic) for haematological malignancy. A patient with acute lymphoblastic leukaemia and co-existent MS underwent auto-HSCT and demonstrated neurologic stability¹⁷ but the follow-up period was short. The literature in this area is sparse and there is a tendency to report positive cases only although there was an abstract reporting the development of new MS lesions in a patient who received an allogeneic HSCT for chronic myeloid leukaemia.¹⁸ In addition, the transplant regimens and methods were not tailored for the autoimmune disease but the underlying haematological disease. In most cases a detailed pre-transplant assessment by a neurologist or rheumatologist is lacking. All this makes anecdotal evidence an unreliable means of evaluating the effect of HSCT on the autoimmune disorder. However, the combination of animal studies and anecdotal evidence has served as an impetus for clinical studies on auto-HSCT for MS.

Clinical Data

Auto-HSCT for MS was suggested as a possible modality of treatment in 1995 and the initial reports date from 1997.^{19,20} It is now the commonest autoimmune disorder for which HSCT is performed in the US and Europe, with over 200 patients treated worldwide. The initial clinical experience was limited to phase I studies and most patients had an advanced degree of disability.²¹⁻²³ The Kurtzke expanded disability status scale (EDSS) is the measure of disability used most often in clinical trials and ranges from 0 (no disability) to 10 (death related to neurologic progression).²⁴ At 6.0, patients are able to ambulate with unilateral support for no more than 100 m while at 7.0, they require bilateral support to walk 10 m.

One of the earliest published reports comes from a pilot

study from Fassas et al,¹⁹ who treated 15 patients with progressive MS and a median EDSS of 6.0. The patients were transplanted after BEAM (BCNU, etoposide, cytarabine arabinoside and melphalan) and antithymocyte globulin (ATG) conditioning and at a median of 6 months follow-up there were no deaths from toxicity of the regimen and no worsening in neurologic disability. There was improvement in the EDSS in 7 of 15 patients although the duration of follow-up then was too short to see if the responses would be durable. Burt et al²⁰ reported their initial experience on 6 patients with MS who received cyclophosphamide and total body irradiation (TBI) conditioning followed by HSCT and were successfully engrafted with minimal regimen-related non-haematopoietic toxicities. Saiz et al²⁵ reported on 5 patients with progressive MS and median EDSS of 6.5 who underwent HSCT after BCNU, cyclophosphamide and ATG conditioning and achieved stabilisation/improvement in 4 patients with improvement in MRI findings. The fifth patient worsened neurologically.

A few centres using different conditioning regimens reported more morbidity from the procedure and less favourable results. Nash et al²⁶ reported on 26 patients with median EDSS of 7.0 who underwent auto-HSCT after cyclophosphamide, TBI and ATG. One patient died of post-transplant lymphoproliferative disease and one had irreversible neurologic deterioration after fever during HSCT. With a median follow-up of 24 months, 27% had an EDSS deterioration of 1.0 at 3 years. Burt et al²⁷ subsequently reported on 21 patients with EDSS from 3.0 to 8.0 who received cyclophosphamide and TBI conditioning for transplant. There were no deaths at 1 year but patients with pre-transplant EDSS scores above 6.0 continued to deteriorate after transplant. The authors concluded that TBI-based regimens were not effective in patients with high pre-transplant EDSS.

The European Group for Blood and Marrow Transplantation (EBMT) Autoimmune Disease Working Party reported on 85 cases from 19 European centres and 1 US centre summarising data from 1997 to 2002.²⁸ This retrospective analysis included patients with secondary progressive MS (70%) and primary progressive MS (26%), with a median EDSS of 6.5 (range, 4.5 to 8.5). They had clinical evidence of disease progression in the preceding 12 months and had failed a number of lines of standard therapy. At a median follow-up of 16 months, the probability of progression-free survival was 74% at 3 years. The median EDSS improved from 6.5 to 6.0 at 2 years post transplant. Eighteen patients had an improvement in EDSS by 1.0 or more, 6 of which subsequently progressed later but only 1 patient beyond baseline. There were 5 deaths from treatment-related causes, 4 of which were due to infection and 1 arising from cardiac failure.

One major contributor to EBMT data is the Thessaloniki group,²⁹ which reported on 35 patients conditioned with either BEAM (n = 25) or a busulfan-based regimen (n = 10). They reported a progression-free survival of 81% at 3 years for patients with secondary-progressive (SP) MS and 67% for primary progressive (PP) MS. MRI findings before and after HSCT have also been reported to show marked improvement. The Italian GITMO-NEURO Intergroup³⁰ reported on 10 patients with SP MS with rapid deterioration who underwent auto-HSCT after BEAM conditioning. Serial MRIs performed within 3 months before, monthly for 6 months after HSCT and 3 monthly thereafter, revealed that gadolinium-enhancing lesions decreased and ultimately completely regressed after HSCT. This effect was sustained for a median follow-up of 15 months. The correlation between clinical benefit and these findings requires longer follow-up as another study showed that while active inflammatory lesions on MRI may be halted with auto-HSCT, axonal atrophy may persist and patients may continue to progress.²¹ Selected data from the larger series are summarised in Table 1.

Table 1. Clinical Data from Selected Studies

Study	No. of patients	Median baseline EDSS	Definition of progression	3-year progression-free survival
Fassas et al ²⁸	85	6.5 (4.5-8.5)	EDSS increase 1.0	78%
Nash et al ²⁶	26	7.0 (5.0-8.0)	EDSS increase 1.0	73%
Fassas et al ³¹	24	6.0 (4.5-8.0)	EDSS increase 1.0 if baseline ≤ 5.0 or 0.5 if baseline ≥ 5.5	RR = 100% SP = 92%
Burt et al ²⁷	14	7.0 (3.0-8.5)	Any EDSS increase	100% (baseline EDSS ≤ 6.0) 35% (baseline EDSS > 6.0)

EDSS: expanded disability status scale

These early studies are heterogeneous in terms of regimens used, patient selection criteria, duration and means of follow-up. However, they have shown that auto-HSCT for MS was feasible and served to assess the toxicity. They also helped shape the proposed selection criteria for protocols studying the efficacy of this modality of treatment and form the basis of ongoing Phase III studies in Europe and USA. A consensus conference held in Milan in 2000 proposed the inclusion criteria listed in Table 2.³² These have been adopted for the ongoing Phase III Autologous Stem Cell Transplantation International Multiple Sclerosis Trial

Table 2. Proposed Inclusion Criteria for Patients with MS for Autologous Stem Cell Transplant (Derived from Milan Consensus Conference)³²

Clinically definite MS according to Poser criteria
Relapsing-remitting, secondary-progressive, progressive relapsing courses
Brain MRI findings typical of MS ³³
Age between 18 and 55 years
Disease duration ≥ 1 year
EDSS between 3.0 and 6.5
Disability progression sustained for at least 6 months in the previous 2 years of:
≥ 1.5 points if entry EDSS between 3.0 and 5.0
≥ 1.0 point if entry EDSS ≥ 5.5
Clinical or MRI activity in the previous year
Unsatisfactory response to other available therapies (based on clinical judgment)
Informed consent

EDSS: expanded disability status scale; MRI: magnetic resonance imaging; MS: multiple sclerosis

(ASTIMS) (<http://www.astims.org>), which randomises patients with severe MS to auto-HSCT or mitoxantrone. In the US, Northwestern University is spearheading a randomised comparison between patients treated with auto-HSCT and standard interferon therapy for relapsing-remitting MS. The results of these trials will serve to clarify the role of auto-HSCT and prove its efficacy in the treatment of patients with severe MS who have failed standard lines of therapy.

In Singapore, auto-HSCT for autoimmune diseases has been the subject of pilot studies since 2002. Patients have had an improvement in their EDSS scores by 1.0 to 1.5 over baseline and have remained clinically stable after a median follow-up of 19.3 months.³⁴

Practical Aspects of Auto-HSCT for MS

The procedure for auto-HSCT first involves the harvesting of haematopoietic stem cells from the peripheral blood. The harvested stem cells are then re-infused following a preparative regimen called the conditioning regimen.

Mobilisation of Haematopoietic Stem Cells

Peripheral blood stem cells (PBSC) are collected via apheresis in well-established methods used for haematological diseases. The mobilisation regimens include granulocyte colony-stimulating factor (G-CSF) used either alone or in combination with a cyclophosphamide or steroids. G-CSF used alone has been associated with flares of MS during mobilisation.³⁵ The addition of steroids to G-CSF has been effective in ameliorating flares, as has the use of cyclophosphamide. Cyclophosphamide has the advantage of aiding in disease control and there have been cases of patients' lesions on MRI regressing after

cyclophosphamide was given, prior to the auto-HSCT.²⁹ However, cyclophosphamide has significant myelotoxicity and neutropenic sepsis is a complication.³⁶ MS symptoms may worsen during episodes of fever and in some cases may even lead to a permanent deterioration in function.

Lymphocyte-depletion of Graft

Most centres would perform *ex vivo* depletion of lymphocytes in the graft in an attempt to decrease the risk of contamination by auto-reactive T-cells, which may theoretically increase the risk of relapse.³⁷ This can be performed either via positive selection of CD-34 cells via a column during apheresis or negative selection with T-cell antibodies. Graft manipulation may, however, slow the rate of immune recovery and increase the risk of opportunistic infections and lymphoproliferative diseases.^{38,39} Animal models seem to support the role of T-cell depletion in transplantation for autoimmune disease⁴⁰ but the only randomised comparison of CD34 selection versus unselected HSCT in rheumatoid arthritis failed to show a difference in outcomes.⁴¹

Conditioning Regimens

Different preparatory regimens have been employed in the various centres worldwide performing auto-HSCT for MS. These had often been "borrowed" from those used in the treatment of haematological malignancies and which haematologists and transplant physicians were thus familiar with. However, worldwide clinical experience has shown that not all regimens are equivalent and the paradigm has shifted toward the importance of immunoablation as opposed to myeloablation in the treatment of autoimmune diseases. In addition, MS is not usually life-threatening in the majority of patients; hence mortality or regimen-related toxicity rates which may be acceptable in treating diseases like leukaemias would not apply. The conditioning regimen selected must thus have the least toxicity possible. TBI may exacerbate neurological disability and has been shown to exacerbate paresis in EAE animals.¹³ Burt et al²⁷ reported that TBI used in conditioning failed to prevent progression in patients with more advanced disability and further concluded that TBI should be avoided as part of the conditioning regimen as it has been reported to cause an engraftment-related fever which may exacerbate neurologic disability.⁴² There have also been reports of patients with co-existent MS who had received radiotherapy for other conditions, and consequently experienced flares of MS.⁴³ There is also the risk of secondary myelodysplasia or malignancies associated with radiation exposure. Accordingly, the Northwestern group has moved away from using TBI for their patients to cyclophosphamide and ATG.

The regimen most commonly applied regimen in Europe is BEAM combined with ATG and accounts for

approximately half of all MS patients who have had an auto-HSCT.^{30,31,44} Treatment-related toxicity has been reported at between 3% and 5%.²⁹ Busulfan has also been used but reports suggest mortality in this group may be as high as 20%.⁴⁵

In our centre, we have opted to use fludarabine in combination with cyclophosphamide, which to our knowledge is a unique regimen for MS.³⁴ The known efficacy of cyclophosphamide in MS prompted our choice, in combination with fludarabine, which is a good lymphodepleting but non-myeloablative drug. The aim is to minimise the number of days of anticipated neutropaenia and attendant risk of sepsis both in terms of toxicity risks and the tendency of fever in MS patients to cause neurologic deterioration.

Patient Selection

As the therapy is still experimental, many of the patients treated in initial studies were severely disabled, with EDSS scores as high as 8.5 (which represents patients who are already wheelchair-bound). Burt et al²⁷ found that patients with EDSS scores above 6.0 failed to improve with auto-HSCT. Patients with SP MS appear to respond better to auto-HSCT compared to those with primary progressive (PP) disease.^{28,31} The presence of Gd-enhancing MRI lesions corresponds with active inflammatory disease and these have shown convincing evidence of regressing following auto-HSCT.³⁰ On the other hand, in the latter stages of MS, the predominant MRI finding is progressive atrophy of the CNS due to axonal loss and correlates clinically with the irreversible neurologic disability seen in these patients. These lesions often persist after transplant and continue to progress.²¹ Patients should thus be considered for auto-HSCT prior to the onset of severe disability if they have failed standard therapy and continue to relapse/progress. The studies currently being conducted in Europe and the US generally include patients with an EDSS of 3.5 to 6.5, with either relapsing-remitting MS with cumulative deficits or SP MS despite standard therapy.^{18,46}

Centre Effect

It has also been shown that the treatment-related toxicity of HSCT depends on the expertise of the transplant team and measures taken to prevent infectious complications.⁴⁷ To this end, it has been suggested that the procedure should be performed in accredited stem cell transplant units with experience in performing allogeneic HSCT.⁴⁸

Potential for Allogeneic HSCT for MS

While there is potential for more longlasting remissions with allogeneic transplantation, the relatively higher transplant-related mortality of such transplants has been a limiting factor. However, the use of reduced intensity

conditioning regimens with low toxicity could reduce the risk and make the use of allogeneic transplantation more practicable.⁴⁹

Conclusion

Auto-HSCT for MS has been made possible by the presence of multidisciplinary teamwork and excellence in stem cell transplantation around the world. With increased experience and adjustment to conditioning regimens and patient selection, improved outcomes are anticipated. Its anti-inflammatory effects measured radiographically appear to be longlasting but clinical correlation and efficacy remains to be proven. This is currently the subject of study in multinational Phase III trials, with results anticipated in the next few years. However, the concept of ASCT is not confined to MS alone; other autoimmune conditions e.g., systemic lupus erythematosus, Crohn's disease, systemic sclerosis and others can also benefit from ASCT.

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REFERENCES

1. Confavreux C, Ainaro G, Devic M. Course and prognosis of multiple sclerosis assessed by the computerized data processing of 349 patients. *Brain* 1980;103:281-300.
2. Cottrell DA, Kremenchutzky M, Rica GP, Koopman WJ, Baskerville J, Ebers GC. The natural history of multiple sclerosis: a geographically based study. 5. The clinical features and natural history of primary progressive multiple sclerosis. *Brain* 1999;122:625-39.
3. Kremenchutzky M, Cottrell D, Rice G, Hader W, Baskerville J, Koopman W, et al. The natural history of multiple sclerosis: a geographically based study. 7. Progressive-relapsing and relapsing-progressive multiple sclerosis: a reevaluation. *Brain* 1999;122:1941-50.
4. Confavreux C, Vukusic S, Moreau T, Adeline P. Relapses and progression of disability in multiple sclerosis. *N Engl J Med* 2000; 343:1430-8.
5. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. *N Engl J Med* 2000;343:938-52.
6. Grossman Z, Singer A. Tuning of activation thresholds explains flexibility in the selection and development of T cells in the thymus. *Proc Natl Acad Sci U S A* 1996;93:14747-52.
7. Grossman Z, Paul WE. Adaptive cellular interactions in the immune system: the tunable activation threshold and the significance of subthreshold responses. *Proc Natl Acad Sci U S A* 1992;89:10365-9.
8. Davidson A, Diamond B. Autoimmune diseases. *N Engl J Med* 2001;345:340-50.
9. Guillaume T, Rubinstein DB, Symann M. Immune reconstitution and immunotherapy after autologous hematopoietic stem cell transplantation. *Blood* 1998;92:1471-90.
10. van Bakkum DW. Stem cell transplantation for autoimmune disorders. Preclinical experiments. *Best Pract Res Clin Haematol* 2004;17:201-22.
11. Singer DE, Moore MJ, Williams RM. EAE in rat bone marrow chimeras: analysis of the cellular mechanism of BN resistance. *J Immunol* 1981;126:1553-7.
12. Van Gelder M, van Bakkum DW. Treatment of relapsing experimental autoimmune encephalomyelitis in rats with allogeneic bone marrow transplantation from a resistant strain. *Bone Marrow Transplant* 1995;16:343-51.
13. Van Gelder M, Mulder AH, van Bakkum DW. Treatment of relapsing experimental autoimmune encephalomyelitis with largely MHC-matched allogeneic bone marrow transplantation. *Transplantation* 1996;62:810-8.

14. van Gelder M, Kinwel-Bohre EPM, van Bekkum DW. Treatment of experimental autoimmune encephalomyelitis in rats with total body irradiation and syngeneic bone marrow transplantation. *Bone Marrow Transplant* 1993;11:223-41.
15. van Gelder M, van Bekkum DW. Effective treatment of relapsing experimental autoimmune encephalomyelitis with pseudoautologous bone marrow transplantation. *Bone Marrow Transplant* 1996;18:1029-34.
16. Mandalfino P, Rice G, Smith A, Klein JL, Rystedt L, Ebers GC. Bone marrow transplantation in multiple sclerosis. *J Neurol* 2000;247:691-5.
17. Meloni G, Capria S, Salvetti M, Cordone I, Mancini M, Mandelli F. Autologous peripheral blood stem cell transplantation in a patient with multiple sclerosis and concomitant Ph+ acute leukemia. *Haematologica* 1999;84:665-7.
18. Jeffrey ER, Alshami E. Allogeneic bone marrow transplant in multiple sclerosis (abstract). *Neurology* 1998;50:A147.
19. Fassas A, Anagnostopoulos A, Kazis A, Kapinas K, Sakellari I, Kimiskidis V, et al. Peripheral blood stem cell transplantation in the treatment of progressive multiple sclerosis: first results of a pilot study. *Bone Marrow Transplant* 1997;20:631-8.
20. Burt RK, Traynor AE, Cohen B, Karin KH, Davis FA, Stefoski D, et al. T-cell depleted autologous haematopoietic stem cell transplantation for multiple sclerosis: report on the first three patients. *Bone Marrow Transplant* 1998;21:537-41.
21. Burt RK, Cohen BA, Lobeck LJ, Oyama Y, Traynor A, Burns WH. Immune suppressive therapy with autologous hematopoietic stem cell transplantation arrests active CNS inflammation but not axonal atrophy in patients with severe disability and progressive multiple sclerosis (abstract). *Blood* 2001;98:687a.
22. Burt RK, Traynor AE, Pope R, Schroeder J, Cohen B, Karlin KH, et al. Treatment of autoimmune disease by intense immunosuppressive conditioning and autologous hematopoietic stem cell transplantation. *Blood* 1998;92:3505-14.
23. Carreras E, Saiz A, Graus F, Marin P, Martinez C, Rovira M, et al. Autologous CD34 selected haematopoietic stem cell transplantation for multiple sclerosis: update of a single center experience in 10 patients (abstract). *Biol Blood Marrow Transplant* 2001;7:69.
24. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;33:1444-52.
25. Saiz A, Carreras E, Berenguer J, Yague J, Martinez C, Marin P, et al. MRI and CSF oligoclonal bands after autologous hematopoietic stem cell transplantation in MS. *Neurology* 2001;56:1084-9.
26. Nash RA, Bowen JD, McSweeney PA, Pavletic SZ, Maravilla KR, Park MS, et al. High-dose immunosuppressive therapy and autologous peripheral blood stem cell transplantation for severe multiple sclerosis. *Blood* 2003;102:2364-72.
27. Burt RK, Cohen BA, Russell E, Spero K, Joshi A, Oyama Y, et al. Haematopoietic stem cell transplantation for progressive multiple sclerosis: failure of a total body irradiation-based conditioning regimen to prevent disease progression in patients with high disability scores. *Blood* 2003;102:2373-8.
28. Fassas A, Passweg JR, Anagnostopoulos A, Kazis A, Kozak T, Havrodova E, et al; Autoimmune Disease Working Party of the EBMT (European Group for Blood and Marrow Transplantation). Hematopoietic stem cell transplantation for multiple sclerosis: A retrospective multicenter study. *J Neurol* 2002;249:1088-97. Erratum in: *J Neurol* 2002;249:1619.
29. Fassas A, Kimiskidis VK. Autologous hemopoietic stem cell transplantation in the treatment of multiple sclerosis: rationale and clinical experience. *J Neurol Sci* 2004;223:53-8.
30. Mancardi GL, Saccardi R, Filippi M, Gualandi F, Murialdo A, Inglese M, et al; Italian GITMO-NEURO Intergroup on Autologous Hematopoietic Stem Cell Transplantation for Multiple Sclerosis. Autologous hematopoietic stem cell transplantation suppresses Gd-enhanced MRI activity in MS. *Neurology* 2001;57:62-8.
31. Fassas A, Anagnostopoulos A, Kazis A, Kapinas K, Sakellari I, Kimiskidis V, et al. Autologous stem cell transplantation in progressive multiple sclerosis – an interim analysis of efficacy. *J Clin Immunol* 2000;20:24-30.
32. Comi G, Kappos L, Clanet M, Ebers G, Fassas A, Fazekas F, et al; BMT-MS Study Group. Guidelines for autologous blood and marrow transplantation in multiple sclerosis: a consensus report written on behalf of the European Group for Blood and Marrow Transplantation and the European Charcot Foundation. *J Neurol* 2000;247:376-82.
33. Barkhof F, Filippi M, Miller DH, Scheltens P, Campi A, Polman CH, et al. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. *Brain* 1997;120:2059-69.
34. Loh SM, Ratnagopal P, Tan HC, Goh YT, Koh BC, Koh LP, et al. Successful autologous haematopoietic stem cell transplants for severe multiple sclerosis with fludarabine and cyclophosphamide conditioning [letter]. *Int J Hematol* 2006;83:368-9.
35. Openshaw H, Stuve O, Antel JP, Nash R, Lund BT, Weiner LP, et al. Multiple sclerosis flares associated with recombinant granulocyte colony-stimulating factor. *Neurology* 2000;54:2147-50.
36. Burt RK, Fassas A, Snowden J, van Laar JM, Kozak T, Wulffraat NM, et al. Collection of hematopoietic stem cells from patients with autoimmune diseases. *Bone Marrow Transplant* 2001;28:1-12.
37. Euler HH, Marmont AM, Bacigalupo A, Fastenrath S, Dreger P, Hoffknecht M, et al. Early recurrence or persistence of autoimmune diseases after unmanipulated autologous stem cell transplantation. *Blood* 1996;88:3621-5.
38. Bomberger C, Singh-Jairam M, Rodney G, Guerriero A, Yeager AM, Fleming WH, et al. Lymphoid reconstitution after autologous PBSC transplantation with FACS-sorted CD34+ hematopoietic progenitors. *Blood* 1998;91:2588-600.
39. Sica S, Salutari P, La Barbera EO, Sora F, Piccirillo N, Leone G. Infectious complications after CD-34 selected autologous peripheral blood stem cell transplantation. *Br J Haematol* 1998;101:592-3.
40. van Bekkum DW. Stem cell transplantation in experimental models of autoimmune diseases. *J Rheumatol* 1997;48(suppl):30-5.
41. Moore J, Brooks P, Milliken S, Biggs J, Ma D, Handel M, et al. A pilot randomized trial comparing CD34-selected versus unmanipulated hematopoietic stem cell transplantation for severe, refractory rheumatoid arthritis. *Arthritis Rheum* 2002;46:2301-9.
42. Omaya Y, Cohen B, Traynor A, Brush M, Rodriguez J, Burt RK. Engraftment syndrome: a common cause for rash and fever following autologous hematopoietic stem cell transplantation for multiple sclerosis. *Bone Marrow Transplant* 2002;29:81-5.
43. Murphy CB, Hashimoto SA, Graeb D, Thiessen BA. Clinical exacerbation of multiple sclerosis following radiotherapy. *Arch Neurol* 2003;60:273-5.
44. Kozak T, Hardova E, Pit'ha J, Gregora E, Pytlík R, Maaloufova J, et al. High-dose immunosuppressive therapy with PBPC support in the treatment of poor risk multiple sclerosis. *Bone Marrow Transplant* 2000;25:525-31.
45. Blanco Y, Saiz A, Carreras E, Graus F. Autologous haematopoietic-stem-cell transplantation for multiple sclerosis. *Lancet Neurol* 2005;4:54-63.
46. Burt RK, Slavín S, Burns WH, Marmont AM. Induction of tolerance in autoimmune diseases by hematopoietic stem cell transplantation: getting closer to a cure? *Blood* 2002;99:768-84.
47. Loberiza FR Jr, Zhang MJ, Lee SJ, Klein JP, LeMaistre CF, Serna DS, et al. Association of transplant center and physician factors on mortality after hematopoietic stem cell transplantation in the United States. *Blood* 2005;105:2979-87.
48. Tyndall A, Gratwohl A. Blood and marrow stem cell transplants in autoimmune disease: a consensus report written on behalf of the European League against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant* 1997;19:643-5.
49. Griffith LM, Pavletic SZ, Tyndall A, Bredeson CN, Bowen JD, Childs RW, et al. Feasibility of allogeneic hematopoietic stem cell transplantation for autoimmune disease: position statement from a National Institute of Allergy and Infectious Diseases and National Cancer Institute-Sponsored International Workshop, Bethesda, MD, March 12 and 13, 2005. *Biol Blood Marrow Transplant* 2005;11:862-70.