

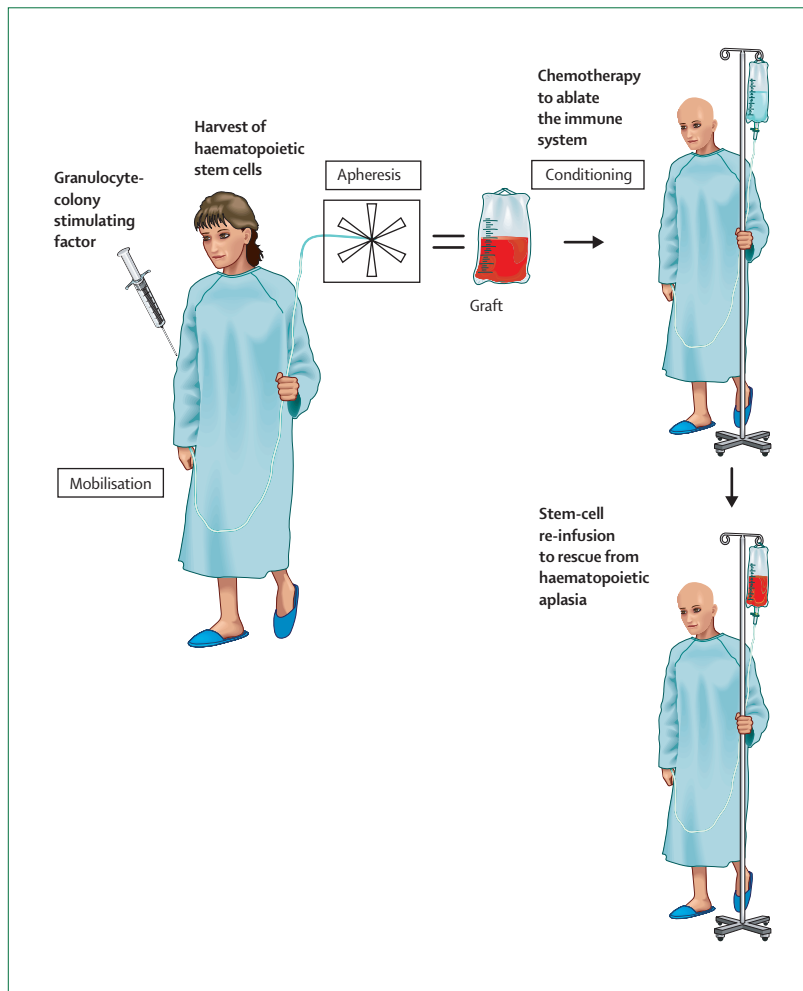
# Autologous haematopoietic-stem-cell transplantation for multiple sclerosis

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Services of Neurology (Y Blanco MD, A Saiz MD, F Graus MD) and Bone Marrow Transplantation Unit (E Carreras MD), Hematology, Hospital Clínic, Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), University of Barcelona, Spain

Correspondence to: Dr Albert Saiz, Service of Neurology, Hospital Clínic Villarroel 170, Barcelona 08036, Spain  
asaiz@clinic.ub.es

Intense immunosuppression followed by autologous haematopoietic-stem-cell transplantation (HSCT) is being assessed as a potential treatment for patients with severe multiple sclerosis (MS). The treatment was developed from research that showed autologous HSCT was as effective as allogeneic HSCT in the treatment of experimental autoimmune encephalomyelitis. The treatment is thought to eradicate the defective immune system, and the infused haematopoietic stem cells reconstitute an immune system that is more tolerant to the nervous system. About 250 patients with MS have been treated with autologous HSCT as part of phase I and phase II open trials. Autologous HSCT seems feasible in MS and assessment with clinical and MRI measures suggests it induces a profound and long-lasting suppression of inflammation. The course of MS seems to be stabilised after autologous HSCT, especially in ambulatory patients with evidence of active disease. Autologous HSCT deserves further study in randomised controlled trials.



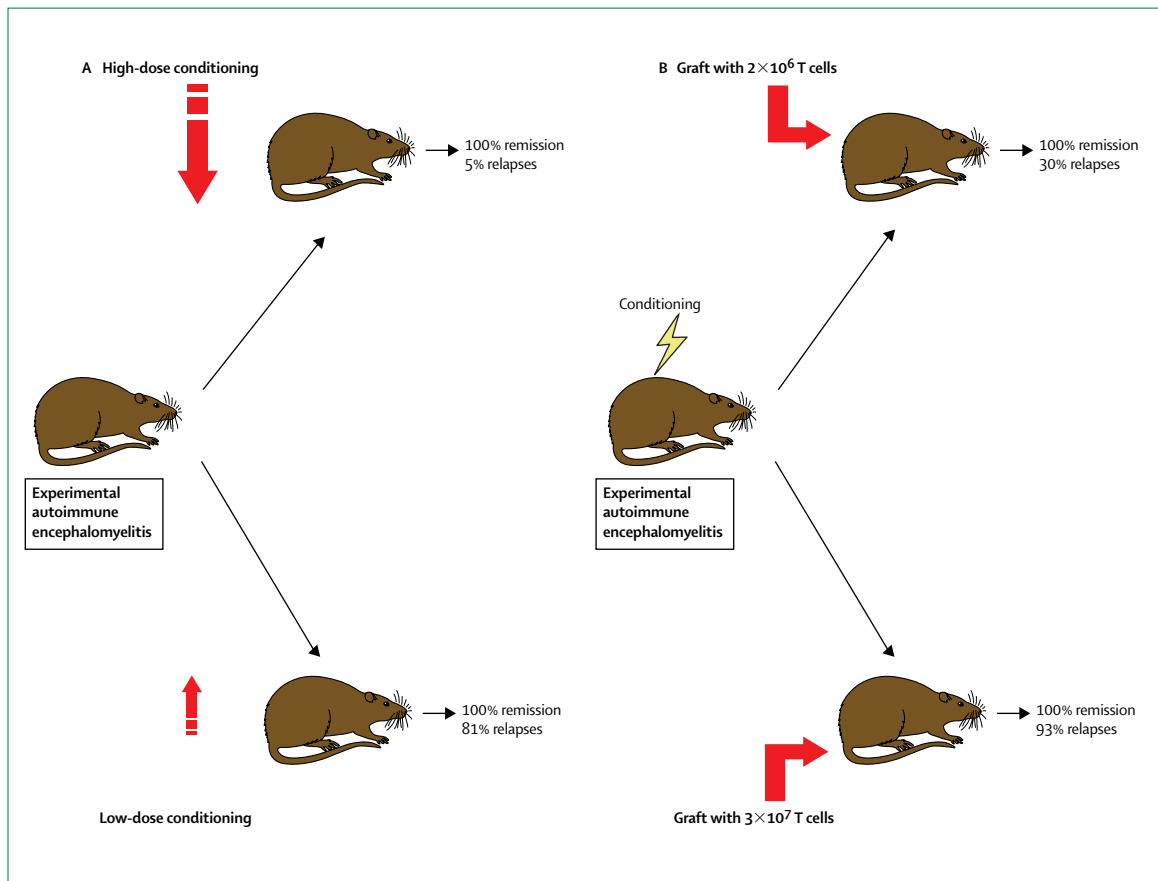
**Figure 1: Autologous HSCT**

The mobilisation of haematopoietic stem cells from bone marrow to peripheral blood is done by subcutaneous injection of granulocyte-colony stimulating factor. Serial peripheral-blood analysis establishes the optimal day (1–2 weeks later) for apheresis (extraction of peripheral blood haematopoietic stem cells with an apheresis machine that uses an immunomagnetic method). The apheresis product is the graft, which will be stored until used. Next, the patient receives the conditioning regimen (chemotherapy with or without total body irradiation) to ablate the immune system. Subsequently, the graft is re-infused to the patient.

Multiple sclerosis (MS) is thought to be an autoimmune disorder in which aberrant immune responses lead to T-cell mediated focal myelin destruction and secondary oligodendrocyte and axonal damage. Although the disease course is highly variable, 50% of patients will not be able to walk independently within 15 years of onset.<sup>1,2</sup> Current treatments for MS include immunomodulatory and immunosuppressive drugs. Interferon beta and glatiramer acetate reduce the number of relapses, but if these therapies are not successful or the disease develops into a progressive phase there are no effective treatments for modification of the course of the disease.<sup>3</sup> Mitoxantrone showed the clinical progression of secondary progressive MS in a randomised clinical trial, although its long-term clinical effect is unknown.<sup>4</sup> The limited effectiveness of these treatments justifies the assessment of alternative therapeutic strategies in patients with MS with aggressive clinical course.

Bone-marrow transplantation is the standard treatment for several haematological malignant disorders and is being assessed for the treatment of severe forms of several autoimmune disorders including MS.<sup>5</sup> Haematopoietic progenitor cells might re-establish the defective immune system in patients with autoimmune disorders. The cells can be obtained from a sibling or an unrelated donor who is closely matched on HLA (allogeneic transplantation), an identical twin (syngeneic transplantation), or the patient before chemotherapy (autologous transplantation). The haematopoietic progenitor cells can be directly harvested from the bone marrow or collected from peripheral blood; the term haematopoietic-stem-cell transplantation (HSCT) includes both sources (figure 1).

In allogeneic HSCT chronic immunosuppression is needed to prevent graft rejection and graft-versus-host disease. Allogeneic HSCT is also associated with higher mortality rates than autologous HSCT—up to 40% if the donor is not a sibling. By contrast, the mortality from autologous HSCT typically is less than 10%.<sup>5</sup>



**Figure 2: Effect of conditioning and T-cell number on allogeneic and autologous bone marrow transplantation**

(A) Effect of the intensity of conditioning on the efficacy of allogeneic bone marrow transplantation in rats with chronic relapsing-remitting experimental autoimmune encephalomyelitis. The use of a suboptimal or low-dose conditioning (in this experiment 7 Gy of total body irradiation) induces complete remission, but significantly more spontaneous relapses than high-dose conditioning (10 Gy total body irradiation) treatment.<sup>13</sup> These results reflect the importance of the eradication of host T cells to achieve optimal results. (B) Effect of the number of T cells in the graft on the efficacy of autologous bone marrow transplantation in rats with chronic relapsing-remitting experimental autoimmune encephalomyelitis. The use of a T-cell enriched graft (T cells added to those typically present in bone marrow) after the same type of conditioning led to a significantly higher incidence of relapse.<sup>15</sup> These results support that the use of a T-cell depleted graft for autologous HSCT in MS should be recommended.

### Rationale and experimental models

The hypothetical basis for the use of allogeneic HSCT to treat MS is that the procedure will eradicate the abnormal immune system and establish a new one that is more tolerant to the nervous system. Since Morton and Siegel described the development of antinuclear antibodies in normal mice after allogeneic HSCT from NZB mice—a strain of mice that spontaneously develop a systemic-lupus-erythematosus-like disease<sup>6</sup>—experimental and clinical reports have confirmed the possibility of patients developing an autoimmune disorder from the haematopoietic stem cells of affected donors.<sup>7–9</sup> Laboratory animals with autoimmune disorders can be cured or improved by allogeneic HSCT from healthy donors.<sup>7</sup> The complete remissions obtained with allogeneic HSCT in animal models of several autoimmune disorders, including experimental allergic encephalomyelitis, were shown in human beings by anecdotal reports of patients who had malignancies and

a coincidental autoimmune disorder and were treated with allogeneic HSCT.<sup>8–12</sup>

Despite promising reports, allogeneic HSCT cannot be used as a treatment for MS because high transplantation-associated toxicity precludes its application in diseases that are not life threatening. In addition, because we do not understand the mechanisms that lead to failure of tolerance in MS and other autoimmune disorders, we cannot be certain that tolerance will develop in cells grafted into the same or similar environment to that implicated in its loss.

Could autologous HSCT be a logical therapeutic approach for MS? The toxicity of the procedure may be acceptable for the treatment of patients with very aggressive forms of MS. However, the infused haematopoietic stem cells reproduce an immune system with potentially the same genetic defects. If MS is the result of an interaction between environmental factors and genetic predisposition, the rationale for the possible

**Panel 1: Guidelines on autologous HSCT****Consensus****Centre**

Should be done in accredited bone-marrow transplant unit with experience in allogeneic HSCT

**Source of haematopoietic stem cells**

Peripheral blood

**Mobilisation**

Cells mobilised with GCSF plus cyclophosphamide or GCSF given alone\*

**Target cell dose for re-infusion**

$>2 \times 10^6$  CD34<sup>+</sup> cell/kg

**Antithymocyte globulin**

Given if depletion not done†

**No consensus****Ex vivo T-cell depletion**

3 Log (1000 times fewer T cells) to 4 Log (10 000 times fewer T cells) depletion of T cells

**Conditioning regimen**

Several used

GCSF=granulocyte-colony stimulating factor. \*If GCSF is given alone, MS flares prevented by steroids. CD34 is the antigen that identifies haematopoietic-stem cells. †Concomitant use of high-dose steroids (500 mg) to prevent neurological deterioration related to drug-induced fever.

beneficial effect of autologous HSCT would be that self tolerance will not be broken during the development of the new immune system because external factors that were present during the initial development of the immune system are absent.

Studies in animals with experimental allergic encephalomyelitis have shown that autologous HSCT can induce remissions of the disease. However, the occurrence of spontaneous and induced relapses was higher after autologous than after allogeneic HSCT.<sup>13–15</sup> To induce long-lasting remissions in MS and other autoimmune diseases autologous HSCT must eradicate all of the autoreactive lymphocyte population of the patient, including those in the target organ, and ensure minimum number of autoreactive T cells in the graft that is re-infused (figure 2).

**Practical issues in autologous HSCT**

As in other medical procedures, the immediate toxicity of the autologous HSCT depends on the expertise of the transplant team and the measures taken to prevent infectious complications. To guarantee the lowest rate of complications in patients with MS who have autologous HSCT, they must be treated by transplant teams with approved protocols for allogeneic HSCT and in isolated rooms with laminar airflow.<sup>16</sup>

Autologous HSCT is a complicated procedure with several steps that have not been done uniformly among

different centres with approved protocols.<sup>16,17</sup> These steps may be important for outcome, including the success of improving the MS clinical course. A general consensus has not been achieved for all the issues (panel 1).<sup>18</sup>

**Source of haematopoietic stem cells**

Haematopoietic stem cells are mainly found in the bone marrow but they can be mobilised to the peripheral blood, in large numbers, by the administration of recombinant granulocyte colony-stimulating factor. Haematological (neutrophil, red cell, and platelet) and immune recovery is faster with peripheral blood cells than with bone marrow cells, owing to more rapid engraftment.<sup>19</sup>

Flares of MS, rheumatoid arthritis, and exacerbation of neurological symptoms in systemic lupus erythematosus have occurred while patients were taking granulocyte colony-stimulating factor.<sup>20–22</sup> Although the mechanism is unclear, it seems to be related to lymphocyte activation by release of cytokines. The concomitant use of steroids or cyclophosphamide with the granulocyte colony-stimulating factor is probably effective to suppress or at least reduce the risk of this complication.<sup>20,23</sup>

**Graft manipulation**

Experimental studies suggest the use of an unmanipulated graft increases the risk of disease recurrence because more autoreactive T cells are infused in the graft.<sup>15,24</sup> Furthermore, the number of T cells in the peripheral-blood transplant are much higher than in transplants where the haematopoietic stem cells are obtained from the bone marrow.<sup>25</sup> The number of re-infused T cells can be reduced ex vivo by T-cell depletion of the graft or in vivo by use of antithymocyte globulin, a potent lymphocytotoxic drug.<sup>18,26</sup> The convenience of T-cell depletion of the graft and the intensity of the depletion is debated. Data from allogeneic HSCT suggests that depletion should achieve a T-cell dose less than  $10^5$ /kg to prevent re-expansion of autoreactive T cells (panel 1).<sup>16</sup> Although haematological recovery is not different between T-cell depleted and unmanipulated grafts,<sup>27</sup> the former slow down quantitative T-cell reconstitution, especially the CD4 T-cell counts.<sup>28–31</sup> In addition, aggressive T-cell depletion may result in increased opportunistic infections and lymphoproliferative disorders.<sup>32–34</sup>

The effect of T-cell depletion of the graft in the development of MS is unknown. In a pilot randomised trial comparing T-cell depleted with unmanipulated autologous HSCT for severe rheumatoid arthritis, a similar outcome was observed in the two arms after a follow-up of 1 year.<sup>35</sup> This result is not unexpected given that, in contrast to what happens in experimental allergic encephalomyelitis,<sup>15</sup> the addition of T cells to the graft does not increase the relapse rate in an adjuvant arthritis experimental model.<sup>36</sup> An alternative explanation is that

the conditioning was insufficient to eradicate the host's autoreactive T cells. The absence of randomised studies to address this issue in patients with MS prevents any statement on the advantage of T-cell depleted grafts in autologous HSCT.

### Conditioning regimens

Conditioning regimens use a combination of cytotoxic drugs, and sometimes radiotherapy, to eliminate the immune cells, including those in the target organ. In MS, the conditioning regimen must destroy the autoreactive T cells in the nervous system, which are probably protected by the blood-brain barrier. There is no consensus on the ideal conditioning regimen to use in autologous HSCT for MS (table 1).<sup>17,37-46</sup>

Some protocols include total body irradiation as part of the conditioning regimen. In experimental allergic encephalomyelitis, total body irradiation as a single treatment has proved effective in the control of the disease and destroys the immune cells in the brain.<sup>13-15,47,48</sup> However, the use of radiotherapy in MS is controversial because it has been associated with worsening of disease activity in experimental allergic encephalomyelitis and may induce MS relapses in patients who receive cranial radiotherapy.<sup>13,49-51</sup> Use of total body irradiation at a dose of 800-1200 cGy as conditioning for MS was not associated with severe neurological toxicity.<sup>42,43</sup> Total body irradiation has been associated with high risk of solid tumours.<sup>52</sup>

The most common conditioning regimen used in autologous HSCT to treat patients with MS is BEAM (carmustine, etoposide, cytarabine, and melphalan), a standard treatment of patients with lymphomas who receive autotransplants (table 1).<sup>37</sup> This regimen, without T-cell depletion of the graft, and treatment with antithymocyte globulin after re-infusion of the graft has been selected for use in the open, randomised, phase III trial that will compare the efficacy of autologous HSCT with that of mitoxantrone to treat severe forms of MS.<sup>53</sup>

Besides the effectiveness of the conditioning regimen in T-cell depletion, there are other considerations in the selection of a regimen: the potential mortality risk of the treatment to patients in whom the expected risk of death from the disease is only slightly increased<sup>54</sup> and the long-term risk of cancer associated with the treatment.<sup>52</sup>

### Clinical studies

Worldwide about 250 patients with MS have been treated with autologous HSCT. The Autoimmune Disease Working Party registry of the European Group for Blood and Marrow Transplantation<sup>53</sup> collected 168 cases up to June 23, 2004 (Dr A Tyndall for the European Group for Blood and Marrow Transplantation, personal communication). Data on toxicity and clinical outcome for the first 85 patients are available as result of a multicentre retrospective observational analysis.<sup>17</sup> In

Study	Regimen	Number of patients	T-cell depletion of the graft
Fassas et al <sup>17</sup>	BEAM ± ATG	54	In 40 patients
Fassas et al <sup>17,38</sup>		25	In some patients*
Kozak et al <sup>39,40</sup>		10	In 7 patients
Mancardi et al <sup>41</sup>		10	No
Nash et al <sup>42</sup>	Cy + TBI + ATG	26	Yes
Burt et al <sup>43</sup>	Cy + TBI	21	Yes
Carreras et al <sup>44,45</sup>	Carm + Cy + ATG	14	Yes
Fassas et al <sup>17,38</sup>	Busulfan + ATG	10	In some patients*
Openshaw et al <sup>46</sup>	Busulfan + Cy + ATG	5	Yes
Fassas et al <sup>17</sup>	Fludarabine + ATG	1	Unknown*

BEAM=BCNU, etoposide, cytosine arabinoside, melphalan; ATG=antithymocyte globulin; Cy=cyclophosphamide; TBI=total body irradiation; Carm=carmustine; \*Not stated in the paper

**Table 1: Conditioning regimens used in autologous HSCT for MS**

addition, several single-centre series from Europe and North America have been reported since 1997. Several patients described in these series were also included in the European Group for Blood and Marrow Transplantation report (table 1).

All the reported studies were phase I and phase II clinical trials assessing the feasibility and toxicity of the procedure rather than the efficacy. The series were heterogeneous for eligibility criteria and the procedures of stem-cell mobilisation, graft manipulation, and transplantation. The mean follow-up in some series was too short. Despite these shortcomings, the studies helped to improve the clinical criteria for the selection of patients with MS for the procedure (panel 2) and provided information on the morbidity of the autotransplant, the effect of this treatment on the immune reconstitution, and on the outcome of MRI and CSF variables.

#### Panel 2: Proposed selection criteria in protocols of autologous HSCT for MS

##### Inclusion criteria\*

- Relapsing-remitting MS with cumulative deficits
- Secondary-progressive MS with or without relapses
- Age 18-50 years
- Current EDSS 3.5-6.5
- Increase of the EDSS in the last year:
  - at least 1.5 points if EDSS is 3.5-5.0, or at least 1.0 point if EDSS is ≥5.5
  - or
  - at least 1.0 point if EDSS is 3.5-5.0, or at least 0.5 point if EDSS is ≥5.5 with at least one enhancing lesion in brain MRI
- Progression despite immunomodulating treatment

\*Inclusion criteria of the ASTIMS trial (phase III study to compare efficacy and safety of autologous HSCT vs mitoxantrone therapy). These criteria are from the guidelines of the Milan consensus conference<sup>18</sup> and from the cumulative experience on autologous HSCT for MS.

Study	Number of patients	MS type	EDSS median	Conditioning regimen	Number and cause of deaths			
					Disease	Treatment-related progression		Overall mortality
						Infection	Other	
Fassas et al <sup>17</sup>	85	3 RR 60 SP 22 PP	6.5 (4.5–8.5)	Several	2 (at 63 and 81 days)	4	1	8.2%
Fassas et al <sup>37,38</sup>	35	2 RR 19 SP 14 PP	6.0 (4.5–8.0)	BEAM+ATG or ± T-cell depletion	Busulfan+ATG	1	1	5.7%
Nash et al <sup>42</sup>	26	1 RR 17 SP 8 PP	7.0 (5.0–8.0)	Cy+TBI+ATG + T-cell depletion	1 (at 23 months)	1		7.7%
Burt et al <sup>43</sup>	21	1 RR 14 SP 6 PR	6.5 (3.0–8.5)	Cy+TBI + T-cell depletion	2 (at 13 and 23 months)			9.5%
Saiz et al <sup>45</sup>	14	9 SP 5 RR	6.0 (4.5–6.5)	Carm+Cy+ATG + T-cell depletion				0%
Kozak et al <sup>39,40</sup>	10	SP	6.5 (5.5–7.5)	BEAM+ATG or BEAM+ T-cell depletion				0%
Mancardi et al <sup>41</sup>	10	SP	6.5 (5.0–6.5)	BEAM+ATG unmanipulated				0%

RR=relapsing-remitting; SP=secondary-progressive; PP=primary-progressive; PR= progressive-relapsing; BEAM=carmustine+etoposide+cytarabine+melfalan; ATG=antithymocyte globulin; Cy=cyclophosphamide; TBI=total body irradiation; Carm=carmustine

**Table 2: Mortality of patients with MS after HSCT**

### Toxicity

The mortality in the European Group for Blood and Marrow Transplantation report, defined as death by any cause, was 8.2%.<sup>17</sup> There were five toxic deaths—four patients died from infection and one patient died from cardiac failure—and two patients died 3 months after the transplant from disease progression. Similar data were found in two North American series of patients treated with a combination of total body irradiation and cyclophosphamide with extensive T-cell depletion. Four of 47 patients (8.5%) treated with this regimen died. Three patients died from disease progression and one died from Epstein-Barr virus-related post-transplant lymphoproliferative disorder (table 2).<sup>42,43</sup>

When interpreting the European Group for Blood and Marrow Transplantation report, some limitations should be noted.<sup>17</sup> The report states that five of the seven dead patients did not fulfil the Milan consensus on old age and high expanded disability status scale (EDSS) score inclusion criteria.<sup>18</sup> Furthermore, three of seven patients were treated with a busulfan-based regimen (mortality in this group of patients was 20%) and another patient was the only one (of the 85 patients in this study) that was treated with a fludarabine-based regimen. All patients were not treated in institutions with the same expertise in HSCT. In our opinion, mortality risks might be lowered by better selection of patients and treatment in centres accredited for allogeneic transplantation.

Short-term toxicity after autologous HSCT in patients with MS is related to an occurrence of infections and engraftment syndrome—a non-infectious episode of fever

and sometimes skin rash at the time of haematological recovery—compared with autologous HSCT for other indications.<sup>37,55</sup> Up to 27% of patients given HSCT for MS have a neurological deterioration that is associated with fever and infections and is commonly transient but can be irreversible (7%).<sup>17</sup> The use of higher doses of prednisone to prevent the fever induced by ATG or the flares associated with G-CSF, if cyclophosphamide is not included in the mobilisation, probably will reduce the incidence of these complications.<sup>42,44,46</sup>

Although autologous HSCT causes a profound immunosuppression, few long-term opportunistic infections have been reported.<sup>23,38</sup> The occurrence of permanent amenorrhoea was observed in 30% of women older than 37 years at the time of the transplant,<sup>45</sup> a percentage that is not different from that reported in patients treated with mitoxantrone.<sup>4</sup> After the autologous HSCT, some patients with MS develop other autoimmune disorders such as autoimmune thyroiditis,<sup>17,38,42</sup> coagulopathy due to factor VIII-inhibitor,<sup>38</sup> uveitis,<sup>44</sup> lymphocytic gastritis, and brachial neuritis.<sup>42</sup> The transient presence of organ-specific autoantibodies is a well-known event but clinically evident autoimmune disorders are rarely observed after autologous HSCT.<sup>56</sup> Possible explanations include inhibition of the thymus-dependent clonal deletion of autoreactive T lymphocytes and an increased threshold of peripheral autoregulation.<sup>57</sup>

### Clinical outcome

At the Milan consensus conference on the role of autologous HSCT in MS,<sup>18</sup> the procedure was suggested to be effective only if the rate of treatment failure at 3 years is less than 20%. This means that 80% of the

Study	Number of patients	MS type	Median baseline EDSS	Median follow-up	Definition of progression	3 year progression-free survival	Definition of activity	3-year activity-free survival
Fassas et al <sup>17</sup>	85	3 RR 60 SP 22 PP	6.5 (4.5–8.5)	16 months (3–59)	Increase of 1.0 point	SP+RR=78% if basal EDSS ≤5.0 or 0.5 points if basal EDSS ≥5.5	Progression PP=66% Progression after improvement	55% Relapse
Nash et al <sup>42</sup>	26	1 RR 17 SP 8 PP	7.0 (5.0–8.0)	24 months (3–36)	1.0 point basal EDSS increase	73%		Not analysed
Fassas et al <sup>37</sup>	24	3 PR 13 SP 8 PP	6.0 (4.5–8.0)	40 months (21–55)	Increase of 1.0 point if basal EDSS ≤5.0 or 0.5 points if basal EDSS ≥5.5 PP=39%	RR=100% SP=92%	Progression Relapse	PR: 0% SP: 12% PP: 0%
Saiz et al <sup>45</sup>	14	9 SP 5 RR	6.0 (4.5–6.5)	36 months (19–55)	Any basal EDSS increase	85.7%	Progression Relapse Progression after improvement AI worsening of 1 point	46.4%

RR=relapsing-remitting; SP=secondary-progressive; PP=primary-progressive; PR= progressive-relapsing; AI=ambulation index

**Table 3: Clinical outcome of patients with MS after autologous HSCT**

patients who had a transplant should not have confirmed progression as measured by the EDSS score. This proportion was derived from a meta-analysis of the progression-free survival in placebo groups included in treatment trials of progressive MS.

Only four series have reported their clinical outcome in comparable terms: probability of confirmed progression-free survival by Kaplan-Meier estimator (table 3). In all of them, the results were close to the established objective of efficacy of the Milan consensus conference. Another method to assess the clinical efficacy of the autologous HSCT is by recording any event after transplantation that indicates disease activity. This secondary outcome, disease-activity-free survival, defined as the probability of being alive without progression of any type, including no increase of the EDSS score, no increase of the EDSS score after initial improvement (even when the final EDSS is lower than the baseline EDSS score), and absence of relapses, was analysed in three studies. These series showed that less than 50% of patients achieved disease activity-free survival (table 3). These data suggest that autologous HSCT cannot be deemed a curative treatment for MS but support the view that it may cause prolonged stabilisation or change the aggressive course of the disease in the patients who are treated.

The studies of autologous HSCT in MS have not been blinded or have not randomly assigned patients to an alternative therapy, for example mitoxantrone. Therefore they have not answered the important question of whether the procedure is effective in modifying the progressive course of the disease. However, they provided important information on which patients may benefit from the autotransplant

and when the treatment should be indicated in future prospective randomised protocols to test the efficacy of the procedure. Primary-progressive MS probably does not benefit from autologous HSCT. Less than 70% of patients with primary progressive MS had a stabilisation of the neurological deficit measured by the EDSS (table 3) and no patients were disease-activity free at 3 years.<sup>17,37</sup> Autologous HSCT probably will not help patients with high EDSS at entry. In one study, none of the nine patients with pre-transplant EDSS scores of 6.0 or lower progressed at least 1 point in the baseline EDSS score after a median follow-up of 18 months, in contrast with four out of 12 (33.3%) patients with pre-transplant EDSS scores of at least 6.5.<sup>43</sup> Lastly, the treatment has been very effective in reducing the number of relapses in those patients with MS who did not benefit from other immunomodulatory therapies.<sup>45</sup> These data suggest that the efficacy of autologous HSCT should be tested in patients with evidence of active MS, defined as patients with frequent relapses that are not controlled by accepted treatment and who have neurological dysfunction that has not exceeded an EDSS score of 6.0.

#### MRI variables

MRI assessment has been included in most series as an additional outcome measure after autologous HSCT.<sup>17,40–43,45,46</sup> All studies included the number of gadolinium-enhancing T1 lesions and new T2-weighted lesions as a measure of disease activity, but only a few studies provided additional information on measures of disease burden or brain atrophy.<sup>42,45,58</sup>

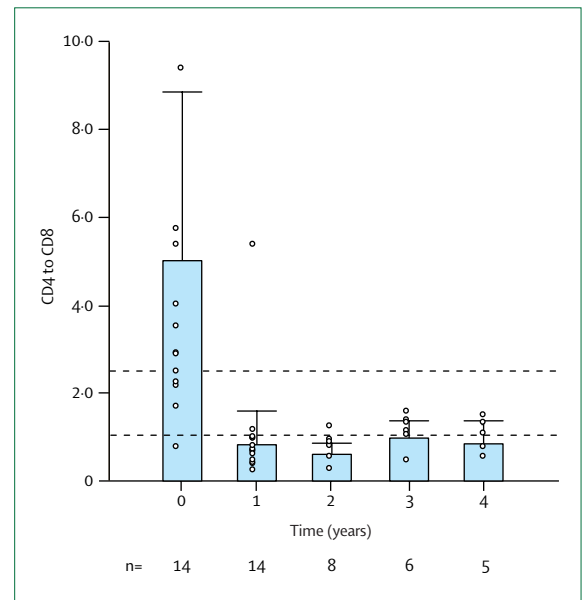
The most impressive finding in all these studies is that autologous HSCT induces a profound and long-

lasting suppression of gadolinium-enhancing lesions. New gadolinium-enhanced lesions were rarely seen in the serial MRIs done after autologous HSCT.<sup>17,40–43,45,46</sup> In a series of ten patients, serial monthly MRI during the 3 month pretreatment period detected a total of 341 gadolinium-enhancing lesions. Eight of the ten patients did not present with gadolinium-enhancing lesions in the serial MRIs done after transplantation. The other two patients presented with five gadolinium-enhancing lesions during the first 3 months post-transplantation that disappeared over the next 24 months.<sup>41</sup> This effect seems to persist more than 3 years after the transplantation.<sup>45</sup> The positive effect on gadolinium-enhanced lesions was also observed in other inflammatory variables as the T2 lesion load that had a median decrease higher<sup>45</sup> than that reported in trials of immunomodulatory therapies in MS.<sup>59</sup>

The issue of long-term progression of brain atrophy after autologous HSCT has been analysed in only two studies, with conflicting results.<sup>45,58</sup> We<sup>45</sup> reported a mean decrease of 12.71% in the corpus callosum area at 3 years post-transplant compared with baseline. The atrophy was highest during the first year, the time of the greatest reduction of T2 lesion load. Over the ensuing years, the percentage of reduction of the corpus callosum area was lower than that reported in patients with MS treated with interferon or placebo.<sup>60,61</sup> The association between the reduction of the T2 lesion load and brain atrophy suggested that the resolution of oedema and inflammation due to the autologous HSCT could explain part of the increase in the measures of brain atrophy.<sup>45</sup> By contrast, in another study<sup>58</sup> there was a mean yearly decrease of about 1.9% in brain volume over the 2 year follow-up. A percentage that is about two-times higher than those reported in studies of brain atrophy in MS.<sup>62</sup> These data would suggest that the degenerative process is not halted by autologous HSCT despite the effect on improving the inflammatory MRI variables. None of these studies assessed the rate of brain atrophy in the year before the autotransplant and the direct effect of autologous HSCT on brain atrophy is not well known.<sup>63</sup> These limitations prevent any unambiguous statement on the role of autologous HSCT in the development of axonal loss and other non-inflammatory mechanisms implicated in MS. Future protocols should be designed to specifically address this issue and measure the clinical effect with serial neuropsychological assessments.

### Oligoclonal IgG bands

The persistence of the CSF oligoclonal IgG bands after autologous HSCT has been reported in most studies.<sup>42,46,64</sup> In the single longitudinal study<sup>64</sup> on the development of oligoclonal bands in the CSF and serum after the autotransplant, CSF oligoclonal IgG bands



**Figure 3:** Change in CD4/CD8 ratio after autologous HSCT in 14 patients with MS<sup>45</sup>

Mean results are presented and error bars represent 95% CI of the mean. Dotted lines show the 25th and 75th percentiles in the control population; n=number of patients analysed at each point in time.

persisted at 3 months post-transplant. In addition, there were multiple bands in the serum and the albumin index was high suggesting a disruption of the blood–brain barrier. Some of the observed oligoclonal bands in the baseline CSF were also identified in the serum, commonly with a lower intensity; this suggests the B cells that synthesise IgG were in the CNS and the bands diffused to the serum owing to the disruption of the blood–brain barrier. At 12 months, all patients had the same pattern of oligoclonal bands identified in the baseline CSF and most of the serum bands had disappeared.

The development of serum bands after autologous and allogeneic HSCT is transient and seems to be related to the recovery of normal B-cell function.<sup>65</sup> The longitudinal analysis of the CSF oligoclonal IgG bands supports the idea that the B cells and presumably also the T cells located in the CNS at the time of the treatment, survived the conditioning regimen.<sup>64</sup> The persistence of the CSF bands after autologous HSCT with different protocols<sup>42,46</sup> suggests that the conditioning regimens used cannot completely eradicate the T-cells in the nervous system. The clinical significance of this observation is unclear.

### Immune reconstitution after transplantation

Information on immune reconstitution after autologous HSCT for MS is scarce and mostly limited to the first year post-transplant.<sup>17,39,42,44,66</sup> The results of the general immune reconstitution are similar to those observed after autologous HSCT for other

indications.<sup>67,68</sup> Thus, the number of B cells, natural killer cells, and CD3 T cells reach normal values in 3 months. However, the subset of CD4 T cells is decreased and that of CD8 T cells increased resulting in an inverted CD4/CD8 ratio that lasts during the first year post-transplant<sup>44,66</sup> and beyond the third year (figure 3). In the early (3–6 month) period post-transplant most of the CD4 T cells are exclusively CD45RO (memory) T cells consistent with selective T-cell expansion from pre-existing T cells that survived the transplantation.<sup>44,66</sup> The number of myelin-basic-protein-reactive T cells greatly diminishes in this early period.<sup>69</sup> CD45RA (naive) T cells (thymus-dependent regeneration) gradually increase after 6 months.<sup>44,66</sup> In this second phase of thymus-dependent pathway recovery there is an expansion of myelin-basic-protein-reactive T cells that recognised a broad repertoire of epitopes including those recognised before the transplant. If confirmed, these findings, obtained from a few patients,<sup>69</sup> support the hypothesis that clonal composition of the reconstituted immune system is not substantially different from the original immune system before transplantation. These data coupled with the profound and long-lasting immunosuppression of the treatment, suggest that immunosuppression may justify the effect of autologous HSCT on the improvement of the MRI inflammatory variables and, if proven in the future, the clinical efficacy.

## Conclusion

Phase I and phase II clinical trials on autologous HSCT for MS have provided important insights on the morbidity and the outcome of MRI and CSF variables. The findings suggest that the treatment is feasible in severe forms of MS provided that strict eligibility criteria are applied to patients and centres. Although the treatment induces a profound and long-lasting suppression of MRI activity associated with inflammation, whether the procedure is really effective in modifying the progressive course of the disease deserves further assessment in the setting of randomised controlled trials. Preliminary studies suggest that autologous HSCT causes an important and persistent immunosuppression rather than a change in the reconstituted immune system.

### Search strategy and selection criteria

Studies were identified by searches of PubMed from 1996 to June 2004 with the terms “multiple sclerosis”, “magnetic resonance imaging”, and “oligoclonal bands” and combining these terms with “haematopoietic-stem-cell transplantation” or “bone-marrow transplantation”. Studies were also identified from the personal files of the authors. Abstracts and reports from meetings were not included.

### Authors' contributions

YB and AS did the reference search, selected the references, and wrote the first draft of the review. EC gave his expert opinion and reviewed the haematological parts of the review. FG critically read and revised the review.

### Conflicts of interest

We have no conflicts of interest.

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