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ORIGINAL ARTICLE

Brazilian experience with two conditioning regimens in patients with multiple sclerosis: BEAM/horse ATG and CY/rabbit ATG

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Studies have shown that autologous hematopoietic SCT (HSCT) can be used as an intensive immunosuppressive therapy to treat refractory patients and to prevent the progression of multiple sclerosis (MS). This is a prospective multicentric Brazilian MS trial comparing two conditioning regimens: BEAM/horse ATG and CY/rabbit ATG. Most (80.4%) of the 41 subjects in the study had the secondary progressive MS subtype and the mean age was 42 years. The baseline EDSS score in 58.5% of the subjects was 6.5 and 78% had a score of 6.0 or higher, respectively. The complication rate during the intra-transplantation period was 56% for all patients: 71.4% of the patients in the BEAM/hATG group and 40% in the CY/rATG group ($P = 0.04$). Three subjects (7.5%) died of cardiac toxicity, sepsis and alveolar hemorrhage, all of them in the BEAM/ATG group. EFS was 58.54% for a ll patients: 47% in the BEAM/hATG group and 70% in the CY/rATG group ($P = 0.288$). In conclusion, the CY/rATG regimen seems to be associated with similar outcome results, but presented less toxicity when compared with the BEAM/hATG regimen. Long-term follow-up would be required to fully assess the differences in therapeutic effectiveness between the two regimens.
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Introduction

Multiple sclerosis (MS) is an autoimmune disease mediated by autoreactive T lymphocytes that enter the central nervous system through small vessels and trigger an immunological cascade, which in turn induces further inflammatory and immune events.[1–4]

There are four clinical subtypes of MS. In the 'relapsing-remitting' subtype, patients suffer from acute attacks of neurological dysfunction that last for days to weeks, followed by remission periods during which they usually (although not always) recover from most or all loss of function. The 'primary progressive' subtype is characterized by gradual and irreversible functional decline from onset. 'Secondary progressive' MS begins as a relapsing-remitting subtype, but evolves over time into progressive neurological decline, with or without acute attacks. The less common subtype, 'progressive-relapsing' MS, shows functional decline between acute attacks.[5]

Multiple sclerosis treatment includes primary immunomodulators (β-IFN, copolymer, i.v. Ig),[1,5] corticosteroid therapy for the acute relapse phase, and after that, immunosuppressors (CYA, CY, azathioprine and mitoxantrone). However, some patients do not respond to treatment and therefore need therapies with more toxic drugs to reach or maintain remission, whereas other patients relapse despite therapy. The two latter categories of patients need alternative therapeutic approaches. Recently, the use of natalizumab, an α-4-selective adhesion antagonist, showed promising results in the reduction of risk for progression of sustained disability and the rate of clinical relapse in patients with relapsing MS when compared with placebo[6,7] as a first-line treatment and also as second-line treatment in relapse-remitting MS.[8] Rituximab reduces inflammatory brain lesions and clinical relapses in relapse-remitting MS.[9] However, attention

should also be given to the rise of opportunistic infections with the use of these new immunosuppressive drugs.

On the other hand, studies published since 1990 have presented animal models and theoretical considerations about autologous hematopoietic SCT (HSCT) for the prevention and treatment of MS.[5,10–18] Some clinical data suggest that high-dose chemotherapy followed by hematopoietic stem cell rescue could 'reset' the immune disorder by controlling autoreactive clones and by inducing self-tolerance after immunological recovery.[13,18–20]

Since the first HSCT reported by Fassas *et al*.,[21] in 1997, more than 300 HSCTs have been performed in MS patients worldwide.[22–24] The EBMT (European Group for Blood and Marrow Transplantation) has published the largest samples. Initially, the EBMT Group undertook a retrospective study, in which 74% of 85 patients remained stable (free of disease progression) for up to 3 years after HSCT. The authors found that patients with relapsing-remitting and secondary progressive MS subtypes, which have more inflammatory characteristics, had a progression-free survival rate of $78 \pm 13\%$, and among those with primary progressive MS, which is more degenerative, this rate was $66 \pm 23\%$ during 3 years.[25] In 2006, the same group published an updated analysis of 143 cases with a 41.7-month follow-up: the disease remained stable or improved in 63% of patients and progressed in 37%.[26]

Regimens that ablate the entire BM hematopoietic compartment are by definition myeloablative. By contrast, non-myeloablative regimens selectively target the immune compartment for ablation without irreversible erasure of the BM's ability to regenerate hematopoiesis. Intense myeloablative regimens used to treat MS, such as BU/CY[27] and CY/TBI/antilymphocyte globulin (CY/TBI/ATG),[28] showed significant treatment-related mortality. An intermediate-intensity conditioning regimen, BEAM (BCNU, cytosine arabinoside, melphalan and etoposide) is better tolerated and has a lower morbidity and mortality rate.[26,29] By contrast, a truly non-myeloablative regimen of CY and anti-lymphocyte antibodies has been used by Burt *et al*.[30,31] for HSCT in patients with MS and has also been used safely in numerous autoimmune diseases, including systemic lupus erythematosus,[32] type I diabetes[33] and systemic sclerosis.[34]

The mortality rate of HSCT for MS in studies with relatively large samples is described in Table 1.[25–30,35–45] Although these studies use myeloablative conditioning regimens of varying intensity, non-myeloablative regimens have been advocated for autologous HSCT of autoimmune diseases.[46] Recently, one of these latter studies was published using CY/rATG and it reported no deaths among 21 patients with relapsing-remitting MS.[31] There is a controversy regarding the analysis of the immune system modifications as increases in naive CD4 + T cells over memory cells[20] and the fact that more intensive regimens seem to present better results than less intensive regimens.[19,26,47] On the other hand, some studies have shown that non-myeloablative conditioning regimens containing CY +/−anti-T-cell antibodies induce deep changes in the immune system of patients with autoimmune diseases, with an increase in regulatory subsets of cells and of naive T cells, improvement in TCR diversity and re-establishment of the auto-tolerant state.[48,49] The implications of these findings in clinical studies are still to be seen. Herein, we compare the toxicity and outcomes of BEAM/hATG vs CY/rATG regimens for autologous HSCT in patients with MS in Brazil.

The objectives of this study are to describe the experience of the Brazilian Group of HSCT for Auto-immune Diseases with 41 HSCTs performed in MS patients during 5 years using two different conditioning regimens, and to compare them by evaluating the clinical response to treatment using Expanded Disability Status Scale (EDSS)[50] and with magnetic resonance imaging (MRI) before and after HSCT; complication and mortality rates after HSCT; and quality of life (QOL) before and after HSCT.

Patients, materials and methods

Between 2001 and 2006, 41 HSCTs for MS were performed in five Brazilian centers (Araújo Jorge Hospital in Goiânia; Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo in Ribeirão Preto; Hospital de Clínicas de Curitiba, Universidade Federal do Paraná in Curitiba; Faculdade de Medicina da Santa Casa de São Paulo and Hospital Israelita Albert Einstein, both in São Paulo). The Research Ethics Committees of all the institutions involved and the National Investigational Review Board (CONEP) approved the study protocol and all patients signed an informed consent form.

Between January 2001 and June 2004, subjects received the BEAM conditioning regimen including BCNU, cytosine arabinoside, melphalan and etoposide, with horse ATG, as described below (group 1). However, on account of the toxicity observed in the beginning of this study (three patients died of cardiac toxicity, sepsis or alveolar hemorrhage), this regimen was modified to the use of CY and rabbit ATG. The eligibility criteria remained the same. This study is an analysis of two groups of patients treated with different conditioning regimens. We had to modify the initially planned protocol (BEAM group) after an interim analysis of the first 21 patients, three of whom died, an unacceptable death rate according to the initial protocol, which allowed a maximum 10% mortality rate. The treatment was then modified to a potentially less-toxic regimen (CY group).

Between June 2004 and April 2006, patients were treated with high-dose CY and rabbit antilymphocyte serum (group 2). A total of 21 patients were included in group 1 and 20 patients in group 2, which allowed a comparative analysis between both groups. All subjects were followed up until November 2007.

Inclusion and exclusion criteria

Patients clinically diagnosed with MS according to Poser's criteria,[51] between 18 and 60 years old and evaluated by MRI were included in the study. Neurological disability was determined using the EDSS scale. For inclusion in the study, subjects had to have either relapsing-remitting, primary or secondary progressive, or progressive-relapsing

Table 1 Literature review of multiple sclerosis treatment with autologous hematopoietic SCT[25–30,35–45]

Site	*Regimen*	N	*Positive outcome*	*Failure (%)*	*Treatment-related mortality*	*Overall mortality (%)*
European multicentric 2002[25]	BEAM—16% BEAM/ATG—47% CY/ATG/other drugs—12% CY/TBI/ATG—6% BU/CY/ATG/other drug—18% Fludara/ATG—1%	85	PFS: 74% DAPFS: 55%	Worse: 7.0% Probability of disease progression: 20%	6%	8.2
European multicentric 2006[26]	BEAM/ATG—41% BEAM—17% BCNU/CY/ATG—11% TBI/CY/ATG—9% BU/ATG—6% Others—11% Unknown—5%	178	Stable or improve: 63.0%	Worse: 37%	5.3	8.8
Italian[29]	BEAM/ATG	19	PFS: 95% DAFS: 64%	Worse: 15.7%	0	0
Update[41]		21	PFS: 58.16% at 8.5 month	Worse: 38%		
Chicago[30]	CY/TBI	21	Stable or better: 62.0%	Worse: 38.1%	0	9.5
Barcelona[35]	BCNU/CY/ATG	15	Stable or improve: 80.0%	Worse: 13.3%	0	0
Update[39]		14	Stable or improve: 64% at 6 years	Worse: 35%		
US multicentric[28]	CY/TBI/ATG	26	Stable or improve: 76%	Worse: 24%	3.8	7.6
Prague[36]	BEAM/ATG or *in vitro* purged graft	10	Stable: 90.0%	Worse: 10.0%	0	0
Update[40]		33	Stable: 69% Better: 3.3% Lost follow-up: 6% At median 60 month	Worse: 27%		
Los Angeles[27]	BU/CY/ATG	5	Stable: 60%	Worse: 20%	20	40
Rotterdam[37]	CY/TBI/ATG	14	Stable or improve: 35.7%	Worse: 64.3%	0	7.1
Russia[38]	BEAM/ATG	45	PFS: 72%	Progression: 8.8%	0	2.2
Canada[42]	BU/CY/ATG	15	Stable or improve: 60%	Worse: 26%	6.6%	6.6%
China-(Peking/Beijin)[44,45]	BEAM/ATG	22	PFS: 77% Better: 59% Stable: 18%	Worse: 23%	0	0
(Shangai/Nanjing)[43]	CY/TBI or BEAM/ATG	21	PFS: 75% DAFS: 33.3%		9.5%	9.5%

Abbreviations: ATG = antilymphocyte globulin; Fludara = fludarabine monophosphate; BEAM = BCNU, cytosine arabinoside, melphalan, etoposide; DAFS = disease active-free survival; DAPFS = disease active progression-free survival, PFS = progression-free survival.

subtypes of MS with the course of the disease equal to or longer than a year; EDSS between 3.0 and 6.5; and documented disease progression over the previous 6 months despite therapy (with β-IFN, copolymers, Igs and/or immunosuppressors). Progression was defined as at least a 1.0-point deterioration in the EDSS in the last 6 months, when the patient's scores ranged between 3.0 and 6.0, or 0.5-point or more when it ranged between 6.0 and 6.5.

Patients were excluded when they had major comorbidities, such as kidney, heart, hepatic, pulmonary, hematological dysfunction or infectious disease; psychiatric disorders or cognitive disturbances affecting their ability to provide informed consent or to comply with the treatment; as well as pregnancy, β-IFN or copolymer therapy within less than a month or disease relapse within less than a month before the study.[51]

Mobilization of peripheral hematopoietic stem cells, conditioning regimens and hematological recovery

Mobilization of peripheral hematopoietic stem cells was obtained with the administration of CY 2 g/m^2, in one single dose, followed by G-CSF (filgrastim-granulocyte CSF), 10-μg/kg daily until reaching 1000 WBCs per mm^3 and/or at least 10 CD34 positive cells/mm^3 in peripheral blood counts. To avoid the cytokine release syndrome, methylprednisolone, at a dose of 1 mg/kg, was administered together with G-CSF. The minimum CD34-positive cell count in the final product was 3.0×10^6 cells/kg. Unmanipulated PBSCs were cryopreserved in 10% DMSO and autologous plasma at −196 °C or in 10% DMSO and HES (hydroxyethylstarch) at −80 °C following the standard procedures.[52]

Subjects underwent conditioning regimen therapy for 15 or more days after PBSC mobilization. Group 1 received a

BEAM/ATG conditioning regimen: BCNU at a dosage of 300 mg/m^2 on day −7; cytarabine at a dosage of 200 mg/m^2 and etoposide at a dosage of 200 mg/m^2 from day −6 to day −3, melphalan at a dosage of 140 mg/m^2 on day −2; and horse ATG (Lymphoglobuline, IMTIX-Sangstat, Lyon, France) at a dosage of 15 mg/kg on days −5, −3, −1, +1, +2 and +3, together with methylprednisolone at a dosage of 2 mg/kg/day. Group 2 patients were treated with a non-myeloablative regimen of CY, at a dosage of 50 mg/kg of body weight, used on days −5, −4, −3 and −2, together with rabbit anti-thymocyte globulin (Thymoglobulin, Genzyme, Cambridge, MA, USA) at a dosage of 0.5 mg/kg on day −6 and 1 mg/kg/day on days −5, −4, −3 and −2 followed by methylprednisolone 2 mg/kg. In both groups, on day 0, frozen PBSCs were thawed and reinfused intravenously. Steroid treatment was continued concurrent with G-CSF, and thereafter suspended. Both groups received an identical standard of care; anti-infectious prophylaxis with acyclovir 10 mg/kg from admission until day +35; fluconazole 200 mg/day from neutropenia until day +60; ciprofloxacin 200 mg twice a day from admission until reaching a granulocyte count of 500 cells/mm^3; sulfamethoxazole and trimethoprim twice a day from admission until day −1 and then from engraftment (defined as 2 days with more than 500 granulocytes/mm^3) up to a CD4 cell count above 250 cells/mm^3; thiabendazole 500 mg twice a day for 3 days before conditioning. During fever peaks (temperature >38 °C), blood and urine were collected for culture, chest X-rays or computed tomography (CT) were performed as needed and broad-spectrum antibiotic therapy was started and subsequently adjusted on the basis of culture results.

Granulocyte-CSF at a dose of 5 μg/kg/day was administered on day +5 until the granulocyte count was >1000/mm^3. All the blood components used were irradiated and leukocyte depleted. The criteria for blood component transfusion were hemoglobin <8.5 g per 100 ml and plts <20 000/μl. Granulocyte engraftment was defined as polymorphonuclear cell counts above 500/mm^3 on 2 consecutive days and plt counts above 20 000/mm^3 without requiring transfusion.

Clinical neurological and imaging assessment

Clinical neurological assessment (using EDSS score),[50,51] according to the European guidelines,[51] was performed in the initial evaluation (screening), in the evaluation just before hospital admission (baseline), 1 month after stem cell infusion and then every 6 months during the following 3 years. A neurology specialist who was aware of the study protocol, and who could or could not be involved with the patients' treatment, carried out the neurological evaluations.

Besides the clinical neurological evaluation, imaging assessment was scheduled before mobilization and 30 days, 6 months and yearly after transplantation. MRI assessment used triple-dose gadolinium for the enhancement of lesions on brain scans.[53,54]

On the basis of EDSS, clinical improvement was defined as at least a 0.5-point score reduction compared with baseline. Progression was defined as a 0.5-point score increase compared with baseline. Relapse of disease was defined as new symptom onset or aggravation of pre-existing symptoms for more than 24 h in those subjects with no other concurrent factors, such as fever and/or infection. EFS was defined as the likelihood of living with no clinical disease progression, that is, no disease progression based on EDSS, no relapse-related events (exacerbation or relapse) and no new lesions on the MRI, even if not associated with EDSS deterioration or relapse.

The effects of transplantation were assessed through MRI (Magneton Vision Erlangen Equipment, Erlangen, Germany) with the i.v. paramagnetic contrast agent (gadopentate dimeglumine at 0.2–0.6 ml/kg body weight, Magnevistan, Schering, Berlin, Germany, or Viewgam, Bacon Laboratories S.A.I.C., Buenos Aires, Argentine) using a standard procedure for all hospitals involved in the study. MRI results were divided into inactivity (no contrast enhancement) and activity (contrast enhancement of any lesion).

Assessment of QOL

Quality of life was assessed by means of SF-36 (Short Form-36), a generic health-related QOL questionnaire. It consists of 36 items grouped into eight domains: physical function, social function, physical role limitations, emotional role limitations, pain, energy/fatigue, mental health and general health. Patients answered the questionnaire before transplantation and 100 days later.

Statistical analysis

The BEAM/ATG and CY/ATG groups were compared with Student's *t*-test or the Mann–Whitney test for quantitative variables and with the Chi-square or Fisher's exact test for categorical variables. Two-way repeated measurement ANOVA (analysis of variance) models were performed to evaluate differences between the groups regarding the QOL before and after transplantation. EFS curves were analyzed by the Kaplan–Meier method and compared with the log-rank test. The significance level was considered to be 5%. SAS software v. 9.1.3 was used in the statistical analysis (Statistical Analysis System, Cary, NC, USA).

Results

Patients

Among 41 subjects, 24 (58.5%) were females, and the mean age was 42 years (ages 27–53). Most subjects (80.4%) had the secondary progressive MS subtype. The subjects' characteristics and conditioning regimens are summarized in Table 2.

Extended Disability Status Scores at baseline examination are also shown in Table 2. Most (24 subjects, or 58.5%) had a score of 6.5 in EDSS, and 78% had a score of 6.0 or higher. No significant difference was seen between the 21 subjects (51.2%) who received the BEAM conditioning regimen and the 20 subjects (48.8%) who received the CY regimen regarding age, sex, disease presentation and EDSS scores, as shown in Table 3. As for the MRI results, fewer cases with signs of disease activity were found in the CY group ($P = 0.04$).

Table 2 Characteristics of multiple sclerosis patients submitted to autologous hematopoietic SCT in Brazil ($n=41$)

Types of multiple sclerosis	
Primary progressive	4 (9.8%)
Secondary progressive	33 (80.4%)
Relapsing-remitting	4 (9.8%)
Conditioning	
BEAM	21 (51.2%)
CY	20 (48.8%)
Initial EDSS	
4.0	1 (2.4%)
5.0	2 (4.8%)
5.5	6 (14.6%)
6.0	6 (14.6%)
6.5	24 (58.5%)
7.0	2[a] (4.8%)
Initial MRI (disease activity)[b]	
Absent	27 (77.14%)
Present	8 (22.85%)

Abbreviations: BEAM = cytosine arabinoside, etoposide and melphalan; EDSS = Expanded Disability Status Scale; MRI = magnetic resonance imaging.
[a]At the time they were included in this study, these patients had an EDSS score of 6.5; however, at the time of transplantation, EDSS reached 7.0.
[b]Exams of six patients were not included because of technical problems, as their MRI scans were performed in other institutions (before patient inclusion in the study).

Table 3 Demographics of patients undergoing two therapeutical regimens for the treatment of multiple sclerosis patients with autologous hematopoietic SCT in Brazil

	BEAM/ATG	*CY/ATG*	
n	21	20	
Age (years)—median	42 (29–52)	41 (27–53)	$P=0.42$
Male	10 (48%)	7 (35%)	$P=0.41$
Female	11 (52%)	13 (65%)	
Primary progressive	2 (9.5%)	2 (10%)	
Secondary progressive	18 (86.7%)	15 (75.0%)	$P=0.53$
Relapsing-remitting	1 (4.8%)	3 (15.0%)	
Duration of disease (year)[a]—median (min–max)	8 (2–22)	7 (3–14)	$P=0.441$
Duration of progressive disease	5.3 (±4.8)	4.7 (±2.6)	$P=0.636$
Initial EDSS ⩾6.0	17 (80.95%)	15 (75.00)	$P=0.71$
Initial EDSS <6.0	4 (19.05%)	5 (25.0%)	
Median EDSS initial	6.5 (5–7)	6.5 (4.5–7)	
Initial MRI showing activity	7 (35.0%)	1 (5.26%)	$P=0.04$

Abbereviations: BEAM = cytosine arabinoside, etoposide and melphalan; EDSS = Expanded Disability Status Scale; MRI = magnetic resonance imaging.
[a]BEAM/ATG with 19 patients and CY/ATG with 20 patients analisados.

Mobilization and stem cell collection

Mobilization was successful in all subjects with only one apheresis session. The mean CD34+ cell count in the apheresis product was 8.8×10^6/kg (range 2.5–25.13). No serious adverse events occurred during PBSC mobilization. Two patients (one of each conditioning regimen arm) had a relapse or disease aggravation in the interval between mobilization and HSCT.

Table 4 Complications during and after autologous hematopoietic SCT in multiple sclerosis patients in Brazil

Complication/toxicity	*All patients*	*BEAM/ATG*	*CY/ATG*	P-*value (BEAM/ATG vs CY/ATG)*
During HSCT				
Febrile neutropenia[a]	18 (46.2%)	12 (57.1%)	6 (33.3%)	$P=0.137$
Pneumonia[b]	8 (20.0%)	6 (28.6%)	2 (10.5%)	$P=0.241$
Allergy to thymoglobulin or lymphoglobulin[b]	5 (12.5%)	5 (23.8%)	0	$P=0.049$
All complications during HSCT	23 (56%)	15 (71.4%)	8 (40%)	$P=0.04$
After HSCT				
Urinary infection[b]	7 (18.4%)	4 (21.1%)	3 (19.8%)	$P>0.949$
Deep vein thrombosis and pulmonary embolism[b]	3 (8.0%)	2 (10.5%)	1 (5.3%)	$P>0.999$
Depression[a]	3 (7.5%)	3 (19%)	0	$P=0.231$
Death	3 (7.5%)	3 (14.3%)	0	$P=0.232$
All complications post HSCT	19 (48%)	12 (63%)	7 (35%)	$P=0.08$

[a]Two patients without information.
[b]One patient without information.

Engraftment, mortality and major adverse events

No adverse reactions such as hypertension, fever or allergic manifestations were seen during graft infusion in any subject. Median neutrophil recovery over 500/mm^3 (engrafting) was 9 days (7–10 days) in the BEAM arm, and also 9 days in the CY arm ($P=0.905$). In the BEAM arm, plt recovery time was 13 days (8–35) and in the CY arm it was 10 days (7–15), a statistically significant difference ($P=0.021$). Transfusional support was on the average 8.94 units (2–63 range) of red cell concentrate in the BEAM group and 2.6 (0–8 range) in the CY group ($P<0.0011$). The mean plt concentrate units transfused were 60 (14–197) in the BEAM group and 14.9 IU (0–40) in the CY group ($P<0.0005$).

Table 4 shows the rate of complications during the intra-transplantation period (56% in the whole group) and the time between conditioning and engraftment. When the two conditioning regimens are compared, it can be seen that 71% of the patients in the BEAM/ATG group and 40% in the CY/ATG group had some complication during HSCT ($P=0.04$). There was no statistical difference between the two groups in relation to febrile neutropenia and pneumonia. Of the patients, 23.8% in the BEAM/ATG group were allergic to lymphoglobulin and none of the CY/ATG group was allergic to thymoglobulin ($P=0.049$). The febrile neutropenia observed in 18 patients (46.0%) was successfully treated with broad-spectrum antibiotics, except in one subject of the BEAM/hATG group, who developed sepsis and died. In the post-transplantation period, the rate of complications was 48%, most frequently related to urinary tract infection (7 subjects, 18.4%). The proportion of complications was also higher after transplantation in group 1, although it was not statistically significant ($P=0.08$).

Table 5 Indirect indicators of aggressiveness of the conditioning regimens used for autologous hematopoietic SCT in the treatment of multiple sclerosis patients

Conditioning regimens	*Length of hospital stay in days*	*Plts transfusion random in IU*	*Hemoglobin transfusion in IU*	*Plts engraftment day from SC infusion*	*WBC engraftment day from SC infusion*
BEAM	35.47 (20–168)	60 (14–197)	8.94 (2–63)	13 (8–35)	9 (7–10)
CY	20.15 (14–32)	14.9 (0–40)	2.6 (0–8)	10 (7–15)	9 (7–10)
	$P=0.0001$	$P=0.005$	$P=0.0011$	$P=0.021$	$P=0.905$

Abbreviations: BEAM = cytosine arabinoside, etoposide and melphalan.

Table 6 Evolution of EDSS scores and MRI after autologous hematopoietic SCT in subjects who received the BEAM/ATG or CY/ATG conditioning regimens ($P=0.596$)

Condition	*Total*	*BEAM/ATG*	*CY/ATG*	P-*value*
EDSS				
Worse	14 (36.8%)	8 (44.4%)	6 (30.0%)	$P=0.596$
Stable	9 (23.7%)	3 (16.7%)	6 (30%)	
Better	15 (39.5%)	7 (38.9%)	8 (40%)	
Stable or better	24 (63.2%)	10 (55.6%)	14 (70%)	$P=0.357$
MRI				
No. of enhanced lesions	0	0	0	—

Abbreviations: ATG = antilymphocyte globulin; BEAM = cytosine arabinoside, etoposide and melphalan; MRI = magnetic resonance imaging.

The average hospital stay was 35.47 days (range 20–168) in the BEAM/hATG group and 20.15 days (14–32 range) in the CY/rATG group ($P<0.0001$). A comparison between both conditioning regimens showed a higher rate of complications during transplantation in the BEAM/ATG (71.4%) than in the CY/rATG regimen group (40%; $P=0.04$). Three subjects (7.5%) died as a result of the conditioning regimen toxicity related to sepsis, cardiac toxicity and alveolar hemorrhage. The three deaths in the study protocol were seen in the BEAM/ATG group. As shown in Table 5, BEAM/hATG proved to be a more toxic conditioning regimen requiring a significantly longer hospital stay.

Clinical evolution

During the mobilization and transplantation periods, two subjects (one from each conditioning regimen) had disease progression (persistent or increased neurological disability). EDSS-based disease progress was seen in 14 patients (36.8%), 8 (44.4%) from the BEAM/hATG group and 6 (30.0%) from the CY/rATG group, but no significant difference was found ($P=0.596$). The EDSS result was stable or better in 24 (63.2%) patients, with no difference between the groups ($P=0.357$, Table 6). EDSS scores obtained at different times were compared (because first the BEAM and then the CY/rATG regimens were used), and this analysis showed that one patient in the BEAM group and one patient in the CY/rATG group had a drop in score: from 5.5 to 2.5 and from 5.0 to 3.5. The EFS was 585:47.62% in the BEAM/hATG group in 3 years and 70% in the CY/rATG group in 2 years ($P=0.288$; Figure 1).

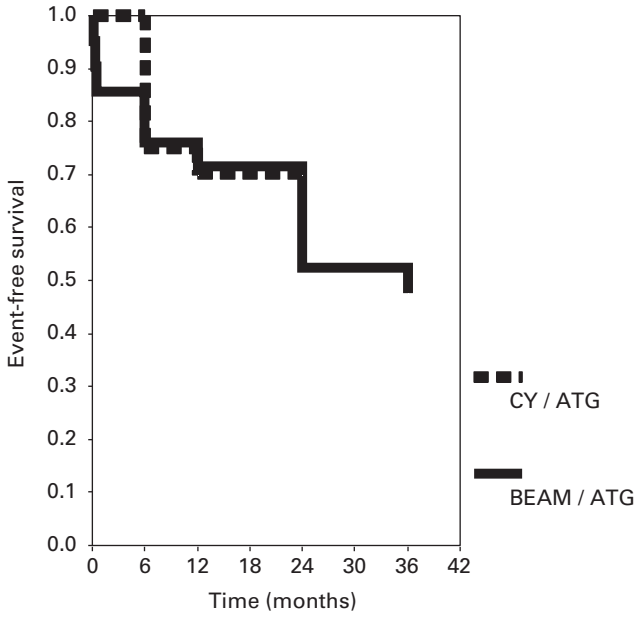

Figure 1 EFS rate at 3 years (BEAM/hATG) and 2 years (CY/rATG) after autologous hematopoietic SCT in multiple sclerosis patients.

Among 35 subjects who underwent MRI assessments, 17 were in the BEAM group and 18 in the CY group ($P=0.48$). Six patients had their results compromised because of technical reasons, as their scans were not performed in hospitals involved in the study. Eight had pre-enrollment disease activity (gadolinium enhancement) shown on the MRI, of which seven were in the BEAM group and only one in the CY group. The enhancement of these lesions disappeared and there were no new lesions seen in all the patients studied after the hematopoietic SCT.

Quality of life

A total of 20 patients completed the SF-36 assessment after transplantation. In the CY/ATG group, they perceived the physical aspects domain as improved during the study period compared with the pre-transplant period ($P=0.0334$), but the differences were not statistically significant in the remaining areas (Table 7). The general health domain seemed to be more positively evaluated in both groups, comparing the periods before and after transplantation, but this difference was NS ($P=0.0961$ in the BEAM/hATG and $P=0.0731$ in the CY/rATG group), probably because of the short period of time between the analysis and the small number of subjects who responded to the SF-36 assessment.

Table 7 Mean and s.d. of the Short Form-36 questionnaire scores in multiple sclerosis patients before and 100 days after autologous hematopoietic SCT

	BEAM/ATG mean (s.d.)	*CY/ATG mean (s.d.)*
FC		
Before	9.17 (10.84)	13.75 (14.33)
After	11.67 (16.00)	23.13 (24.92)
P-value[a]	0.6004	0.1199
PA		
Before	16.67 (22.19)	9.38 (18.6)
After	14.58 (34.47)	40.63 (37.65)
P-value[a]	0.8529	0.0334
Pain		
Before	50.33 (20.40)	63.38 (35.15)
After	57.42 (29.43)	67.63 (32.74)
P-value[a]	0.4233	0.6928
GH		
Before	64 (27.54)	65.63 (23.79)
After	75.58 (11.93)	81.00 (16.89)
P-value[a]	0.0961	0.0731
Vit		
Before	61.67 (25.88)	52.50 (19.09)
After	53.42 (14.27)	53.75 (15.53)
P-value[a]	0.2506	0.8849
SA		
Before	58.33 (28.29)	34.38 (25.66)
After	57.38 (30.70)	56.38 (39.46)
P-value[a]	0.9248	0.0894
EA		
Before	47.23 (38.85)	45.88 (50.22)
After	41.83 (40.58)	66.63 (39.92)
P-value[a]	0.7491	0.3210
MH		
Before	68.67 (27.65)	66.50 (33.33)
After	66.50 (16.07)	68.00 (21.70)
P-value[a]	0.7861	0.8780

Abbreviations: FC = functional capacity; PA = physical aspects; GH = general health; Vit = vitality; SA = social aspects; EA = emotional aspects; MH = mental health.
[a]After × before.

Discussion

High-dose chemotherapy with autologous hematopoietic stem cell rescue shows promising results in the treatment of autoimmune diseases. Controversy exists as to the efficacy and safety of non-myeloablative vs myeloablative HSCT regimens for autoimmune diseases. We therefore compared an intermediate-intensity conditioning regimen (BEAM), pioneered in by Fassas *et al*.[21] in Europe, with a non-myeloablative approach proposed by Burt *et al*.[55] at Northwestern University (Chicago, IL, USA). We reported the results of MS treatment with HSCT in five Brazilian centers, focusing on the comparison of these two conditioning regimens and their impact on the clinical outcomes of the patients, given that the choice of the most adequate conditioning regimen is still being studied and is constantly changing.

Among our first 21 transplant patients who received the BEAM regimen, three died (mortality rate of 7.5%), and this led to a change to the CY/ATG regimen for the following 20 patients. This mortality rate was slightly higher than that of 5.3% found by the European Group.[26] It is worth noting that in the European study, all deaths occurred by the year 2000[26]—this rate has recently been changed to 3.3%,[56] and no deaths were seen in the last 62 patients treated with BEAM. This has been attributed to the learning curve and experience gained by the transplant center, better patient selection and the use of a less intense conditioning regimen.[26,56] The same factors may explain in part the mortality rate that was seen in our study. In our experience, the BEAM/ATG regimen proved to be more toxic than CY/ATG. The length of hospitalization, number of plt and RBC transfusions, plt engraftment and early complications were all significantly higher in the BEAM/ATG group. The different types of ATG regimens and different dosages can also be seen as possible factors involved in the worse results in the BEAM group, such as allergic reactions to ATG, plt engraftment and consequently a greater need for transfusion, with longer periods of hospitalization. However, we did not observe any significant differences with regard to effectiveness between the two regimens, despite these differences in the ATG sources. Although the follow-up period was too short to allow definitive conclusions, we believe that a longer follow-up would corroborate our findings.

Although BEAM/hATG was more toxic, as expected, the CY/rATG regimen did not show inferiority with regard to neurological progression during the follow-up period in this study. Disease progression was seen in half of the patients in the BEAM/hATG group and in a quarter of the CY/rATG group through EDSS scores, but the difference was NS. In our study, there were no new lesions captured by MRI in either conditioning regimen groups, showing the effectiveness in the suppression of MR-enhanced activity, as in the Italian study of the BEAM regimen.[57] Lesion enhancement by MRI was used in our study only as a marker for inflammatory activity for patient selection. In our sample, we did not find a correlation between improved MRI scans and the level of neurological disability (EDSS), as opposed to the Italian group.[29] This could be explained by the reduced number of patients with lesions before transplantation: seven in the BEAM group and one in the CY/ATG group. A more formal MRI study would be needed to verify disease progression by means of lesion burden as determined by MRI brain volume, number of lesions and other markers, especially in reduced intensity regimens.

It should be stressed that in our study, three patients had significant EDSS score deterioration, two in the BEAM group and one in the CY group. On the other hand, the worst outcomes were seen among those with higher EDSS scores. Among the two patients with an EDSS score of 7, one remained stable and the other progressed to an EDSS score of 9 in 6 months. Similar data were found in the European group analysis.[26] When they compared patients aged $\leqslant 50$ years, EDSS $\leqslant 6.5$, and progressive secondary or progressive remitting subtypes with patients aged >50 who did not meet these criteria, the TRM rate was 6.9%

compared with the 3.6% observed in patients who met the criteria.

The EFS was 70% in the CY/ATG group and 47.62% in the BEAM/ATG group, with no significant difference between them, a result that is similar to that obtained by Saiz *et al*.[39] 71% at 4.5 years and 62% at 6 years. However, the follow-up period in our study was shorter and the results could decrease with time, as shown by the Italian group: in their study, EFS dropped from 84% at 3 years to 58% after 8.5 years.[42,56] The same trend was observed by other groups.[56] A longer period of time is needed to corroborate the results of the use of the non-myeloablative regime as it was used in this study, to compare with the results in the literature in terms of having the same clinical efficacy, in spite of the progressive reduction in the results.

Our study was not designed to show the efficacy of HSCT over conventional therapy for progressive forms of MS, which can be performed only with randomized clinical trials. Other limitations of our study are the use of two different sources of ATG, the involvement of various transplant centers, the analysis of two different conditioning regimens used in different time periods, and the relatively short period of follow-up. In our analysis, the CY-ATG conditioning was not inferior to BEAM-ATG, and it had less toxicity.

In conclusion, in our study with 41 MS patients treated with HSCT, the CY/rATG rabbit regimen seems to be associated with less toxicity and to be as effective as the BEAM/hATG horse regimen. Long-term follow-up would be required to fully assess the differences in therapeutic effectiveness.

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References

1 Hafler DA. Multiple sclerosis. *J Clin Invest* 2004; **113**: 788–794.
2 Hemmer B, Archelos JJ, Hartung HP. New concepts in the immunopathogenesis of multiple sclerosis. *Nat Rev Neurosci* 2002; **3**: 291–301.
3 Dyment DA, Ebers GC, Sadovnick AD. Genetics of multiple sclerosis. *Lancet Neurol* 2004; **3**: 104–110.
4 Sospedra M, Martin R. Immunology of multiple sclerosis. *Annu Rev Immunol* 2005; **23**: 683–747.
5 Compston A, Coles A. Multiple sclerosis. *Lancet* 2002; **359**: 1221–1231.
6 Havrdova E, Galetta S, Hutchinson M, Stefoski D, Bates D, Polman CH *et al*. Effect of natalizumab on clinical and radiological disease activity in multiple sclerosis: a retrospective analysis of the Natalizumab Safety and Efficacy in Relapsing-Remitting Multiple Sclerosis (AFFIRM) study. *Lancet Neurol* 2009; **8**: 254–260.
7 Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH *et al*. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2006; **354**: 899–910.
8 Putzki N, Kollia K, Woods S, Igwe E, Diener HC, Limmroth V. Natalizumab is effective as second line therapy in the treatment of relapsing remitting multiple sclerosis. *Eur J Neurol* 2009; **16**: 424–426.
9 Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ *et al*. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med* 2008; **358**: 676–688.
10 Ikehara S, Yasumizu R, Inaba M, Izui S, Hayakawa K, Sekita K *et al*. Long-term observations of autoimmune-prone mice treated for autoimmune disease by allogeneic bone marrow transplantation. *Proc Natl Acad Sci USA* 1989; **86**: 3306–3310.
11 van Bekkum DW. Autologous stem cell transplantation for treatment of autoimmune diseases. *Stem Cells* 1999; **17**: 172–178.
12 Bekkum DW. Immune ablation and stem-cell therapy in autoimmune diseases. Experimental basis for autologous stem-cell transplantation. *Arthritis Res* 2000; **2**: 281–284.
13 van Bekkum DW. Experimental basis of hematopoietic stem cell transplantation for treatment of autoimmune diseases. *J Leukoc Biol* 2002; **72**: 609–620.
14 van Gelder M, van Bekkum DW. Treatment of relapsing experimental autoimmune encephalomyelitis in rats with allogeneic bone marrow transplantation from a resistant strain. *Bone Marrow Transplant* 1995; **16**: 343–351.
15 Burt RK, Traynor AE. Hematopoietic stem cell transplantation: a new therapy for autoimmune diseases. *Oncologist* 1999; **4**: 77–83.
16 Burt RK, Padilla J, Dal Canto MC, Miller SD. Viral hyperinfection of the central nervous system and high mortality after hematopoietic stem cell transplantation for treatment of Theiler's murine encephalomyelitis virus-induced demyelinating disease. *Blood* 1999; **94**: 2915–2922.
17 Burt RK, Padilla J, Begolka WS, Canto MC, Miller SD. Effect of disease stage on clinical outcome after syngeneic bone marrow transplantation for relapsing experimental autoimmune encephalomyelitis. *Blood* 1998; **91**: 2609–2616.
18 van Bekkum DW. Stem cell transplantation in experimental models of autoimmune disease. *J Clin Immunol* 2000; **20**: 10–16.
19 Herrmann MM, Gaertner S, Stadelmann C, van den Brandt J, Böscke R, Budach W *et al*. Tolerance induction by bone marrow transplantation in a multiple sclerosis model. *Blood* 2005; **106**: 1875–1883.
20 Muraro PA, Douek DC, Packer A, Chung K, Guenaga FJ, Cassiani-Ingoni R *et al*. Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. *J Exp Med* 2005; **201**: 805–816.
21 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**: 631–638.
22 Burt RK, Burns W, Hess A. Bone marrow transplantation for multiple sclerosis. *Bone Marrow Transplant* 1995; **16**: 1–6.
23 Burt RK, Traynor AE, Cohen B, Karlin KH, Davis FA, Stefoski D *et al*. T cell-depleted autologous hematopoietic stem cell transplantation for multiple sclerosis: report on the first three patients. *Bone Marrow Transplant* 1998; **21**: 537–541.

24 Blanco Y, Saiz A, Carreras E, Graus F. Autologous haematopoietic-stem-cell transplantation for multiple sclerosis. *Lancet Neurol* 2005; **4**: 54–63.
25 Fassas A, Passweg JR, Anagnostopoulos A, Kazis A, Kozak T, Havrdova E *et al*. Hematopoietic stem cell transplantation for multiple sclerosis. A retrospective multicenter study. *J Neurol* 2002; **249**: 1088–1097.
26 Saccardi R, Kozak T, Bocelli-Tyndall C, Fassas A, Kazis A, Havrdova E *et al*. Autologous stem cell transplantation for progressive multiple sclerosis: update of the European Group for Blood and Marrow Transplantation autoimmune diseases working party database. *Mult Scler* 2006; **12**: 814–823.
27 Openshaw H, Lund BT, Kashyap A, Atkinson R, Sniecinski I, Weiner LP *et al*. Peripheral blood stem cell transplantation in multiple sclerosis with busulfan and cyclophosphamide conditioning: report of toxicity and immunological monitoring. *Biol Blood Marrow Transplant* 2000; **6**: 563–575.
28 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**: 2364–2372.
29 Saccardi R, Mancardi GL, Solari A, Bosi A, Bruzzi P, Di Bartolomeo P *et al*. Autologous HSCT for severe progressive multiple sclerosis in a multicenter trial: impact on disease activity and quality of life. *Blood* 2005; **105**: 2601–2607.
30 Burt RK, Cohen BA, Russell E, Spero K, Joshi A, Oyama Y *et al*. Hematopoietic 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**: 2373–2378.
31 Burt RK, Loh Y, Cohen B, Stefosky D, Balabanov R, Katsamakis G *et al*. Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study. *Lancet Neurol* 2009; **8**: 244–253.
32 Burt RK, Traynor A, Statkute L, Barr WG, Rosa R, Schroeder J *et al*. Non-myeloablative hematopoietic stem cell transplantation for systemic lupus erythematosus. *JAMA* 2006; **295**: 527–535.
33 Voltarelli JC, Couri CE, Stracieri AB, Oliveira MC, Moraes DA, Pieroni F *et al*. Autologous non-myeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA* 2007; **297**: 1568–1576.
34 Oyama Y, Barr WG, Statkute L, Corbridge T, Gonda EA, Jovanovic B *et al*. Autologous non-myeloablative hematopoietic stem cell transplantation in patients with systemic sclerosis. *Bone Marrow Transplant* 2007; **40**: 549–555.
35 Carreras E, Saiz A, Marín P, Martínez C, Rovira M, Villamor N *et al*. CD34+ selected autologous peripheral blood stem cell transplantation for multiple sclerosis: report of toxicity and treatment results at one year of follow-up in 15 patients. *Haematologica* 2003; **88**: 306–314.
36 Kozák T, Havrdová E, Pit'ha J, Gregora E, Pytlík R, Maaloufová J *et al*. Immunoablative therapy with autologous stem cell transplantation in the treatment of poor risk multiple sclerosis. *Transplant Proc* 2001; **33**: 2179–2181.
37 Samijn JP, te Boekhorst PA, Mondria T, van Doorn PA, Flach HZ, van der Meché FG *et al*. Intense T cell depletion followed by autologous bone marrow transplantation for severe multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2006; **77**: 46–50.
38 Shevchenko YL, Novik AA, Kuznetsov AN, Afanasiev BV, Lisukov IA, Kozlov VA *et al*. High-dose immunosuppressive therapy with autologous hematopoietic stem cell transplantation as a treatment option in multiple sclerosis. *Exp Hematol* 2008; **36**: 922–928.
39 Saiz A, Blanco Y, Berenguer J, Gómez-Choco M, Carreras E, Arbizu T *et al*. Resultado clínico a 6 años del trasplante autólogo de progenitores hematopoyéticos en la esclerosis múltiple. [Clinical outcome 6 years after autologous hematopoietic stem cell transplantation in multiple sclerosis]. *Neurologia* 2008; **23**: 405–407.
40 Kozak T, Havrdova E, Pitha J, Mayerova K, Novakova L, Trneny M *et al*. Immunoablative therapy with autologous PBPC transplantation in the treatment of poor-risk multiple sclerosis. *Bone Marrow Transplant* 2008; **41**(Suppl 1): S18 (abstract). Available from: http://registration.akm.ch/einsicht.php?XNABSTRACT_ID=68025&XNSPRACHE_ID=2&XNKONGRESS_ID=69&XNMASKEN_ID=900, Accessed in 2009 (Mar 27).
41 Saccardi R, Mancardi G, Bosi A, Bruzzi P, Di Bartolomeo P, Donelli A *et al*. Autologous HSCT for severe progressive multiple sclerosis in the Italian prospective, multicentre GITMO-Neuro trial: long-term follow-up. *Bone Marrow Transplant* 2008; **41**(Suppl 1): S17 (abstract). Available from: http://registration.akm.ch/einsicht.php?XNABSTRACT_ID=68382&XNSPRACHE_ID=2&XNKONGRESS_ID=69&XNMASKEN_ID=900. Accessed in 2009 (Mar 27).
42 Freedman MS, Atkins HL, Arnold DL, Bar-Or A, on behalf of the Canadian BMT Study Group. Immune ablation and autologous stem cell transplantation for aggressive multiple sclerosis: interim 5-year report. *Mult Scler* 2007; **13** (Supp 2): S22 (abstract). Available from: http://registration.akm.ch/einsicht.php?XNABSTRACT_ID=52501&XNSPRACHE_ID=2&XNKONGRESS_ID=63&XNMASKEN_ID=900. Accessed in 2009 (Mar 27).
43 Ni XS, Ouyang J, Zhu WH, Wang C, Chen B. Autologous hematopoietic stem cell transplantation for progressive multiple sclerosis: report of efficacy and safety at three yr of follow up in 21 patients. *Clin Transplant* 2006; **20**: 485–489.
44 Su L, Xu J, Ji BX, Wan SG, Lu CY, Dong HQ *et al*. Autologous peripheral blood stem cell transplantation for severe multiple sclerosis. *Int J Hematol* 2006; **84**: 276–281.
45 Xu J, JI B, Su L, Dong HQ, Sun XJ, Liu CY. Clinical outcomes after autologous haematopoietic stem cell transplantation in patients with progressive multiple sclerosis. *Chin Med J (Engl)* 2006; **119**: 1851–1855.
46 Burt RK, Marmont A, Oyama Y, Slavin S, Arnold R, Hiepe F *et al*. Randomized controlled trials of autologous hematopoietic stem cell transplantation for autoimmune diseases: the evolution from myeloablative to lymphoablative transplant regimens. *Arthritis Rheum* 2006; **54**: 3750–3760.
47 de Kleer I, Vastert B, Klein M, Teklenburg G, Arkesteijn G, Yung GP *et al*. Autologous stem cell transplantation for autoimmunity induces immunologic self-tolerance by reprogramming autoreactive T cells and restoring the CD4+CD25+ immune regulatory network. *Blood* 2006; **107**: 1696–1702.
48 Farge D, Henegar C, Carmagnat M, Daneshpouy M, Marjanovic Z, Rabian C *et al*. Analysis of immune reconstitution after autologous bone marrow transplantation in systemic sclerosis. *Arthritis Rheum* 2005; **52**: 1555–1563.
49 Alexander T, Thiel A, Rosen O, Massenkeil G, Sattler A, Kohler S *et al*. Depletion of autoreactive immunologic memory followed by autologous hematopoietic stem cell transplantation in patients with refractory SLE induces long-term remission through *de novo* generation of a juvenile and tolerant immune system. *Blood* 2009; **113**: 214–223.
50 Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an Expanded Disability Status Scale (EDSS). *Neurology* 1983; **33**: 1444–1452.
51 Comi G, Kappos L, Clanet M, Ebers G, Fassas A, Fazekas F *et al*. Guidelines for autologous blood and marrow stem cell transplantation in multiple sclerosis: a consensus report written on behalf of the European Group for Blood

and Marrow Transplantation and the European Charcot Foundation. BMT-MS Study Group. *J Neurol* 2000; **247**: 376–382.
52 Rowley SD. Hematopoietic stem cell cryopreservation. In: Thomas ED, Blume KG, Forman SJ (eds). *Hematopoietic Cell Transplantation*, 2nd edn. Blackewell Science: Boston, MA, USA, 1999, pp 481–492.
53 Simon JH, Li D, Traboulsee A, Coyle PK, Arnold DL, Barkhof F *et al*. Standardized MR imaging protocol for multiple sclerosis: consortium of MS centers consensus guidelines. *AJNR Am J Neuroradiol* 2006; **27**: 455–461.
54 Barkhof F, Filippi M, van Waesberghe JH, Molyneux P, Rovaris M, Lycklama à Nijeholt G *et al*. Improving interobserver variation in reporting gadolinium-enhanced MRI lesions in multiple sclerosis. *Neurology* 1997; **49**: 1682–1688.
55 Burt RK, Cohen B, Rose J, Petersen F, Oyama Y, Stefoski D *et al*. Hematopoietic stem cell transplantation for multiple sclerosis. *Arch Neurol* 2005; **62**: 860–864.
56 Mancardi G, Saccardi R. Autologous haematopoietic stem-cell transplantation in multiple sclerosis. *Lancet Neurol* 2008; **7**: 626–636.
57 Mancardi GL, Saccardi R, Filippi M, Gualandi F, Murialdo A, Inglese M *et al*. Autologous hematopoietic stem cell transplantation suppresses Gd-enhanced MRI activity in MS. *Neurology* 2001; **57**: 62–68.