

ORIGINAL INVESTIGATION

Long Term Outcomes after Autologous Hematopoietic Stem Cell Transplantation for Multiple Sclerosis

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ABSTRACT

Importance: Autologous hematopoietic stem cell transplantation may be effective in aggressive forms of multiple sclerosis that failed to respond to standard therapies.

Objective: To evaluate long-term outcomes after autologous hematopoietic stem cell transplantation for treatment of multiple sclerosis.

Design, Setting and Participants: Data was collected in a multicenter observational retrospective cohort study. Eligibility criteria were having received autologous hematopoietic stem cell transplantation for the treatment of multiple sclerosis during 1995-2006 and availability of a pre-specified minimum dataset, comprising the disease subtype at baseline; the expanded disability status scale (EDSS) score at baseline; information on the administered conditioning regimen and graft manipulation; and availability of at least one follow-up visit/report after transplantation. Last patient last visit was on July 1, 2012. To avoid biases, all eligible patients were included in the analysis regardless the duration of follow-up.

Exposures: Demographic, disease- and treatment-related exposures were considered as variables of interest. These included age, disease subtype, baseline EDSS score, number of previous disease modifying treatments, and intensity of transplantation conditioning regimen.

Main Outcomes and Measures: The primary outcomes were multiple sclerosis progression-free survival and overall survival. The probabilities of progression-free and overall survival were calculated using Kaplan-Meier survival curves and Cox regression multivariate analysis models.

Results: Valid data was collected from 25 centers in 13 countries for 281 evaluable patients with median follow up of 6.6 years (range 0.2-16 y). The majority of patients had progressive forms of multiple sclerosis (78%). The median EDSS score prior to mobilization was 6.5 (range 1.5-9.0). The five-year probability of EDSS progression free survival was 46% (95% confidence interval [CI], 42-54 %) and overall survival was 93% (95% CI, 89-96 %). Factors associated with neurological progression post-transplantation were age (HR=1.03, 95% CI, 1.00-1.05), progressive vs. relapsing forms of multiple sclerosis (HR=2.33, 95%CI , 1.27-4.28) and >2 previous disease-modifying therapy (HR=1.65, 95%CI, 1.10-2.47). EDSS score was associated with worse overall survival (HR 2.03, 95%CI=1.40-2.95).

Conclusions and Relevance: In this observational study of MS patients treated with autologous hematopoietic stem cell transplantation, nearly half survived free from neurological progression for 5 years after transplantation. Younger age, relapsing multiple sclerosis, less prior immunotherapies and lower disability score were factors associated with better outcomes. The results support the rationale for further, randomized controlled studies of AHSCT for treatment of MS.

INTRODUCTION

Approximately 3 million people in the world have multiple sclerosis (MS).¹ MS typically presents in young adulthood and can cause severe neurological disability, a major socio-economic burden².

Patients with an aggressive course of MS often fail to respond to several lines of disease-modifying treatment and deteriorate within a few years.

Autologous hematopoietic stem cell transplantation (AH SCT) is being investigated as treatment for aggressive MS³. The rationale of this approach is to allow the use of high dose immunosuppressive therapy to abrogate the autoimmune inflammatory process. Infusion of autologous hematopoietic cells boosts bone marrow recovery and promotes immune reconstitution. The procedure has been shown to induce a degree of immune 'resetting'^{4,5}. The treatment goals are to arrest worsening of neurologic disability, induce a prolonged medication-free interval and potentially an improvement in neurological function. Early clinical trials established the proof of principle that AH SCT could induce disease remissions in patients with severe MS⁶. More recent studies have shown that autologous AH SCT is effective at suppressing clinical and magnetic resonance imaging (MRI) disease reactivations⁷⁻⁹, can result in neurological improvement in patients with relapsing-remitting MS^{7,8,10-12} and can halt all detectable CNS inflammatory activity for a prolonged period of time¹³. However, outcome assessments in the majority of studies were limited to a relatively short follow-up, and longer-term outcomes have been reported only from small case series¹⁴⁻¹⁶. It would therefore be important to examine in a large patient population the course of MS after AH SCT and the rates of risks and complications over longer term.

The objective of this study was to evaluate long-term outcomes in patients who underwent AH SCT for treatment of MS in a large multi-center cohort by analyzing progression-free survival, evolution of neurological disability, overall survival, transplant related mortality and late effects, including new autoimmune and malignant disorders; and to examine the association of demographic, MS disease- and treatment-related variables with the long-term outcomes.

METHODS

Study Design, Setting and Data Sources

This study was an observational retrospective cohort study on autologous AHSCT for treatment of MS and was performed through collaboration between the Center for International Blood and Marrow Transplant Research (CIBMTR) Autoimmune Disease Working Committee and the European Blood and Marrow Transplant Group (EBMT) Autoimmune Disease Working Party. The CIBMTR is a voluntary working group of more than 450 transplant centers worldwide that contribute detailed data on consecutive marrow transplants to a Statistical Center located at the Medical College of Wisconsin in Milwaukee and at the National Marrow Donor Program Coordinating Center in Minneapolis.¹⁷ The EBMT is a non-profit organization comprising 640 transplant centers mainly from Europe. All transplant centers have been required to obtain written informed consent to report data to the CIBMTR and to the EBMT database in accordance with the Helsinki Declaration 1975. Institutional Review Board approval for data collection and use of data for research purposes was obtained locally by each center. Only fully anonymised data were transferred to the study database. Following review by the study Steering Committee, the study protocol (Supplementary Appendix 1) was approved by the CIBMTR and the EBMT Board in agreement with the rules for retrospective studies of both organisations. MS pre-transplant and follow-up data had been prospectively collected from participating transplant centers on disease specific forms, which were harmonized between the two registries.¹⁸ For the purposes of this study, all bone marrow transplantation centers that had reported at least one autologous AHSCT for MS to CIBMTR or EBMT between 1995 and 2006 were sent an invitation to participate in the study together with a protocol summary. The centers that agreed to participate were asked to identify a transplant physician and a neurologist to oversee all patient data for accuracy and completeness at each site. To better describe disease activity before and after transplant and extend the follow up for our study, additional data collection was undertaken retrospectively. To this end, the Study team developed a supplemental data collection form that was pre-populated with the previously reported data in order to facilitate additional data collection and concurrently verify the accuracy of existing information. The overall completeness of enrollment in

our study, calculated as percentage of all the procedures reported to the two registries during the time period, was 281/493 (57%). A CONSORT diagram of enrollment and screening of the potentially eligible cases is provided in eFigure 1). Our study is reported according to the STROBE guidelines (checklist in supplementary Appendix).

Patients and Treatments

For each case to be included in the study a minimum dataset was required, which comprised: the MS course classification at baseline (relapsing remitting, RR; primary- PP or secondary progressive, SP; progressive relapsing, PR); the expanded disability status scale (EDSS; ranging from 0 signifying no disability through 7 for wheelchair bound to 10 for death due to MS; see eTable 1 for a detailed description) score at baseline; information on the administered conditioning regimen and graft manipulation; and availability of at least one follow-up visit/report after transplantation. Mobilization of Peripheral Blood Stem Cells (PBSC) was carried out by the administration of a hematopoietic Growth Factor (GF), with or without chemotherapy; type of both GF and chemotherapy were sought. Manipulation of the graft aimed to reduce the content of immune cells was also requested. Conditioning regimens including either Busulphan or Total Body Irradiation (TBI) were classified as high intensity; regimens including Cyclophosphamide alone or associated to Anti-Thymocyte Globulin (ATG) or Fludarabine were classified as reduced intensity; all the others were considered as intermediate intensity.

Study End Points

Progression-free survival was defined as survival in the absence of progression of MS. Progression of MS was defined clinically as an increase of 1 point in the Expanded Disability Status Scale (EDSS) confirmed at 12 months (0.5 points if baseline EDSS score was ≥ 5.5) compared to the pre-treatment baseline. The pre-treatment baseline was defined as the last assessment before mobilization (for peripherally mobilized autologous grafts) or before immunosuppressive conditioning (for bone

marrow autologous grafts). EDSS increases that were detected on the last visit and therefore cannot be confirmed were considered as events, according to a more conservative approach. A sensitivity analysis was run censoring these last visits events. Death by any cause was considered MS progression in this analysis. Overall survival was time to death by any cause. For all the patients who died after the AH SCT the cause of death was examined. Early deaths that occurred within 100 days from transplant, which are considered treatment-related, were described separately. Surviving patients were censored at time of last follow-up. Information regarding the incidence of late effects was collected, including malignancies and secondary autoimmune diseases.

Statistical Analysis

Data collected from both the CIBMTR and EBMT were summarized in descriptive tables of demographic information of all the population. Continuous variables were reported as medians and ranges or means and standard deviations, while categorical variables were reported as absolute numbers and percent of total patients. The probability of progression-free survival was calculated using the Life Table estimator and the overall survival was calculated using the Kaplan-Meier Estimator. A multivariate analysis assessing the association of baseline characteristics and transplant methodology on progression free and overall survival was run using Cox proportional hazards regression models, adjusted for center. Proportionality of hazard was checked by plotting log-log transformation of the KM survival curve, namely, $\log\text{-}\log(S(t))$, versus time t for each level of covariates; the assumption of proportional hazard is tenable if the difference between the two log-log KM curves is constant over time.

Variables significantly associated with each outcome event at univariate analysis were included as covariate in the multivariate model, which selected the independent set of variables using a stepwise approach. For each patient having an EDSS assessment 1 year before and 1 year after transplant, the yearly EDSS changes pre and post-transplant were calculated. These changes were compared by a repeated measures analysis of variance with 2 time points (change pre vs change post-transplant), including also disease type (relapsing vs progressive forms) and an interaction term (period by disease

type) to evaluate whether the EDSS change pre and post-transplant was different between relapsing and progressive patients.

A Loess smoothing technique ¹⁹ was applied to describe the EDSS trend over time in relapsing and in progressive patients, for those subjects having EDSS date of assessment reported. The Loess technique is a non-parametric, graphical tool to fit a smooth curve to the points in a scatterplot, based on local weighted regression analyses ¹⁹.

A two-sided significance level of 5% was used. We used R version 3.2 and SPSS version 19 software for the analysis.

RESULTS

Patients Demographics and Procedures

Valid data was obtained for 281 patients. Demographic and clinical data at the time of AHSCT are summarized in Table 1. The median disease duration calculated from diagnosis of MS to AHSCT was 81 months (range 1-413). At the time of AHSCT, 171 patients (61%) had received 2 or more prior lines of disease-modifying treatment for MS. At the assessment preceding mobilization the most represented disease subtype was SPMS, contributing 186 of the 281 patients (66%), and the median EDSS score was 6.5 (range 1.5-9) indicating moderately advanced disability on average. A few differences existed in the subsets of patients reported to CIBMTR and EBMT reflecting different patient selection practices in the two groups of countries. Compared to the CIBMTR cohort, patients from the EBMT cohort were younger (median age 35 years vs. 40 years, $p<0.001$), had more often >2 lines of therapy prior to transplant (52% vs. 32%, $p=0.002$), had less patients in SP (61% vs. 75 %, $p=0.001$), shorter time from diagnosis to transplant (median time of 77 months vs. 91 months, $p=0.04$) and had a greater proportion of patients transplanted during the first half (1995-2000) of the 12-year period qualifying for inclusion in our study (42% vs. 22%, $p<0.01$). In total, however, two thirds of the patients underwent AHSCT during the second half (2001-06). Mobilisation, graft manipulation and conditioning regimens details are also summarized in Table 1. The proportions of patients who received high-, intermediate- and low-intensity conditioning regimens were evenly split (approximately one third each) in CIBMTR, whereas 89% of patients reported to EBMT received an intermediate intensity regimen (most commonly BEAM+ATG). The percentage of patients treated with high-intensity regimens was higher in progressive (20%) than in relapsing (10%) patients ($p=0.05$). The median duration of follow-up post-AHSCT was 6.6 years (range 0.2-16).

Progression-free survival

Progression-free survival as assessed by EDSS was considered the primary neurological endpoint. Patients with yearly EDSS assessments post transplant enabling this analysis were 239 (85%). MS progression-free survival in all evaluable patients was 46% at 5 years post-AHSCT (CI 42-54%; Fig. 1A). Progression-free survival in the subgroup with relapsing MS was 82% at 3 years (95% CI 71-93%), 78% at 4 years (95% CI 66-91%) and 73% at 5 years post-AHSCT (95% CI 57-88%). Amongst patients with SPMS, the largest subgroup in our study (n=162), 33% (95% CI 24-42%) remained free from EDSS deterioration at 5 years post-AHSCT. When applying a Cox regression analysis, the assumption of proportional hazard was tenable. Factors associated with the risk of EDSS progression as identified by univariate Cox analysis were: age; progressive vs. relapsing phase/subtype of MS; and number of prior treatments (Table 2). The significance of these factors was confirmed at multivariate analysis (Table 2; Fig. 1 B, C, D). Younger age and relapsing forms of MS were independently associated with better progression-free survival (Fig. 1B, C). There was no statistical difference in the risk of progression between PPMS patients and SPMS (HR=1.09, p=0.63)(Fig. 1C). Patients who had received 3 or more immune-suppressive/modulatory treatments had a higher probability of progression than those who received 1-2 treatments before AHSCT (Fig. 1D). The results did not change when the unconfirmed progressions on the last visits were considered censored observations.

EDSS change in the period preceding and after AHSCT

It was important to also consider the evolution of neurological disability in the patients before they underwent AHSCT and the information was available in a subset of patients in the cohort. There were 111 patients who met the minimum requirement for this evaluation consisting of availability of at least one EDSS score during the three years prior to as well as after AHSCT with the respective dates of assessment. In the evaluable subgroup, the mean EDSS increased by 0.94 points (95%CI= 0.77-1.11) during the 12 months preceding transplant, as compared to a mean decrease of -0.32 EDSS points (95%CI= -0.15, -0.49) in the 12 months following transplant (p<0.001). A test for interaction demonstrated that the evolution of EDSS change pre and post-transplant was significantly different

between patients with relapsing MS [EDSS change 1 year pre-transplant = +1.42 (95%CI= 0.98-1.86), EDSS change 1 year post-transplant = -0.76 (95%CI=-1.08-0.34)] and progressive MS forms [EDSS change 1 year pre-transplant= +0.73 (95%CI= 0.59-0.87), EDSS change 1 year post-transplant = -0.14 (95%CI=-0.28 - 0.01)] patients ($p<0.001$). Figure 2 shows the evolution of EDSS score recorded before and after AHST in the 111 patients, divided by disease course in relapsing (A) and progressive (B) MS types. This representation allows visualizing the rapid neurological deterioration occurring in both patient subgroups before AHST. Post-transplantation, the integrated line suggests a reduction of the rate of accrual of disability in the subgroup with relapsing MS (Figure 2A).

Overall survival

Overall survival was 93% (95% CI,=89-96%) at 5 years and 84% (95% CI= 78-89%) at 10 years from transplant (Figure 3A). When applying a Cox regression analysis, the assumption of proportional hazard was tenable. Factors associated with worse overall survival at univariate Cox analysis were: age; baseline EDSS score; high vs. low intensity of the conditioning regimen; progressive vs. relapsing form of MS (Table 2). At multivariate analysis only a higher baseline EDSS score remained significantly associated with a higher risk of death over time with HR 2.03 per EDSS point (95%CI=1.40-2.95; Table 2). When stratifying by disability levels, Kaplan-Meier analysis revealed worse survival in patients with baseline EDSS ≥ 7 ($p = 0.004$, Fig. 3B).

Mortality and late adverse events

Overall, 37 deaths from any cause (treatment related or not) out of 281 patients were reported during the entire follow-up. Eight deaths (2.8%, CI=1.0-4.9%) were reported within 100 days from transplant and were considered transplant related mortality. Data on factors associated with lower overall survival at univariate analysis are presented for the patients who died within and after day 100 post-transplant against the whole cohort in eTable2. Among the patients who died during follow-up, progressive MS type and high intensity conditioning regimens were overrepresented compared to the frequency of these factors in the whole cohort. However, the small number of events precludes a formal statistical evaluation. Individual causes of death and details on previous immune suppressive/modulatory treatments for MS are provided in eTable 3.

Late adverse events including the new onset of malignancies and autoimmune diseases are reported in eTable 4. Additionally, one case of Monoclonal Gammopathy of Unknown Significance (MGUS) was reported. Of the 3 cases of myelodysplastic syndrome two received a total body irradiation-based regimen and the other received cyclophosphamide+ATG. In the small number of events occurred, there was no clear evidence to suggest association of any of the late events with specific treatment regimens.

DISCUSSION

Previous studies of AHSCT for treatment of MS often reported detailed assessments, some including MRI endpoints, yet most had relatively short duration of follow-up: for example in the largest published prospective study (n = 145) median follow-up was 2 years¹¹. The few studies with truly long-term follow-up (i.e. including outcomes at 5 years) included small numbers of patients, the largest reporting on 35 patients over a median follow-up period of 11 years¹⁵. We analyzed a large cohort of patients undergoing AHSCT for treatment of MS (n = 281) over long term (median follow up of 6.6 years). Compared to the largest previously published cohort (Burt et al¹¹) that included 118 RRMS (81.4%) and 27 SPMS patients (18.6%) our study includes a different proportion of MS types where 63 patients had relapsing types (RRMS and PRMS totaling 22.4%) and 218 had progressive types (PPMS and SPMS totaling 77.5%), thus it provides more information on outcomes after AHSCT in progressive MS, an area of unmet need²⁰. Burt and colleagues noted that their criteria selecting patients with active inflammation and excluding those with late secondary-progressive MS may have prevented them from detecting associations that may exist with baseline EDSS score, older age, or prior number of immune-modulation or suppression regimens with a worse outcome¹¹. In our study, not imposing any criteria to select a disease phenotype enabled us to demonstrate significant associations of these factors with worse outcomes. Lastly, all previous studies focused on specific AHSCT protocols utilized at those centers whereas in our study, for the first time to our knowledge in a long-term cohort, we report outcomes after a wide range of regimens, including low- (17%), intermediate- (64%) and high-intensity regimens (19%) and include conditioning intensity as a variable in the statistical analyses.

Our primary neurological outcome was progression-free survival, as assessed by systematic EDSS neurological disability scoring. In our cohort 78% of patients had PP- or SPMS and the observed progression-free survival in the evaluable patients (239/281, 85%) was 46% at 5-year follow-up. Because long-term stability of neurological disability is not an expected feature of the natural course of aggressive forms of relapsing or progressive MS²¹ these data raise the possibility that AHSCT may

have reduced the risk of progression in the treated patients, yet in the absence of a control group demonstration is lacking. Neurological outcomes in our study, however were considerably better in patients with relapsing- than in those with progressive MS, consistent with recent evidence of good efficacy in RRMS^{7,11}. By using multivariate analysis we identified relapsing MS as a factor robustly associated with progression-free survival (HR=2.33), which remained >70% at 5 years post-AHSCT in this patient subgroup. In the report by Burt et al, 81.4% of the patients had RRMS and progression-free survival was 87% at 4 years¹¹. In the HALT-MS trial patients were all RRMS by inclusion criteria and progression-free survival was 90.9% at 3 years⁷. Inclusion criteria selecting patients with early RRMS may explain the higher progression-free rates observed in those studies. Additional factors that were significantly associated with better progression-free survival in our study were younger age and less than 3 prior MS disease-modifying treatments. Some of the previous studies considered age in subgroup analyses²² but none to our knowledge demonstrated their significance through formal statistical evaluation. Furthermore, we analyzed in a subset of evaluable patients the trajectory of neurological disability as measured by EDSS during the periods preceding and following AHSCT. The mean accumulation of disability during the 12 months pre-transplant (+0.94 EDSS points) was partially reversed post-transplant (-0.32 EDSS points) and the reversal was significantly greater in the patients with the relapsing compared to the progressive MS forms. This comparison extends previous observations of improvements in EDSS scores after AHSCT in studies including predominantly or exclusively RRMS patients^{7,8,10,11} and in the subgroup of patients with RRMS of the Italian AHSCT database¹².

We also examined the association of variables with overall survival. Univariate analysis identified age, baseline EDSS score, intensity of the conditioning regimen and progressive vs. relapsing form of MS as factors significantly associated with lower overall survival rate. Of these, only baseline EDSS score was confirmed as significant in multivariate analysis, with a HR=2 per EDSS point. However, the low rate of events limits the power to detect at multivariate analysis all the variables underlying

mortality and we cannot conclude that factors like conditioning intensity or disease stage do not affect survival.

Transplant-related death is a major concern in a non-immediately life-threatening disease such as MS. In the present study the 100-day mortality, which in hematological practice is considered a surrogate of transplant related mortality, was 2.8%, a high rate that likely reflects the early AHSCT experience captured in our study that only included transplants performed until 31/12/2006. Indeed, a retrospective analysis of the EBMT Registry performed in 2007 reported a decrease of treatment-related mortality from 7.3% to 1.3% in transplants for MS carried out before and after the year 2000, respectively³. In a 2010 update²³, the 100-day mortality in the whole Registry was 2%, half of that in the 2005 report (4%)²⁴. The reduction over the years is likely related to improved selection of patients with the exclusion of patients with advanced disability who are at higher risk of complications, and to the less frequent use of intensive conditioning regimens²². In our study the causes of deaths within day 100 were partly related to the immunosuppression, as expected²² although the small number of events prevents a reliable analysis. Beyond day 100, the incidence of death is scattered throughout the follow-up (Fig. 3 and data not shown) and the causes can be attributed in large part to progression of MS disability and its attendant complications, which often include infection even in patients who have not been treated with immunosuppressant therapies. The conditioning regimen was more frequently a high-intensity one in the patients who died during follow-up than in the whole cohort, yet non-random allocation of treatment and small numbers prevent us from making definitive conclusions about this association.

The analysis of late events included malignancies and new onset of autoimmune disease. With regard to malignancies, 3 patients (1%) were reported with a myelodysplastic syndrome, a disorder associated with prior treatment with cytotoxic drugs; the other neoplasms reported in this cohort are usually not associated to previous chemotherapy. The incidence of new autoimmune disease was not negligible (5%), in line with a survey recently carried out by EBMT²⁵, yet considerably lower than after lymphocyte-depleting treatment with alemtuzumab that approaches a risk of 50%²⁶.

The main limitation of our study is its partially retrospective nature. Although some of the data was collected retrospectively from clinical records, we took many steps to optimize the analysis. As most database studies, the reported outcomes mirror the practice for MS treatment in many countries.

EDSS assessments were not rater-blinded and not systematically performed for the duration of follow-up in every patient, thus we limited the analysis of progression-free survival to the large subset of patients (85%) who had yearly EDSS rating. The number of patients with enough data points for the different analyses was variable and sometimes low, which reduced statistical power. In a retrospective study incomplete reporting and loss to follow-up may result in underestimating the frequency of late adverse events. We also acknowledge the limitation that although our analysis includes the majority (57%) of the transplants registered with CIBMTR and EBMT during the time period, more than one third of the activity was not captured by our study. However, the reason for 166/212 (78%) of the unavailable cases was that the centers where the patients were treated declined to participate in the study; 43 (74%) out of 58 centers which did not join the study had performed less than 3 transplants and lack of incentive for the clinicians to contributing few cases to a large study was stated in many centers' responses. Based on this information we do not expect that the unavailability of those cases could represent a significant source of bias.

In summary, in this large observational cohort of MS patients with predominantly progressive forms of MS treated with AHSCT and followed long-term, almost half survived free from neurological progression for 5 years after transplantation. Taken together, the multivariate statistics indicate that the profile of a patient who is more likely to survive free from neurological progression is that of a younger subject with relapsing MS who has failed no more than two disease-modifying treatments and has not reached high levels of disability. These associations strengthen the case for an evaluation of safety and efficacy of AHSCT in a randomized controlled trial against approved therapies of high efficacy as first- or second line of treatment in patients with highly active relapsing MS, as suggested by experts' consensus ²⁷. Furthermore, our results raise the question whether AHSCT may attenuate

the progression of disability in patients with progressive forms of MS, a possibility that is more plausible in patients with MRI evidence of CNS inflammatory activity pre-transplant^{8,12,15} and that could be addressed in a randomized trial of AHST controlled against standard care.

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Author Contributions: Drs Muraro and Sormani had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Muraro, Pasquini, Saccardi.

Acquisition, analysis, or interpretation of data: Muraro, Pasquini, Atkins, Bowen, Farge, Fassas, Freedman, Georges, Gualandi, Hamerschlag, Havrdova, Kimiskidis, Kozak, Mancardi, Massacesi, Moraes, Nash, Pavletic, Ouyang, Rovira, Saiz, Simoes, Trneny, Zhu, Badoglio, Zhong, Sormani and Saccardi.

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Study supervision: Muraro.

Declaration of interests

PAM declares honoraria for speaking and travel support from Merck Serono, Biogen, Bayer, and Novartis. JB declares financial relationships as Consultant for Acorda Therapeutics, Biogen IDEC, Genzyme, Genentech, Pfizer/ EMD Serono, Novartis, Teva Neuroscience; as speaker for corda Therapeutics, Biogen IDEC, Pfizer/ EMD Serono, Novartis, Teva Neuroscience; and as recipient of Grant/Research support from Acorda Therapeutics, Alexion, Avanir, Biogen, EMD Serono, Genzyme, Glaxo Smith Kline, Medimmune, Novartis, Osmotica, Roche, Sanofi-Aventis, Synthon, Vaccinex, Xenoport; and as stock shareholder with Amgen. MSF received honoraria or consultation fees from BayerHealthcare, BiogenIdec, Chugai, EMD Canada, Genzyme, Merck Serono, Novartis,

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FIGURE LEGENDS

Figure 1. MS progression-free survival. Probabilities of EDSS progression-free survival after AHSCT are shown by Kaplan-Meier analysis (**A**) in the whole patient cohort and in subgroups stratified according to the factors identified by multivariate analysis as affecting progression-free survival, respectively: (**B**) in quartiles according to age ($p = 0.022$ for trend); (**C**) in patients with relapsing-remitting (RR), secondary progressive (SP) and primary progressive (PP) forms of MS ($p = 0.007$ for heterogeneity); and (**D**) in patients who received 1-2 or 3 previous disease-modifying treatments ($p = 0.008$ for heterogeneity). The different shades of grey represent 95% Confidence Intervals for each K-M line.

Figure 2. Evolution of EDSS scores before and after AHSCT in relapsing and progressive MS.

The individual (colored dotted) and integrated (solid black) lines depict the evolution of EDSS scores in the subset of patients who had both longitudinal pre-transplantation and post-transplant EDSS data and the date of EDSS assessment documented ($n = 111$). These are subdivided in relapsing (**A**; $n = 32$) and progressive (**B**; $n = 79$) forms of MS at the time of transplant. Rapid worsening of disability was observed prior to transplant in both subgroups, as expected for patients with aggressive forms of MS who were selected for AHSCT. The integrated line suggests that on average the accrual of disability was stopped in relapsing MS patients during the first 2 years post-transplant.

Figure 3. Overall survival. The probability of survival after AHSCT is shown as Kaplan-Meier analysis in the whole patient cohort (**A**). Since higher baseline EDSS score was found by multivariate analysis to be independently associated with worse survival (see Table 2 for details) we show in (**B**) the probabilities of survival after AHSCT in three strata of patients with different levels of disability at baseline assessment (Expanded Disability Status Scale, EDSS brackets: 0-5.5; 6-6.5; ≥ 7), which differed significantly for the highest EDSS bracket ($p = 0.004$ for heterogeneity). The grey shades represent 95% Confidence Intervals for each K-M line.

TABLES

Table 1: Demographic and clinical characteristics of patients

	CIBMTR	EBMT	TOTAL
Number of patients	111	170	281
Number of centers	8	17	25
Age, median (range), years	40 (26 - 60)	35 (15-65)	37 (15-65)
10-19	0 (0%)	6 (4%)	6 (2%)
20-29	11 (10%)	47 (28%)	58 (21%)
30-39	40 (36%)	67 (39%)	107 (38%)
40-49	44 (40%)	41 (24%)	85 (30%)
50+	16 (14%)	9 (5%)	25 (9%)
Gender			
Male	48 (43%)	69 (41%)	117 (42%)
Female	63 (57%)	101 (59%)	164 (58%)
EDSS prior to mobilization			
N evaluated	111 (100%)	170 (100%)	281(100%)
median (range)	6.5 (2.5-9)	6.5 (1.5-9)	6.5 (1.5-9)
EDSS prior to conditioning			
Missing	62 (56%)	39 (20%)	101 (36%)
N evaluated	49 (44%)	131 (80%)	180 (64%)
median (range)	6.0 (3.5-9.0)	6.5 (1.5-9.5)	6.0 (1.5-9.5)
Number of MS treatments¹ prior to transplant			
Missing	5	27	32
N evaluated	106	143	249
1 treatment	42 (40%)	36 (25%)	78 (28%)
2 treatments	30 (28%)	33 (26%)	63 (22%)
>2 treatments	34 (32%)	74 (52%)	108 (38%)
Disease status at baseline			
Relapsing remitting	12 (11%)	34 (20%) ²	46 (16%)
Progressive relapsing	--	17 (10%)	17 (6%)
Primary progressive	16 (14%)	16 (9%)	32 (11%)
Secondary progressive	83 (75%)	103 (61%)	186 (66%)
Time from diagnosis to transplant			
N evaluated	110 (99%)	170 (100%)	280 (99%)
median (range), months	91 (<1-413)	77 (2-340)	81(<1-413)
Time from mobilization to transplant			
N evaluated	79 (72%)	169 (99%)	248 (88%)
median (range), months	1 (<1-7)	2 (<1-9)	2 (<1-9)

Year of transplant

1995-2000	24 (22%)	71 (42%)	95 (34%)
2001-2006	87 (78%)	99 (58%)	186 (66%)
Chemo- mobilization³	101 (91%)	162 (95%)	263 (93%)

Graft manipulation⁴

Yes	53 (48)	70 (41%)	123(44%)
No	58 (52)	100 (59%)	158(56%)

Conditioning regimen

High Intensity	43 (39%)	10 (6%)	53 (19%)
CY+TBI+ATG	28 (25%)	0	28 (10%)
BU+CY+ATG	15 (14%)	0	15 (6%)
BU+ATG	0	10 (6%)	10 (3%)
Intermediate Intensity	28 (25%)	151 (89%)	179 (64%)
BEAM+ATG	23 (21%)	86 (51%)	109 (39%)
BEAM	0	40 (24%)	40 (14%)
CY+THIO	0	7 (4%)	7 (3%)
TLI+Melphalan	5 (4%)	0	5 (2%)
BCNU+CY+ATG	0	18 (10%)	18 (6%)
Low Intensity	40 (36%)	9 (5%)	49 (17%)
CY+ATG	37 (33%)	9 (5%)	46 (16%)
CY+FLUD	3 (3%)	0	3 (1%)
ATG	104 (94%)	128 (75%)	232 (83%)

1: Indicates the number of disease modifying therapies received for treatment of MS prior to AHSCT

2: Includes one case reported as Marburg-type MS

3: Indicates whether a chemotherapy was associated to Growth Factors to mobilize Hematopoietic Stem Cells

4: Indicates whether manipulation of the graft was carried out; either with CD34 selection or T cell depletion

Abbreviations: EDSS=Expanded Disability Status Scale, range 0-10, 0=no disability, 10=dead; ATG, antithymocyte globulin; BEAM, carmustine, etoposide, cytarabine and melphalan; BCNU, carmustine; Bu, busulfan; CY, cyclophosphamide; FLUD, fludarabine; TBI, total body irradiation; THIO, thiotepa; TLI, total lymphoid irradiation.

Key Points

Question: What are the long-term outcomes following autologous hematopoietic stem cell transplantation for treatment of multiple sclerosis?

Findings: In this multicenter observational retrospective cohort study of 281 patients with predominantly (78%) progressive forms of multiple sclerosis who underwent autologous hematopoietic stem cell transplantation during 1995-2006, transplant-related mortality was 2.8% and neurological progression-free survival was 46% at 5 years. Relapsing multiple sclerosis, younger age, less prior immunotherapies and lower neurological disability score were significantly associated with better outcomes.

Meaning: The results support the rationale for further, randomized controlled studies of autologous hematopoietic stem cell transplantation for treatment of multiple sclerosis.

Word count: 99

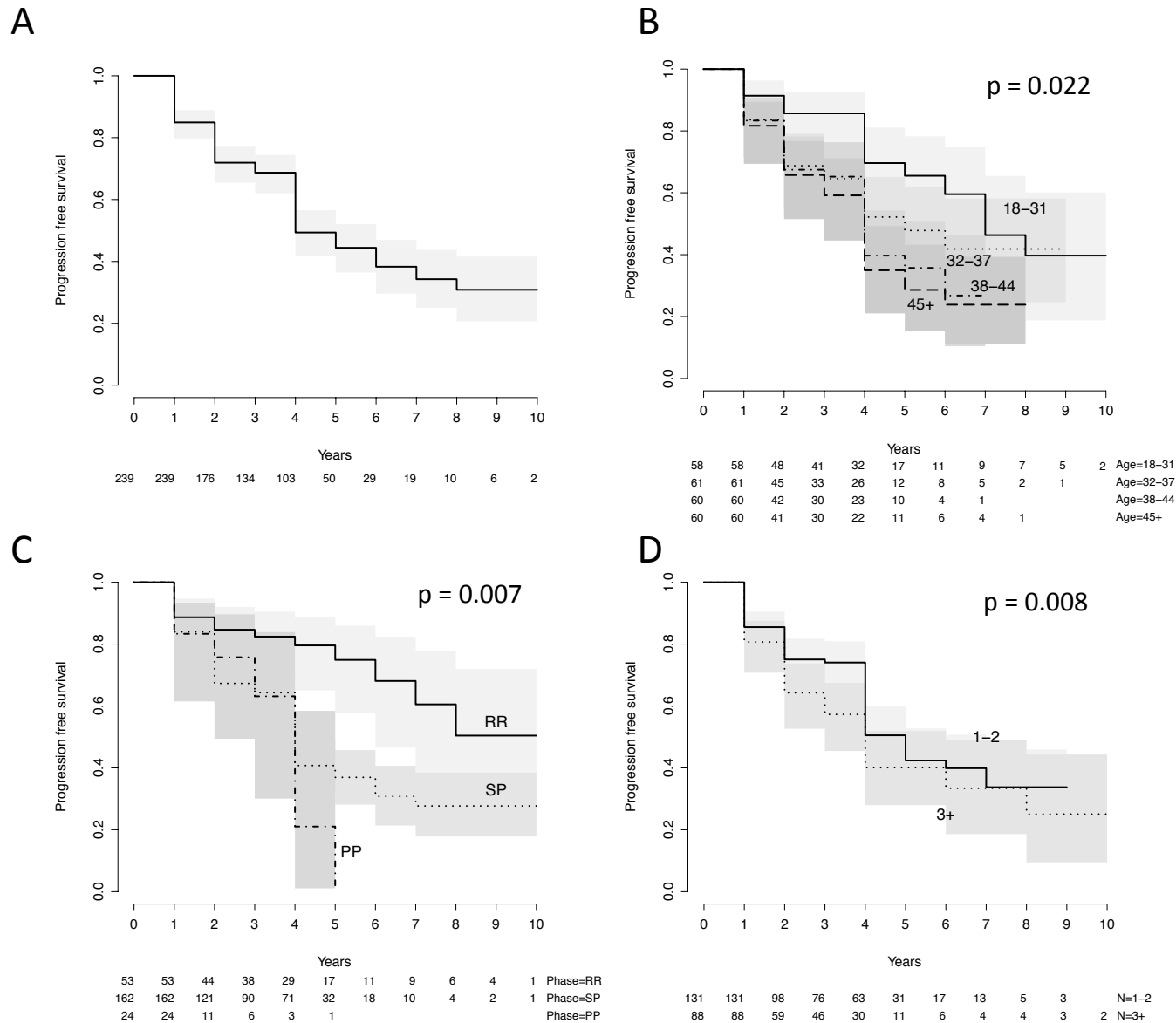


Figure 1. MS progression-free survival. Probabilities of EDSS progression-free survival after AHSCT are shown by Kaplan-Meier analysis **(A)** in the whole patient cohort and in subgroups stratified according to the factors identified by multivariate analysis as affecting progression-free survival, respectively: **(B)** in quartiles according to age ($p = 0.022$ for trend); **(C)** in patients with relapsing-remitting (RR), secondary progressive (SP) and primary progressive (PP) forms of MS ($p = 0.007$ for heterogeneity); and **(D)** in patients who received 1-2 or 3 previous disease-modifying treatments ($p = 0.008$ for heterogeneity). The different shades of grey represent 95% Confidence Intervals for each K-M line.

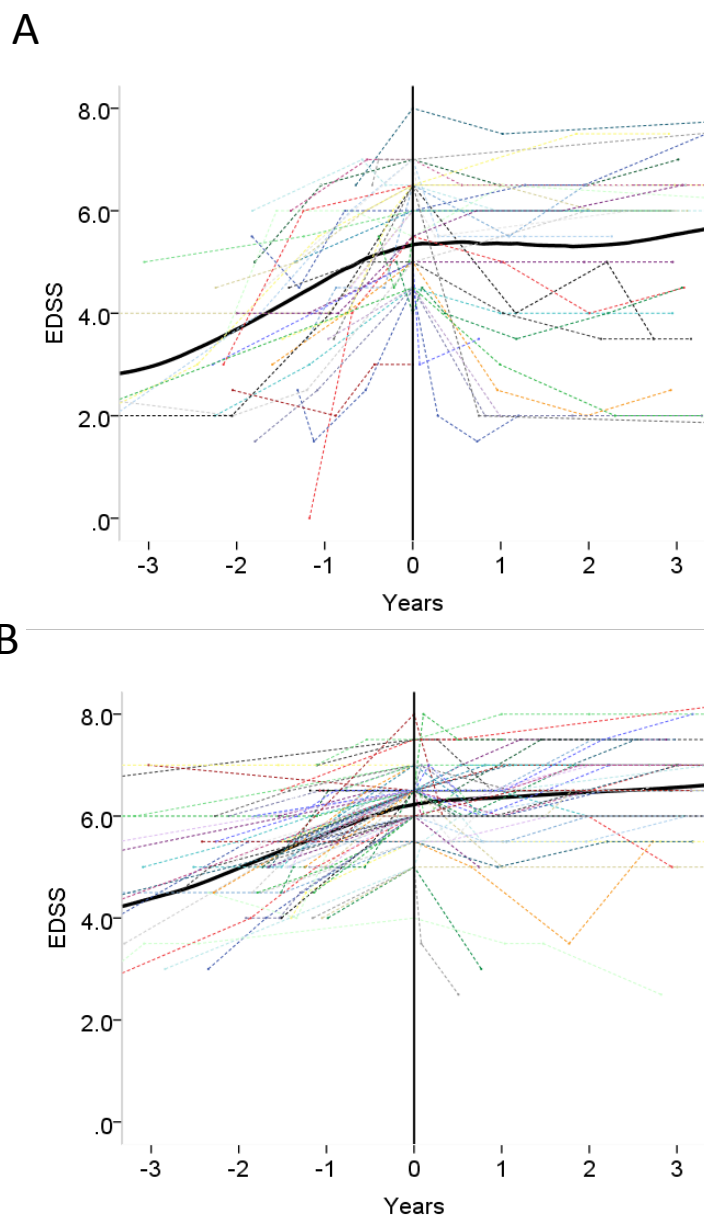


Figure 2. Evolution of EDSS score before and after AHSCT in relapsing and progressive MS.

The individual (colored dotted) and integrated (solid black) lines depict the evolution of EDSS scores in the subset of patients who had both longitudinal pre-transplantation and post-transplant EDSS data and the date of EDSS assessment documented (n =111). These are subdivided in relapsing (**A**; n = **XX**) and progressive (**B**; n = **XX**) forms of MS at the time of transplant. Rapid worsening of disability was observed prior to transplant in both subgroups, as expected for patients with aggressive forms of MS who were selected for AHSCT. The integrated line suggests that on average the accrual of disability was stabilized in relapsing MS patients during the first 2 years post-transplant.

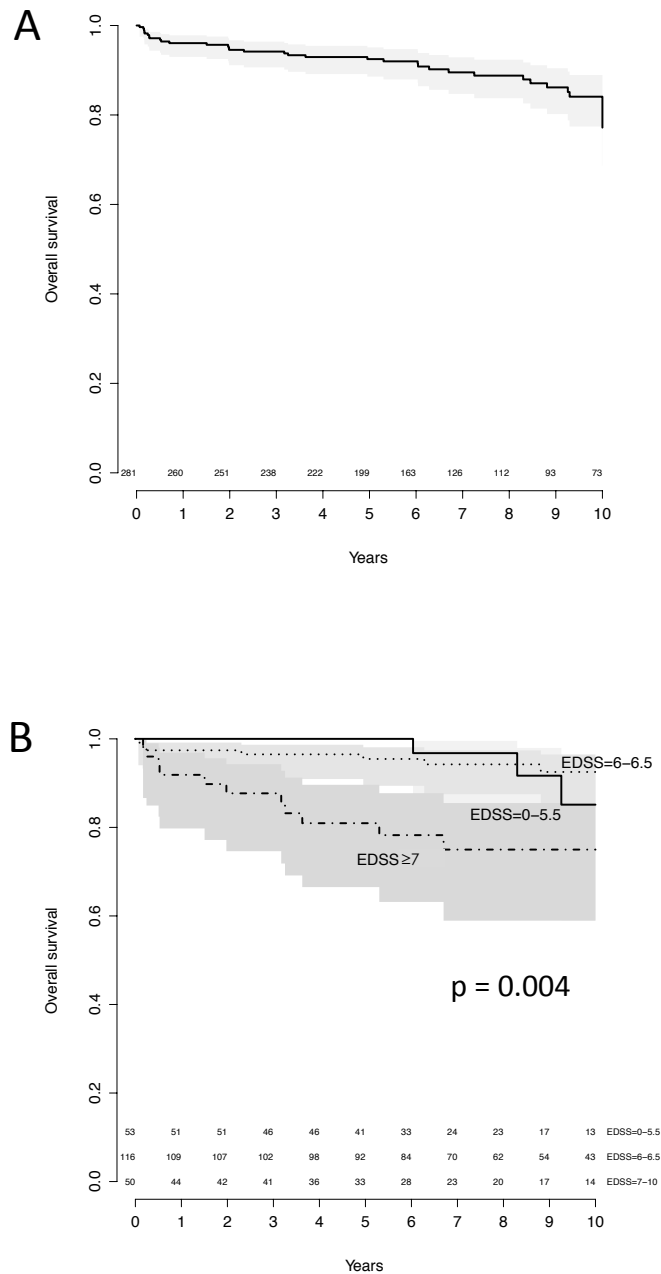


Figure 3. Overall survival. The probability of survival after AHSCT is shown as Kaplan-Meier analysis in the whole patient cohort (**A**). Since higher baseline EDSS score was found by multivariate analysis to be independently associated with worse survival (see Table 2 for details) we show here in (**B**) the probabilities of survival after AHSCT in three strata of patients with different levels of disability at baseline assessment (Expanded Disability Status Scale, EDSS brackets: 0-5.5; 6-6.5; ≥ 7), which differed significantly for the highest EDSS bracket ($p = 0.004$ for heterogeneity). The grey shades represent 95% Confidence Intervals for each K-M line.

ONLINE SUPPLEMENTAL MATERIAL TO:

Muraro et al., Long Term Outcomes after Autologous Hematopoietic Stem Cell Transplantation for Multiple Sclerosis

List of supplemental content:

1 Supplementary Figure:

eFigure 1: CONSORT type diagram of enrollment in the study database

4 Supplementary Tables:

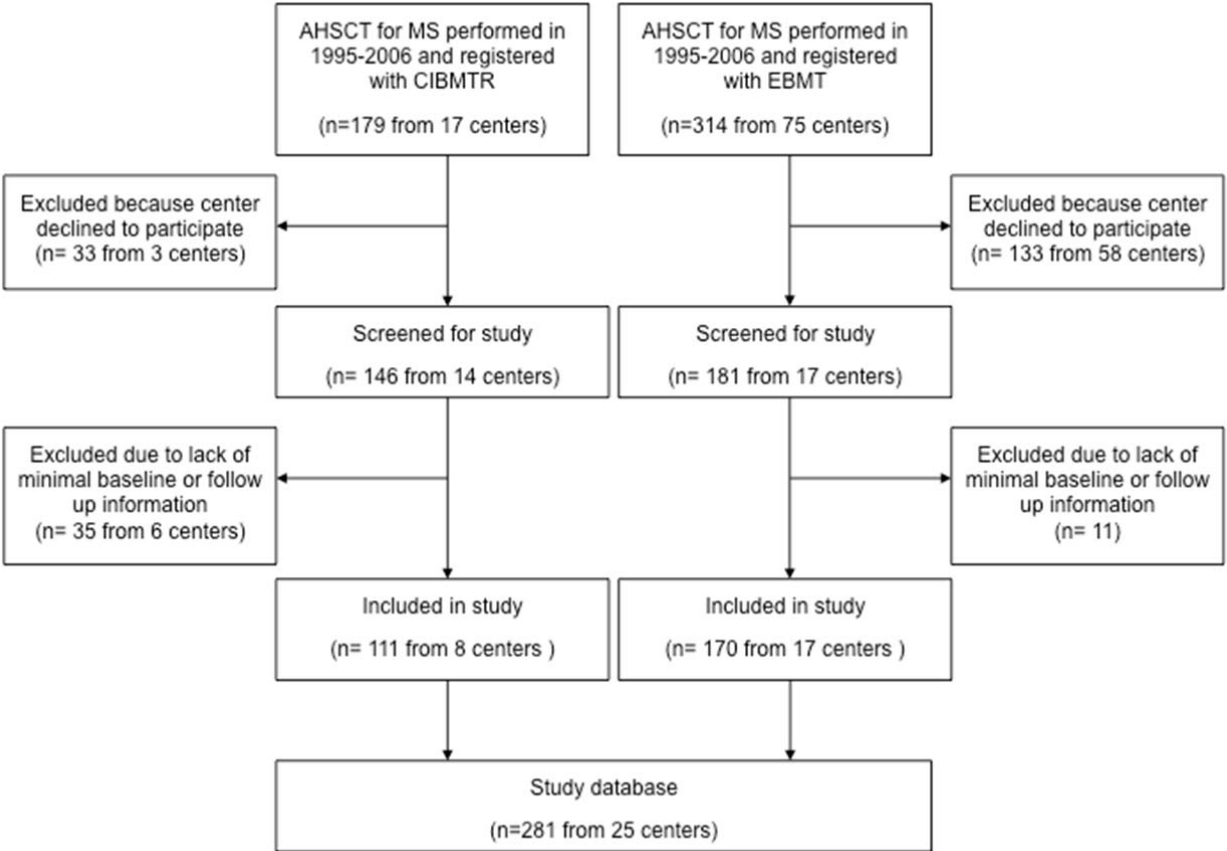
eTable 1: Kurtzke Expanded Disability Status Scale (EDSS)

eTable 2: Data on factors associated with lower overall survival in the patients who died during follow-up

eTable 3: Causes of death and prior MS treatments

eTable 4: Late adverse events

eFigure 1: CONSORT type diagram of enrollment in the study database



eTable 1: : Kurtzke Expanded Disability Status Scale (EDSS)

Reference: Kurtzke J.F. (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 33, 1444-1452

The EDSS provides a total score on a scale from 0 to 10. Scores of 1.0 to 4.5 refer to people with a high degree of ambulatory ability and subsequent levels 5.0 to 9.5 refer to loss of ambulatory ability. In addition, it quantifies disability in eight functional systems (FS) and allows neurologists to assign a Functional System Score (FSS) in each of these.

The functional systems are: (1) Pyramidal (2) Cerebellar (3) Brainstem (4) Sensory (5) Bowel and Bladder (6) Visual and (7) Cerebral or Mental.

The Functional systems are scored on a scale of 0-5 to best reflect the level of disability observed clinically. The 'other' category is not rated numerically.

EDSS scores below 4.0 are determined by the FSS alone. Scores between 4.0 and 9.5 are determined by both gait abilities and the FSS.

0	Normal neurological exam (all grade 0 in Functional Systems (FS); cerebral grade 1 acceptable).
1	No disability, minimal signs in one FS (i.e. one grade 1 excluding cerebral grade 1).
1.5	No disability, minimal signs in more than one FS (more than one grade 1 excluding cerebral grade 1).
2.0	Minimal disability in one FS (one FS grade 2, others 0 or 1).
2.5	Minimal disability in two FS (two FS grade 2, others 0 or 1).
3.0	Moderate disability in one FS (one FS grade 3, others 0 or 1), or mild disability in three or four FS (three-four FS grade 2, others 0 or 1).
3.5	Fully ambulatory but with moderate disability in one FS (one grade 3 and one or two FS grade 2) or two FS grade 3, others 0 or 1, or five FS grade 2, others 0 or 1.
4.0	Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest some 500 meters or 0.3 miles.
4.5	Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability. (Usually consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest for some 300 meters or 975 ft).
5.0	Ambulatory without aid or rest for about 200 meters or 650 ft; disability severe enough to impair full daily activities (e.g., to work full day without special provisions). (Usual FS equivalents are one grade 5 alone (others 0 or 1); or combinations of lesser grades usually exceeding specifications for step 4.0.)
5.5	Ambulatory without aid or rest for about 100 meters or 325 ft; disability severe enough to impair full daily activities. (Usual FS equivalents are one grade 5 alone (others 0 or 1); or combinations of lesser grades usually exceeding specifications for step 4.0.)
6.0	Intermittent or constant unilateral assistance (cane, crutch, or brace) required to walk about 100 meters or 325 ft. with or without resting. (Usual FS equivalents are combinations with more than two FS grade 3+.)

6.5	Constant bilateral assistance (canes, crutches, or braces) required to walk about 20 meters or 65 ft. (Usual FS equivalents are combinations with more than two FS grade 3+.)
7.0	Unable to walk beyond about 5 meters or 16 ft. even with aid, essentially restricted to wheelchair, wheels self in standard wheelchair a full day and transfers alone; up and about in wheelchair some 12 hours a day. (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely pyramidal grade 5 alone.)
7.5	Unable to take more than a few steps; restricted to wheelchair; may need aid in transfers, wheels self but cannot carry on in standard wheelchair a full day; may require motorised wheelchair. (Usual FS equivalents are combinations with more than one FS grade 4+.)
8.0	Essentially restricted to bed or chair or perambulated in wheelchair; but may be out of bed much of the day; retains may self-care functions; generally has effective use of arms. (Usual FS equivalents are combinations, generally grade 4+ in several systems.)
8.5	Essentially restricted to bed for much of the day; has some effective use of arm(s); retains some self-care functions. (Usual FS equivalents are combinations, generally grade 4+ in several systems.)
9.0	Helpless bed patient; can communicate and eat. (Usual FS equivalents are combinations, mostly grade 4.)
9.5	Totally helpless bed patient; unable to communicate or effectively eat/swallow. (Usual FS equivalents are combinations, almost all grade 4+.)
10	Death due to MS.

eTable 2: Data on factors associated with lower overall survival in the patients who died during follow-up

	Death within day 100	Death after day 100	Whole Cohort
Number of patients	8	29	281
Age, median (range), years	40 (38 - 57)	42 (20-60)	37 (15-65)
N aged 10-19	0	0	6 (2%)
N aged 20-29	0	3 (10%)	58 (21%)
N aged 30-39	4 (50%)	10 (35%)	107 (38%)
N aged 40-49	3 (38%)	13 (45%)	85 (30%)
N aged 50+	1 (12%)	3 (10%)	25 (9%)
EDSS prior to mobilization			
N evaluated	5 (63%)	21 (72%)	281(100%)
Median EDSS (range)	6.5 (6.0-7.5)	7.0 (5.0-9.0)	6.5 (1.5-9.0)
MS disease type at baseline			
Relapsing	1 (12%)	2 (7%)	63 (22%)
Progressive	7 (88%)	27 (93%)	218 (78%)
Conditioning regimen			
High Intensity	2 (25%)	11 (38%)	53 (19%)
CY+TBI+ATG	1 (12%)	8 (28%)	28 (10%)
BU+CY+ATG	1 (12%)	3 (10%)	15 (6%)
BU+ATG	0	0	10 (3%)
Intermediate Intensity	6 (75%)	15 (52%)	179 (64%)
BEAM+ATG	6 (75%)	9 (31%)	109 (39%)
BEAM	0	6 (21%)	40 (14%)
CY+THIO	0	0	7 (3%)
TLI+Melphalan	0	0	5 (2%)
BCNU+CY+ATG	0	0	18 (6%)
Low Intensity	0	3 (10%)	49 (17%)
CY+ATG	0	2 (7%)	46 (16%)
CY+FLUD	0	1 (3%)	3 (1%)

eTable 3: Causes of death and prior MS treatments**A. Causes of death within 100 days from transplant**

	n	Prior MS treatments*
<i>Patients at risk</i>	281	
Infection	2	IFNb (n=2), AZA (n=1)
Accident	1	IFNb1a, mitox
Veno-occlusive disease	1	IFNb1a
Hemorrhage	1	IFNb
EBV lympho- proliferative disorder	1	IFNb1a, IFNb1b, GA, mitox
Not reported	2	IFNb (n=2), mitox (n=1)
Total	8 (2.8%)	

B. Causes of death after post-transplant day 100

	n	Prior MS treatments
<i>Patients at risk</i>	273	
MS progression	9	IFNb (n=7)
Infection	5	IFNb (n=3), CY, mitox
Malignancies	3	IFNb (n=2), CY (n=1), AZA (n=1)
Accident	1	IFNb1a
Diverticulitis	1	Methotrexate
Organ failure	1	Corticosteroids only
Rejection/poor graft function	1	AZA, IvIG, IFNb1a, IFNb1b, mitox
Arterial thromboembolism lower limbs	1	None reported
Interstitial pneumonia	1	IFNb, AZA
Sepsis	1	IFNb
Gastrointestinal bleeding	1	IFNb
Not reported	4	IFNb (n=3)
Total	29 (10%)	

* Immune-modifying treatments given for MS are listed for each cause of death. Numbers indicate how many patients within each subgroup received the stated treatment. Individual patients may, and in most cases will, have received >1 treatment. Abbreviations: AZA, azathioprine; CY, cyclophosphamide; GA, glatiramer acetate; IFNb, interferon beta; mitox, mitoxantrone.

eTable 4: Late adverse events**A. Malignancies**

Myelodysplastic syndrome	3
Breast cancer	2
Squamous cell carcinoma	1
Prostate cancer	1
Cervical Carcinoma	1
Glioblastoma Multiforme	1
Total	9

B. New Autoimmune Disease

Autoimmune thyroid disease	8
Autoimmune thrombocytopenia	3
Acquired haemophilia	2
Crohn's disease	1
Total	14