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Long-Term Clinical Outcomes of Hematopoietic Stem Cell Transplantation in Multiple Sclerosis

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Abstract

Objective: To determine whether autologous hematopoietic stem cell transplantation (aHSCT) is able to induce durable disease remission in people with multiple sclerosis (MS), we analyzed the long-term outcomes after transplant in a large cohort of MS patients.

Methods: To be included, a minimum data set (consisting of age, MS phenotype, EDSS at baseline, information on transplant technology and at least 1 follow-up visit after transplant) was required.

Results: 210 patients were included [relapsing-remitting (RR)MS=122(58%)]. Median baseline EDSS was 6(1-9), mean follow-up was 6.2(±5.0) years. Among RRMS patients, disability worsening-free survival (95%CI) was 85.5%(76.9-94.1%) at 5 years and 71.3%(57.8-84.8%) at 10 years. In patients with progressive MS, disability worsening-free survival was 71.0%(59.4-82.6%) and 57.2%(41.8-72.7%) at 5 and 10 years, respectively. In RRMS patients, EDSS significantly reduced after aHSCT [p=0.001; mean EDSS change per year -0.09 (95%CI=-0.15 to -0.04%)]. In RRMS patients, the use of the BEAM+ATG conditioning protocol was independently associated with a reduced risk of NEDA-3 failure [HR=0.27(0.14-0.50), p<0.001]. Three patients died within 100-days from aHSCT (1.4%); no deaths occurred in patients transplanted after 2007.

Conclusions: aHSCT prevents disability worsening in the majority of patients and induces durable improvement in disability in patients with RRMS. The BEAM+ATG conditioning protocol is associated with a more pronounced suppression of clinical relapses and MRI inflammatory activity.

Classification of Evidence: This study provides Class IV evidence that for people with MS, aHSCT induces durable disease remission in most patients.

Introduction

Several disease modifying therapies have been shown to reduce disease activity in people with multiple sclerosis (MS). However long-term disease remission remains elusive[1] and approved therapies have not demonstrated consistent effects in preventing long-term disability progression. Despite treatment, more than half of relapsing-onset MS patients accumulate disability over 10 years[2]. The early abrogation of relapses and MRI inflammatory activity has little impact on neurological outcomes at 10 years[2,3], questioning the utility of short term outcomes to assess the long-term effect of treatment on disability progression.

Disease control is particularly relevant for aggressive MS[4], characterized by accelerated accrual of irreversible disability. Intense immunosuppression followed by autologous hematopoietic stem cell transplantation (aHSCT) has been extensively explored as a treatment strategy for aggressive MS[5–12]. The rationale of aHSCT in MS is to eliminate self-reacting cell clones and to induce self-tolerance through a profound renewal of the immune system[13–16]. To date, outcome assessment after aHSCT is limited to a short follow-up and it's still unclear whether aHSCT is able to induce long-term drug-free disease remission. The largest registry-based study on aHSCT in MS[17] has reported that almost half of transplanted patients remained free from neurological progression in the following 5 years. Against this background, in Italy aHSCT has been extensively used for MS since 1996[8]. To determine whether aHSCT is able to prevent long-term disability worsening, we analyzed the outcomes in a large cohort of people with aggressive MS who underwent aHSCT for the treatment of MS in Italy.

Methods

Study Design

This study was an observational, retrospective, multicenter cohort study on aHSCT for the treatment of MS, collecting data from MS patients transplanted in Italy from 1997 to 2019.

In July 1998, five Italian neurologic teams, together with the Italian Cooperative Group for Bone Marrow and Blood Transplantation (GITMO), initiated a phase I/II trial on the use of aHSCT in MS[18]. Thereafter, other Italian MS centers developed local transplant programs for MS patients, (mostly identical to those developed by the two leading haemato-neurological centers in Italy - Florence and Genoa-). Although no formal guidelines on patients selection for aHSCT exist, all treated patients had aggressive MS, characterized by the occurrence of severe relapses or MRI inflammatory activity or accelerated accrual of neurological disability despite active treatment. Patients were treated with aHSCT according to the European Group for Blood and Marrow Transplantation (EBMT) guidelines, following the decision of the treating physician and approval of the local Ethics Committee.

To be included in the present retrospective study, a minimum data set [consisting of age, MS phenotype, expanded-disability-status-scale (EDSS) at baseline, information on the transplant technology and at least 1 follow-up visit after transplant] was required. For the analysis of MRI disease activity, only patients with yearly brain MRI records were considered.

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained from all patients. All participants provided consent to use their medical history for publication. This retrospective study was approved by the ethical standards committee of the coordinating center (protocol number 61/08).

Conditioning regimens and transplant care

Peripheral hematopoietic stem cells (PBSCs) were mobilized with cyclophosphamide (CY) (4 or 2g/m^2 iv) and filgrastim (5-10 μg/kg/day sc). PBSCs were collected with a leuko-apheresis procedure and an unmanipulated graft targeted to 3-8x10^6 CD34$^+$ cells/kg was cryopreserved. Patients were transplanted using different conditioning regimens, according to center experience and preference: (i) BEAM+ATG regimen (74.8%), which includes BCNU (carmustine, 300 mg/m^2

at day -6), cytosine-arabinoside (200 mg/m^2) and etoposide (200 mg/m^2) from day -5 to day -2 and melphalan (140 mg/m^2) at day -1, followed by rabbit anti-thymocyte globulin (ATG) (3.75-5 mg/kg/day) at days +1 and +2; (ii) BEAM regimen as above described without rabbit ATG (4.8%); (iii) FEAM regimen (1.9%), substituting fotemustine (150 mg/m^2 on days -7, -6) instead of BCNU in the BEAM regimen; (iv) CY+ATG regimen I (8.1%), containing CY (60mg/kg at day –3 and –2) followed by rabbit ATG (3.75 mg/kg/d at day +1 and +2); (v) CY+ATG regimen II (4.8%), containing CY (50 mg/Kg/d at days -5 to day -2) and rabbit ATG (2.5 mg/Kg/d at day -4 and -2); (vi) Thiothepa+CY regimen (4.8%), consisting of thiothepa 10 mg/kg for 5 days and CY 50 mg/kg at day -3 and -2. One patient was transplanted with a conditioning regimen made of BCNU and melphalan (0.5%) and one patient was transplanted with a conditioning regimen made of bortezomib, cyclophosphamide, dexamethasone and melphalan (0.5%). Anti-herpetic and anti-pneumocistis jirovecii prophylaxes were performed with Acyclovir and Sulphamethoxazol-Trimetoprim, respectively, according to centers protocols. After aHSCT, patients did not receive immune-based therapies unless they experienced clinical relapse, new lesions on MRI, or EDSS progression, based on decision by the treating neurologist.

Study endpoints

The primary endpoint was to analyze the long-term 6 months-confirmed disability worsening as measured by EDSS. Secondary objectives were the evaluation of (i) the evolution of the EDSS scores after transplant, (ii) the occurrence of relapses, (iii) the occurrence of MRI inflammatory activity, (iv) the proportion of patients achieving “no-evidence-of-disease-activity (NEDA) status”, a composite endpoint which includes the absence of clinical relapses, EDSS worsening and MRI inflammatory activity (v) the effect of the different conditioning regimens on long-term outcomes and (vi) the early transplant-related mortality. The analysis of the primary and the secondary end-points generate class IV evidence of the long-term effects of transplant in people with aggressive MS. Disability worsening was defined as an increase of 1 point in the EDSS score (0.5 points if the

baseline EDSS score was ≥5.5) confirmed after 6 months. Baseline was defined as the last neurological assessment before the administration of mobilizing therapy. All relapses were clinically-assessed by treating neurologists. Follow-up for any component of NEDA score was not censored by earlier events so that each has an independent interpretation. MRI activity was defined as the presence of new/enlarging T2 lesions or T1 gadolinium-enhancing lesions detected by radiologists on routine follow up MRI. The baseline brain MRI (acquired within 3 months before the aHSCT procedure) was the pre-treatment reference scan for assessment of treatment failure and no re-baseline was performed. All deaths occurring in the first 100 days after transplant were reported and considered likely transplant-related[19].

Statistical analyses

The probability of disability worsening-free survival, relapse-free survival, MRI-activity free-survival and NEDA-3 status was calculated with the Kaplan-Meier estimator. Univariate and multivariate analyses assessing the association of disease- and treatment-related characteristics with survival endpoints were performed using Cox proportional hazards regression analysis models. Variables significantly associated with each outcome event on univariate analysis were included as covariates in the multivariate model. A linear mixed model with random intercept and random slope was carried out in order to detect changes in the EDSS scores before *vs* after transplant. A two-sided $p<0.05$ was used for statistical significance. All analyses were performed using SPSS 23 (IBM; version 23.0) and R software.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Patients demographics and procedures

Patients from 20 Italian MS centers who underwent transplant from 1997 to 2019 were identified (n=210). Demographic, clinical and hematological characteristics of the study cohort are summarized in Table 1. Out of 210 patients, n=196 (93.3%) were eligible for the analysis of the primary endpoint. As for relapse occurrence, data were available for 198 (94.3%) patients. Serial brain MRI radiology records were available for 167 (79.5%) patients. At the time of transplant, 122 patients (58%) had a relapsing-remitting (RR) phenotype of MS (RRMS), 86 patients (41%) had secondary progressive (SP) MS and 2 patients (1%) had primary-progressive MS. Data on previous treatment history is available for 175 patients (83.3%). 118 patients had been exposed to interferon-beta, 55 to natalizumab, 54 to pulsed cyclophosphamide, 53 to mitoxantrone, 39 to azathioprine, 38 to glatiramer acetate, 29 to fingolimod, 7 to alemtuzumab and 6 to rituximab. Among patients with RRMS, those who were transplanted with the BEAMT+ATG protocol were older (34.0 years versus 28.3 years; p<0.0001), had longer disease duration (10.3 years versus 7.1 years; p=0.029) and had a shorter follow-up (5.1 years versus 7.2 years; p=0.027). Among patients with progressive MS, the BEAM+ATG subgroup had higher EDSS scores one year before transplant (median EDSS of 6 versus 5; p=0.027).

Disability worsening-free survival and the evolution of neurological disability

The probabilities of disability-worsening free survival for the entire study cohort and according to disease phenotype are reported in Figure 1A and 1B, respectively. In the entire study cohort, disability worsening-free survival was 79.5% (72.0-86.6%) and 65.5% (55.3%-75.7%) at 5 and 10 years. The RRMS phenotype was associated with a reduced risk of disability worsening [HR (95%CI)= 0.46 (0.24-0.86), p=0.015], with disability worsening-free survival rates of 85.5% (76.9%-94.1%) at 5 years and 71.3% (57.8%-84.8%) at 10 years. In RRMS, a higher treatment exposure before aHSCT was associated with a higher risk of disability worsening [HR=1.57 (1.12-2.20), p=0.009] (Table 2). Among patients with progressive MS, disability worsening-free survival

was 71.0% (59.4%-82.6%) and 57.2% (41.8%-72.7%) at 5 and 10 years, respectively. A higher number of relapses in the year before aHSCT was associated with a lower risk of disability worsening [HR=0.56 (0.34-0.92), p=0.022]. The use of the BEAM+ATG conditioning protocol did not influence the probabilities of disability worsening free-survivals. Progression-free survival in RRMS patients who were transplanted with the BEAM+ATG protocol was 81.9% (70.1%-93.7%) at 5 and 10 years.

Figure 1C shows the evolution of EDSS scores recorded after aHSCT in patients with RRMS and progressive MS. Among patients with RRMS, median EDSS scores significantly reduced after transplant over 10 years [p=0.001, mean EDSS change per year -0.09 (95%CI= -0.15 to -0.04)]. EDSS stabilized in patients with progressive MS, with no significant increase over time [p=0.42, mean EDSS change per year=0.02 (95%CI= -0.03 to 0.07)].

Secondary endpoints

The probabilities of relapse-free survival, MRI inflammatory activity-free survival and NEDA-3 status are reported in Figure 2 (RRMS) and Figure 3 (progressive MS), according to the conditioning regimen used in the transplant technology. For RRMS patients, relapse-free survival was 78.1% (68.5%-87.7%) and 63.5% (49.4%-77.6%) at 5 and 10 years after aHSCT. In RRMS patients treated with the BEAM+ATG protocol, relapse-free survival was 86.4% (75.8%-97.0%) and 77.0% (61.5%-92.5%) at 5 and 10 years. The use of the BEAM+ATG conditioning protocol [HR= 0.21 (0.09-0.49), p<0.0001] and an older age at transplant [HR=0.94 (0.88-0.99), p=0.034] were independently associated with a reduced risk of relapses (Table 2). Among patients with progressive MS, relapse-free survival was 88.3% (80.7%-96.0%) and 78.9% (63.4%-91.4%) at 5 and 10 years, respectively. The use of the BEAM+ATG conditioning protocol [HR=0.25 (0.71-0.86), p=0.029] was associated with a reduced risk of a relapse. In the entire study cohort, relapse-free survival was 82.9% (76.6%-89.2%) and 71.2% (61.8%-80.6%) 5 and 10 years after aHSCT, respectively.

Probabilities for MRI inflammatory activity-free survival for patients with RRMS were 74.6% (63.2%-85.6%) at 5 years and 52.7% (35.6%-69.7%) after 10 years. When the BEAM+ATG was used, the MRI inflammatory activity-free survival was 82.0% (68.5%-95.5%) and 65.5% (45.3%-85.7%) at 5 and 10 years, respectively. The use of the BEAM+ATG conditioning regimen [HR=0.24 (0.11-0.54), p=0.001] and an older age [HR=0.93 (0.88-1.00), p=0.041] were independently associated with a reduced risk of MRI inflammatory activity after aHSCT (Table 2). In the subgroup of patients with progressive MS, the MRI inflammatory activity-free survival was at 84.0% (74.2%-93.8%) and 78.7% (65.2%-92.2%) at 5 and 10 years, respectively. The use of the BEAM+ATG protocol was found to be associated with a higher probability of suppression of MRI inflammatory activity [HR=0.28 (0.08-1.00), p=0.048]. In the entire study cohort, the percentages of patients free of MRI inflammatory activity were 78.7% (71.1%-86.3%) at 5 years and 64.3% (52.7%-75.9%) at 10 years.

For patients with RRMS, probabilities of achieving NEDA-3 status were 62.2% (50.6%-73.8%) at 5 years and 40.5% (30.0%-55.0%) at 10 years. In the subgroup of RRMS patients who underwent aHSCT with the BEAM+ATG conditioning protocol, NEDA-3 status was achieved in 67.7% (53.2%-82.2%) and 54.9% (37.3%-72.5%) of patients at 5 and 10 years, respectively. In RRMS patients, the use of the BEAM+ATG protocol [HR=0.27 (0.14-0.50), p<0.001] was associated with a higher probability of maintaining NEDA-3 status (Table 2). In patients with progressive MS, NEDA-3 status estimates were 50.8% (37.3%-64.3%) and 37.3% (22.8%-52.6%) at 5 and 10 years respectively, and no baseline characteristics were found to be associated with the probability of NEDA-3 status. In the entire study cohort, NEDA-3 status was achieved in 57.9% of patients (49.1%-66.7%) at 5 years and in 39.8% of patients (29.2%-50.4%) 10 years after aHSCT.

When comparing the BEAM+ATG conditioning regimen with the cyclophosphamide-based protocols alone, we confirmed that, in patients with RRMS, the use of the BEAM+ATG was associated with a lower risk of relapse [HR=0.12 (0.05-0.32), p<0.001], MRI inflammatory activity [HR=0.18 (0.07-0.48), p=0.001] and with a higher probability of maintaining NEDA-3 status

[HR=0.18 (0.09-0.38), p<0.001] over the entire follow-up. In patients with progressive MS we did not find any difference between BEAM+ATG and cyclophosphamide-based regimens on treatment response.

Thirty-two patients (15.2%) started a new DMT after transplant. Median number of new DMTs was 1 (range 1-3, IQR 1-2), mean time to re-treatment was 3.7 years (SD=3.0) and median time was 2.08 years (range=0.54–13.0). DMTs initiated after aHSCT are listed in Table 3.

Three deaths occurred within 100 days following aHSCT (1.4% of the entire study population). Extensive data from these patients have already been reported[8]. Patient #1, a 38 years-old secondary-progressive MS patient, developed pulmonary thrombo-embolism, which caused a syncope with head trauma 56 days after aHSCT. He was treated with fibrinolytic treatment and died 48 hours later after intracranial hemorrhage. Patient #2, a 39 years-old RRMS patient, had engraftment failure and died 24 days after transplant due to an opportunistic infection caused by *Actinomyces sp*. Patient #3, a 48 years-old RRMS patient, died 1 month after transplantation from a Wernicke's like encephalopathy. All deceased patients have been transplanted with the BEAM+ATG conditioning regimen. No transplant-related deaths occurred in patients transplanted after 2007.

Discussion

Multiple sclerosis-related disability might take many years or decades to develop and very long follow-up periods are required in order to understand the role of treatments for MS.

We herein report the long-term outcomes in a large cohort of MS patients who underwent aHSCT in Italy in the last two decades, showing that 65.5% of patients were free of disability worsening 10 years after transplant, with a disability worsening-free survival greater than 70% in patients with RRMS. Our data extend previous studies at 5 years[5–8,17], demonstrating that the effects of aHSCT persist for over a decade. These results are of particular relevance considering that patients treated with aHSCT were affected by extremely aggressive forms of MS, which is not the case in available

randomized clinical trials. Of note, the 5-years progression-free survival rate in our cohort of RRMS (85.5%) is higher than those reported with other highly active treatments for MS, such as natalizumab[20] and alemtuzumab[21]. In line with previous observations[17], disability worsening-free survival in our cohort was higher in RRMS patients with lower treatment exposure, confirming the notion that aHSCT should be performed early in the course of the disease.

Based on our data, patients with progressive MS still benefit from aHSCT. Indeed, we found a disability worsening-free survival of 71% at 5 years, which was maintained in 57.2% of progressive MS patients at 10 years. Although a control group was not available, such low rates of disability worsening are an unexpected feature in progressive MS patients and deserve some consideration. Accrual of neurological disability in progressive MS seems to be associated with compartmentalized inflammation behind the blood–brain-barrier and recent data have demonstrated that targeting inflammation within the CNS slow the course of progressive MS[22,23]. All the different drugs used in the transplant technology share the ability to cross the blood-brain-barrier and to penetrate in the CNS, where they can halt compartmentalized inflammation slowing neurological deterioration. In line with this hypothesis, we found that a higher number of relapses in the year before aHSCT, indicating residual ongoing CNS inflammation[24], was associated with an increased probability of disability worsening-free survival. We did not find any association between disease duration and treatment effect. One possible explanation is that some patients of our cohort with relatively long disease duration experienced dramatic disease exacerbations after withdrawal of specific DMTs (especially natalizumab and fingolimod) and had excellent response to aHSCT, possibly hiding the effect of disease duration on treatment response.

According to other independent groups[5,11], we observed sustained EDSS reduction after transplant in RRMS patients. When speculating on the possible effects of aHSCT in improving MS-related disability, it's noteworthy that most of transplanted patients had experienced MS attacks right before aHSCT and the reduction in disability could represent the expected gradual recovery from

relapses. In our cohort neurological improvement was sustained over 10 years and EDSS scores continued to ameliorate beyond the first years following aHSCT, when recovery from relapses no longer occurs, suggesting a robust effect of aHSCT in improving neurological status. It's arguable that after CNS inflammation is completely suppressed, endogenous structural and functional plasticity mechanisms eventually reemerge[25], resulting in sustained clinical improvement.

The optimal intensity of the conditioning regimen for the treatment of MS remains an open question[26]. This is the first study suggesting that the use of the BEAM+ATG conditioning regimen is independently associated with a reduced probability of relapses, MRI activity and NEDA-3 failure in patients with RRMS. Our results are in line with the evidence that a high-intensity, busulfan-based[6], but not a low-intensity cyclophosphamide-based[27], conditioning regimen was able to completely abrogate MRI activity and clinical relapses. These results are also in line with the evidence that the bone marrow is the major site of memory helper T cells[28] and memory plasma cells which are resistant to treatment with cyclophosphamide[29] and that could be responsible for the maintenance of the autoimmune process over time. However, our results should be interpreted with caution because of the relatively small number of patients transplanted with cyclophosphamide-based regimens. Moreover, the cyclophosphamide protocols analyzed in this study are slightly different to the one used by Burt and colleagues[11], preventing direct comparisons. Finally, it's important to note that in our work, as in published studies[19], no transplant related mortality has been observed after cyclophosphamide-based aHSCT. We believe that, far from being a weakness, the distinct safety and efficacy profiles of the many conditioning regimens used in the transplant technology allow treatment tailoring on individual patient's disease course and profile risk, representing an advantage over available DMTs.

In this study we had the opportunity to analyze serial MRI records from 167 patients. Available long-term longitudinal MRI data after aHSCT are scarce and limited by small sample sizes[6,30,31]. In

our cohort of RRMS patients treated with BEAM+ATG, 65.5% of patients were free of MRI inflammatory activity at 10 years. These results are quite impressive, considering that MRI activity is seen in 50-60% of patients treated with alemtuzumab[21] and ocrelizumab[32] in a typical 2-years follow-up. Similarly, percentages of NEDA-3 status at 5 and 10 years in the subgroup of patients with RRMS treated with BEAM+ATG (67.7% and 54.9% respectively) are higher than those reported in randomized clinical trials for available therapies[26]. However, these data should be interpreted with caution because patient populations and the follow-up schedules, as well as the use of a re-baseline MRI scan for MRI activity assessment, differ greatly between clinical studies.

Limitations

Our work suffers from several methodological limitations. First, the EDSS raters were not blinded to treatment and this could have introduced some bias. However, the long-term design of this study has partially mitigated this measurement bias. Second, we had no information about the time between last clinical relapse and transplant start and we could not correct for this confounder when analyzing EDSS improvement over time, that can be thus overestimated. Third, clinical and MRI assessments were not systematically performed throughout the study. To overcome this bias, only patients with 6-months confirmed EDSS assessment and yearly MRI records were included in the analysis of treatment effects.

Conclusions

Findings from this study demonstrate that the benefits of aHSCT persist for over 10 years. Although patients with RRMS are those who benefit the most from transplant, aHSCT has been also shown to prevent disability worsening in a large proportion of patients with active progressive MS. The BEAM+ATG conditioning protocol, although associated with a higher transplant mortality rate, was associated with a more pronounced suppression of clinical relapses and MRI inflammatory activity, allowing complete disease control in a higher proportion of patients.

We suggest that aHSCT should be considered as a treatment strategy for MS not responding to conventional therapy.

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Tables

Table 1. Demographic, disease-related and treatment-related characteristics.

	Study Cohort (n=210)	Relapsing-remitting MS (n=122)		Progressive MS (n=88)	
		BEAM+ATG (n=90)	Other conditioning protocols (n=32)	BEAM+ATG (n=67)	Other conditioning protocols (n=21)
Age, mean (SD), y	34.8 (8.6)	34.0 (8.7)	28.3 (5.7)	38.0 (7.3)	37.8 (9.6)
Females, n (%)	148 (70.5)	64 (71.1)	24 (75.0)	48 (71.6)	12 (57.1)
Disease duration, mean (SD), y	11.0 (6.7)	10.3 (6.7)	7.1 (3.5)	13.2 (6.7)	13.2 (7.2)
EDSS, median (IQR)	6.0 (4.5-6.5)	5.0 (3.0-6.0)	6 (3.0-6.0)	6.5 (6.0-7.0)	6.5 (5.5-7.0)
EDSS one year before aHSCT					
Median (IQR)	5.0 (3.0-6.0)	4 (2.5-5.5)	3.5 (2.0-5.0)	6 (5.0-6.5)	5.0 (3.5-6.0)
Missing, n (%)	*19 (9.0)*	*11 (12.2)*	*0 (0)*	*4 (6.0)*	*2 (9.5)*
Delta EDSS in the year before aHSCT					
Mean (SD)	0.8 (1.7)	0.9 (2.0)	1.0 (2.1)	0.6 (0.7)	0.9 (1.2)
Missing, n (%)	*17 (9.0)*	*11 (12.2)*	*0 (0)*	*4 (6.0)*	*2 (9.5)*
Number of relapses in the year before aHSCT					
Mean (SD)	1.8 (1.6)	2.2 (1.6)	2.5 (1.8)	1.1 (1.1)	1.5 (1.7)
Missing, n (%)	*19 (8.1)*	*9 (10.0)*	*2 (6.2)*	*7 (10.4)*	*1 (4.8)*
Number of patients with active MRI scan at baseline					
Number (%)	112 (73.2)	37 (75.5)	19 (73.1)	30 (85.7)	11 (57.9)
Missing, n (%)	*57 (27.1)*	*41 (45.6)*	*6 (18.8)*	*32 (47.8)*	*2 (9.5)*
Number of DMTs before aHSCT					
Median (IQR)	3 (2-4)	3 (2-4)	3 (2-4)	2 (1-3)	3 (2-4)
Missing, n (%)	*8 (3.8)*	*3 (3.3)*	*0 (0)*	*4 (6.0)*	*1 (4.8)*
Follow-up, mean (SD), y	6.2 (5.0)	5.1 (4.4)	7.2 (4.6)	7.6 (5.7)	5.1 (3.6)
Follow-up, median (IQR), y	4.2 (2.1-10.7)	3.5 (2.1-6.9)	6.6 (3.0-12.0)	6.9 (2.3-11.8)	4.9 (1.6-5.1)
Conditioning regimes, n (%)					
BEAM+ATG	157 (74.8)	90 (100)	/	67 (100)	/
BEAM	10 (4.8)	/	6 (18.8)	/	4 (19.0)
FEAM	4 (1.9)	/	4 (12.5)	/	0 (0)

CY+ATG	27 (12.9)	/	15 (46.9)	/	12 (57.1)
Thiothepa+CY	10 (4.8)	/	6 (18.8)	/	4 (19.0)
Others	2 (1.0)	/	1 (3.3)	/	1 (4.8)

Table 2. Univariate and Multivariate Analyses of Factors Influencing Long-Term Outcomes.

	Disability worsening			**Occurrence of a relapse**			**MRI-inflammatory activity**			**NEDA-3 status**		
Relapsing-remitting MS												
	Eligible, n	HR (95% CI)	p value	Eligible, n	HR (95% CI)	p value	Eligible, n	HR (95% CI)	p value	Eligible, n	HR (95% CI)	p value
Age	112	1.05 (1.00-1.11)	0.054	113	0.932 (0.88-0.98)	0.011#	102	0.93 (0.88-0.99)	0.015^	106	0.98 (0.94-1.02)	0.978
Disease duration	111	1.04 (0.96-1.11)	0.321	112	0.96 (0.89-1.03)	0.281	101	0.94 (0.87-1.01)	0.113	105	0.98 (0.93-1.04)	0.588
Baseline EDSS score	112	0.96 (0.77-1.21)	0.747	113	0.89 (0.73-1.10)	0.284	102	0.91 (0.75-1.10)	0.33	106	0.89 (0.76-1.04)	0.160
Number of treatments before aHSCT	112	1.57 (1.12-2.20)	0.009°	112	1.24 (0.91-1.67)	0.167	101	1.15 (0.87-1.52)	0.326	105	1.23 (0.98-1.54)	0.074
Number of relapses in the year before aHSCT	104	0.85 (0.61-1.18)	0.328	105	1.04 (0.82-1.33)	0.725	96	1.10 (0.88-1.38)	0.381	100	0.95 (0.78-1.16)	0.627
BEAM+ATG vs others conditioning regimens	112	0.76 (0.28-2.06)	0.595	113	0.19 (0.08-0.43)	<0.0001*	102	0.22 (0.10-0.49)	<0.0001§	106	0.27 (0.14-0.50)	<0.0001
Active baseline MRI scan	70	1.83 (0.63-5.29)	0.264	71	1.29 (0.52-3.21)	0.587	62	0.66 (0.24-1.81)	0.425	65	1.69 (0.85-3.36)	0.135
Progressive MS												
	Eligible, n	HR (95% CI)	p value	Eligible, n	HR (95% CI)	p value	Eligible, n	HR (95% CI)	p value	Eligible, n	HR (95% CI)	p value
Age	81	1.01 (0.96-1.07)	0.658	82	0.99 (0.92-1.09)	0.988	64	0.97 (0.89-1.06)	0.525	67	1.03 (0.98-1.09)	0.200
Disease duration	81	0.99 (0.93-1.06)	0.885	82	1.03 (0.93-1.13)	0.584	64	0.98 (0.89-1.09)	0.779	67	1.02 (0.96-1.07)	0.536
Baseline EDSS score	81	0.91 (0.59-1.41)	0.671	82	1.61 (0.76-3.44)	0.217	64	1.49 (0.65-3.44)	0.345	67	1.35 (0.85-2.12)	0.200
Number of treatments before aHSCT	77	0.96 (0.71-1.31)	0.812	78	1.13 (0.70-1.83)	0.607	63	1.07 (0.63-1.80)	0.806	66	1.05 (0.79-1.38)	0.724
Number of relapses in the year before aHSCT	75	0.56 (0.34-0.92)	0.022	76	1.13 (0.72-1.78)	0.590	63	1.19 (0.71-1.98)	0.505	66	0.71 (0.49-1.03)	0.076
BEAM+ATG vs others conditioning regimens	81	2.30 (0.69-7.74)	0.118	82	0.25 (0.71-0.86)	0.029	64	0.28 (0.08-1.00)	0.048	67	0.99 (0.42-2.32)	0.975
Active baseline MRI scan	42	1.52 (0.16-14.4)	0.713	44	0.69 (0.08-5.84)	0.731	37	1.03 (0.19-5.43)	0.974	39	0.86 (0.24-3.10)	0.817

Multivariate analisis HR (95%CI)=0.94 (0.88-0.99), p=0.034

* Multivariate analisis HR (95%CI)=0.21 (0.09-0.49), p<0.0001

^ Multivariate analisis HR (95%CI)=0.93 (0.88-1.00), p=0.041

§ Multivariate analisis HR (95%CI)=0.24 (0.11-0.54), p=0.001

Table 3. Disease modifying therapies after aHSCT.

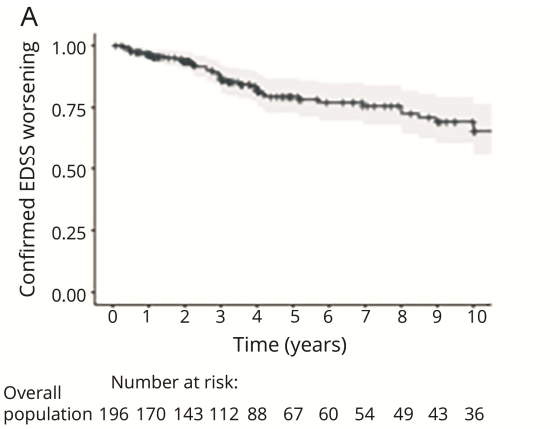
Therapy name	Number (%)
Natalizumab	12 (25.5)
Fingolimod	8 (17.0)
Dimethyl-fumarate	7 (14.9)
Interferon beta 1a	7 (14.9)
Glatiramer Acetate	6 (12.8)
Ocrelizumab	3 (6.4)
Cyclophosphamide	2 (4.3)
Alemtuzumab	1 (2.1)
Rituximab	1 (2.1)

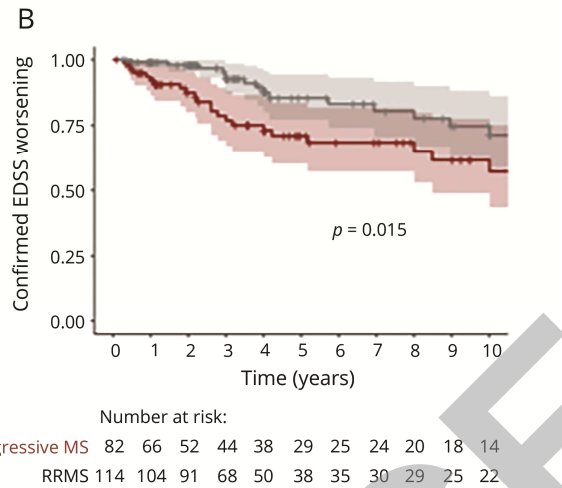
Figures' captions

Figure 1. Disability worsening-free survival and the evolution of the neurological disability.

Panel A shows the probabilities of disability worsening-free survival after aHSCT for the entire study cohort. Panel B shows disability worsening-free survival curves according to the MS phenotype. Panel C shows the evolution of the neurological disability in patients with RRMS and with progressive MS.

EDSS= expanded disability status scale; MS= multiple sclerosis; RRMS= relapsing-remitting multiple sclerosis.





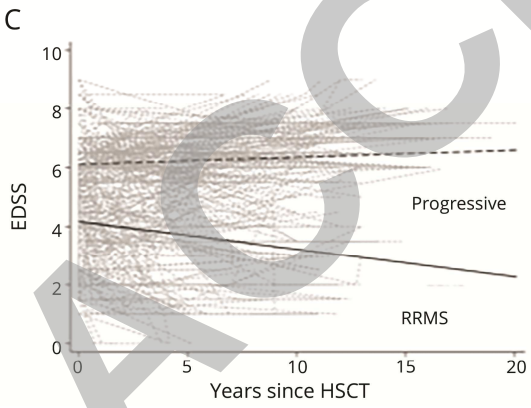


Figure 2. Relapse-free survival, MRI inflammatory activity-free survival and No Evidence of Disease Activity (NEDA-3) status in patients with RRMS.

Panels 2A, 2C and 2E show the probabilities of relapse-free survival, MRI inflammatory activity-free survival and NEDA-3 percentages for patients with relapsing-remitting MS. Panel 2B, 2D and 2F show the survival curves according to the conditioning regimen used within the transplant technology.

BEAM+ATG=carmustine, etoposide, cytarabine and melphalan plus rabbit anti-thymocyte globulin; MRI= magnetic resonance imaging; NEDA-3= No Evidence of Disease Activity-3

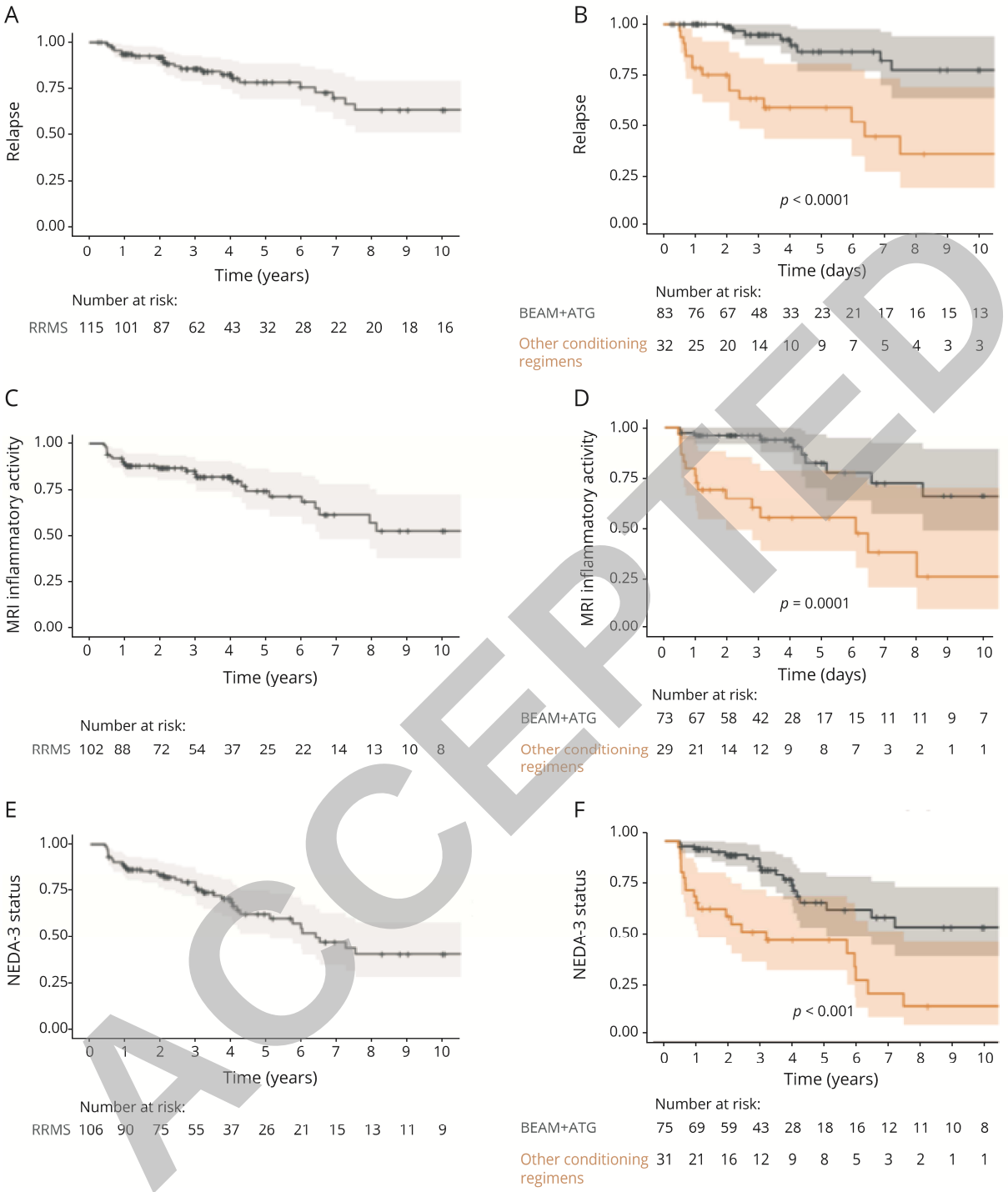
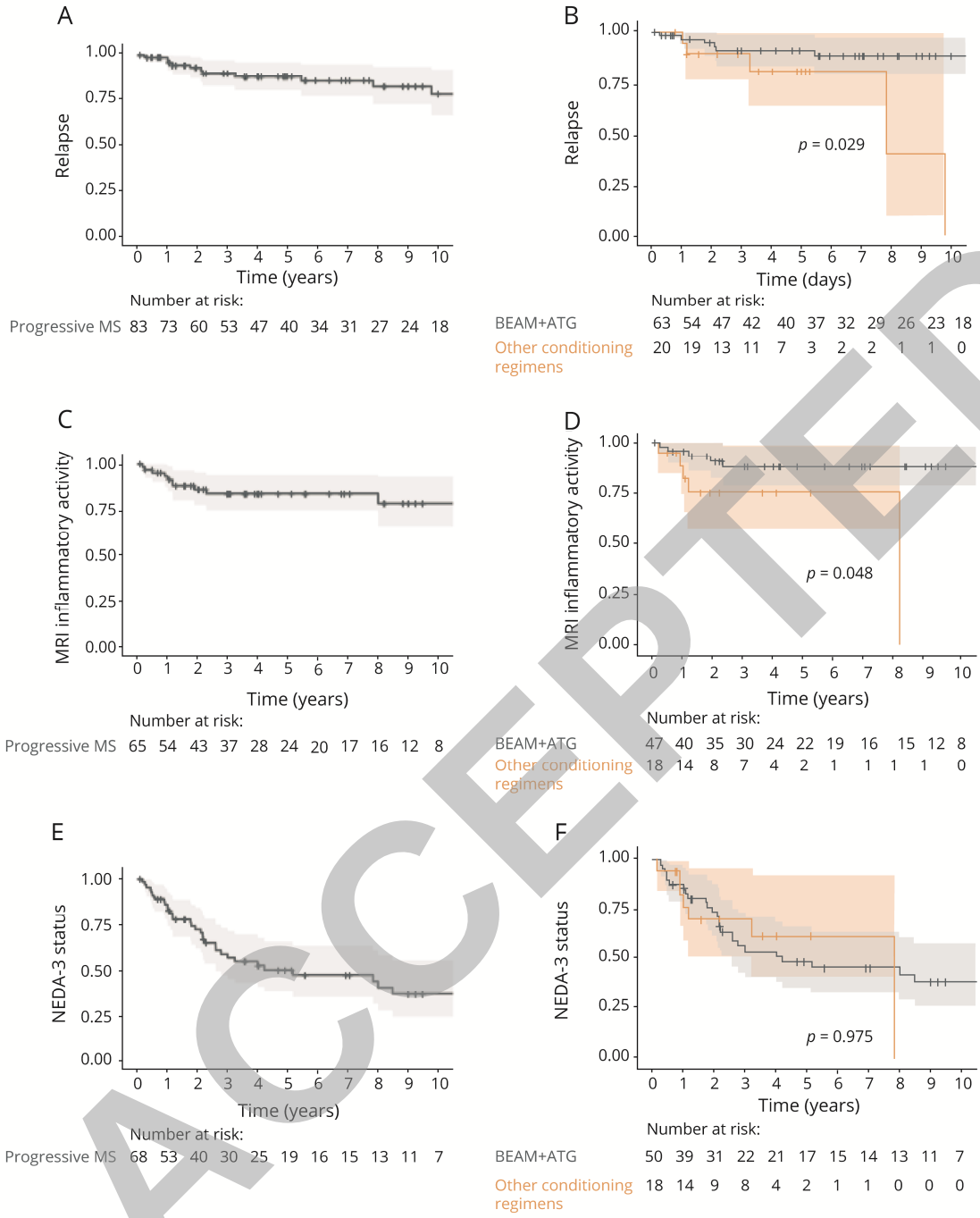


Figure 3. Relapse-free survival, MRI inflammatory activity-free survival and No Evidence of Disease Activity (NEDA-3) status in patients with progressive MS.

Panels 3A, 3C and 3E show the probabilities of relapse-free survival, MRI inflammatory activity-free survival and NEDA-3 percentages for patients with progressive MS. Panel 3B, 3D and 3F show the survival curves according to the conditioning regimen used within the transplant technology. *BEAM+ATG=carmustine, etoposide, cytarabine and melphalan plus rabbit anti-thymocyte globulin; MRI= magnetic resonance imaging; NEDA-3= No Evidence of Disease Activity-3*



Appendix 1: Authors

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Long-Term Clinical Outcomes of Hematopoietic Stem Cell Transplantation in Multiple Sclerosis

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