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

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1 **Abstract**

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

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

7 **Results:** 210 patients were included [relapsing-remitting (RR)MS=122(58%)]. Median baseline
8 EDSS was 6(1-9), mean follow-up was 6.2(±5.0) years. Among RRMS patients, disability
9 worsening-free survival (95%CI) was 85.5%(76.9-94.1%) at 5 years and 71.3%(57.8-84.8%) at 10
10 years. In patients with progressive MS, disability worsening-free survival was 71.0%(59.4-82.6%)
11 and 57.2%(41.8-72.7%) at 5 and 10 years, respectively. In RRMS patients, EDSS significantly
12 reduced after aHSCT [p=0.001; mean EDSS change per year -0.09 (95%CI=-0.15 to -0.04%)]. In
13 RRMS patients, the use of the BEAM+ATG conditioning protocol was independently associated
14 with a reduced risk of NEDA-3 failure [HR=0.27(0.14-0.50), p<0.001]. Three patients died within
15 100-days from aHSCT (1.4%); no deaths occurred in patients transplanted after 2007.

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

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

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26

27 **Introduction**

28 Several disease modifying therapies have been shown to reduce disease activity in people with
29 multiple sclerosis (MS). However long-term disease remission remains elusive¹ and approved
30 therapies have not demonstrated consistent effects in preventing long-term disability progression.
31 Despite treatment, more than half of relapsing-onset MS patients accumulate disability over 10
32 years². The early abrogation of relapses and MRI inflammatory activity has little impact on
33 neurological outcomes at 10 years^{2,3}, questioning the utility of short term outcomes to assess the
34 long-term effect of treatment on disability progression.

35 Disease control is particularly relevant for aggressive MS⁴, characterized by accelerated accrual of
36 irreversible disability. Intense immunosuppression followed by autologous hematopoietic stem cell
37 transplantation (aHSCT) has been extensively explored as a treatment strategy for aggressive MS⁵⁻
38 ¹². The rationale of aHSCT in MS is to eliminate self-reacting cell clones and to induce self-
39 tolerance through a profound renewal of the immune system¹³⁻¹⁶. To date, outcome assessment after
40 aHSCT is limited to a short follow-up and it's still unclear whether aHSCT is able to induce long-
41 term drug-free disease remission. The largest registry-based study on aHSCT in MS¹⁷ has reported
42 that almost half of transplanted patients remained free from neurological progression in the
43 following 5 years. Against this background, in Italy aHSCT has been extensively used for MS since
44 1996⁸. To determine whether aHSCT is able to prevent long-term disability worsening, we analyzed
45 the outcomes in a large cohort of people with aggressive MS who underwent aHSCT for the
46 treatment of MS in Italy.

47

48 **Methods**

49 ***Study Design***

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

52 In July 1998, five Italian neurologic teams, together with the Italian Cooperative Group for Bone
53 Marrow and Blood Transplantation (GITMO), initiated a phase I/II trial on the use of aHSCT in
54 MS¹⁸. Thereafter, other Italian MS centers developed local transplant programs for MS patients,
55 (mostly identical to those developed by the two leading haemato-neurological centers in Italy -
56 Florence and Genoa-). Although no formal guidelines on patients selection for aHSCT exist, all
57 treated patients had aggressive MS, characterized by the occurrence of severe relapses or MRI
58 inflammatory activity or accelerated accrual of neurological disability despite active treatment.
59 Patients were treated with aHSCT according to the European Group for Blood and Marrow
60 Transplantation (EBMT) guidelines, following the decision of the treating physician and approval
61 of the local Ethics Committee.

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

66

67 *Standard Protocol Approvals, Registrations, and Patient Consents*

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

71

72 *Conditioning regimens and transplant care*

73 Peripheral hematopoietic stem cells (PBSCs) were mobilized with cyclophosphamide (CY) (4 or
74 2g/m² iv) and filgrastim (5-10 µg/kg/day sc). PBSCs were collected with a leuko-apheresis
75 procedure and an unmanipulated graft targeted to 3-8x10⁶ CD34⁺ cells/kg was cryopreserved.
76 Patients were transplanted using different conditioning regimens, according to center experience
77 and preference: (i) BEAM+ATG regimen (74.8%), which includes BCNU (carmustine, 300 mg/m²

78 at day -6), cytosine-arabioside (200 mg/m²) and etoposide (200 mg/m²) from day -5 to day -2 and
79 melphalan (140 mg/m²) at day -1, followed by rabbit anti-thymocyte globulin (ATG) (3.75-5
80 mg/kg/day) at days +1 and +2; (ii) BEAM regimen as above described without rabbit ATG (4.8%);
81 (iii) FEAM regimen (1.9%), substituting fotemustine (150 mg/m² on days -7, -6) instead of BCNU
82 in the BEAM regimen; (iv) CY+ATG regimen I (8.1%), containing CY (60mg/kg at day -3 and -2)
83 followed by rabbit ATG (3.75 mg/kg/d at day +1 and +2); (v) CY+ATG regimen II (4.8%),
84 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);
85 (vi) Thiothepa+CY regimen (4.8%), consisting of thiothepa 10 mg/kg for 5 days and CY 50 mg/kg
86 at day -3 and -2. One patient was transplanted with a conditioning regimen made of BCNU and
87 melphalan (0.5%) and one patient was transplanted with a conditioning regimen made of
88 bortezomib, cyclophosphamide, dexamethasone and melphalan (0.5%). Anti-herpetic and anti-
89 pneumocistis jirovecii prophylaxes were performed with Acyclovir and Sulphamethoxazol-
90 Trimetoprim, respectively, according to centers protocols. After aHSCT, patients did not receive
91 immune-based therapies unless they experienced clinical relapse, new lesions on MRI, or EDSS
92 progression, based on decision by the treating neurologist.

93

94 ***Study endpoints***

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

104 baseline EDSS score was ≥ 5.5) confirmed after 6 months. Baseline was defined as the last
105 neurological assessment before the administration of mobilizing therapy. All relapses were
106 clinically-assessed by treating neurologists. Follow-up for any component of NEDA score was not
107 censored by earlier events so that each has an independent interpretation. MRI activity was defined
108 as the presence of new/enlarging T2 lesions or T1 gadolinium-enhancing lesions detected by
109 radiologists on routine follow up MRI. The baseline brain MRI (acquired within 3 months before
110 the aHSCT procedure) was the pre-treatment reference scan for assessment of treatment failure and
111 no re-baseline was performed. All deaths occurring in the first 100 days after transplant were
112 reported and considered likely transplant-related¹⁹.

113

114 *Statistical analyses*

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

124

125 **Results**

126 *Patients demographics and procedures*

127 Patients from 20 Italian MS centers who underwent transplant from 1997 to 2019 were identified
128 (n=210). Demographic, clinical and hematological characteristics of the study cohort are
129 summarized in Table 1. Out of 210 patients, n=196 (93.3%) were eligible for the analysis of the

130 primary endpoint. As for relapse occurrence, data were available for 198 (94.3%) patients. Serial
131 brain MRI radiology records were available for 167 (79.5%) patients. At the time of transplant, 122
132 patients (58%) had a relapsing-remitting (RR) phenotype of MS (RRMS), 86 patients (41%) had
133 secondary progressive (SP) MS and 2 patients (1%) had primary-progressive MS. Data on previous
134 treatment history is available for 175 patients (83.3%). 118 patients had been exposed to interferon-
135 beta, 55 to natalizumab, 54 to pulsed cyclophosphamide, 53 to mitoxantrone, 39 to azathioprine, 38
136 to glatiramer acetate, 29 to fingolimod, 7 to alemtuzumab and 6 to rituximab. Among patients with
137 RRMS, those who were transplanted with the BEAMT+ATG protocol were older (34.0 years
138 versus 28.3 years; $p < 0.0001$), had longer disease duration (10.3 years versus 7.1 years; $p = 0.029$)
139 and had a shorter follow-up (5.1 years versus 7.2 years; $p = 0.027$). Among patients with progressive
140 MS, the BEAM+ATG subgroup had higher EDSS scores one year before transplant (median EDSS
141 of 6 versus 5; $p = 0.027$).

142

143 *Disability worsening-free survival and the evolution of neurological disability*

144 The probabilities of disability-worsening free survival for the entire study cohort and according to
145 disease phenotype are reported in Figure 1A and 1B, respectively. In the entire study cohort,
146 disability worsening-free survival was 79.5% (72.0-86.6%) and 65.5% (55.3%-75.7%) at 5 and 10
147 years. The RRMS phenotype was associated with a reduced risk of disability worsening [HR
148 (95%CI)= 0.46 (0.24-0.86), $p = 0.015$], with disability worsening-free survival rates of 85.5%
149 (76.9%-94.1%) at 5 years and 71.3% (57.8%-84.8%) at 10 years. In RRMS, a higher treatment
150 exposure before aHSCT was associated with a higher risk of disability worsening [HR=1.57 (1.12-
151 2.20), $p = 0.009$] (Table 2). Among patients with progressive MS, disability worsening-free survival
152 was 71.0% (59.4%-82.6%) and 57.2% (41.8%-72.7%) at 5 and 10 years, respectively. A higher
153 number of relapses in the year before aHSCT was associated with a lower risk of disability
154 worsening [HR=0.56 (0.34-0.92), $p = 0.022$]. The use of the BEAM+ATG conditioning protocol did
155 not influence the probabilities of disability worsening free-survivals. Progression-free survival in

156 RRMS patients who were transplanted with the BEAM+ATG protocol was 81.9% (70.1%-93.7%)
157 at 5 and 10 years.

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

163

164 *Secondary endpoints*

165 The probabilities of relapse-free survival, MRI inflammatory activity-free survival and NEDA-3
166 status are reported in Figure 2 (RRMS) and Figure 3 (progressive MS), according to the
167 conditioning regimen used in the transplant technology. For RRMS patients, relapse-free survival
168 was 78.1% (68.5%-87.7%) and 63.5% (49.4%-77.6%) at 5 and 10 years after aHSCT. In RRMS
169 patients treated with the BEAM+ATG protocol, relapse-free survival was 86.4% (75.8%-97.0%)
170 and 77.0% (61.5%-92.5%) at 5 and 10 years. The use of the BEAM+ATG conditioning protocol
171 [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]
172 were independently associated with a reduced risk of relapses (Table 2). Among patients with
173 progressive MS, relapse-free survival was 88.3% (80.7%-96.0%) and 78.9% (63.4%-91.4%) at 5
174 and 10 years, respectively. The use of the BEAM+ATG conditioning protocol [HR=0.25 (0.71-
175 0.86), p=0.029] was associated with a reduced risk of a relapse. In the entire study cohort, relapse-
176 free survival was 82.9% (76.6%-89.2%) and 71.2% (61.8%-80.6%) 5 and 10 years after aHSCT,
177 respectively.

178 Probabilities for MRI inflammatory activity-free survival for patients with RRMS were 74.6%
179 (63.2%-85.6%) at 5 years and 52.7% (35.6%-69.7%) after 10 years. When the BEAM+ATG was
180 used, the MRI inflammatory activity-free survival was 82.0% (68.5%-95.5%) and 65.5% (45.3%-
181 85.7%) at 5 and 10 years, respectively. The use of the BEAM+ATG conditioning regimen

182 [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
183 independently associated with a reduced risk of MRI inflammatory activity after aHSCT (Table 2).
184 In the subgroup of patients with progressive MS, the MRI inflammatory activity-free survival was
185 at 84.0% (74.2%-93.8%) and 78.7% (65.2%-92.2%) at 5 and 10 years, respectively. The use of the
186 BEAM+ATG protocol was found to be associated with a higher probability of suppression of MRI
187 inflammatory activity [HR=0.28 (0.08-1.00), p=0.048]. In the entire study cohort, the percentages
188 of patients free of MRI inflammatory activity were 78.7% (71.1%-86.3%) at 5 years and 64.3%
189 (52.7%-75.9%) at 10 years.

190 For patients with RRMS, probabilities of achieving NEDA-3 status were 62.2% (50.6%-73.8%) at 5
191 years and 40.5% (30.0%-55.0%) at 10 years. In the subgroup of RRMS patients who underwent
192 aHSCT with the BEAM+ATG conditioning protocol, NEDA-3 status was achieved in 67.7%
193 (53.2%-82.2%) and 54.9% (37.3%-72.5%) of patients at 5 and 10 years, respectively. In RRMS
194 patients, the use of the BEAM+ATG protocol [HR=0.27 (0.14-0.50), p<0.001] was associated with
195 a higher probability of maintaining NEDA-3 status (Table 2). In patients with progressive MS,
196 NEDA-3 status estimates were 50.8% (37.3%-64.3%) and 37.3% (22.8%-52.6%) at 5 and 10 years
197 respectively, and no baseline characteristics were found to be associated with the probability of
198 NEDA-3 status. In the entire study cohort, NEDA-3 status was achieved in 57.9% of patients
199 (49.1%-66.7%) at 5 years and in 39.8% of patients (29.2%-50.4%) 10 years after aHSCT.

200 When comparing the BEAM+ATG conditioning regimen with the cyclophosphamide-based
201 protocols alone, we confirmed that, in patients with RRMS, the use of the BEAM+ATG was
202 associated with a lower risk of relapse [HR=0.12 (0.05-0.32), p<0.001], MRI inflammatory activity
203 [HR=0.18 (0.07-0.48), p=0.001] and with a higher probability of maintaining NEDA-3 status
204 [HR=0.18 (0.09-0.38), p<0.001] over the entire follow-up. In patients with progressive MS we did
205 not find any difference between BEAM+ATG and cyclophosphamide-based regimens on treatment
206 response.

207 Thirty-two patients (15.2%) started a new DMT after transplant. Median number of new DMTs was
208 1 (range 1-3, IQR 1-2), mean time to re-treatment was 3.7 years (SD=3.0) and median time was
209 2.08 years (range=0.54–13.0). DMTs initiated after aHSCT are listed in Table 3.

210 Three deaths occurred within 100 days following aHSCT (1.4% of the entire study population).
211 Extensive data from these patients have already been reported⁸. Patient #1, a 38 years-old
212 secondary-progressive MS patient, developed pulmonary thrombo-embolism, which caused a
213 syncope with head trauma 56 days after aHSCT. He was treated with fibrinolytic treatment and died
214 48 hours later after intracranial hemorrhage. Patient #2, a 39 years-old RRMS patient, had
215 engraftment failure and died 24 days after transplant due to an opportunistic infection caused by
216 *Actinomyces sp.* Patient #3, a 48 years-old RRMS patient, died 1 month after transplantation from a
217 Wernicke's like encephalopathy. All deceased patients have been transplanted with the
218 BEAM+ATG conditioning regimen. No transplant-related deaths occurred in patients transplanted
219 after 2007.

220

221 **Discussion**

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

224 We herein report the long-term outcomes in a large cohort of MS patients who underwent aHSCT
225 in Italy in the last two decades, showing that 65.5% of patients were free of disability worsening 10
226 years after transplant, with a disability worsening-free survival greater than 70% in patients with
227 RRMS. Our data extend previous studies at 5 years^{5-8,17}, demonstrating that the effects of aHSCT
228 persist for over a decade. These results are of particular relevance considering that patients treated
229 with aHSCT were affected by extremely aggressive forms of MS, which is not the case in available
230 randomized clinical trials. Of note, the 5-years progression-free survival rate in our cohort of
231 RRMS (85.5%) is higher than those reported with other highly active treatments for MS, such as
232 natalizumab²⁰ and alemtuzumab²¹. In line with previous observations¹⁷, disability worsening-free

233 survival in our cohort was higher in RRMS patients with lower treatment exposure, confirming the
234 notion that aHSCT should be performed early in the course of the disease.

235 Based on our data, patients with progressive MS still benefit from aHSCT. Indeed, we found a
236 disability worsening-free survival of 71% at 5 years, which was maintained in 57.2% of progressive
237 MS patients at 10 years. Although a control group was not available, such low rates of disability
238 worsening are an unexpected feature in progressive MS patients and deserve some consideration.
239 Accrual of neurological disability in progressive MS seems to be associated with
240 compartmentalized inflammation behind the blood–brain-barrier and recent data have demonstrated
241 that targeting inflammation within the CNS slow the course of progressive MS^{22,23}. All the different
242 drugs used in the transplant technology share the ability to cross the blood-brain-barrier and to
243 penetrate in the CNS, where they can halt compartmentalized inflammation slowing neurological
244 deterioration. In line with this hypothesis, we found that a higher number of relapses in the year
245 before aHSCT, indicating residual ongoing CNS inflammation²⁴, was associated with an increased
246 probability of disability worsening-free survival. We did not find any association between disease
247 duration and treatment effect. One possible explanation is that some patients of our cohort with
248 relatively long disease duration experienced dramatic disease exacerbations after withdrawal of
249 specific DMTs (especially natalizumab and fingolimod) and had excellent response to aHSCT,
250 possibly hiding the effect of disease duration on treatment response.

251

252 According to other independent groups^{5,11}, we observed sustained EDSS reduction after transplant
253 in RRMS patients. When speculating on the possible effects of aHSCT in improving MS-related
254 disability, it's noteworthy that most of transplanted patients had experienced MS attacks right
255 before aHSCT and the reduction in disability could represent the expected gradual recovery from
256 relapses. In our cohort neurological improvement was sustained over 10 years and EDSS scores
257 continued to ameliorate beyond the first years following aHSCT, when recovery from relapses no
258 longer occurs, suggesting a robust effect of aHSCT in improving neurological status. It's arguable

259 that after CNS inflammation is completely suppressed, endogenous structural and functional
260 plasticity mechanisms eventually reemerge²⁵, resulting in sustained clinical improvement.

261

262 The optimal intensity of the conditioning regimen for the treatment of MS remains an open
263 question²⁶. This is the first study suggesting that the use of the BEAM+ATG conditioning regimen
264 is independently associated with a reduced probability of relapses, MRI activity and NEDA-3
265 failure in patients with RRMS. Our results are in line with the evidence that a high-intensity,
266 busulfan-based⁶, but not a low-intensity cyclophosphamide-based²⁷, conditioning regimen was able
267 to completely abrogate MRI activity and clinical relapses. These results are also in line with the
268 evidence that the bone marrow is the major site of memory helper T cells²⁸ and memory plasma
269 cells which are resistant to treatment with cyclophosphamide²⁹ and that could be responsible for the
270 maintenance of the autoimmune process over time. However, our results should be interpreted with
271 caution because of the relatively small number of patients transplanted with cyclophosphamide-
272 based regimens. Moreover, the cyclophosphamide protocols analyzed in this study are slightly
273 different to the one used by Burt and colleagues¹¹, preventing direct comparisons. Finally, it's
274 important to note that in our work, as in published studies¹⁹, no transplant related mortality has been
275 observed after cyclophosphamide-based aHSCT. We believe that, far from being a weakness, the
276 distinct safety and efficacy profiles of the many conditioning regimens used in the transplant
277 technology allow treatment tailoring on individual patient's disease course and profile risk,
278 representing an advantage over available DMTs.

279

280 In this study we had the opportunity to analyze serial MRI records from 167 patients. Available
281 long-term longitudinal MRI data after aHSCT are scarce and limited by small sample sizes^{6,30,31}. In
282 our cohort of RRMS patients treated with BEAM+ATG, 65.5% of patients were free of MRI
283 inflammatory activity at 10 years. These results are quite impressive, considering that MRI activity
284 is seen in 50-60% of patients treated with alemtuzumab²¹ and ocrelizumab³² in a typical 2-years

285 follow-up. Similarly, percentages of NEDA-3 status at 5 and 10 years in the subgroup of patients
286 with RRMS treated with BEAM+ATG (67.7% and 54.9% respectively) are higher than those
287 reported in randomized clinical trials for available therapies²⁶. However, these data should be
288 interpreted with caution because patient populations and the follow-up schedules, as well as the use
289 of a re-baseline MRI scan for MRI activity assessment, differ greatly between clinical studies.

290

291 **Limitations**

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

300

301 **Conclusions**

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

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

310

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317

318 **Data availability statement**

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

321

322

323 **Tables**

324 *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)
Thiohepa+CY	10 (4.8)	/	6 (18.8)	/	4 (19.0)
Others	2 (1.0)	/	1 (3.3)	/	1 (4.8)

325

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

	Disability worsening			Occurrence of a relapse			MRI-inflammatory activity			NEDA-3 status		
	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
Relapsing-remitting MS												
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 regimes	112	0.76 (0.28-2.06)	0.595	113	0.19 (0.08-0.43)	<0.001*	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												
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

327

328

329 # Multivariate analysis HR (95%CI)=0.94 (0.88-0.99), p=0.034

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

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

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

333

334

335 **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)

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356 **Figures' captions**

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

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

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

364

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

367 Panels 2A, 2C and 2E show the probabilities of relapse-free survival, MRI inflammatory activity-
368 free survival and NEDA-3 percentages for patients with relapsing-remitting MS. Panel 2B, 2D and
369 2F show the survival curves according to the conditioning regimen used within the transplant
370 technology.

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

373

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

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

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

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383 **Authors' statement**

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Matilde Inglese	MD, PhD	University of Genoa, San	Corresponding Author	design and conceptualized

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386 **Co-investigators statement**

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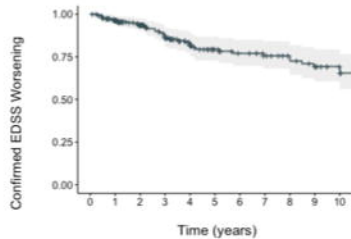
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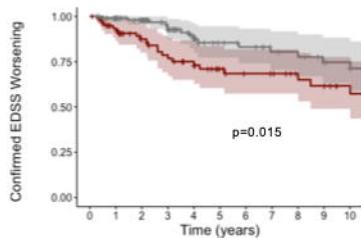
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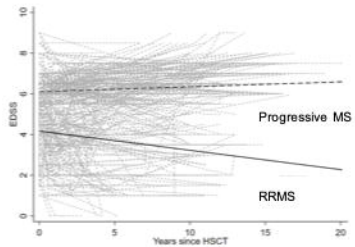
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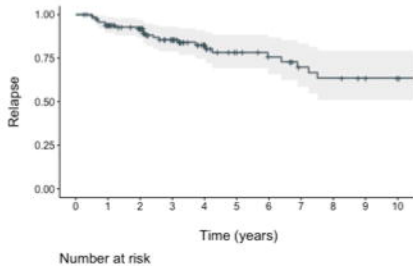
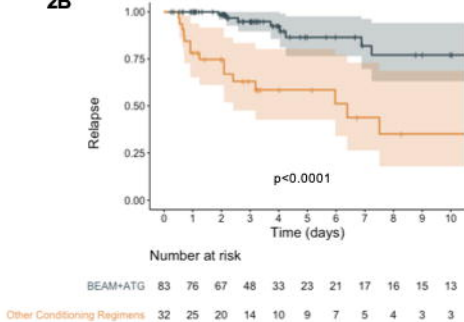
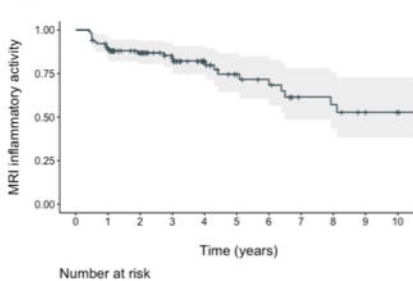
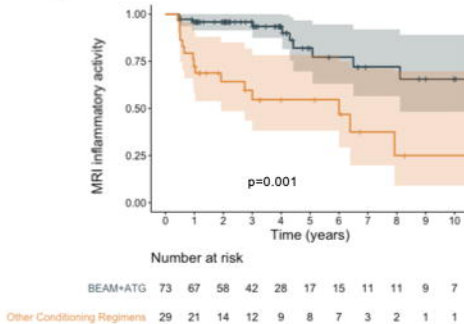
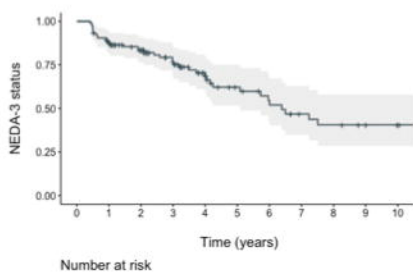
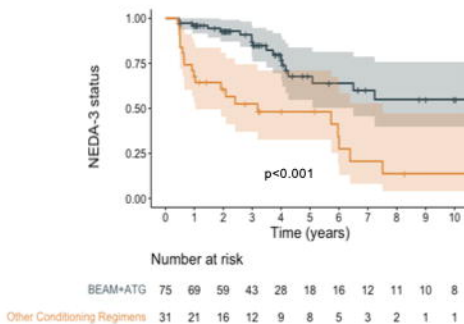
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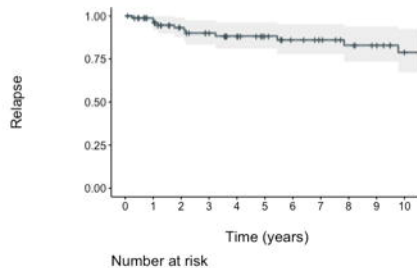
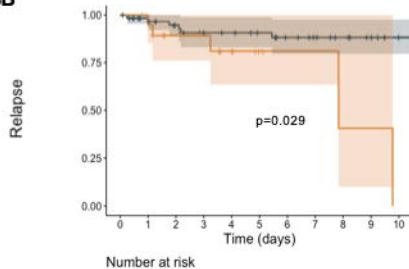
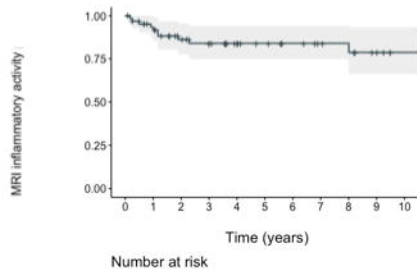
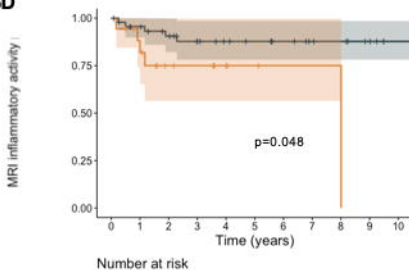
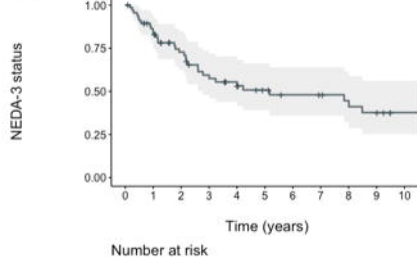
B

Progressive MS 82 66 52 44 38 29 25 24 20 18 14

RRMS 114 104 91 68 50 38 35 30 29 25 22

C

2A**2B****2C****2D****2E****2F**

3A**3B****3C****3D****3E****3F**