

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

### **This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1770694> since 2021-02-01T18:18:39Z

*Published version:*

DOI:10.1212/WNL.0000000000011461

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

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

## Author(s):

Giacomo Boffa, MD; Luca Massacesi, MD; Matilde Inglese, MD, PhD; Alice Mariottini, MD; Marco Capobianco, MD; Moiola Lucia, MD; Maria Pia Amato, MD; Salvatore Cottone, MD; Francesca Gualandi, MD; Marco De Gobbi, MD; Raffaella Greco, MD; Rosanna Scimè, MD; Jessica Frau, MD; Giovanni Bosco Zimatore, MD; Antonio Bertolotto, MD; Giancarlo Comi, MD; Antonio Uccelli, MD; Alessio Signori, PhD; Emanuele Angelucci, MD; Chiara Innocenti, MD; Fabio Ciceri, MD; Anna Maria Repice, MD; Maria Pia Sormani, PhD; Riccardo Saccardi, MD; Gianluigi Mancardi, MD on behalf of the Italian BMT-MS study group

## Corresponding Author:

Matilde Inglese

m.inglese@unige.it

**Affiliation Information for All Authors:** Giacomo Boffa, Department of Neurology, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, San Martino Hospital, Genoa/Italy; Luca Massacesi Department of Neurosciences Drugs and Child Health and Department of Neurology 2, Careggi University Hospital, Florence, Italy; Matilde Inglese Department of Neurology, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa and Ospedale Policlinico San Martino, IRCCS, Genoa, Italy; Alice Mariottini Department of Neurosciences Drugs and Child Health and Department of Neurology 2, Careggi University Hospital, Florence, Italy; Marco Capobianco Department of Neurology, San Luigi Gonzaga Hospital, Orbassano, Italy; Lucia Moiola Department of Neurology, Vita-Salute San Raffaele University, San Raffaele Scientific Institute, Milan/Italy; Maria Pia Amato Department NEUROFARBA, Section Neurological Sciences University of Florence IRCCS Fondazione Don Carlo Gnocchi, Florence, Italy; Salvatore Cottone Department of Neurology, Villa Sofia Hospital, Palermo/Italy; Francesca Gualandi Department of Haematology and Bone Marrow Transplant Unit, Policlinico San Martino IRCCS, Genoa/Italy; Marco De Gobbi Department of Clinical and Biological Sciences, Haematopoietic Stem Cell Transplant Unit, University of Turin, San Luigi Gonzaga Hospital, Orbassano/Italy; Raffaella Greco Department of Haematology and Bone marrow transplant, Vita-Salute San Raffaele University, San Raffaele Scientific Institute, Milan/Italy; Rosanna Scimè Department of Haematology, Villa Sofia Hospital, Palermo/Italy; Jessica Frau Multiple Sclerosis Center, Department of Medical Sciences and Public Health University of Cagliari, Binaghi Hospital Cagliari/Italy; Giovanni Bosco Zimatore Department of Neurology, Ospedale Generale Regionale "F. Miulli", Acquaviva delle Fonti, BA, Italy; Antonio Bertolotto Department of Neurology, San Luigi Gonzaga Hospital, Orbassano, Italy; Giancarlo Comi Department of Neurology, Vita-Salute San Raffaele University, San Raffaele Scientific Institute, Milan/Italy; Antonio Uccelli Department of Neurology, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa and Ospedale Policlinico San Martino, IRCCS, Genoa, Italy; Alessio Signori Biostatistics Unit, University of Genoa, Genoa/Italy; Emanuele Angelucci Department of Haematology and Bone Marrow Transplant Unit, Policlinico San Martino IRCCS, Genoa/Italy; Chiara Innocenti Cell Therapy and Transfusion Medicine Unit, Careggi University Hospital, Florence/Italy; Fabio Ciceri Department of Haematology and Bone marrow transplant, Vita-Salute San Raffaele University, San Raffaele Scientific Institute, Milan/Italy; Anna Maria Repice Department of Neurosciences Drugs and Child Health and Department of Neurology 2, Careggi University Hospital, Florence, Italy; Maria Pia Sormani Biostatistics Unit, University of Genoa, Genoa/Italy; Riccardo Saccardi Cell Therapy and Transfusion Medicine Unit, Careggi University Hospital, Florence/Italy; Gianluigi Mancardi Department of Neurology, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa and Ospedale Policlinico San Martino, IRCCS, Genoa, Italy and IRCCS Scientific Clinical Institutes Maugeri, Pavia-Genoa

Nervi/Italy.

**Contributions:**

Giacomo Boffa: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Luca Massacesi: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Matilde Inglese: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data

Alice Mariottini: Major role in the acquisition of data

Marco Capobianco: Major role in the acquisition of data

Moiola Lucia: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Maria Pia Amato: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Salvatore Cottone: Major role in the acquisition of data

Francesca Gualandi: Major role in the acquisition of data

Marco De Gobbi: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Raffaella Greco: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Rosanna Scimè: Major role in the acquisition of data

Jessica Frau: Major role in the acquisition of data

Giovanni Bosco Zimatore: Major role in the acquisition of data

Antonio Bertolotto: Major role in the acquisition of data

Giancarlo Comi: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Antonio Uccelli: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Alessio Signori: Analysis or interpretation of data

Emanuele Angelucci: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Chiara Innocenti: Major role in the acquisition of data

Fabio Ciceri: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the

acquisition of data

Anna Maria Repice: Major role in the acquisition of data

Maria Pia Sormani: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data

Riccardo Saccardi: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Gianluigi Mancardi: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Number of characters in title: 92

Abstract Word count: 235

Word count of main text: 3479

References: 32

Figures: 3

Tables: 3

**Statistical Analysis performed by:** Alessio Signori, PhD Maria Pia Sormani, PhD Biostatistics Unit, University of Genoa, Genoa/Italy

**Search Terms:** [ 23 ] Clinical trials Observational study (Cohort, Case control), [ 41 ] Multiple sclerosis, [ 131 ] All Immunology

The authors report no targeted funding

**Disclosures:** Dr. Giacomo Boffa has nothing to disclose. Dr. L. Massacesi received educational grants and/or research funds from Fondazione Cassa di Risparmio di Firenze, Biogen, Merck-Serono, Genzyme, Roche; received honoraria or consultation fees from Biogen, Roche, Mylan, Merck-Serono, Genzyme, Novartis. Dr. M. Inglese received grants NIH, NMSS, FISM; received fees for consultation from Roche, Genzyme, Merck, Biogen and Novartis. Dr. A. Mariottini has nothing to disclose. Dr. M. Capobianco received personal compensation for speaking honoraria or participating in advisory board from Almirall, Biogen, Merck, Novartis, Roche, Sanofi, Teva. Dr. L. Moiola received personal compensation for consulting, serving on a scientific advisory board, speaking, or other activities with Sanofi-Genzyme, Novartis, Teva, Merck-Serono, Biogen, Roche, Excemed. Dr. Amato received research grants and honoraria as a speaker and member of advisory boards by Bayer, Biogen, Merck, Novartis, Sanofi Genzyme, Teva, Almirall, Roche. Dr. S. Cottone has nothing to disclose. Dr. F. Gualandi has nothing to disclose. Dr. De Gobbi M has nothing to disclose. Dr. R. Greco has nothing to disclose. Dr. R. Scimè has nothing to disclose. Dr. J Frau serves on scientific advisory boards for Biogen, received honoraria for speaking from Merck Serono, Biogen and Teva and received research grant from Serono. Dr. G.B. Zimatore has nothing to disclose. Dr. Bertolotto received honoraria for serving on the scientific advisory boards of Biogen, Merck, Mylan, and Sanofi Genzyme, and received speaker honoraria from Biogen, Genzyme, Novartis, and TEVA. Dr. G. Comi received consulting fees from Actelion, Bayer, Merck Serono, Novartis, Sanofi, and Teva and lecture fees from Bayer, Biogen Dompé, Merck Serono, Novartis, Sanofi, Serono, Symposia International Foundation, and Teva. Dr. A. Uccelli received grants and contracts from FISM, Novartis, Fondazione Cariplo, Italian Ministry of Health; received honoraria or consultation fees from Biogen, Roche, Teva, Merck, Genzyme, Novartis. Dr. A. Signori has nothing to disclose. Dr. E. Angelucci received honoraria from Novartis and Celgene, Jazz Pharmaceuticals and Roche for involvement in local advisory boards and participation in DMC for Celgene and Vertex Pharmaceuticals Incorporated and CRISPR Therapeutics. Dr. C. Innocenti has nothing to disclose. Dr. F. Ciceri has nothing to disclose. Dr. Repice has received personal compensation from Biogen Idec, Genzyme, Novartis and Merck Serono for public speaking and advisory boards. Dr. Sormani received consulting fees from Biogen Idec, Merck Serono, Teva, Genzyme, Roche, Novartis, GeNeuro and Medday. Dr. R. Saccardi reports honoraria from Jazz Pharmaceuticals and Sanofi Genzyme. Dr. G.L. Mancardi received support from Biogen Idec (honoraria for lecturing, travel expenses for attending meetings and financial support for research), Genzyme (honorarium for lecturing), Merck Serono, Novartis, Teva (financial support for research) and Sanofi Aventis (honorarium for speaking).

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.

21

22

23

24

25

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 elusive<sup>1</sup> 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 years<sup>2</sup>. The early abrogation of relapses and MRI inflammatory activity has little impact on  
33 neurological outcomes at 10 years<sup>2,3</sup>, 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 MS<sup>4</sup>, 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 MS<sup>5-</sup>  
38 <sup>12</sup>. 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 system<sup>13-16</sup>. 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 MS<sup>17</sup> 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 1996<sup>8</sup>. 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 MS<sup>18</sup>. 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/m<sup>2</sup> iv) and filgrastim (5-10 µg/kg/day sc). PBSCs were collected with a leuko-apheresis  
75 procedure and an unmanipulated graft targeted to 3-8x10<sup>6</sup> CD34<sup>+</sup> 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/m<sup>2</sup>

78 at day -6), cytosine-arabioside (200 mg/m<sup>2</sup>) and etoposide (200 mg/m<sup>2</sup>) from day -5 to day -2 and  
79 melphalan (140 mg/m<sup>2</sup>) 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/m<sup>2</sup> 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  $\geq 5.5$ ) 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-related<sup>19</sup>.

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 reported<sup>8</sup>. 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<sup>5-8,17</sup>, 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 natalizumab<sup>20</sup> and alemtuzumab<sup>21</sup>. In line with previous observations<sup>17</sup>, 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<sup>22,23</sup>. 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 inflammation<sup>24</sup>, 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<sup>5,11</sup>, 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 reemerge<sup>25</sup>, resulting in sustained clinical improvement.

261

262 The optimal intensity of the conditioning regimen for the treatment of MS remains an open  
263 question<sup>26</sup>. 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-based<sup>6</sup>, but not a low-intensity cyclophosphamide-based<sup>27</sup>, 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 cells<sup>28</sup> and memory plasma  
269 cells which are resistant to treatment with cyclophosphamide<sup>29</sup> 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 colleagues<sup>11</sup>, preventing direct comparisons. Finally, it's  
274 important to note that in our work, as in published studies<sup>19</sup>, 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<sup>6,30,31</sup>. 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 alemtuzumab<sup>21</sup> and ocrelizumab<sup>32</sup> 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 therapies<sup>26</sup>. 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



311 **Acknowledgements**

312 Autologous haematopoietic stem cell transplantation in Italy was partially funded and supported by  
 313 the Italian Multiple Sclerosis Foundation (FISM) with grants 2000/R/43, 2001/R/38 and 2002/R/36  
 314 to GLM. RS and CI activity was partially supported by a grant of Elena Pecci Research Fund. This  
 315 work was developed within the framework of the DINOEMI Department of Excellence of MIUR  
 316 2018-2022 (legge 232 del 2016).

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)
<b>EDSS one year before aHSCT</b>					
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)
<b>Delta EDSS in the year before aHSCT</b>					
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)
<b>Number of relapses in the year before aHSCT</b>					

<b>Mean (SD)</b>	1.8 (1.6)	2.2 (1.6)	2.5 (1.8)	1.1 (1.1)	1.5 (1.7)
<b>Missing, n (%)</b>	19 (8.1)	9 (10.0)	2 (6.2)	7 (10.4)	1 (4.8)
<b>Number of patients with active MRI scan at baseline</b>					
<b>Number (%)</b>	112 (73.2)	37 (75.5)	19 (73.1)	30 (85.7)	11 (57.9)
<b>Missing, n (%)</b>	57 (27.1)	41 (45.6)	6 (18.8)	32 (47.8)	2 (9.5)
<b>Number of DMTs before aHSCT</b>					
<b>Median (IQR)</b>	3 (2-4)	3 (2-4)	3 (2-4)	2 (1-3)	3 (2-4)
<b>Missing, n (%)</b>	8 (3.8)	3 (3.3)	0 (0)	4 (6.0)	1 (4.8)
<b>Follow-up, mean (SD), y</b>	6.2 (5.0)	5.1 (4.4)	7.2 (4.6)	7.6 (5.7)	5.1 (3.6)
<b>Follow-up, median (IQR), y</b>	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)
<b>Conditioning regimens, n (%)</b>					
<b>BEAM+ATG</b>	157 (74.8)	90 (100)	/	67 (100)	/
<b>BEAM</b>	10 (4.8)	/	6 (18.8)	/	4 (19.0)
<b>FEAM</b>	4 (1.9)	/	4 (12.5)	/	0 (0)
<b>CY+ATG</b>	27 (12.9)	/	15 (46.9)	/	12 (57.1)
<b>Thiohepa+CY</b>	10 (4.8)	/	6 (18.8)	/	4 (19.0)
<b>Others</b>	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
<b>Relapsing-remitting MS</b>												
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)	<0.001*	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
<b>Progressive MS</b>												
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)

336

337

338

339

340

341

342

343

344

345

346

347  
348  
349  
350  
351  
352  
353  
354  
355

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*

381

382

383 **Authors' statement**

<b>Name</b>	<b>Degree</b>	<b>Location</b>	<b>Role</b>	<b>Contribution</b>
Giacomo Boffa	MD	University of Genoa	Author	design and conceptualized study; acquisition of data; analyzed the data; drafted the manuscript.
Luca Massacesi	MD	University of Florence, Careggi University Hospital	Author	acquisition of data; revised the manuscript for intellectual content.
Matilde Inglese	MD, PhD	University of Genoa, San	Corresponding Author	design and conceptualized

		Martino Hospital IRCCS		study; acquisition of data; revised the manuscript for intellectual content.
Alice Mariottini	MD	University of Florence, Careggi University Hospital	Author	acquisition of data
Marco Capobianco	MD	San Luigi Gonzaga Hospital, Orbassano	Author	acquisition of data
Lucia Moiola	MD	San Raffaele Hospita, Milan	Author	acquisition of data; revised the manuscript for intellectual content
Maria Pia Amato	MD	University of Florence, IRCCS Fondazione Don Carlo Gnocchi	Author	acquisition of data; revised the manuscript for intellectual content
Salvatore Cottone	MD	Villa Sofia Hospital, Palermo	Author	acquisition of data
Francesca	MD	San Martino	Author	acquisition of data

Gualandi		Hospital IRCCS, Genoa		
Marco De Gobbi	MD	San Luigi Gonzaga Hospital, Orbassano	Author	acquisition of data; revised the manuscript for intellectual content
Raffaella Greco	MD	San Raffaele Hospital, Milan	Author	acquisition of data; revised the manuscript for intellectual content
Rosanna Scimè	MD	Villa Sofia Hospital, Palermo	Author	acquisition of data
Jessica Frau	MD	University of Cagliari	Author	acquisition of data
Giovanni Bosco Zimatore	MD		Author	acquisition of data
Antonio Bertolotto	MD	San Luigi Gonzaga Hospital, Orbassano	Author	acquisition of data
Giancarlo Comi	MD	San Raffaele Hospital, Milan	Author	acquisition of data; revised the manuscript for intellectual content
Antonio Uccelli	MD	University of	Author	acquisition of data;

		Genoa, San Martino Hospital IRCCS		revised the manuscript for intellectual content
Alessio Signori	PhD	University of Genoa	Author	analyzed the data
Emanuele Angelucci	MD	San Martino Hospital IRCCS, Genoa	Author	acquisition of data; revised the manuscript for intellectual content
Chiara Innocenti	MD	University of Florence	Author	acquisition of data
Fabio Ciceri	MD	San Raffaele Hospital, Milan	Author	acquisition of data; revised the manuscript for intellectual content
Anna Maria Repice	MD	University of Florence, Careggi University Hospital	Author	acquisition of data
Maria Pia Sormani	PhD	University of Genoa	Author	analyzed the data; revised the manuscript for intellectual content.
Riccardo	MD	University of	Author	design and



Saccardi		Florence		conceptualized study; acquisition of data; revised the manuscript for intellectual content.
Gianluigi Mancardi	MD	University of Genoa	Author	design and conceptualized study; acquisition of data; revised the manuscript for intellectual content.

384  
385

386 **Co-investigators statement**

387

<b>Name</b>	<b>Degree</b>	<b>Location</b>	<b>Role</b>	<b>Contribution</b>
M. Radaelli	MD	Papa Giovanni XXIII Hospital, Bergamo	Co-investigator	acquisition of data
Vincenzo Pavone	MD	<i>Ospedale Cardinale Giovanni Panico, Tricase</i>	Co-investigator	Acquisition of data
C. Gasperini	MD	Ospedale San	Co-investigator	acquisition of data

		Camillo- Forlanini, Roma		
V. Zoli	MD	Ospedale San Camillo- Forlanini, Roma	Co-investigator	acquisition of data
L.M. Caniatti	MD	Sant'Anna Corona Hospital, Ferrara	Co-investigator	acquisition of data
F. Lanza	MD	Santa Maria delle Croci Hospital, Ravenna	Co-investigator	acquisition of data
S. Meletti	MD	S.Agostino Estense Hospital, Modena	Co-investigator	acquisition of data
M. Onofrj	MD	University of Chieti	Co-investigator	acquisition of data
G. Meucci	MD	USL6 Hospital, Livorno	Co-investigator	acquisition of data
E. Scarpini	MD	University of Milan	Co-investigator	acquisition of data
S. Montepietra	MD	Santa Maria Nuova Hospital, Reggio Emilia	Co-investigator	acquisition of data
U. Aguglia	MD	Bianchi Melacrino	Co-investigator	acquisition of data

		Morelli, Reggio Calabria		
F. Granella	MD	University of Parma	Co-investigator	acquisition of data
D. Guidetti	MD	Guglielmo Da Saliceto Hospital, Piacenza	Co-investigator	acquisition of data
L. Ruiz	MD	SS.Antonio e Biagio e Cesare Arrigo Hospital, Alessandria	Co-investigator	acquisition of data
A.M. Raiola	MD	San Martino Hospital IRCCS, Genoa	Co-investigator	acquisition of data
R. Varaldo	MD	San Martino Hospital IRCCS, Genoa	Co-investigator	acquisition of data
E. Capello	MD	San Martino Hospital IRCCS, Genoa	Co-investigator	acquisition of data
E. Sbragia	MD	University of Genoa	Co-investigator	acquisition of data
D. Currò	MD	San Paolo Hospital, Savona	Co-investigator	acquisition of data
A. Barilaro	MD	Careggi	Co-investigator	acquisition of data

		University Hospital, Florence		
--	--	-------------------------------------	--	--

388  
389

390

391  
392

393 **References**

- 394 1. Rotstein DL, Healy BC, Malik MT, Chitnis T, Weiner HL. Evaluation of No Evidence of  
395 Disease Activity in a 7-Year Longitudinal Multiple Sclerosis Cohort. *JAMA Neurol.*  
396 2015;72(2):152. doi:10.1001/jamaneurol.2014.3537
- 397 2. Cree BAC, Gourraud P-A, Oksenberg JR, et al. Long-term evolution of multiple sclerosis  
398 disability in the treatment era. *Ann Neurol.* 2016;80(4):499-510. doi:10.1002/ana.24747
- 399 3. Cree BAC, Hollenbach JA, Bove R, et al. Silent progression in disease activity-free  
400 relapsing multiple sclerosis. *Ann Neurol.* 2019;85(5):653-666. doi:10.1002/ana.25463
- 401 4. Rush CA, MacLean HJ, Freedman MS. Aggressive multiple sclerosis: proposed definition  
402 and treatment algorithm. *Nat Rev Neurol.* 2015;11(7):379-389. doi:10.1038/nrneurol.2015.85
- 403 5. Nash RA, Hutton GJ, Racke MK, et al. High-dose immunosuppressive therapy and  
404 autologous HCT for relapsing-remitting MS. *Neurology.* 2017;88(9):842-852.  
405 doi:10.1212/WNL.0000000000003660
- 406 6. Atkins HL, Bowman M, Allan D, et al. Immunoablation and autologous haemopoietic stem-  
407 cell transplantation for aggressive multiple sclerosis: a multicentre single-group phase 2 trial.  
408 *Lancet.* 2016;388(10044):576-585. doi:10.1016/S0140-6736(16)30169-6
- 409 7. Burman J, Iacobaeus E, Svenningsson A, et al. Autologous haematopoietic stem cell  
410 transplantation for aggressive multiple sclerosis: The Swedish experience. *J Neurol*  
411 *Neurosurg Psychiatry.* 2014;85(10):1116-1121. doi:10.1136/jnnp-2013-307207

- 412 8. Mancardi GL, Sormani MP, Di Gioia M, et al. Autologous haematopoietic stem cell  
413 transplantation with an intermediate intensity conditioning regimen in multiple sclerosis: The  
414 Italian multi-centre experience. *Mult Scler J*. 2012;18(6):835-842.  
415 doi:10.1177/1352458511429320
- 416 9. Mancardi GL, Sormani MP, Gualandi F, et al. Autologous hematopoietic stem cell  
417 transplantation in multiple sclerosis: A phase II trial. *Neurology*. 2015;84(10):981-988.  
418 doi:10.1212/WNL.0000000000001329
- 419 10. Moore JJ, Massey JC, Ford CD, et al. Prospective phase II clinical trial of autologous  
420 haematopoietic stem cell transplant for treatment refractory multiple sclerosis. *J Neurol*  
421 *Neurosurg Psychiatry*. 2019;90(5):514-521. doi:10.1136/jnnp-2018-319446
- 422 11. Burt RK, Balabanov R, Burman J, et al. Effect of Nonmyeloablative Hematopoietic Stem  
423 Cell Transplantation vs Continued Disease-Modifying Therapy on Disease Progression in  
424 Patients With Relapsing-Remitting Multiple Sclerosis. *Jama*. 2019;321(2):165.  
425 doi:10.1001/jama.2018.18743
- 426 12. Kvistad SAS, Lehmann AK, Trovik LH, et al. Safety and efficacy of autologous  
427 hematopoietic stem cell transplantation for multiple sclerosis in Norway. *Mult Scler J*.  
428 December 2019;135245851989392. doi:10.1177/1352458519893926
- 429 13. Harris KM, Lim N, Lindau P, et al. Extensive intrathecal T cell renewal following  
430 hematopoietic transplantation for multiple sclerosis. *JCI Insight*. 2020;5(2).  
431 doi:10.1172/jci.insight.127655
- 432 14. Muraro PA, Robins H, Malhotra S, et al. T cell repertoire following autologous stem cell  
433 transplantation for multiple sclerosis. *J Clin Invest*. 2014;124(3):1168-1172.  
434 doi:10.1172/JCI71691
- 435 15. Sellner J, Rommer PS. Immunological consequences of “immune reconstitution therapy” in  
436 multiple sclerosis: A systematic review. *Autoimmun Rev*. 2020;19(4):102492.  
437 doi:10.1016/j.autrev.2020.102492

- 438 16. Lünemann JD, Ruck T, Muraro PA, Bar'Or A, Wiendl H. Immune reconstitution therapies:  
439 concepts for durable remission in multiple sclerosis. *Nat Rev Neurol*. 2019.  
440 doi:10.1038/s41582-019-0268-z
- 441 17. Muraro PA, Pasquini M, Atkins HL, et al. Long-term Outcomes After Autologous  
442 Hematopoietic Stem Cell Transplantation for Multiple Sclerosis. *JAMA Neurol*.  
443 2017;74(4):459. doi:10.1001/jamaneurol.2016.5867
- 444 18. Mancardi GL, Saccardi R, Filippi M, et al. Autologous hematopoietic stem cell  
445 transplantation suppresses Gd-enhanced MRI activity in MS. *Neurology*. 2001;57(1):62-68.  
446 doi:10.1212/WNL.57.1.62
- 447 19. Sormani MP, Muraro PA, Schiavetti I, et al. Autologous hematopoietic stem cell  
448 transplantation in multiple sclerosis: A meta-analysis. *Neurology*. 2017;88(22):2115-2122.  
449 doi:10.1212/WNL.0000000000003987
- 450 20. Dekker I, Leurs CE, Hagens MHJ, et al. Long-term disease activity and disability  
451 progression in relapsing-remitting multiple sclerosis patients on natalizumab. *Mult Scler*  
452 *Relat Disord*. 2019;33:82-87. doi:10.1016/j.msard.2019.05.017
- 453 21. Coles AJ, Cohen JA, Fox EJ, et al. Alemtuzumab CARE-MS II 5-year follow-up. *Neurology*.  
454 2017;89(11):1117-1126. doi:10.1212/WNL.0000000000004354
- 455 22. Kappos L, Bar-Or A, Cree BAC, et al. Siponimod versus placebo in secondary progressive  
456 multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study. *Lancet*.  
457 2018;391(10127):1263-1273. doi:10.1016/S0140-6736(18)30475-6
- 458 23. Naegelin Y, Naegelin P, von Felten S, et al. Association of Rituximab Treatment With  
459 Disability Progression Among Patients With Secondary Progressive Multiple Sclerosis.  
460 *JAMA Neurol*. 2019:1-8. doi:10.1001/jamaneurol.2018.4239
- 461 24. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis:  
462 The 2013 revisions. *Neurology*. 2014;83(3):278-286. doi:10.1212/WNL.0000000000000560
- 463 25. Di Filippo M, Sarchielli P, Picconi B, Calabresi P. Neuroinflammation and synaptic

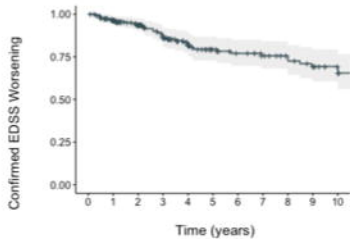
- 464 plasticity: theoretical basis for a novel, immune-centred, therapeutic approach to neurological  
465 disorders. *Trends Pharmacol Sci.* 2008;29(8):402-412. doi:10.1016/j.tips.2008.06.005
- 466 26. Muraro PA, Martin R, Mancardi GL, Nicholas R, Sormani MP, Saccardi R. Autologous  
467 haematopoietic stem cell transplantation for treatment of multiple sclerosis. *Nat Rev Neurol.*  
468 2017;13(7):391-405. doi:10.1038/nrneurol.2017.81
- 469 27. Curro D, Vuolo L, Gualandi F, et al. Low intensity lympho-ablative regimen followed by  
470 autologous hematopoietic stem cell transplantation in severe forms of multiple sclerosis: A  
471 MRI-based clinical study. *Mult Scler J.* 2015;21(11):1423-1430.  
472 doi:10.1177/1352458514564484
- 473 28. Tokoyoda K, Zehentmeier S, Hegazy AN, et al. Professional Memory CD4+ T Lymphocytes  
474 Preferentially Reside and Rest in the Bone Marrow. *Immunity.* 2009;30(5):721-730.  
475 doi:10.1016/j.immuni.2009.03.015
- 476 29. Mumtaz IM, Hoyer BF, Panne D, et al. Bone marrow of NZB/W mice is the major site for  
477 plasma cells resistant to dexamethasone and cyclophosphamide: Implications for the  
478 treatment of autoimmunity. *J Autoimmun.* 2012;39(3):180-188.  
479 doi:10.1016/j.jaut.2012.05.010
- 480 30. Mariottini A, Filippini S, Innocenti C, et al. Impact of autologous haematopoietic stem cell  
481 transplantation on disability and brain atrophy in secondary progressive multiple sclerosis.  
482 *Mult Scler J.* February 2020:135245852090239. doi:10.1177/1352458520902392
- 483 31. Fassas A, Kimiskidis VK, Sakellari I, et al. Long-term results of stem cell transplantation for  
484 MS: A single-center experience. *Neurology.* 2011;76(12):1066-1070.  
485 doi:10.1212/WNL.0b013e318211c537
- 486 32. Hauser SL, Bar-Or A, Comi G, et al. Ocrelizumab versus Interferon Beta-1a in Relapsing  
487 Multiple Sclerosis. *N Engl J Med.* 2017;376(3):221-234. doi:10.1056/NEJMoa1601277  
488  
489

490

491

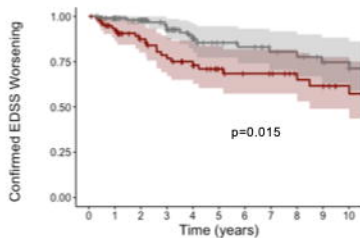
492



**A**

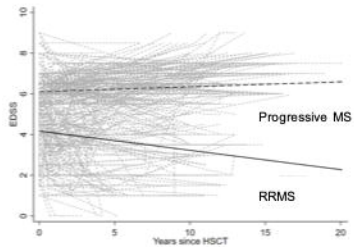
Number at risk

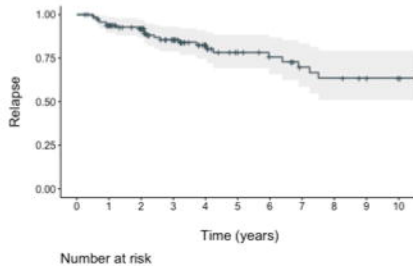
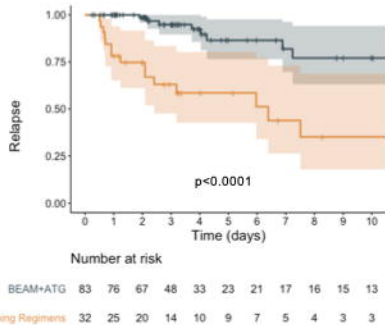
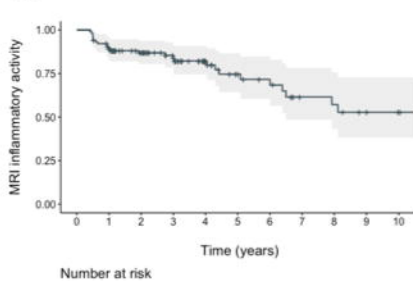
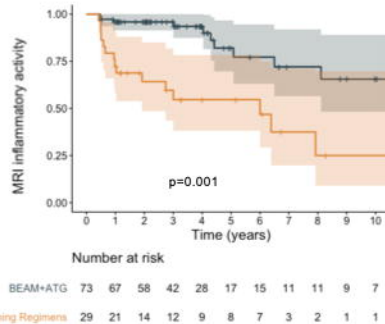
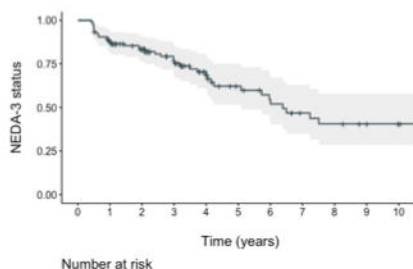
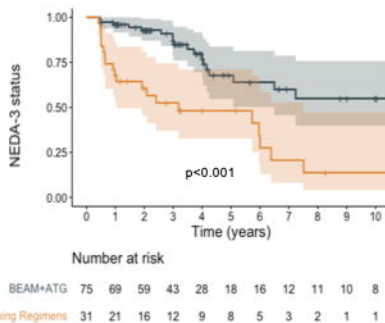
Overall population 196 170 143 112 88 67 60 54 49 43 36

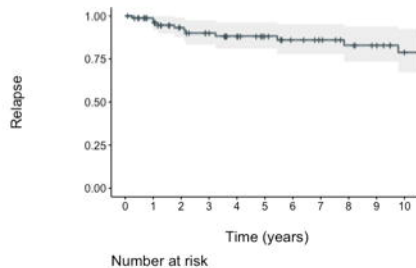
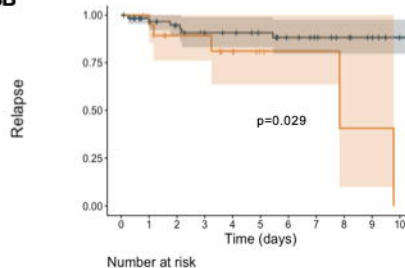
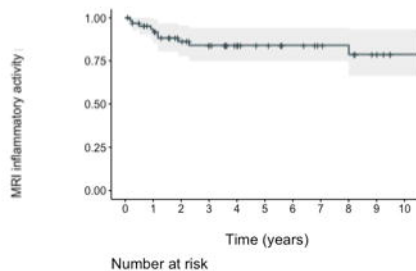
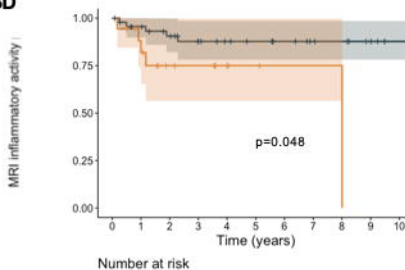
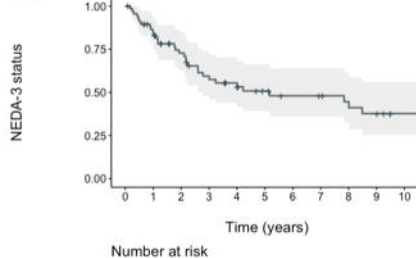
**B**

Number at risk

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**