

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 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

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) |
| 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) |

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**

| Name | Degree | Location | Role | Contribution |
|-----------------|---------------|---|----------------------|--|
| 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 Hospital, 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

| Name | Degree | Location | Role | Contribution |
|-----------------|---------------|--|-----------------|---------------------|
| 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

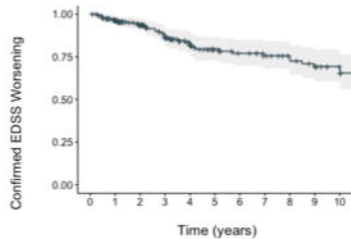
- 438 16. Lünemann JD, Ruck T, Muraro PA, Bar'Or A, Wiendl H. Immune reconstitution therapies:
439 concepts for durable remission in multiple sclerosis. *Nat Rev Neurol*. 2019.
440 doi:10.1038/s41582-019-0268-z
- 441 17. Muraro PA, Pasquini M, Atkins HL, et al. Long-term Outcomes After Autologous
442 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

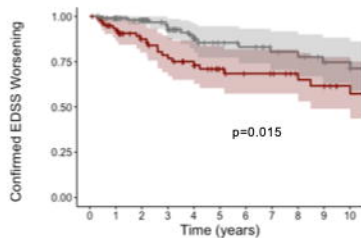
490

491

492

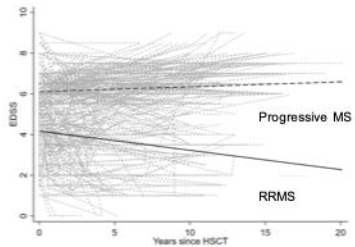
A

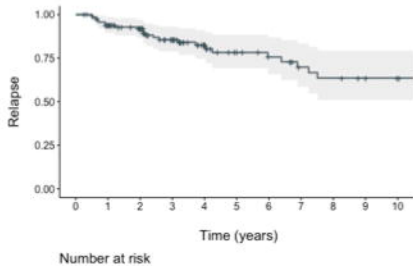
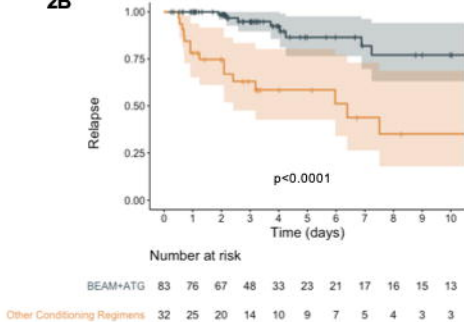
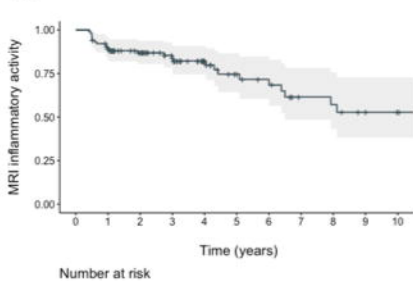
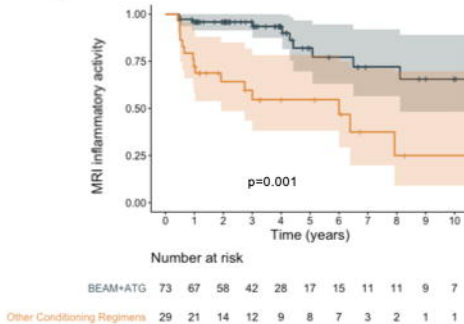
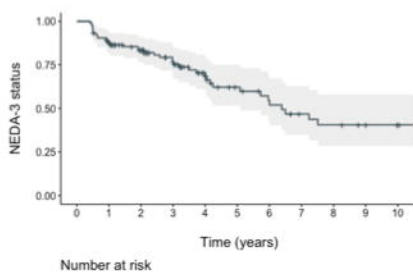
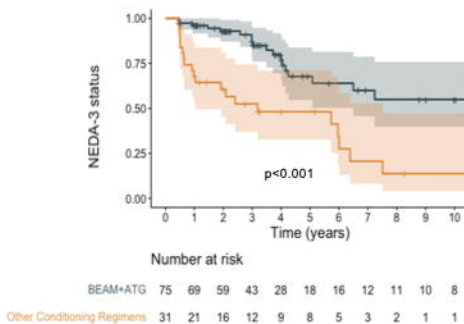
Overall population 196 170 143 112 88 67 60 54 49 43 36

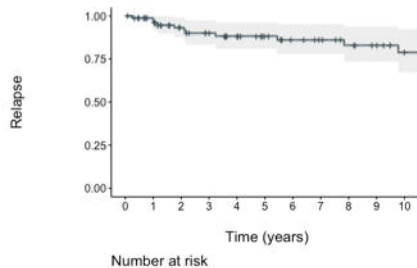
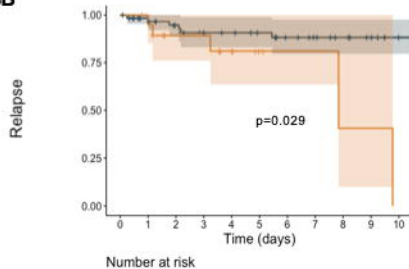
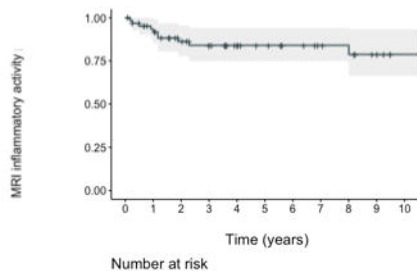
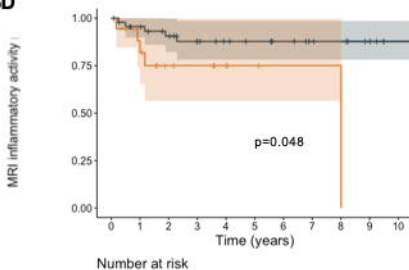
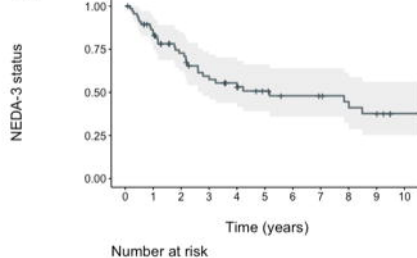
B

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**