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Autologous hematopoietic stem cell transplantation in multiple sclerosis

A phase II trial

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ABSTRACT

Objective: To assess in multiple sclerosis (MS) the effect of intense immunosuppression followed by autologous hematopoietic stem cells transplantation (AHSCT) vs mitoxantrone (MTX) on disease activity measured by MRI.

Methods: We conducted a multicenter, phase II, randomized trial including patients with secondary progressive or relapsing-remitting MS, with a documented increase in the last year on the Expanded Disability Status Scale, in spite of conventional therapy, and presence of one or more gadolinium-enhancing (Gd+) areas. Patients were randomized to receive intense immunosuppression (mobilization with cyclophosphamide and filgrastim, conditioning with carmustine, cytosine-arabinoside, etoposide, melphalan, and anti-thymocyte globulin) followed by AHSCT or MTX 20 mg every month for 6 months. The primary endpoint was the cumulative number of new T2 lesions in the 4 years following randomization. Secondary endpoints were the cumulative number of Gd+ lesions, relapse rate, and disability progression. Safety and tolerability were also assessed. Twenty-one patients were randomized and 17 had postbaseline evaluable MRI scans.

Results: AHSCT reduced by 79% the number of new T2 lesions as compared to MTX (rate ratio 0.21, $p = 0.00016$). It also reduced Gd+ lesions as well as the annualized relapse rate. No difference was found in the progression of disability.

Conclusion: Intense immunosuppression followed by AHSCT is significantly superior to MTX in reducing MRI activity in severe cases of MS. These results strongly support further phase III studies with primary clinical endpoints. The study was registered as EUDRACT No. 2007-000064-24.
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GLOSSARY

AHSCT = autologous hematopoietic stem cell transplantation; **ARR** = annualized relapse rate; **ASTIMS** = Autologous Haematopoietic Stem Cell Transplantation trial in MS; **CI** = confidence interval; **EBMT** = European Group for Blood and Marrow Transplantation; **EDSS** = Expanded Disability Status Scale; **Gd** = gadolinium; **ITT** = intention-to-treat; **LOCF** = last observation carried forward; **MS** = multiple sclerosis; **MTX** = mitoxantrone; **NB** = negative binomial; **PBSC** = peripheral hematopoietic stem cells; **PP** = per protocol; **RR** = relapsing-remitting; **SAE** = serious adverse events; **SP** = secondary progressive.

In multiple sclerosis (MS), for patients who continue to deteriorate in spite of treatment with approved therapies, as well as for patients with other severe autoimmune disorders, a new strategy has been proposed in recent years,[1–3] characterized by intense immunosuppression followed by autologous hematopoietic stem cell transplantation (AHSCT). The target of this treatment is

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Editorial, page XXX

Supplemental data at Neurology.org

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the eradication of a self-reactive abnormal immune system by intense immunosuppression, followed by the infusion of autologous hematopoietic stem cells aimed to restore the hemato-lymphopoietic system. This procedure, besides its certain immunosuppressive properties, is believed to reset the immune system and induce a prolonged tolerance toward self-antigens.[4–9] Over 1,500 patients diagnosed with an autoimmune disorder were reported to the Registry of the European Group for Blood and Marrow Transplantation (EBMT) and more than 800 MS cases have been treated worldwide with this procedure in recent years,[10,11] usually within small phase I/II studies.[12–18] Although AHSCT appears to be very effective, especially in selected MS cases,[19–22] currently there are no published prospective phase II or phase III studies comparing AHSCT with the conventional treatments. The Autologous Haematopoietic Stem Cell Transplantation trial in MS (ASTIMS) is a multicenter, randomized, phase II study promoted by the EBMT designed to assess the effect of AHSCT vs mitoxantrone (MTX) on the disease activity in MS, measured by MRI in the 4 years following treatment. Safety and the effect on relapse rate and clinical progression of the disease are also reported.

METHODS

Patients. Patients eligible for the study had clinically defined MS,[23] a secondary progressive (SP) or relapsing-remitting (RR) form that accumulates disability between relapses, with a documented worsening during the last year (1 step of Expanded Disability Status Scale [EDSS], or 0.5 when EDSS is between 5.5 and 6.5), in spite of conventional therapy (interferon-β or glatiramer acetate or immunosuppressive therapy), and presence of one or more gadolinium (Gd)-enhancing areas on MRI. The EDSS score had to be between 3.5 and 6.5.

Standard protocol approvals, registrations, and patient consents. The study was conducted in accordance with the Guidelines for Good Clinical Practice and the Declaration of Helsinki. The protocol and its amendments were approved by the competent authorities and the ethics committees for each center. Enrolled patients provided written informed consent. The study protocol was registered as EUDRACT No 2007-000064-24.

Study design. ASTIMS is a proof of concept academic study endorsed by the EBMT Autoimmune Disease Working Party. ASTIMS, originally designed as a phase III study, started in May 2004 but the accrual rate was much lower than expected. Two years after opening recruitment, only 13 patients were randomized and therefore the Steering Committee amended the protocol, switching the primary endpoint from confirmed EDSS progression to the cumulative number of new T2 MRI lesions in the 4 years following treatment. Fewer than 30 patients were estimated to be sufficient to detect a reduction of at least 70% in the number of new T2 lesions in the AHSCT arm vs the MTX arm, with 80% power at 95% significance level. An interim analysis after the first 10 patients included was run.

Study procedures and endpoints assessment. Patients were followed by a treating neurologist and an examining physician. Clinical and neurologic evaluations were carried out just before randomization, at baseline, after 6 months from randomization, and then every 6 months for the following 48 months. Neurologic evaluation was done each time the patient complained of symptoms or signs suggestive of a relapse. Safety was evaluated on the basis of adverse events reported by the investigators according to National Cancer Institute criteria (CTCAE v 4.0, www.cancer.gov).

MRI. MRI examinations were performed at each participating center with MRI scanners operating at 1.5T using a standardized protocol. Contrast agent–enhanced T1-weighted images were obtained for all patients after IV injection of 0.1 mmol/kg body weight Gd-based contrast agent. MRI of the brain was obtained at screening and subsequently at baseline, and then every 12 months for 48 months. MRI scans were centralized to the coordinating center and examined by a single operator, blinded to the treatment assignment.

Treatment. After the screening and baseline evaluation, patients were randomized to receive either AHSCT or MTX. In the transplant arm, peripheral hematopoietic stem cells (PBSC) were mobilized by cyclophosphamide (4 g/m^2) in 1 day. After 5 days, filgrastim was administered daily (5 μg/kg body weight SC) until the completion of the stem cells harvest. Hematopoietic stem cells were then collected with a leukapheresis procedure and an unmanipulated graft containing between 3 and 8 × 10^6 CD34+/kg cells was cryopreserved. The conditioning regimen was BEAM, which includes BCNU (carmustine, 300 mg/m^2 at day −6); cytosine-arabinoside (200 mg/m^2); etoposide (200 mg/m^2 from day −5 to day −2); and melphalan (140 mg/m^2 at day −1). PBSC were then thawed and infused through the central venous catheter. Rabbit ATG was added at a total dose of 3.75 mg/kg/d at day +1 and +2. Acyclovir and sulfamethoxazole/trimethoprim from discharge until day +180 were recommended. The MTX arm consisted of an IV infusion of 20 mg plus methylprednisolone 1 g diluted in 250 mL 0.9 saline once every month for 6 months.[24] After AHSCT and MTX, patients were treated only with symptomatic therapy.

Endpoints. The primary endpoint of ASTIMS was to determine the effect of AHSCT vs MTX on disease activity measured by the cumulative number of new T2 MRI lesions in the 4 years following randomization. Secondary endpoints were the activity of AHSCT vs MTX on the cumulative number of Gd-enhancing lesions on T1-weighted MRI from baseline to year 4. Secondary clinical endpoints also included the cumulative number of relapses from baseline to year 4 and the time to disability progression confirmed after 6 and 12 months. Progression was defined as an increase of 1 or more EDSS points when baseline EDSS is between 3.5 and 5.5 or of 0.5 EDSS points when baseline EDSS is between 5.5 and 6.5. Other secondary endpoints were AHSCT-related mortality, safety, side effects, early adverse events, and serious adverse events (SAE) in the 2 arms. Additional analyses included comparisons between treatment arms in the cumulative number of new T2 lesions over the first, second, third, and fourth year after therapy, as well as in the time of appearance of the first new T2 MRI lesion.

Table 1 Baseline demographic characteristics and disease history of patients

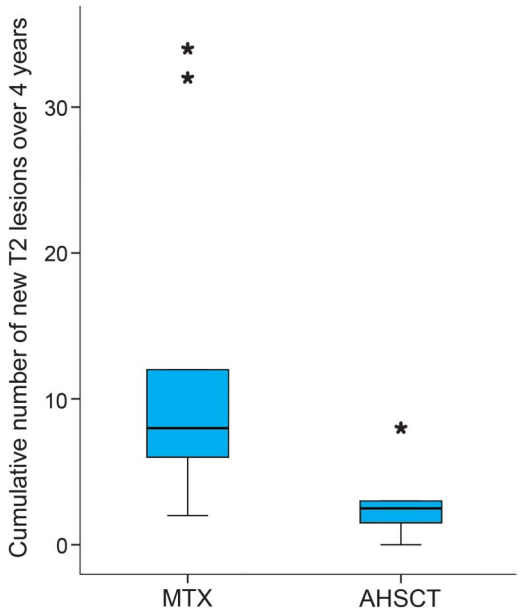
	AHSCT, n = 9	MTX, n = 12	Overall, n = 21
Mean (range) age, y	36 (22-46)	35 (19-43)	35.5 (19-46)
Women, n (%)	5 (24)	9 (43)	14 (67)
Median (range) EDSS	6.5 (5.5-6.5)	6 (5.5-6.5)	6 (5.5-6.5)
Median (range) EDSS 1 year before	5 (3-6)	4 (2-6)	4.5 (2-6)
Disease course, n (%)			
RR	2 (22)	5 (42)	7 (33)
SP	3 (33)	3 (25)	6 (29)
SP with relapses	4 (45)	3 (25)	7 (33)
RP	0	1 (8)	1 (5)
Disease duration (range), y	10.5 (5-20)	9.8 (2-23)	10.2 (2-23)

Abbreviations: AHSCT = autologous hematopoietic stem cell transplantation; EDSS = Expanded Disability Status Scale; MTX = mitoxantrone; RP = relapsing progressive; RR = relapsing-remitting; SP = secondary progressive.

Statistical analysis. The analysis was performed according to the intention-to-treat principle (ITT), to stick to the original protocol; the population comprised in the ITT analysis was defined as the one including patients having baseline MRI and at least one postbaseline MRI examination within 4 years from treatment. Imputation rules for the primary endpoint were conducted according to the last observation carried forward (LOCF) approach; all the specific procedures for the imputation rules are detailed in the statistical analysis plan (e-appendix on the *Neurology*® Web site at Neurology.org). The cumulative number of new T2 and Gd+ MRI lesions from baseline to year 4 was compared between treatment arms using a negative binomial (NB) regression model. Supplementary analyses were performed adjusting for the number of baseline T1 Gd+ lesions in the NB model, using the Wilcoxon rank sum test, using the per protocol (PP) population adjusted for the different number of scans/patient with an offset in the NB model, and running a mixed-effect model using all the available MRI scans over time. Sensitivity analyses for the primary endpoint were performed also using a best and worst case scenario for missing/invalid new T2 MRI lesions imputation, and also fully imputing the 4 missing patients. In the worst case scenario, all the missing scans in the control (MTX) arm were imputed as having 0 new T2 lesions, while all the missing scans in the experimental (AHSCT) arm were imputed by the LOCF approach; in the best case scenario, all the missing scans in the AHSCT arm were imputed as having 0 new T2 lesions, and all the missing scans in the MTX arm were imputed by the LOCF approach. The 4 missing patients were imputed as all having 0 new T2 lesions and as all having the maximum number of new T2 lesions counted in the cohort during follow-up. Also, an analysis excluding the 2 outliers in the MTX arm was run. Sensitivity analysis methodology is detailed in the e-appendix. Time to disease progression was compared between treatment arms by Kaplan-Meier survival curves and the log-rank test, while the Wilcoxon test was used to compare EDSS changes between treatment arms. The annualized relapse rate (ARR) was computed by dividing the total number of relapses by the person-years in each treatment arm. The relapse rate was compared between treatment arms using a NB regression model with the relapse counts as the dependent variable and the treatment arm as covariate, with the duration of follow-up as an offset. The analysis was run also adjusting for previous year relapses.

RESULTS **Patient characteristics.** From 2004 to 2009, 21 patients were recruited from 7 centers in 2 countries, Italy and Spain. All the patients had a follow-up of 4 years, and only 2 cases were followed for 3 years. Nine patients were randomized in the AHSCT and 12 in the MTX arm. No relevant difference was observed in baseline demographic and disease

Figure 1 Primary endpoint, intent-to-treat population



The cumulative number of new T2 MRI lesions counted over 4 years is significantly reduced in the autologous hematopoietic stem cell transplantation (AHSCT) arm as compared to the mitoxantrone (MTX) arm: MTX = median 8 (range 2-34), AHSCT = median 2.5 (range [illegible]-3), rate ratio = 0.21 (95% confidence interval 0.10-[illegible]), $p = 0.00016$. Black lines: medians; box = interquartile range. *Rate ratio, negative binomial regression analysis.

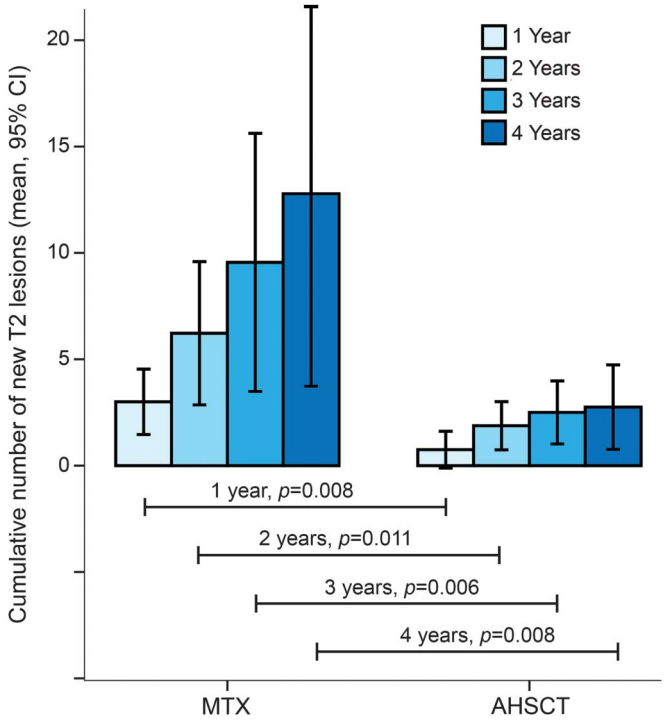
characteristics in the 2 groups (table 1), excluding a slightly higher proportion of RR subjects in the MTX arm vs the AHSCT arm (42% vs 22%). Among the recruited patients, 33% were in the RR phase and 67% in the progressive period of the disease, with or without relapses. Mean age at transplantation was 35.5 years (range 19–46) and median EDSS at baseline was 6 (range 5.5–6.5). All patients clinically deteriorated in the previous year with an increase of EDSS of at least 1 point. All the included patients had failed previous treatments with glatiramer acetate, interferon-β, or azathioprine, cyclophosphamide, and methotrexate, in different combinations.

MRI outcomes. Out of 21 enrolled patients, technical problems (an informatics crash during the transfer of MRI images from one to another computer) caused the loss of baseline MRI data of 3 cases in one center (2 patients randomized in the MTX and 1 in the AHSCT arm); the last randomized patient was assigned to the MTX arm and refused to continue the study just after the first dose of MTX, being then excluded from further clinical and MRI data analysis. Therefore the final MRI analysis was performed on 17 patients (25 scans in the MTX and 26 in the AHSCT group). Data were imputed for 17 missing scans (8%), 2 at year 1, 3 at year 2, 3 at year 3, and 9 at year 4.

AHSCT significantly reduced the number of new T2 MRI lesions counted over 4 years, compared to MTX. In the AHSCT arm, a median number of 2.5 lesions appeared in the evaluated period (mean 2.75, rang[illegible]25–3) as compared to 8 (mean 12.75, rang[illegible]34) in the MTX-treated group (rate ratio = 0.21, 95% confidence interval [CI] 0.10–0.48, $p = 0.00016$, figure 1). This result was maintained in all the sensitivity analyses: the rate ratio was 0.32 (95% CI 0.16–0.66, $p = 0.002$) in the worst case scenario and was 0.19 (95% CI 0.09–0.41, $p < 0.0001$) in the best case scenario for imputation (see details in the e-appendix). The difference in the rate of new T2 lesions was significant adjusting for baseline Gd+ lesions (rate ratio = 0.19, 95% CI 0.09–0.41, $p < 0.0001$), using the PP population with an offset variable for the number of scans (rate ratio = 0.18, 95% CI 0.06–0.52, $p < 0.0001$), applying a mixed effects model with new T2 lesions on each MRI scan over time as the dependent variable (rate ratio = 0.28, 95% CI 0.13–0.62, $p = 0.002$), imputing the 4 missing patients as having 0 new T2 lesions (rate ratio = 0.25, 95% CI 0.05–0.74, $p = 0.012$) and imputing them as having 35 new T2 lesions (rate ratio = 0.34, 95% CI 0.15–0.82, $p = 0.016$), and excluding the 2 outliers in the MTX arm ([illegible] ratio = 0.08, 95% CI 0.02–0.25, $p < 0.000$[illegible]ore details in the e-appendix). The difference between the 2 groups was already significant in the first year ($p = 0.008$) and increased constantly, maintaining the same significant difference over the follow-up (figure 2). In all the patients treated with MTX, at least 1 new T2 lesion appeared at year 1, while 50% of AHSCT treated cases were free from new T2 lesions at year 1; 3 cases developed new T2 lesions at year 2; and 1 patient was free from T2 activity at the end of follow-up (log-rank test, $p = 0.019$). None of the patients treated with AHSCT (100%) had Gd+ lesions during the 4 years of follow-up, while 56% of the MTX patients had at least 1 Gd+ lesion during the follow-up ($p = 0.029$, Fisher exact test). The results did not change when adjusting for disease phase (RR vs progressive).

Clinical outcome, disability, and relapses. Clinical data were available for 20 out of 21 randomized patients. AHSCT significantly reduced the ARR as compared to MTX: ARR was 0.6 for the MTX arm and 0.19 for the AHSCT treatment group and the difference was statistically significant, despite the low power of the study for this endpoint (rate ratio = 0.36, 95% CI 0.15–0.88, $p = 0.026$). Progression occurred at the end of follow-up in 48% of cases in the MTX arm and in 57% of the AHSCT-treated group. There was no statistical difference between the 2 groups (log-rank test $p = 0.50$). No difference in EDSS change at year 1, 2, 3, and 4 was found between the treatment arms.

Figure 2 Cumulative number of new T2 MRI lesions over 1, 2, 3, and 4 years



AHSCT = autologous hematopoietic stem cell transplantation; CI = confidence interval; MTX = mitoxantrone.

Safety. Early adverse events were those expected and occurred at least in 80% of treated cases and are reported in table 2. SAE occurred in the AHSCT arm only and resolved without sequelae.

T2

DISCUSSION ASTIMS is a multicenter, randomized controlled study evaluating the activity of AHSCT vs immunosuppressive therapy on MRI measures in MS. It is well known from the literature that either AHSCT or MTX has a profound effect on MRI activity in MS.[25–28] In this study, AHSCT was shown to be significantly superior to MTX in reducing the MRI activity: patients in the AHSCT arm experienced 79% fewer new T2 lesions as compared to patients in the MTX arm. This effect was already evident in the first year and was sustained through the 4-year follow-up. Treatment with AHSCT also resulted in a complete suppression of active inflammatory lesions, as demonstrated by the absence of new Gd+ lesions during the 4-year follow-up in the AHSCT arm while inflammatory activity was still present in 56% of patients treated with MTX.

MTX was used at a dosing commonly utilized in Europe in rapidly worsening MS cases with MRI signs of inflammation[24] and not the standard dosage of 12 mg/m^2 every 3 months.[29] The goal of this study was to compare an intense immunosuppression scheme of therapy followed by AHSCT given in a short period of a few months vs a commonly utilized immunosuppressive therapy administered for a similar period of time and therefore the results of ASTIMS should be referred to the dosage of MTX used in this study.

Table 2 Distribution of adverse events and severe adverse events in AHSCT and control arms

Patient no.	Arm	Adverse events (description, grade)	Severe adverse events (description, grade)
001	AHSCT	Febrile neutropenia (2), leukopenia (2), diarrhea (1), anemia (1), cystitis (2), herpes zoster (1)	None
002	AHSCT	Febrile neutropenia (3), leukopenia (3), diarrhea (2), anemia (3), platelets count decreased (3), diarrhea (2)	None
003	AHSCT	Febrile neutropenia (2), leukopenia (2), diarrhea (1), anemia (2)	Sepsis (4)
004	AHSCT	Febrile neutropenia (3), leukopenia (3), diarrhea (1), anemia (3), pneumothorax (2)	None
005	MTX	Anemia (1), neutrophil count decreased (3), lymphocyte count decreased (1)	None
006	AHSCT	Febrile neutropenia (2), leukopenia (4), diarrhea (1), anemia (4), platelets count decreased (4)	Late engraftment (3); prolonged hospitalization
007	MTX	Amenorrhea (3)	None
008	MTX	Amenorrhea (3)	None
009	MTX	Leukopenia (3), lymphocyte count decreased (3), gastrointestinal toxicity (2)	None
010	MTX	None	None
011	MTX	Leukopenia (3)	None
012	AHSCT	Amenorrhea (3), leukopenia (3), diarrhea (1), anemia (3), platelets count decreased (3), mucositis (2)	None
013	MTX	Neutrophil count decreased (3), lymphocyte count decreased (1)	None
014	MTX	Arthritis (1)	None
015	AHSCT	Amenorrhea (3), febrile neutropenia (4), leukopenia (4), diarrhea (1), anemia (3), platelets count decreased (4)	Systemic candidiasis (4), CMV reactivation (4), engraftment failure (4); life-threatening
016	AHSCT	Amenorrhea (3), febrile neutropenia (3), leukopenia (4), diarrhea (1), anemia (2), platelets count decreased (3)	None
017	MTX	Neutrophil count decreased (4)	None
018	MTX	None	None
019	AHSCT	Febrile neutropenia (3), leukopenia (3), diarrhea (1), anemia (2), platelets count decreased (3)	ATG reaction (dyspnea [2], bradycardia [3], hypoxemia [2]); life-threatening
020	MTX	None	None
021	MTX	Not applicable	Not applicable

Abbreviations: AHSCT = autologous hematopoietic stem cell transplantation; CMV = cytomegalovirus; MTX = mitoxantrone.

ASTIMS is an academic study that has intrinsic limitations. First, it was designed as a phase III study, which became a phase II study with a primary laboratory endpoint when it was clear that the number of enrolled patients was lower than expected. Second, the number of cases included was small. Third, being a spontaneous study, some accessory data, such as information on the quality of life and on brain atrophy, could not be collected. In spite of these limitations, ASTIMS was able to reach the primary endpoint, demonstrating that the difference on MRI measures of activity between the 2 treatment arms is of particular magnitude.

In ASTIMS, treatment with AHSCT resulted in a significantly reduced rate of clinical relapses. On the contrary, in our study we were not able to detect any difference between the 2 groups in disability progression. The reasons for this negative result can be partly explained by the low power of this study to detect even large effects of AHSCT on disability progression. A post hoc power calculation reveals that with 20 patients followed for 4 years, and a probability of progression of 50% in the control group, the study would have had a power of 26% to detect a complete suppression of disability accumulation and a power of 20% to detect a halving of this proportion. Moreover, 67% of included cases were already in the SP phase of the disease and it is now widely accepted that there is a window of therapeutic opportunity in MS,[30] and in AHSCT there are recent publications[31–36] that underline that patients still in the RR phase of the disease have a better clinical outcome.

The incidence and relevance of adverse events and SAE were in the expected range and similar to the data reported in the literature. There were no deaths or late SAE.

The effect of a therapy on clinical relapses and, in the long run, on progression of disability can be predicted by the effect of that therapy on MRI lesions.[37,38] The superiority of AHSCT vs MTX is of a such a large magnitude that it is possible to speculate that AHSCT can also profoundly impact the clinical course of the disease, if this treatment is reserved for a population of patients still in the RR phase of disease.

ASTIMS unequivocally demonstrates the superiority of AHSCT vs an immunosuppressive approved therapy on MRI measures of activity in MS. The effect is related to the intense immunosuppression and, possibly, also to a reset of the immune system following the infusion of autologous hematopoietic stem cells. In spite of some weakness of the study, the obtained data are robust and confirmed by sensitivity analyses. Therefore, the results of the ASTIMS study support the design of phase III studies aimed to evaluate the superiority of AHSCT vs the approved therapies on clinical endpoints. A phase III study randomizing patients who failed interferon-β in a non-myeloablative AHSCT regimen[31] compared to the Food and Drug Administration–approved standard of care is already running in Chicago, Sweden, and Brazil (www.clinicaltrials.gov NCT00273364). In Europe and North America, a study is currently in preparation that compares AHSCT vs the best approved therapy in severe MS cases, poorly responding to approved drugs, still in the RR phase of the disease, and showing clinical and MRI activity.[39]

AUTHOR CONTRIBUTIONS

G.L. Mancardi: design or conceptualization of the study, analysis and interpretation of the data, and drafting or revising the manuscript. M.P. Sormani: analysis and interpretation of the data and drafting or revising the manuscript. F. Gualandi: design or conceptualization of the study and analysis and interpretation of the data. A. Saiz: design or conceptualization of the study and analysis and interpretation of the data. E. Carreras: design or conceptualization of the study. E. Merelli: design or conceptualization of the study. A. Donelli: design or conceptualization of the study. A. Lugaresi: analysis and interpretation of the data and drafting or revising the manuscript. P. Di Bartolomeo: design or conceptualization of the study. M.R. Rottoli: analysis and interpretation of the data. A. Rambaldi: design or conceptualization of the study. M.P. Amato: analysis and interpretation of the data. L. Massacesi: analysis and interpretation of the data. M. Di Gioia: analysis and interpretation of the data and drafting or revising the manuscript. L. Vuolo: analysis and interpretation of the data. D. Currò: analysis and interpretation of the data and drafting or revising the manuscript. L. Roccatagliata: analysis and interpretation of the data and drafting or revising the manuscript. M. Filippi: analysis and interpretation of the data and drafting or revising the manuscript. U. Aguglia: design or conceptualization of the study. P. Iacopino: design or conceptualization of the study. D. Farge: drafting or revising the manuscript. R. Saccardi: design or conceptualization of the study, analysis and interpretation of the data, and drafting or revising the manuscript.

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