Immunoablation and autologous haemopoietic stem-cell transplantation for aggressive multiple sclerosis: a multicentre single-group phase 2 trial

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Summary

Background **Strong immunosuppression, including chemotherapy and immune-depleting antibodies followed by autologous haemopoietic stem-cell transplantation (aHSCT), has been used to treat patients with multiple sclerosis, improving control of relapsing disease. We addressed whether near-complete immunoablation followed by immune cell depleted aHSCT would result in long-term control of multiple sclerosis.**

Methods **We did this phase 2 single-arm trial at three hospitals in Canada. We enrolled patients with multiple sclerosis, aged 18–50 years with poor prognosis, ongoing disease activity, and an Expanded Disability Status Scale of 3·0–6·0. Autologous CD34 selected haemopoietic stem-cell grafts were collected after mobilisation with cyclophosphamide and filgrastim. Immunoablation with busulfan, cyclophosphamide, and rabbit anti-thymocyte globulin was followed by aHSCT. The primary outcome was multiple sclerosis activity-free survival (events were clinical relapse, appearance of a new or Gd-enhancing lesion on MRI, and sustained progression of Expanded Disability Status Scale score). This study was registered at ClinicalTrials.gov, NCT01099930.**

Findings **Between diagnosis and aHSCT, 24 patients had 167 clinical relapses over 140 patient-years with 188 Gd-enhancing lesions on 48 pre-aHSCT MRI scans. Median follow-up was 6·7 years (range 3·9–12·7). The primary outcome, multiple sclerosis activity-free survival at 3 years after transplantation was 69·6% (95% CI 46·6–84·2). With up to 13 years of follow-up after aHSCT, no relapses occurred and no Gd enhancing lesions or new T2 lesions were seen on 314 MRI sequential scans. The rate of brain atrophy decreased to that expected for healthy controls. One of 24 patients died of transplantation-related complications. 35% of patients had a sustained improvement in their Expanded Disability Status Scale score.**

Interpretation **We describe the first treatment to fully halt all detectable CNS inflammatory activity in patients with multiple sclerosis for a prolonged period in the absence of any ongoing disease-modifying drugs. Furthermore, many of the patients had substantial recovery of neurological function despite their disease's aggressive nature.**

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Introduction

Multiple sclerosis is an acquired inflammatory autoimmune disease of the CNS resulting in loss of myelin and axon degeneration.[1] Treatments target inflammation, yet many patients continue to relapse or progress and to date no treatment has produced substantial and sustained neurological recovery.[2]

Use of increasingly intense treatments to suppress or eliminate immune mechanisms responsible for CNS destruction, with or without autologous haemopoietic stem-cell transplantation, has varying results.[3,4] Usually refractory to previous treatments, most patients have transient benefits with reduced clinical relapses, MRI activity, or delayed disease progression, but ultimately the disease reactivated. Even the highest doses of chemotherapy do not fully abolish inflammatory disease.[5,6] We did a phase 2 trial of busulfan, cyclophosphamide, and anti-thymocyte globulin, used to ablate the destructive immune system and eliminate immunological memory, followed by aHSCT with a graft depleted of immune cells to reconstitute an immune system that no longer causes CNS-directed autoimmunity (ie, is self-tolerant) but is fully capable of providing protective immunity.

Methods

Study design and participants

We did this single-arm trial at three hospitals in Canada. We enrolled patients with a poor prognosis, defined as a high probability of significant disease progression during the next 10 years based on the natural history dataset for London, ON, Canada.[7] Inclusion criteria were: age 18–50 years; multiple early relapses; early development of sustained disability measured by the Expanded Disability Status Scale[8] (EDSS) affecting motor control with cerebellar or pyramidal Kurtzke Functional System score of at least 3·0 within 5 years of disease onset; evidence of ongoing clinical disease activity despite at least 1 year of immunomodulatory or

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Research in context

Evidence before this study

Inspired by observations of autoimmune remissions for patients undergoing haemopoietic stem-cell transplantation (HSCT) for a concurrent malignancy, and animal models supporting the biological basis of the treatment effect, investigators in the 1990s began to explore HSCT for the treatment of multiple sclerosis. We searched MEDLINE, Embase, and the Cochrane Register of Controlled Trials for reports of the use of HSCT for multiple sclerosis using the terms "multiple sclerosis", "hematopoietic stem cell transplantation", "bone marrow transplantation", "stem cell transplantation", "transplantation", and "transplantation conditioning". No restrictions were placed on patient characteristics, study design, study duration, endpoints measured, or language of publication. We found five reports of three studies of ten or more patients (44 patients in total) published before the start of our trial in 2001. All were single-arm cohort studies of patients with progressive disease that had failed previous treatment with BEAM chemotherapy, anti-thymocyte globulin, and autologous HSCT. One treatment-related death (2%) was reported, nine (20%) of 44 patients had further progression, 18 (41%) had stable disabilities, and 16 (36%) had some degree of improvement in their disabilities over a follow-up period of 30 months. Some patients had relapses and MRI activity after HSCT. Given mixed benefit after short follow-up, the question remained whether this complex treatment was warranted. We postulated that the failure to control multiple sclerosis resulted from the inability of a mild conditioning regimen to achieve adequate immunodepletion and that greater immunoablation would lead to better outcomes.

Evidence from this study

This study is the first showing the complete long-term suppression of all inflammatory activity in a cohort of patients with active and progressing multiple sclerosis who have received a myeloablative HSCT regimen. The frequent, planned, comprehensive clinical and MRI follow-up lends strength to our conclusion. With a median follow-up of 6·7 years (range 3·9–12·7), 16 (70%) of 23 patients were free from further progression and many patients had improvements in disability.

Implications of all the available evidence

As of 2015, 30 reports of 16 studies document the outcome of more than 650 transplantation recipients but only three studies report outcomes after a median follow-up of greater than 5 years. By contrast with our study, disease activity after HSCT occurred in more than 70% of the 75 patients reported in three previous studies. These results strongly indicate that a more aggressive conditioning regimen can result in long-term disease remission in patients with unrelenting, refractory multiple sclerosis and warrant testing in a prospective randomised trial.

immunosuppressive treatment (either at least two disabling relapses in the year before enrolment, three disabling relapses in the 2 years before enrolment, or deterioration of 1 point or more on the EDSS in the 18 months before enrolment if the baseline EDSS score was ≤5·0, or of 0·5 points on the EDSS if the baseline score was >5·5); an EDSS score of 3·0–6·0 with a cerebellar or pyramidal Kurtzke Functional System score of at least 3·0; and brain MRI satisfying the Paty[9] or Fazekas[10] criteria for diagnosing multiple sclerosis. We excluded patients with substantial cardiac, renal, pulmonary, or hepatic dysfunction, or those with active infection or other medical problems that could increase the risk of morbidity or mortality. Individuals previously treated with cytotoxic drugs had to have bone marrow morphology and cytogenetics assessed to exclude myelodysplasia. The study began in 2000 when treatments for multiple sclerosis in Canada included interferon-beta, glatiramer acetate, mitoxantrone, and cyclophosphamide.

The study was approved by the research ethics boards of participating institutions. An independent data and safety monitoring committee reviewed potential participants, acceded to each enrolment, and reviewed unexpected serious adverse events. The protocol contained stopping rules to terminate the study if undue toxic effects were encountered or if the treatment was deemed ineffective. All participants provided written informed consent.

Procedures

A detailed outline of the procedure has been published previously (figure 1A).[11] Briefly, an autologous bone marrow graft was harvested and cryopreserved in case of non-engraftment or persistent immunodeficiency. Then, autologous haemopoietic stem cells were mobilised into circulation with cyclophosphamide (4·5 g/m² intravenous) and filgrastim (10 μg/kg per day, subcutaneously for 10 days) with appropriate supportive care and collected by peripheral vein leucopheresis using a COBE Spectra apheresis system (Terumo BCT; Lakewood, CO, USA). CD34 immunomagnetic selection, on a CliniMACS Stem Cell Selection Device (Miltenyi Biotec; Auburn, CA, USA), depleted the graft of mature immune cells.

The transplant conditioning regimen consisted of busulfan, with monitoring of first dose pharmacokinetics, administered every 6 h for 16 doses, cyclophosphamide (50 mg/kg per day, intravenously for 4 days), and rabbit anti-thymocyte globulin (1·25 mg/kg per day, intravenously for 4 days). The CD34-selected autologous haemopoietic stem-cell graft was infused 48 h after the final dose of chemotherapy. Patients were admitted to a stem-cell transplantation ward for standard supportive care and anti-infective prophylaxis. Treatment-related toxic effects were assessed each day during admission with the Bearman Regimen-Related Toxicity Score.[12] Participants were not scheduled

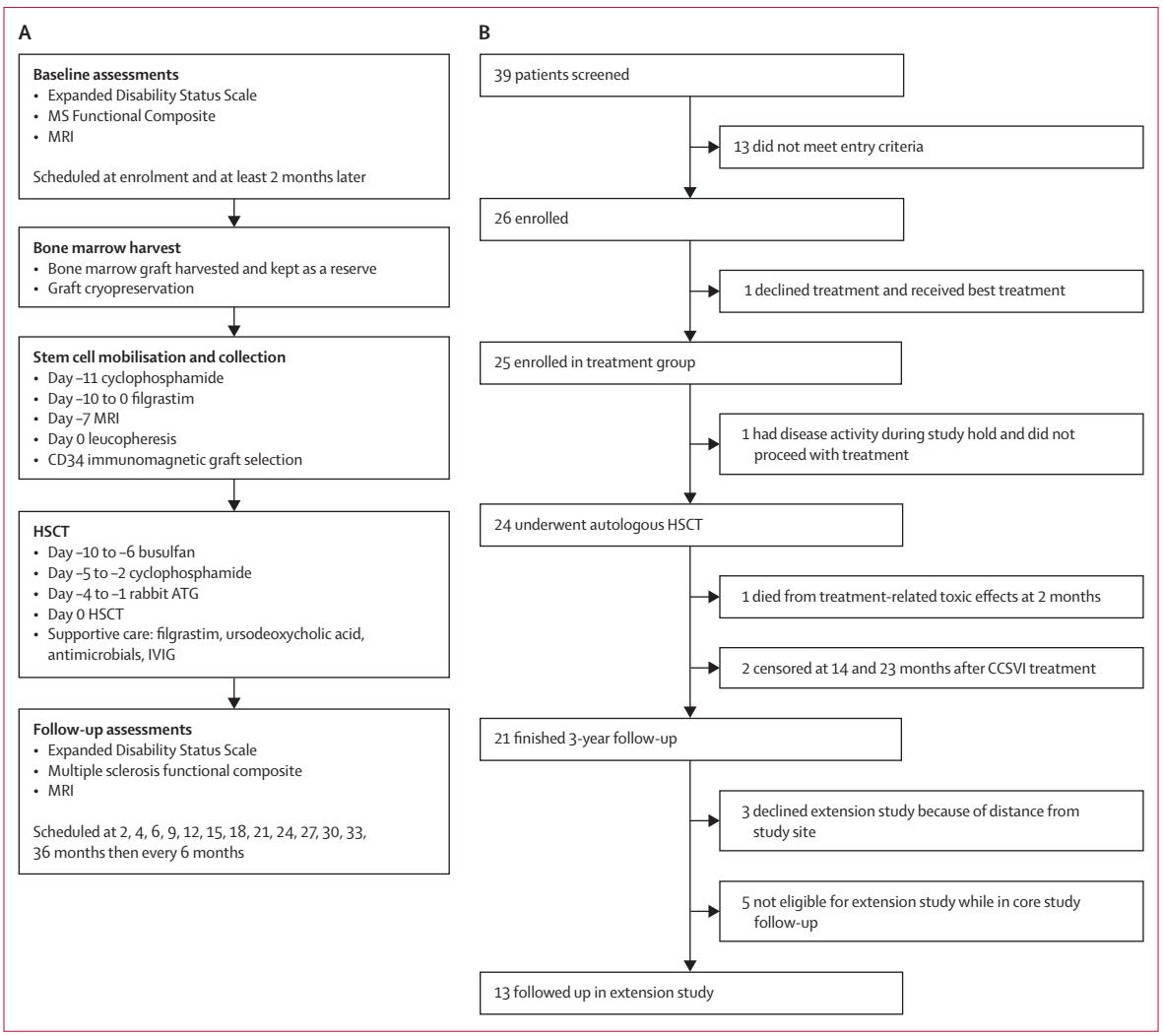

Figure 1: **Structure of the trial**
(A) The timing of assessments and treatments, and (B) trial profile. ATG=anti-thymocyte globulin. IVIG=intravenous immunoglobulin. HSCT=haemopoeitc stem-cell transplantation. CCSVI=chronic cerebrospinal venous insufficiency.

to receive disease-modifying drugs after stem-cell transplantation.

Transplantation care visits and assessments were scheduled in accordance with the standard practices of the participating stem-cell transplantation programmes with unscheduled visits to assess unanticipated medical issues. Patients were censored from further trial assessments if they began a conventional, experimental, or unproven treatment for multiple sclerosis.

Disease activity and disability were assessed with predefined scheduled clinical, laboratory, and MRI tests. All patients underwent validated standardised assessments of their neurological condition. An independent neurologist and an investigator scored the EDSS using the modified Neurostatus Scale[13] at least twice before treatment, every 2 months for a year after stem-cell transplantation, then every 3 months for the next 2 years, and every 6 months thereafter. Because of high inter-rater reliability of paired EDSS scores (appendix p 1), the protocol was amended so a single neurologist scored the EDSS after the first 12 patients completed 3 years of follow-up.

See Online for appendix

The Multiple Sclerosis Function Composite was tested at the same timepoints as EDSS and quality of life was assessed with a validated tool every 6 months for the 3 years after transplantation. The data from these tests will be reported separately.

MRI brain scans were done at least twice before treatment, 7 days after administration of cyclophosphamide for stem-cell mobilisation and at 1 month, 2 months, 4 months, 6 months, 9 months, 12 months, 15 months, and 18 months, then every 6 months until 36 months, and then every 6–12 months after stem-cell transplantation. The scans were done with a previously described standardised acquisition protocol,[14] including: T2-weighted, proton density-weighted, T1-weighted

measurements (before and after Gd infusion), and FLAIR sequences. Lesion-based measurements included: new or enlarging T2-weighted lesion count and new T1-weighted lesion count at all scans after baseline; T2-weighted lesion volume; Gd-enhanced lesion count and volume; and total volume of non-enhancing T1-weighted lesions on all MRI scans. We estimated longitudinal percentage change in brain volume between baseline and each MRI visit with SIENA software from the Functional Magnetic Resonance Imaging of the Brain software library.[15]

Outcomes

The primary study outcome was multiple sclerosis activity-free survival at 3 years after stem-cell transplantation. The events for the primary outcome were: clinical relapse, appearance of a new or Gd-enhancing lesion on MRI, or sustained progression of EDSS score. Secondary outcomes were time to treatment failure (relapse or progression), overall survival, transplantation-related mortality, transplantation-related morbidity, immunological reconstitution, haemopoietic reconstitution, MRI-related changes in disease activity (new and Gd-enhancing lesions as well as atrophy).

Post-hoc analysis included time to improvement in EDSS, relations between baseline characteristics and changes in disease activity after stem-cell transplantation, and social wellbeing after stem-cell transplantation.

Statistical analysis

We present baseline characteristics as means (SDs) for data with a normal distribution and as medians (IQRs) for data with a non-normal distribution. We report percentages for categorical data. We calculated incidence with person-time measured in years, with 95% CI, for multiple sclerosis activity-free survival and Gd and T2 lesions. We calculated proportions of patients reaching these endpoints with 95% CIs using Fisher's exact test. We used Kaplan-Meier statistics to assess time-to-event outcomes. Brain atrophy is presented as yearly rates per patient. We calculated differences in EDSS scores from pre-treatment baseline to 1·5 years, 3 years, and 6 years after treatment. EDSS scores were determined as the average of treating physician and independent reviewer assessment. We assessed inter-rater reliability for EDSS scores by calculating intra-class correlations and their 95% CIs. Toxic effects and safety endpoints are presented as percentages.

This study was registered at ClinicalTrials.gov, NCT01099930.

Role of the funding source

The funder had no role in study design, data collection, analysis, or interpretation, writing of the report, or the decision to submit for publication. HA and MF had access to all the data and were responsible for the decision to submit the report.

Results

We screened 39 patients, of whom 26 met the eligibility criteria (figure 1B). One patient, who declined stem-cell transplantation and received mitoxantrone followed by interferon beta-1b had disease progression 103 months after finishing mitoxantrone. During a study hold following serious adverse events, one patient had substantial deterioration of disability and was withdrawn from the study before transplantation. Four additional patients had disease activity during study holds but continued on the study and underwent stem-cell transplantation.

The 24 remaining patients had stem-cell transplantation, between October, 2001, and December, 2009. The table shows baseline characteristics. Compared with the last 12 patients to be enrolled, the first 12 patients enrolled had a higher EDSS (median score 5·8 *vs* 4·3), were further from diagnosis (median time 6·2 *vs* 4·8 years), but were still having relapses although more likely to be categorised as secondary progressive multiple sclerosis (11 of 12 *vs* one of 12). The appendix shows treatment and follow-up intervals (p 2).

16 (67%) of 24 patients had febrile events during mobilisation. Three (13%) patients had urinary tract infections. Graft products were collected by peripheral vein leucopheresis after one procedure for 22 patients (92%) and after two procedures for two patients (8%). CD34 selection of the graft successfully depleted the product of residual immune cells before cryopreservation (appendix p 10).

Two patients had a grade 3 or 4 regimen-related toxic effect resulting in safety holds, during which enrolment and stem-cell transplantation were postponed while the protocol was reviewed by the data and safety monitoring board. One patient, who received 14·9 mg/kg of busulfan, died from massive hepatic necrosis following sinusoid obstruction syndrome and *Klebsiella* sepsis 62 days after transplantation. A second patient, given 12·7 mg/kg of busulfan, developed sinusoid obstruction syndrome requiring admission to intensive care before recovering fully. The dose and route of busulfan was changed during the study to reduce severe regimen-related toxic effects (appendix p 11).

Eight (33%) of 24 patients had a maximum grade 2 toxic effect and 14 (58%) patients had a maximum grade 1 transplantation-related toxic effect, similar to patients with lymphoma who underwent stem-cell transplantation at The Ottawa Hospital (appendix p 3). All patients had febrile neutropenia, for a median of 4 days (range 1–22), and 14 patients (58%) had 29 positive cultures during the admission for stem-cell transplantation. Overall survival plateaued at 95% beyond 62 days after stem-cell transplantation (appendix p 4).

Engraftment was prompt, with an absolute neutrophil count of at least $0·5\times10^9$ per L reached by a median of 10 days (range 9–17) and a transfusion-independent platelet count of more than 20×10^9 per L reached by a median of 11 days (range 6–45) after stem-cell

transplantation (appendix p 5). No patient needed back-up bone marrow graft infusion. Patients were admitted to hospital for a median of 29 days (range 21–170), which included 11 days of conditioning before stem-cell transplantation.

Late post-transplantation viral infections included six patients (26%) with shingles, two patients (9%) with plantar warts, and one patient (4%) with human herpesvirus-6 pneumonia. Secondary autoimmune events occurred in six patients (26%). Three patients (13%) became hypothyroid, at 1 year, 3 years, and 3 years, after stem-cell transplantation and one patient (4%) became hyperthyroid, 8 months after stem-cell transplantation. Another patient developed asymptomatic thyroid hypofunction (high thyroid-stimulating hormone and low normal T4 concentrations) 4 months after transplantation that lasted 7 years before spontaneously resolving. One patient (4%) developed post-infectious immune thrombocytopenia 4·5 years after transplantation and needed prednisone treatment for 4 months but has had a normal platelet count over the subsequent 8 years.

Pre-existing immune responses were reduced or eliminated after stem-cell transplantation. Skin tests became negative 6 months after transplantation for all seven patients who had positive skin reactions to an intradermal antigen (five patients for candida, two for tetanus, and one for BCG) before receiving chemotherapy. Serum antibodies to measles, mumps, or rubella were undetectable when examined 18 months after transplantation in ten (50%) of 20 patients who had paired serum samples available. Passive prophylactic immunoglobulin immunisation administered for 12 months after transplantation could account for seropositivity in the remaining patients. Additional data on immune reconstitution have been reported.[16]

The primary outcome, multiple sclerosis activity free survival at 3 years after transplantation was 69·6% (95% CI 46·6–84·2). This was driven by sustained progression of disability in seven patients.

The treatment abolished all clinical and radiological hallmarks of disease-related CNS inflammation, without the use of disease-modifying drugs, during the entire extended follow-up period. From diagnosis to transplantation, the 24 patients had 167 relapses (mean 1·2 relapses per year, 95% CI 1·0–1·5) over 146 patient-years of follow-up (median 5·8 years, range 1·3–11·2). Clinical relapses did not occur in any of the 23 surviving patients after stem-cell transplantation with 179 patient-years of follow-up (0·0% patients, 95% CI 0·0–11·8%; figure 2A). These clinical outcomes were mirrored by freedom from detectable new disease activity on 327 MRI images after haemopoietic stem-cell transplantation (figure 2B). There were 93 Gd-enhancing lesions on 24 initial baseline MRI scans (mean 3·9 lesions per scan, 95% CI 1·3–6·5) and 95 Gd-enhancing lesions on 24 second pretreatment scans (mean 4·0 lesions per scan, 95% CI 1·2–6·7). There were 94 T2 lesions on the second pretreatment MRI scan not seen on the initial pretreatment MRI scan (mean 3·9 lesions per scan, 95% CI 1·3–6·5). Overall, 26 of 53 pretreatment scans (49·1%, 95% CI 35·1–63·2) had Gd-enhancing lesions and 19 of 29 pretreatment

	Data (n=24)
Age at transplantation (years)	34 (24–45)
Women	14 (58%)
Ever smoked	10 (42%)
Family history of multiple sclerosis	7 (29%)
Relapsing-remitting multiple sclerosis	12 (50%)
Secondary progressive multiple sclerosis	12 (50%)
HLA-DRB*1501	
Heterozygous	9 (38%)
Homozygous	4 (17%)
Time from first symptoms	
To EDSS 3·0	
Mean (SD)	4·1 (3·2)
Median (range)	3·0 (0·6–14·7)
To HSCT	
Mean (SD)	6·5 (3·8)
Median (range)	7·5 (1·7–21·2)
Time from diagnosis	
To EDSS 3·0	
Mean (SD)	2·7 (2·3)
Median (range)	2·2 (0·2–7·8)
To HSCT	
Mean (SD)	6·1 (2·5)
Median (range)	5·8 (1·3–11·2)
Previous disease modifying treatments	
One	10 (42%)
Two	7 (29%)
Three or more	7 (29%)
Subcuntaneous interferon beta-1a	20 (83%)
Intramuscular interferon beta-1a	1 (4%)
Subcutaneous interferon beta-1b	6 (25%)
Subcutaneous glatiramer acetate	6 (25%)
Intravenous mitoxantrone	7 (29%)
Intravenous cyclophosphamide	1 (4%)
Intravenous immunoglobulin	1 (4%)
Other	4 (17%)
EDSS at enrolment	
3·0	2 (8%)
3·5	1 (4%)
4·0	6 (25%)
4·5	2 (8%)
5·0	2 (8%)
5·5	2 (8%)
6·0	9 (38%)

Data are median (range) or n (%) unless stated otherwise. EDSS=Expanded Disability Status Scale. HSCT=haemopoietic stem-cell transplantation.

Table: **Patient characteristics**

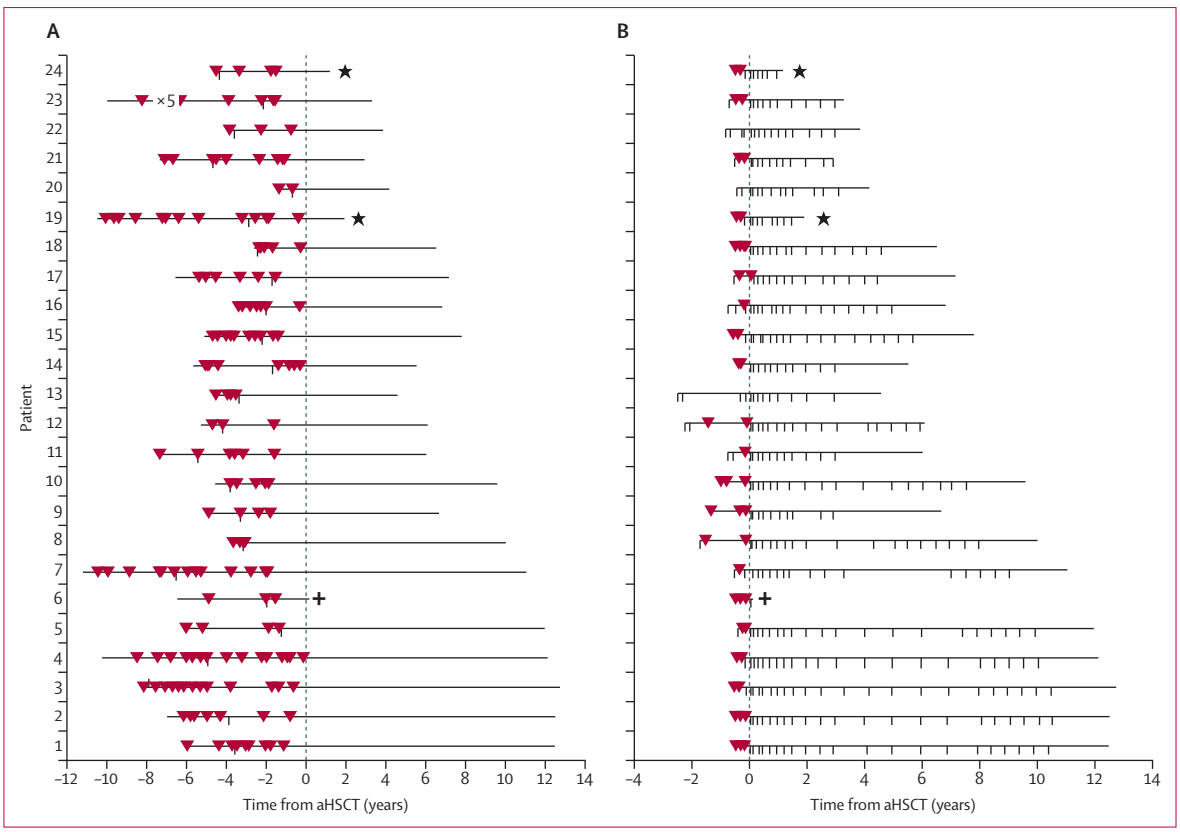

Figure 2: **Timeline of clinical relapses (A) and active MRI scans (B)**
The lines continue until the latest follow-up assessment, death (cross), or censoring because the patient received a conventional, experimental, or unproven treatment (star). For A, the lines begin at diagnosis of multiple sclerosis, inverted triangles denote a clinical relapse, tick marks are the time when a sustained EDSS of 3·0 was reached. For B, the line begins with the first study scan, inverted triangles denote an active scan with Gd-enhancing lesions or new T2 lesions, tick marks indicate a scan without new activity. Both censored patients had EDSS progression in the absence of relapse or new MRI lesions before being removed from the study. aHSCT=autologous haemopoietic stem-cell transplantation

scans (65·5%, 95% CI 45·7–82·1) had new T2 lesions. After haemopoietic stem-cell transplantation, none of 327 scans showed Gd-enhancing lesions (0% of patients, 95% CI 0–14·8) and one scan, taken 1 month after haemopoietic stem-cell transplantation had four new T2 lesions (mean 0·013 lesions per scan, 95% CI 0·005–0·032) that had not been seen on the MRI scan taken 5 months previously.

All patients had progressive loss of functional abilities before transplantation manifesting as increasing EDSS (figure 3A). After haemopoietic stem-cell transplantation, 17 patients (70%) had no further EDSS progression (figure 3B) with a median follow-up of 6·7 years (3·9–12·7; appendix p 2). EDSS progression was similar in both halves of the cohort (27% of first 11 patients *vs* 33% of last 12 patients). In a post-hoc analysis, baseline characteristics did not predict EDSS progression (appendix p 6) but EDSS stabilised or improved in ten (91%) of 11 patients with a baseline Multiple Sclerosis Severity Score[17] of 8·3 or less, whereas six (50%) of 12 patients with a score of more than 8·3 progressed (p=0·0686; figure 3C). The mean yearly rate of brain atrophy increased in the first 6 months after transplantation but subsequently slowed and stabilised at –0·32 (SD –0·67) beyond 24 months after transplantation (figure 4).

The cumulative incidence of improvement in EDSS was 40% after 7·5 years when the cumulative incidence curve plateaued after haemopoietic stem-cell transplantation (figure 5A). Measurable improvement was first recorded as much as 3 years after transplantation. The size of the improvement varied and ranged from a sustained improvement of 0·5 to 3·0 points (figure 5B–D). Durable improvements occurred for all functional neurological domains (appendix p 7). Improvements in at least one functional domain occurred in four (50%) of the eight patients who continued to have disease progression. Unexpected recoveries included: resolution of longstanding primary nystagmus, loss of ataxia, and substantial recovery in strength. Improvements in functional capacity were reflected by positive social wellbeing with six (37%) of 16 patients able to come fully or partly off disability insurance returning to work or school, five patients (31%) married or becoming engaged, and two had children using previously banked or donated gametes (appendix p 12).

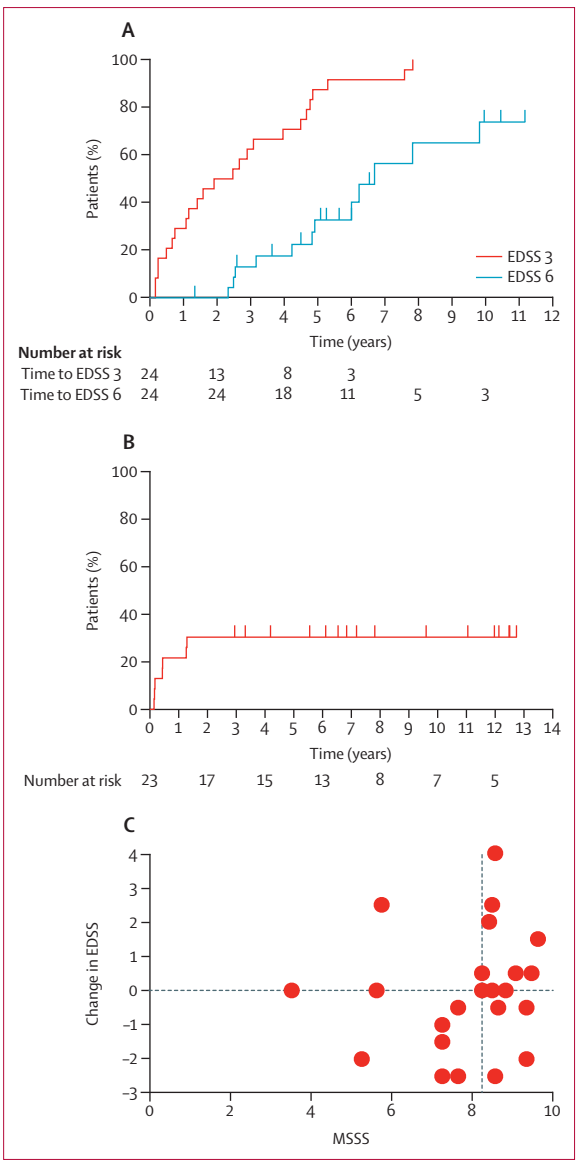

***Figure 3*: Progression of disabilities before and after autologous haemopoietic stem-cell transplantation**

(A) Time from diagnosis to a sustained EDSS of 3·0 and EDSS of 6·0. Patients reached EDSS 3·0 after a median of 2·2 years, and EDSS 6·0 after a median of 6·7 years from diagnosis. (B) Time from autologous haemopoietic stem-cell transplantation to sustained accumulation of additional disability. (C) Relation between the MSSS and change in the EDSS between the latest follow-up assessment and the final assessment before autologous haemopoietic stem-cell transplantation, showing that disability is more likely to improve for patients with an MSSS less than or equal to 8·3 just before autologous haemopoietic stem-cell transplantation; positive predictive value 90·9% (95% CI 58·7–99·8). EDSS=Expanded Disability Status Scale. MSSS=Multiple Sclerosis Severity Score.

Discussion

We describe the first treatment for multiple sclerosis to fully halt all detectable CNS inflammatory activity for a long period in the absence of disease-modifying drugs. In addition, whole brain atrophy slowed to a rate associated with normal aging.[18,19]

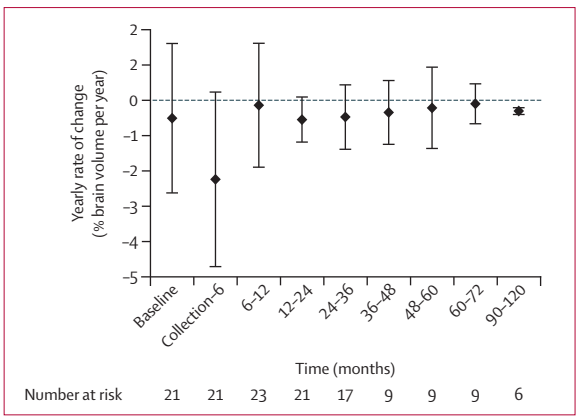

***Figure 4*: Yearly rate of change in brain volume before and after autologous haemopoietic stem-cell transplantation**

Shows the mean and standard deviation at each timepoint. The mean yearly rate of change has been reported to be –0·27% for healthy volunteers,[19] –0·49% for patients with relapsing-remitting multiple sclerosis, and –0·64% for patients secondary progressive multiple sclerosis.[18]

Without a randomly assigned control group, this study relies on a sufficiently long follow-up period to detect renewed disease activity. Other studies, reporting on similar patients with aggressive multiple sclerosis, showed renewed activity within the first 5 years of follow-up. MRI activity, with new enhancing lesions, occurred in 9–25% of patients within the first year after starting mitoxantrone induction, with most having relapses and 12–40% of patients having sustained worsening of EDSS score within 3–5 years.[20,21]

Our procedure differs from other reported treatments for multiple sclerosis based on autologous haemopoietic stem-cell transplantation,[3] using both a high-dose regimen for greater immunoablation and ex vivo immunomagnetic selection to eliminate autoreactive immune cells from the graft because contaminating lymphocytes can adoptively transfer autoimmunity.[22] Mobilisation with cyclophosphamide partly depletes immune cells,[23] but ex vivo depletion can prevent graft-mediated immune effects.[24] Graft selection has not been incorporated into most autologous haemopoietic stem-cell transplantation regimens for multiple sclerosis because of cost, regulatory complexities, and potentially delayed immune reconstitution. The degree of immune depletion affects ongoing disease activity. Relapses occurred within 2 years after alemtuzumab salvage therapy in 35% of patients who failed another disease-modifying drug.[4] Relapses occurred in 20% of patients with relapsing-remitting multiple sclerosis who received a more intense regimen of high-dose cyclophosphamide with alemtuzumab or anti-thymocyte globulin followed by autologous haemopoietic stem-cell transplantation.[25] Further intensification with BEAM and anti-thymocyte globulin conditioning did not suppress relapses in 14% of patients within 3 years[26] and 15% of patients within 5 years[6] of stem-cell transplantation in two studies. Although BEAM and anti-thymocyte

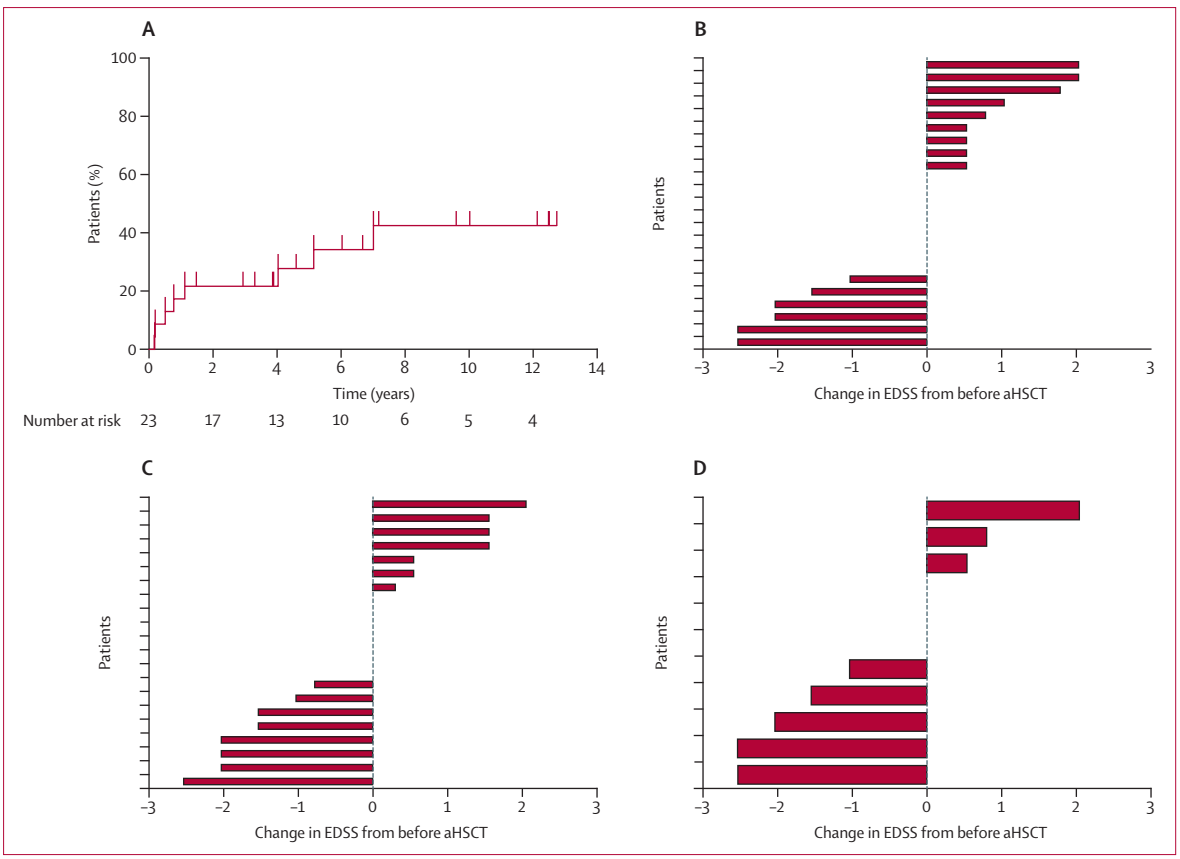

Figure 5: **Sustained Improvement of EDSS after aHSCT**
(A) Time to a sustained improvement of 0·5 in the EDSS score after aHSCT, if the last EDSS before aHSCT was 5·5 or more, or a sustained improvement of 1·0 if the last EDSS before aHSCT was less than 5·5. (B) Difference between the EDSS score 1·5 years after aHSCT and the last EDSS before aHSCT. (C) Difference between the EDSS score 3·0 years after aHSCT and the last EDSS before aHSCT. (D) Difference between the EDSS score 6·0 years after aHSCT and the last EDSS before aHSCT. aHSCT=autologous haemopoietic stem-cell transplantation. EDSS=Expanded Disability Status Scale.

globulin conditioning reduced the number of new T2 lesions on MRI compared with patients assigned to receive mitoxantrone, the number of new T2 lesions increased with the length of follow-up in transplantation recipients.[27] The outcomes after autologous haemopoietic stem-cell transplantation vary but no other strategy can completely halt all detectable clinical and MRI inflammatory events in the entire cohort across more than 5 years of follow-up.

Progression ceased in 70% of patients in our study, signifying an important change in the expected course of their disease—continued progression was expected in most patients.[28] Progression-free survival after autologous haemopoietic stem-cell transplantation varies, with continued accumulation of disabilities reported in 29–70% of patients in cohorts followed up for at least 5 years.[5,6,29–32] Younger patients who undergo transplantation earlier after diagnosis have better outcomes than older patients.[33] Despite the long follow-up, patients with continued progression manifest within 2 years of stem-cell transplantation. Similarly, Mancardi and colleagues noted that disease progression generally started within 5 years of transplantation and those with active baseline MRI scans had a better progression-free survival.[6] Our patients were selected because of ongoing relapses and most had MRI evidence of active inflammation just before transplantation. Those who progressed lacked detectable clinical and MRI evidence of inflammatory activity, suggesting dissociation of the process responsible for advancing disability from ongoing focal inflammatory lesions. Optimally stopping further progression requires targeting patients when they still have active CNS inflammation.

Many of our patients recovered substantially, indicating that repair mechanisms were still active and might have been suppressed by ongoing inflammation. Sustained improvement in EDSS score has been noted in up to 50% of patients after autologous haemopoietic stem-cell transplantation.[6,25,26] It is not possible to predict accurately repair potential and although advanced disability is often considered a predictor of poor outcome,[34] some patients with very severe disability have benefited.[35] An MSSS score of less than 8·3 had good positive predictive value for recovery of disability in our study, but it remains to be seen if this is valid in a larger cohort.

Although many treatments for multiple sclerosis reduce the rate of brain atrophy relative to placebo, the rate rarely approaches that of normal aging especially for patients, such as those in our cohort, with aggressive multiple sclerosis. There was progressive slowing of atrophy in 14 patients treated with autologous haemopoietic stem-cell transplantation followed by MRI,[36] but even at 3 years, the rate of loss of brain volume was still within the range reported in untreated patients[18] and greater than expected for normal aging.[19] Our study showed continued reduction in the rate of brain atrophy with increasing follow-up, ultimately reaching that of normal aging.

Achieving the best outcomes for autologous haemopoietic stem-cell transplantation requires experience for patient selection and specialised care to minimise procedural risks, and is best done in accredited centres with specialised units for multiple sclerosis and haemopoietic stem-cell transplantation and reported to an international haemopoietic stem-cell transplantation registry. The overall survival of 95% in our study is similar to the 93% among 345 patients reported to the European Bone Marrow Transplant Registry[37] and the 97% among 143 patients reported to the Consortium for International Blood and Marrow Transplantation Research.[38]

Caution is necessary before widespread adoption of aggressive immunoablation followed by CD34-selected autologous haemopoietic stem-cell transplantation, because our cohort was small and lacked a control group. The strength of these data lies in the careful selection of patients with aggressive inflammatory disease who were deemed to have a very poor prognosis and long prospectively planned follow-up with methodically collected outcome data from regularly scheduled clinical and MRI assessments. For a substantial number of patients whose disease was not well-controlled with disease-modifying drugs, this procedure led to neurological improvement and long-lasting remission free of ongoing treatment.

Contributors

HA, BH, and MF did the literature search. HA, MB, DLA, ABO, and MF designed the study. HA, MB, DA, DLA, ABO, IB-B, PB, CB, JC, MH, LHa, LHu, HL, PL, YL, LM, SM, PO, MS, LW, and MF collected data. HA, MB, DLA, ABO, JC, DF, BH, and HL analysed the data. HA, MB, DLA, ABO, JC, DF, BH, HL, and TR interpreted the data. HA and HL designed the figures. HA, MB, and MF wrote the first draft of the Article. HA, MB, DA, DLA, ABO, IB-B, PB, CB, JC, DF, MH, LHa, LHu, HL, PL, YL, LM, SM, PO, TR, MS, LW, and MF critically revised the Article. HA and MF had the idea for the study, supervised the study, had full access to all the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final Article.

Declaration of interests

HA has received grants from the Multiple Sclerosis Scientific Research Foundation and non-financial support from Amgen Canada. DLA has received grants from the Multiple Sclerosis Scientific Research Foundation of Canada, personal fees from Biogen, EMD Serono, Genentech, Genzyme, Hoffman LaRoche, Innate Immunotherapy, MedImmune, Mitsubishi, Novartis, Receptos, Acorda, Sanofi-Aventis, Teva, and Alkermes, and grants from Novartis and Biogen. ABO has received personal fees from Biogen Idec, Genentech, GlaxoSmithKline, Guthy-Jackson/GGF, Merck/EMD Serono, Medimmune, Mitsubishi Pharma, Novartis, Ono Pharma, Receptos, Roche, Sanofi-Genzyme, and Teva Neuroscience. BH has received personal fees from Amgen Canada, Eisai Canada, and Cornerstone Research Group. PL has received grants from the Multiple Sclerosis Scientific Research Foundation. LW has received grants from the Multiple Sclerosis Scientific Research Foundation and consulting fees paid to her institution from Novartis Pharmaceuticals and EMD Serono. MF has received personal fees from Genzyme, Merck Serono, Bayer, Biogen Idec, Teva Canada Innovation, Opexa, Novartis, and Chugai. The other authors declare no competing interests.

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