Intensive immunosuppression followed by autologous hematopoietic stem cell transplantation for the treatment of multiple sclerosis

Jan Lycke and Stig Lenhoff

Abstract: Autologous hematopoietic stem cell transplantation (AHSCT) to treat multiple sclerosis (MS) has mostly been used in devastating cases as the last option to stop further neurological deterioration. However, evidence from several retrospective clinical trials indicates that young, less disabled patients with highly inflammatory active MS are the most likely to benefit from AHSCT, and after moving from high-intensity to nonmyeloablative procedures the tolerability of AHSCT has increased and its associated risk and mortality have declined considerably. Recent meta-analyses and randomized clinical trials show that AHSCT is more effective than currently approved disease-modifying therapies (DMTs), with suppression of disease activity in 70–90% of patients and long-term cessation of disease activity in two-thirds of treated patients. The rationale for AHSCT is to eliminate autoimmunity and achieve immune resetting by intense immunosuppression followed by infusion of autologous hematopoietic stem cells. Similar effects on the immune system have been suggested for cladribine and alemtuzumab treatment and, together with AHSCT, they constitute the induction or immune-reconstitution therapies for MS. Although, further randomized controlled trials of AHSCT for MS are needed, it has become clear that improved patient selection and lower intensity conditioning regimens have reduced AHSCT associated risks and mortality and strengthened the position of AHSCT among other DMTs. Do we have enough experience and scientific support for AHSCT in MS to move from an exclusive treatment for aggressive, treatment-resistant MS and acquire broader indications, similar to other effective DMTs?

Keywords: autologous hematopoietic stem cell transplantation, disease-modifying therapies, multiple sclerosis, treatment, trials

Received: 13 December 2019; revised manuscript accepted: 22 March 2020.

Introduction

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating, and degenerative disease of the central nervous system (CNS) and is considered the most common nontraumatic cause of neurological disability in young adults. Although the clinical course of MS is heterogeneous, it is initially relapsing-remitting (RRMS) in 85–90% of patients. Relapses, periods during which the patient has neurological symptoms, alternate with periods of remission. After 20–30 years of RRMS, the disease often acquires a secondary progressive course (SPMS), characterized by progressive disability independent of relapses. In a minority of patients, the course is progressive from the clinical onset and is therefore designated primary progressive MS (PPMS). Inflammatory activity is the dominating feature in RRMS, causing relapses and new lesions on magnetic resonance imaging (MRI), but degeneration with neuro-axonal loss...
and CNS atrophy development is also present from the early stages of MS. It determines the rate and severity of disability during progressive MS (PMS). However, in recent years it has become evident that inflammation also contributes to the pathogenesis of PMS. All existing approved disease-modifying therapies (DMTs) for MS modulate or suppress the immune system and thereby influence the clinical course, disease activity, and the rate of disability development. They are approved for relapsing MS, but recently the B-cell depleting monoclonal antibody ocrelizumab was approved for PPMS, and siponimod, a selective sphingosine-1-phosphate receptor modulator, showed beneficial effects in SPMS, providing evidence that inflammation also influences progression during PMS.

Immunoablation following autologous hematopoietic stem cell transplantation (AHSCT) has been used for over two decades to treat aggressive autoimmune diseases and, among them, MS has become the dominating neurological autoimmune disease, constituting almost 93% of the patients treated with AHSCT, according to the European group for Blood and Marrow Transplantation (EBMT). The first pilot study on MS treated with AHSCT was published in 1997. Since then, the treatment’s beneficial effect, tolerability, and safety have been reported from a growing number of retrospective observational studies, mostly of small size, in both essentially progressive or aggressive relapsing forms of MS. In addition, two randomized controlled prospective trials, two meta-analyses, long-term single-center studies, and a large systematic, multicenter, long-term follow-up study have compared the efficacy and long-term outcome of AHSCT with established DMTs for MS.

**AHSCT in progressive MS**

The early experiences of AHSCT treatment in MS were with disabling PMS. These early attempts were summarized in an EBMT report including 183 patients treated with AHSCT from 1995 to 2006, of which 72% were progressive, 10% relapsing-progressive, 12% RRMS, and 6% of unknown course. The expanded disability status scale (EDSS) decreased or was stable in 63% of patients and worsened in 37% of patients at a median follow up of 41.7 months. The most common side effects were neutropenic fever and infections. Mortality was 4.7%, mostly associated with the highly intense conditioning regimen. A similar, relatively low rate of progression was observed in a single-center study of 99 patients, including 54% of patients with PMS and 46% of patients with relapsing MS, treated with intermediate-intensity AHSCT. Progression after 8 years of follow up was insignificantly higher in patients having progressive (21%) compared with relapsing MS (13%). However, more recent long-term evaluations following AHSCT of PMS showed that disability is seldom halted by AHSCT. In a study of 31 patients, with a mean follow up of 8.4 years, none of the patients with RRMS (n = 22) worsened according to the EDSS, whereas 7 of 9 patients with SPMS increased their EDSS score. A multicenter observational retrospective study included 281 patients, of whom 78% had PMS, and were treated with AHSCT between 1995 and 2006, with a median follow-up time of 6.6 years. The progression-free survival at 5 years after AHSCT was 33% for SPMS compared with 73% in patients with RRMS, and treatment-related mortality (TRM) was 2.8%.

**AHSCT in RRMS**

Since 2005, a shift has occurred in the selection of patients for AHSCT from patients with progressive to those with inflammatory active RRMS, and the AHSCT regimens have moved from high- to intermediate- and low-intensity protocols to improve safety. An increasing number of prospective studies have been published, including two randomized treatment trials, the Autologous Hematopoietic Stem Cell Transplantation Trial (ASTIMS) and the Multiple Sclerosis International Stem Cell Transplant (MIST) trial. Thus, AHSCT efficacy and safety data for RRMS are more reliable than those reported for PMS. Currently, there are seven published studies, one including a 3-year interim report, each consisting of more than 10 patients with ≥50% patients with RRMS, and with a median follow up after AHSCT of 2 years or more (Table 1). The 358 patients included constituted a heterogeneous group (80% with RRMS) treated with a variety of AHSCT regimens. Nevertheless, their reported outcome measures showed similar high efficacy after AHSCT, with relapse-free survival of 68–100%, progression-free survival of 70–91%, and no evidence of disease activity (NEDA; no relapses, no confirmed disability progression, no gadolinium-enhancing lesions and no new or enlarging T2-hyperintense lesions on cranial MRI) of 55–78%, with the lowest numbers seen in cohorts
containing more PMS. Further analyses of outcomes revealed significant improvement of EDSS scores in a substantial percentage of transplanted patients with RRMS.19,22,23,28,31–36 Thus, AHSCT may not only reduce disease activity and stabilize disability, but even improve neurological function. With one exception, the AHSCT regimens used were of intermediate intensity, and the only death was reported in a patient treated with high-intensity AHSCT.34

Comparison of AHSCT with other DMTs for MS

Two randomized controlled trials (RCTs) have been performed comparing AHSCT with other approved treatments for MS.22,23 The ASTIMS trial was an open-label, 4-year, multicenter, phase II study, comparing an intermediate-intensity regimen of AHSCT with mitoxantrone (MTX) in MS.23 Overall, 21 patients were included: 9 were randomized to AHSCT and 12 to MTX, the latter receiving MTX 20 mg intravenously once every month for 6 months. The primary endpoint was the cumulative number of new T2 lesions in the 4 years following randomization. AHSCT significantly reduced the number of new T2 lesions over 4 years, compared with MTX. The median number of new T2 lesions was 2.5 (range 0–8) compared with 8 (range 2–34). None of the AHSCT-treated patients had gadolinium (Gd)-enhancing lesions compared with 56% of MTX-treated patients. The clinical outcomes showed a significantly lower annual relapse rate in the AHSCT arm (0.19) compared with the MTX arm (0.6), but no difference in progression, which occurred in 57% of the AHSCT-treated patients and in 48% of the MTX-treated patients. The MIST trial was an open-label multicenter study with a median follow up of 2 years. It included 110 patients with RRMS who were randomized to nonmyeloablative AHSCT (n = 55) or to an approved DMT (n = 55).22 The DMT treatments consisted of at least seven different treatments of various efficacies. The primary endpoint was disability progression (EDSS score increased ≥1.0), which occurred in 3 AHSCT-treated patients compared with 34 patients in the DMT arm. The proportions of patients who progressed at 1, 3, and 5 years of follow up were 1.9%, 5.2%, and 9.7%, respectively, for the AHSCT group, and 24.5%, 62.5%, and 75.3%, respectively, for the DMT group. The EDSS score improved in the AHSCT group whereas it worsened in the DMT group. The proportions of patients reaching NEDA at 1, 3, and 5 years after randomization were 93.3%, 90.3%, and 78.5%, respectively, in the AHSCT group and 20.8%, 5.9%, and 3%, respectively, in the DMT group. Thus, although these studies have several limitations, they both showed that AHSCT has profound effects in RRMS, halting disease activity and preventing worse disability. Note that no randomized phase II, head-to-head comparison with a single DMT has been performed and that the DMTs in the MIST trial did not include more PMS.

### Table 1. Outcomes in patients with MS [study populations with ≥50% RRMS] treated with AHSCT.

| Authors         | Study | RRMS/PMS/TOTAL | Relapse-free survival (%) | Median follow-up time (years) | Progression-free survival (%) | NEDA (%) | Regimen intensity | TRM |
|-----------------|-------|----------------|---------------------------|-----------------------------|-----------------------------|----------|------------------|------|
| Burman et al.   | RO    | 34/7/41        | 90                        | 4                           | 77                          | 68       | Intermediate     | 0    |
| Burt et al.     | RO    | 118/27/145     | 80                        | 2                           | 87                          | 68       | Intermediate     | 0    |
| Nash et al.     | PO    | 24/0/24        | 87                        | 5                           | 83                          | 69       | Intermediate     | 0    |
| Atkins et al.   | PO    | 12/11/23       | 100                       | 6.7                         | 70                          | 70*      | High             | 4    |
| Burt et al.     | RCT   | 53/0/53        | 85                        | 2                           | 90                          | 78       | Intermediate     | 0    |
| Moore et al.    | PO    | 19/15/34       | 90                        | 3                           | 77                          | 60       | Intermediate     | 0    |
| Casanova et al. | RO    | 28/10/38       | 68                        | 8.4                         | 77                          | 55       | Intermediate     | 0    |

*NEDA not including T2 lesions on magnetic resonance imaging.
AHSCT, autologous hematopoietic stem cell transplantation; MS, multiple sclerosis; NEDA, no evidence of disease activity; PMS, progressive multiple sclerosis (including primary, secondary, and progressive-relapsing MS); PO, prospective observational; RCT, randomized controlled trial; RO, retrospective observational; RRMS, relapsing-remitting multiple sclerosis; TRM, treatment-related mortality.
not include highly effective therapies such as alemtuzumab or ocrelizumab. Thus, in the absence of direct comparisons of the efficacy of AHSCT and that of approved DMTs, two review papers have reported post hoc analyses of clinical trials to assess NEDA in RRMS after 2 years of therapy.38,39 A cross-sectional analysis revealed that the proportion of patients who achieved NEDA after 2 years of treatment with placebo was 7–16%, with interferon beta 1a 13–27%, with other DMTs including those considered highly effective 22–48%, and with AHSCT 70–92%.39 A similar analysis of NEDA status at 5 years found that it was achieved by 60–68% of transplanted patients.38

Long-term outcomes following AHSCT
In many AHSCT trials of MS, the outcome assessment is limited to a relatively short follow up of usually 2–4 years.19,20,22,31,37 However, longer-term outcomes of AHSCT have been reported from some small, mostly retrospective, single-center11,13,26,30,34,40 or two-center30 cohort studies, but from only two relatively large retrospective cohort studies, one single-center27 and one multicenter cohort study.28 A total of 8 studies, including 552 patients with a follow up of more than 4 years, were identified (Table 2). Although a high degree of heterogeneity between these long-term studies is apparent, some conclusions can be drawn. Most had PMS (74%) and moderate-to-severe disability at transplantation. Their progression-free survival varied from 25% to 83%, with worse outcome in studies including more PMS and patients with higher baseline EDSS. The TRM was higher compared with those reported for AHSCT in RRMS. In the multicenter study of 281 patients with mostly progressive course (78%), the median EDSS score was 6.5 (range 1.5–9.0), the 5-year probability of EDSS progression-free survival was 46% [95% confidence interval (CI) 42–54%), and overall survival was 93% (CI 89–96%).28 The factors from the multivariate statistics of this study showed increased risk of progression with age, progressive course, and prior treatment with more than two DMTs. Patients with higher EDSS at baseline had worse overall survival.

The effect from AHSCT on lesion formation and neurodegeneration
AHSCT has a marked effect on lesion formation on MRI and prevented the appearance of new T2 and Gd-enhancing lesions in approximately 85% of patients at 5 years of follow up.19,31,36 There is also evidence that T2 lesion volume may decrease following AHSCT, when comparing the baseline with the last follow-up MRI scans.20,22,31,36,37 These measures of reduced inflammatory activity may also have influenced degeneration, since brain atrophy seemed to stabilize31,34,36 or was reduced post-AHSCT.37 However, these conclusions relied on comparisons with brain-volume measurements from healthy persons and no comparison of brain atrophy rate was performed between treatment cohorts in the RCT studies ASTIMS and MIST.22,34

There are several factors that might influence determination of brain volumes in MS. In AHSCT, the possible neurotoxic effect was investigated in a prospective study of 19 patients, 12 with RRMS and 7 with SPMS, followed for 1.5–10.5 years with serial MRI.41 They were treated with a high-intensity regimen using busulfan for conditioning. The brain atrophy rate was accelerated during the first year following AHSCT, indicating a neurotoxic effect of busulfan. The atrophy rate of both gray and white matter of the brain seemed thereafter to normalize.

Selection of patients for AHSCT
Accumulating evidence from previous AHSCT studies serves to refine the profile of the best candidate for this treatment. It has become obvious from long-term retrospective observational studies that the effect of AHSCT in PMS is often transient, and that an increasing number of patients continue to deteriorate with time.28,30 In contrast, AHSCT causes a pronounced reduction of inflammatory activity in RRMS, and has a marked and sustainable impact on disability development during this stage of the disease.39 Outside clinical trials, current consensus reports and reviews suggest the use of AHSCT in patients with RRMS who are young (<40–45 years old), less disabled and able to ambulate independently, have disease duration of <10 years, and present clinical and/or MRI evidence of concurrent disease activity despite the use of DMTs.9,17,39,42 However, recently ocrelizumab was approved for PPMS7 and siponimod for SPMS.8 In both studies, patients of younger age who were less disabled and had more inflammatory active disease were those that benefited most from these treatments. Thus, some patients with progressive disease can be considered for AHSCT if there is
clear clinical and MRI evidence of significant disease activity, although the benefit in these patients is probably limited. Moreover, patients considered for AHSCT should not have severe comorbidity and their cognition should be preserved enough that they can understand the possible adverse effects and risks with AHSCT.

**Switch from high- to low-dose immunosuppressants in AHSCT for MS**

There are four key steps that encompass the AHSCT procedure: (a) mobilization of CD34+ hematopoietic stem cells (HSCs); (b) HSC collection and preservation; (c) immunoablative conditioning; (d) infusion of cryopreserved HSC 1–2 days postconditioning. Mobilization of HSC from the bone marrow is achieved by granulocyte colony-stimulating factor (G-CSF) together with cyclophosphamide (Cyc). This combined regimen is preferable to G-CSF alone since it improves the HSC yield, reinforces immunosuppression (by using Cyc sequentially at both the mobilizing and conditioning steps), lowers the risk of exacerbation of disease activity, and reduces the risk of carryover of auto-aggressive T cells with the transplant.43 After mobilization, HSCs are harvested from peripheral blood through leukapheresis and cryopreserved until re-infusion. A further CD34+ selection of the leukapheresis product seems not to improve the outcome of AHSCT.44,45 According to the EBMT guidelines, the conditioning regimens for transplantation for autoimmune diseases are classified and defined as high, intermediate, and low intensity9 (Table 3). However, the high-intensity protocols were not recommended in autoimmune diseases due to short- and long-term toxicity with unacceptable morbidity and high TRM, and have been replaced with less toxic regimens.44 In line with this evolution AHSCT in MS has moved from high- to intermediate-intensity and also, in recent years, low-intensity protocols. The most accepted protocol has previously been BEAM, i.e. carmustine (BiCNU), etoposide, cytarabine (cytosine arabinoside), and melphalan, an intermediate-intensity protocol inducing myeloablation. This protocol is often followed by anti-thymocyte globulin (ATG), which further depletes surviving T cells.39,46 However, some centers are now moving to high-dose Cyc and ATG and even to low-intensity protocols, mostly using only high-dose Cyc for conditioning.21 With this regimen the tolerability and safety are improved and immunoablation without myeloablation is achieved.21,33 Thus, the treatment target is limited to the dysregulated lymphocyte clones, which are considered

| Authors           | RRMS/PMS/TOTAL 1–5 years of follow up after AHSCT, n | Median follow-up time (years) | Median baseline EDSS | Progression-free survival, % (follow-up duration) | Regimen intensity | TRM |
|-------------------|-----------------------------------------------------|-------------------------------|----------------------|---------------------------------------------------|-------------------|-----|
| Chen et al.       | 3/22/25                                             | 8                             | 8.0                  | 48 (9 years)                                      | Intermediate      | 8*  |
| Fassas et al.     | 1/34/35                                             | 11                            | 6.0                  | 25 (15 years)                                     | Intermediate      | 6‡  |
| Shevchenko et al. | 43/56/99                                            | 4                             | 3.5                  | 83 (8 years)                                      | Intermediate      | 0   |
| Bowen et al.      | 1/24/25                                             | 4                             | 7.0                  | 48 (6 years)                                      | High              | 4   |
| Casanova et al.   | 28/10/38                                            | 8.4                           | 5.0                  | 77 (9 years)                                      | Intermediate      | 0   |
| Muraro et al.     | 46/235/281                                          | 6.6                           | 6.5                  | 46 (5 years)                                      | High              | 2.8 |
| Krasulová et al.  | 11/15/26                                            | 5.5                           | 6.0                  | 29 (6 years)                                      | Intermediate      | 0   |
| Atkins et al.     | 12/11/23                                            | 6.7                           | 5.0                  | 70 (6.7 years)                                     | High              | 4   |

*One from pneumonia and one from varicella-zoster hepatitis at 4.5 months and 15 months post-transplantation, respectively.

‡One from aspergillosis and one from factor VIII inhibitor at 2 months and 2.5 years post-transplantation, respectively.

AHSCT, autologous hematopoietic stem cell transplantation; EDSS, expanded disability status scale; MS, multiple sclerosis; PMS, progressive multiple sclerosis (including primary, secondary, and progressive-relapsing MS); RRMS, relapsing-remitting multiple sclerosis; TRM, treatment-related mortality.
Therapeutic Advances in Neurological Disorders 13

...responsible for the autoreactive CNS attack in MS. However, based on MRI activity measures from only seven patients, a low-intensity regimen was considered less efficacious than intermediate-intensity regimens.21 Similar differences in safety and efficacy of different intensity regimens of AHSCT have previously been reported from reviews consisting of heterogeneity of autoimmune diseases.47,48

A retrospective review found higher progression-free survival with intermediate-intensity compared with high-intensity regimens in SPMS.50 However, other attempts to find differences in efficacy between conditioning regimens, including retrospective surveys17 and a meta-analysis,25 have so far failed.27 Greater safety is achieved with lower intensity conditioning regimens, but no consensus yet exists regarding the optimal AHSCT protocol.

Adverse effects and safety with AHSCT in MS

AHSCT is associated with TRM which previously has been the main concern limiting the use of AHSCT in MS. The main reasons for AHSCT complications are the intensity of the immunosuppression, particularly in conditioning, and age, disability, clinical course, and comorbidities.25 However, TRM has decreased from 7.3% in 1995–2000 and 1.3% in 2001–2007, to 0.7% in 2008–2016, according to the EBMT registry.28 Similarly, a meta-analysis of 15 published studies showed that the TRM was 0.3% in patients treated with AHSCT after 2005, and no TRM was observed in patients receiving intermediate-intensity conditioning.25 Three identified factors can explain this improvement: (a) patient selection; (b) intensity of conditioning regimen; (c) center experience.47

The common side effects following AHSCT are due to the toxic effect of the conditioning and infections due to immunosuppression, an expected and desirable transient effect of the treatment. The conditioning causes pancytopenia and transient bone-marrow aplasia lasting for approximately 1–2 weeks where mainly bacterial infections, mucocitis, and transient alopecia occur. In the following period of at least 3–6 months, before re-setting and re-population of the immune system, the patient is still in an immunosuppressed state with increased risk of infections. In particular, reactivation of Epstein-Barr virus and cytomegalovirus infection,51 and sometimes reactivation of herpes varicella-zoster infection occur as a late complication. In addition, the patient often experiences a transient increase in neurological symptoms and deterioration during and following AHSCT.17 Fever, in particular, due to ATG and/or infection, may cause such exacerbation of neurological symptoms. These symptoms persist in only a minority of patients.52 However, one study reported poor long-term neurological recovery in patients with peri-transplant-sustained pyrexia.20 Transient amenorrhea is also commonly seen after AHSCT.

| Intensity | Conditioning regimen | Effect | Reference |
|----------|----------------------|--------|-----------|
| High     | Total body irradiation, Cyc and ATG Busulfan, Cyc, and ATG (BuCycATG) | Immunoablative | Bowen et al.11; Burt et al.12; Samijn et al.15; Fassas et al.26; Muraro et al.28; Atkins et al.24; Nash et al.25 |
| Intermediate | BEAM and ATG (BEAM-ATG) Cyc and ATG (Cyc-ATG) | Myeloablative and lymphoablative | Chen et al.13; Xu et al.14; Burman et al.19; Burt et al.20,22; Mancardi et al.23; Shevchenko et al.27, Casanova et al.30; Nash et al.31,36; Moore et al.37; Saiz et al.49 |
| Low      | Cyc alone Melphalan alone Fludarabine-based regimens alone | Lymphoablative | Curro et al.21 |

AHSCT, autologous hematopoietic stem cell transplantation; ATG, anti-thymocyte globulin; BEAM, carmustine (BiCNU), etoposide, cytarabine, and melphalan; Cyc, cyclophosphamide.
Cardiac impairment in MS is rare. Although cardiac toxicity during AHSCT is low, a baseline cardiac functional assessment before the transplant is recommended. Cyc, the most-used agent for mobilization and conditioning, displays dose-dependent cardiotoxicity but no risk for accumulated cardiac toxicity. However, special concern is only recommended in patients with known cardiovascular disorders and in patients already exposed to MTX, an immunosuppressive agent previously used in MS with accumulative risks for cardiotoxicity. AHSCT should not be performed in patients with a left ventricular ejection fraction of less than 40%.

Two long-term adverse effects of AHSCT that should be taken into consideration are the increased risk of secondary autoimmune diseases and infertility.

**Secondary autoimmune diseases**

Although alemtuzumab treatment of MS is associated with increased risk of secondary autoimmune diseases, this is also a concern after AHSCT. In alemtuzumab-treated patients with RRMS, with a median follow up of 7 years (range 33–144 months), 48% developed secondary autoimmunity, predominantly with thyroid disease. Patients with autoimmune disease treated with AHSCT also develop secondary autoimmune diseases but the risk is considerably lower. The different conditioning protocols of AHSCT are all lymphoablative and aim to eliminate autoreactive T-cell clones. The increased risk might be associated with ATG, which is usually included in the conditioning regime to induce rapid and profound lymphopenia and cause durable impairment of T-cell function for at least 12 months. In a retrospective multicenter study, the incidences of at least one secondary autoimmune disease after 3 years and 5 years of follow up were 7.7% and 9.8%, respectively. The most common secondary autoimmune diseases were those affecting the thyroid gland, immune thrombocytopenia, autoimmune hemolytic anemia, and acquired hemophilia. Risk factors were systemic lupus erythematosus as a primary autoimmune disease, conditioning with ATG and CD34+ graft selection, and younger age.

Only a few AHSCT studies in MS reported the risk of secondary autoimmunity. In line with alemtuzumab treatment, the highest risk after AHSCT was found for thyroid disease. In a study of SPMS treated with a high-intensity AHSCT regimen, 2 of 14 patients developed thyroid disease and 8.3% were diagnosed with thyroid disease in a study of intermediate-intensity conditioning of patients with RRMS. In the EBMT report of AHSCT-treated patients with MS from 1995, 3.4% had secondary autoimmune disease. The higher risk of secondary autoimmunity associated with alemtuzumab was also shown in a prospective nonrandomized study of 123 patients with RRMS and 28 with SPMS, treated with Cyc together with either alemtuzumab \((n=22)\) or ATG \((n=129)\) as conditioning regimens. Secondary autoimmune diseases including thyroid disease were reported in 22.7% of alemtuzumab-treated patients and in 6.9% of ATG-treated patients.

**Infertility**

Temporary ovarian and testicular failure is common after AHSCT and the risk of infertility and premature menopause is significantly increased after AHSCT. Counseling is therefore important, and patients should be offered fertility conservation procedures, such as cryopreservation of sperm, eggs, or embryos before treatment. However, age is an important influence on fertility and the risk of infertility and spontaneous abortion increase rapidly in women aged 35 years or older. In a retrospective study on 41 premenopausal women treated for lymphoma with AHSCT and BEAM conditioning, 59% aged 18–25 years, 29% aged 26–30 years, and 12% aged 31–35 years recovered menstruation. In a study based on responses from an online questionnaire of 28 women treated with AHSCT with low- or intermediate-intensity conditioning, menstruation was restored in 38% of women 33–41 years of age, and in all women younger than 32 years. It is important to note that MS increases the risk of infertility in both men and women, and that infertility in MS is influenced by multiple factors, among which the choice of conditioning has crucial impact.

**Immunoreconstitution mechanisms: comparing AHSCT with alemtuzumab and cladribine**

Intensive immunosuppression followed by AHSCT reconstitutes the immune system, which loses its previous harmful auto-aggressivity against CNS tissue and regains tolerance with long-term effects on disease course. The goal is similar with
two approved therapies for RRMS: alemtuzumab, a monoclonal antibody that targets CD52 and thereby depletes T lymphocytes and B lymphocytes,\textsuperscript{66,67} and cladribine (2-chlorodeoxyadenosine), which is activated through phosphorylation and accumulates preferentially in lymphocytes where it disrupts DNA, leading to cell death.\textsuperscript{68} They and AHSCT are considered immune-reconstitution therapies or induction therapies, since after a short-term course all aim to re-build the immune system in order to establish immune tolerance. The main differences in outcome between these therapies are summarized in Table 4. Results from the pivotal studies of alemtuzumab\textsuperscript{66,67} and cladribine\textsuperscript{68} were compared with 2 years of data obtained from study populations consisting of 44% or more RRMS, which were included in one of the meta-analysis of AHSCT.\textsuperscript{25} The heterogeneity among these AHSCT studies was high.

While the AHSCT procedure is carried out only once, alemtuzumab and cladribine therapies are administered as courses, separated by an interval of 1 year. Thus, while the effect of AHSCT occurs early, the full effect of alemtuzumab and cladribine might not be reached before the second course is given. Unfortunately, such 12 months re-base-line data have not been reported, which limits a comparison between these treatments.

### AHSCT

The sustained effects of AHSCT on the immune system appear to be unspecific and result from the broad immunoablation and immune reconstitution that give rise to an immunotolerant state. AHSCT includes ablative chemotherapies that eradicate most of the mature lymphocyte pool. Thereafter, B cells, NK cells, and monocytes repopulate within weeks to 6 months, and CD3+ lymphocyte levels normalize by 6–12 months.\textsuperscript{69,70} The lymphopenia that follows AHSCT induces proliferation of T lymphocytes, and in particular CD8+ lymphocytes.\textsuperscript{71} While the repertoire of circulating T cells seems to be essentially novel for CD4+ cells, the CD8+ cells also derive from the expansion of pre-existing T cells.\textsuperscript{72} These cells may either have survived chemotherapy or been reinfused from the cryopreserved leukapheresis product. When lymphocytes repopulate the immune system, they increase regulatory T-cell numbers, reconstitute NK cells, and reduce the capacity for proinflammatory Th17 responses.\textsuperscript{73,74}

### Alemtuzumab

Alemtuzumab is approved by the European Medicines Agency (EMA) for active MS, but Food and Drug Administration (FDA) approval is limited to patients that have not responded sufficiently on two or more DMTs. However, the indication has recently been further restricted due to severe infusion-related adverse events and the appearance of new secondary autoimmune diseases.\textsuperscript{75} Alemtuzumab is a humanized monoclonal IgG1 antibody against CD52, a cell-surface glycoprotein preferentially expressed on mature lymphocytes. These are rapidly depleted following alemtuzumab infusion by complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and apoptosis. In two phase III RCTs, alemtuzumab was more effective than interferon beta 1a in reducing the relapse rate, disability progression, and MRI activity\textsuperscript{66,67} (Table 4). Alemtuzumab is administered as 12mg infusions over 5 days and, 1 year later, over 3 days. Additional courses may be given if there is breakthrough disease activity at follow up.\textsuperscript{76} Three major adverse events have been recognized: infusion-related reactions, infections, and secondary autoimmune disease, in particular thyroid gland disease. Following alemtuzumab infusion, mature B cells and T cells are depleted, but the reservoir of precursor cells lacks CD52 and is therefore not affected, allowing reconstitution of lymphocytes.\textsuperscript{77,78} The repopulation of lymphocytes starts within weeks and shifts the relative proportions of lymphocyte subsets. The temporal repopulation of B cells and T cells typically shows a faster increase of B cells. Within 3–6 months, CD19+ B cells reach

### Table 4. The effect of immune-reconstitution therapy in MS.

|          | AHSCT\textsuperscript{25} | Alemtuzumab\textsuperscript{65,66} | Cladribine\textsuperscript{67} |
|----------|-----------------------------|----------------------------------|-------------------------------|
| ARR      | 0.04                        | 0.18 and 0.26                    | 0.14                          |
| Relative reduction of 6-month worsening at 2 years | Not reported | 30% and 42%                      | 47%                           |
| Progression-free survival | 92%\textsuperscript{*} | 87% and 91%\textsuperscript{‡} | 80%\textsuperscript{§} |
| NEDA at 2 years | 83%                         | 30% and 32%                      | 47%                           |

AHSCT from meta-analyses; alemtuzumab compared with interferon beta 1a; cladribine compared with placebo; *at 2 years; ‡free of 3-month confirmed EDSS progression; §free of 6-month confirmed EDSS progression. AHSCT, autologous hematopoietic stem cell transplantation; ARR, annual relapse rate; EDSS, expanded disability status scale; NEDA, no evidence of disease activity.
low-normal levels and at 12 months B-cell numbers are 124–165% higher than baseline. CD8+ cytotoxic T cells increase to the lower level of normal within 9–12 months, and CD4+ T cells repopulate more slowly and reach the lower level of normal after 1–2 years. Alemtuzumab preferentially depletes class-switched and unswitched memory B cells. The reconstituted pool of naïve B cells increases while there is a prolonged memory B-cell lymphopenia. In the CD4+ repertoire, regulatory T cell function is regained after 3–4 years. Following anti-CD52 treatment, the number of regulatory B cells increases at 12 months and the proportions of Th1 and Th17 cells are reduced.

### Cladribine

Oral cladribine is approved by the EMA for highly active relapsing MS, and by the FDA for relapsing MS, including relapsing SPMS. In the clinical RCT CLARITY and its extension, cladribine 3.5 mg/kg body weight was superior to placebo and reduced the relapse rate, disability progression, and MRI activity (Table 4). Cladribine treatment was associated with transient, mostly mild to moderate lymphocytopenia (21.6%). Other adverse effects were few, but herpes zoster was more common among the cladribine-treated patients than in the placebo group. The tablets are administered over 4–5 days during weeks 1 and 5, and this procedure is repeated once again after 1 year. Cladribine is incorporated into cells and is phosphorylated by deoxycytidine kinase and inactivated by 5'-nucleotidase. Due to the high ratio of kinase to phosphatase in lymphocytes, activated cladribine accumulates and disrupts DNA, leading to cell depletion. The depletion of B cells is more pronounced than that of T cells, but while the B-cell count returns to normal range within 3–6 months, the modest T-cell reduction is much slower to recover. The B-cell depletion affects mostly class-switched and unswitched memory B cells and the durable effects of cladribine have been hypothesized to be related to a sustained reduction in memory B cells.

### Conclusion and the future position of AHSCT in MS therapies

Attitudes to AHSCT as an alternative therapeutic option in patients with MS have gradually changed, and MS has become the main autoimmune indication for AHSCT. Since the mid-1990s, when the first attempts were made to treat MS with AHSCT, there has been a shift in patient selection from progressive to active RRMS. The safety of AHSCT has improved with less toxic conditioning regimens, and hematology centers have become more skilled at treating adverse events related to transplantation. These are probably the main reasons why AHSCT is now a more attractive therapeutic alternative in highly active RRMS. The recent introduction of alemtuzumab and cladribine as new immune-reconstitution therapies for the treatment of RRMS, might also have increased the awareness of AHSCT as such a therapy for MS. Although they use different mechanisms to restore a dysregulated immune system, their goal is to create durable effects after short-term treatment. However, when these therapies are compared, AHSCT seems to be more effective than alemtuzumab or cladribine (Table 4), with approximately two-thirds of AHSCT-treated patients being free of inflammatory activity after transplantation. The safety of cladribine seems high, but its long-term efficacy has not been proven. The high risk of secondary autoimmune disease after alemtuzumab treatment is not seen with cladribine, but there is a slight increase in risk with AHSCT. Increased risks for acute infusion-related stroke and cardiovascular disease were recently reported in alemtuzumab-treated patients, restricting the selection of patients for this therapy. It is obvious that a careful selection of patients for AHSCT is also needed to minimize risks and improve outcomes. Although a first RCT comparing AHSCT with several DMTs showed superior and impressive efficacy with AHSCT, with relatively preserved safety, there is a need for further RCTs. Initiatives have been taken to compare AHSCT with the best available DMTs in RRMS and there are currently several active phase III trials in which patients are randomized to AHSCT and alemtuzumab, ocrelizumab, or cladribine. There are also ongoing trials comparing different intensities of conditioning regimens to see if the efficacy of AHSCT is preserved with improved safety. Evidence from these trials will certainly have an impact on the position of AHSCT in MS treatment and determine if the indications for AHSCT can be broadened.

### Funding

The authors received no financial support for the research, authorship, and/or publication of this article.
References
1. Compston A and Coles A. Multiple sclerosis. 
   *Lancet* 2002; 359: 1221–1231.
2. Manouchehrinia A, Beiki O and Hillert J. Clinical 
   course of multiple sclerosis: a nationwide cohort 
   study. *Mult Scler* 2017; 23: 1488–1495.
3. Fambiatos A, Jokubaitis V, Horakova D, 
   et al. Risk of secondary progressive multiple 
   sclerosis: a longitudinal study. *Mult Scler.* 
   Epub ahead of print 9 August 2019. DOI: 
   10.1177/1352458519868990.
4. Lublin FD and Reingold SC. Defining the 
   clinical course of multiple sclerosis: results 
   of an international survey. National multiple 
   sclerosis society (USA) advisory committee on 
   clinical trials of new agents in multiple sclerosis. 
   *Neurology* 1996; 46: 907–911.
5. Lublin FD, Reingold SC, Cohen JA, et al. 
   Defining the clinical course of multiple 
   sclerosis: the 2013 revisions. *Neurology* 2014; 
   83: 278–286.
6. Mahad DH, Trapp BD and Lassmann H. 
   Pathological mechanisms in progressive multiple 
   sclerosis. *Lancet Neurol* 2015; 14: 183–193.
7. Montalban X, Hauser SL, Kappos L, et al. 
   Ocrelizumab versus placebo in primary 
   progressive multiple sclerosis. *N Engl J Med* 
   2017; 376: 209–220.
8. Kappos L, Bar-Or A, Cree BAC, et al. Siponimod 
   versus placebo in secondary progressive 
   multiple sclerosis (EXPAND): a double-blind, 
   randomised, phase 3 study. *Lancet* 2018; 391: 
   1263–1273.
9. Sharrack B, Saccardi R, Alexander T, 
   et al. Autologous haematopoietic stem cell 
   transplantation and other cellular therapy 
   in multiple sclerosis and immune-mediated 
   neurological diseases: updated guidelines and 
   recommendations from the EBMT autoimmune 
   diseases working party (ADWP) and the joint 
   accreditation committee of EBMT and ISCT 
   (JACIE). *Bone Marrow Transplant.* Epub ahead of 
   print 26 September 2019. DOI: 10.1038/s41409- 
   019-0684-0.
10. Fassas A, Anagnostopoulos A, Kazis A, et al. 
    Peripheral blood stem cell transplantation in 
    the treatment of progressive multiple sclerosis: first 
    results of a pilot study. *Bone Marrow Transplant* 
    1997; 20: 631–638.
11. Bowen JD, Kraft GH, Wundes A, et al. 
    Autologous hematopoietic cell transplantation 
    following high-dose immunosuppressive therapy 
    for advanced multiple sclerosis: long-term results. 
    *Bone Marrow Transplant* 2012; 47: 946–951.
12. Burt RK, Cohen BA, Russell E, et al. 
    Hematopoietic stem cell transplantation for 
    progressive multiple sclerosis: failure of a total 
    body irradiation-based conditioning regimen to 
    prevent disease progression in patients with high 
    disability scores. *Blood* 2003; 102: 2373–2378.
13. Chen B, Zhou M, Ouyang J, et al. Long-term 
    efficacy of autologous haematopoietic stem 
    cell transplantation in multiple sclerosis at a 
    single institution in China. *Neurol Sci* 2012; 33: 
    881–886.
14. Hamerschlag N, Rodrigues M, Moraes DA, 
    et al. Brazilian experience with two conditioning 
    regimens in patients with multiple sclerosis: 
    BEAM/horse ATG and CY/rabbit ATG. *Bone 
    Marrow Transplant* 2010; 45: 239–248.
15. Samijn JP, te Boekhorst PA, Mondria T, et al. 
    Intense T cell depletion followed by autologous 
    bone marrow transplantation for severe multiple 
    sclerosis. *J Neurol Neurosurg Psychiatry* 2006; 77: 
    46–50.
16. Xu J, Ji BX, Su L, et al. Clinical outcome of 
    autologous peripheral blood stem cell transplantation 
    in optospinal and conventional forms of secondary 
    progressive multiple sclerosis in a Chinese 
    population. *Am J Hematol* 2011; 90: 343–348.
17. Saccardi R, Kozak T, Bocelli-Tyndall C, 
    et al. Autologous stem cell transplantation 
    for progressive multiple sclerosis: update of 
    the European group for blood and marrow 
    transplantation autoimmune diseases working 
    party database. *Mult Scler* 2006; 12: 814–823.
18. Mancardi GL, Sormani MP, Di Gioia M, 
    et al. Autologous haematopoietic stem cell 
    transplantation with an intermediate intensity 
    conditioning regimen in multiple sclerosis: the 
    Italian multi-centre experience. *Mult Scler* 2012; 
    18: 835–842.
19. Burman J, Iacobaeus E, Svenningsson A, 
    et al. Autologous haematopoietic stem cell 
    transplantation for aggressive multiple sclerosis: 
    the Swedish experience. *J Neurol Neurosurg 
    Psychiatry* 2014; 85: 1116–1121.
20. Burt RK, Balabanov R, Han X, et al. Association 
    of nonmyeloablative hematopoietic stem cell 
    transplantation with neurological disability
in patients with relapsing-remitting multiple sclerosis. *JAMA* 2015; 313: 275–284.

21. Curro D, Vuolo L, Gualandi F, *et al.* Low intensity lympho-ablative regimen followed by autologous hematopoietic stem cell transplantation in severe forms of multiple sclerosis: a MRI-based clinical study. *Mult Scler* 2015; 21: 1423–1430.

22. Burt RK, Balabanov R, Burman J, *et al.* Effect of nonmyeloablative hematopoietic stem cell transplantation vs continued disease-modifying therapy on disease progression in patients with relapsing-remitting multiple sclerosis: a randomized clinical trial. *JAMA* 2019; 321: 165–174.

23. Mancardi GL, Sormani MP, Gualandi F, *et al.* Autologous hematopoietic stem cell transplantation in multiple sclerosis: a phase II trial. *Neurology* 2015; 84: 981–988.

24. Ge F, Lin H, Li Z, *et al.* Efficacy and safety of autologous hematopoietic stem-cell transplantation in multiple sclerosis: a systematic review and meta-analysis. *Neurol Sci* 2019; 40: 479–487.

25. Sormani MP, Muraro PA, Schiavetti I, *et al.* Autologous hematopoietic stem cell transplantation in multiple sclerosis: a meta-analysis. *Neurology* 2017; 88: 2115–2122.

26. Fassas A, Kimiskidis VK, Sakellari I, *et al.* Long-term results of stem cell transplantation for MS: a single-center experience. *Neurology* 2011; 76: 1066–1070.

27. Shevchenko JL, Kuznetsov AN, Ionova TI, *et al.* Long-term outcomes of autologous hematopoietic stem cell transplantation with reduced-intensity conditioning in multiple sclerosis: physician’s and patient’s perspectives. *Ann Hematol* 2015; 94: 1149–1157.

28. Muraro PA, Pasquini M, Atkins HL, *et al.* Long-term outcomes after autologous hematopoietic stem cell transplantation for multiple sclerosis. *JAMA Neurol* 2017; 74: 459–469.

29. Kurtzke JF. Rating neurologic impairment in neurological diseases. *J Neurol Neurosurg Psychiatry* 2009; 8: 254–256.

30. Casanova B, Jarque I, Gascon F, *et al.* Autologous hematopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: comparison with secondary progressive multiple sclerosis. *Neurol Sci* 2017; 38: 1213–1221.

31. Nash RA, Hutton GJ, Racke MK, *et al.* High-dose immunosuppressive therapy and autologous hematopoietic cell transplantation for relapsing-remitting multiple sclerosis. (HALT-MS): a 3-year interim report. *JAMA Neurol* 2015; 72: 159–169.

32. Havrdova E, Galetta S, Hutchinson M, *et al.* Effect of natalizumab on clinical and radiological disease activity in multiple sclerosis: a retrospective analysis of the natalizumab safety and efficacy in relapsing-remitting multiple sclerosis (AFFIRM) study. *Lancet Neurol* 2009; 8: 244–253.

33. Burt RK, Loh Y, Cohen B, *et al.* Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase III study. *Lancet Neurol* 2009; 8: 244–253.

34. Atkins HL, Bowman M, Allan D, *et al.* Immunoablation and autologous haemopoietic stem-cell transplantation for aggressive multiple sclerosis: a multicentre single-group phase 2 trial. *Lancet* 2016; 388: 576–585.

35. Nash RA, Bowen JD, McSweeney PA, *et al.* High-dose immunosuppressive therapy and autologous peripheral blood stem cell transplantation for severe multiple sclerosis. *Blood* 2003; 102: 2364–2372.

36. Nash RA, Hutton GJ, Racke MK, *et al.* High-dose immunosuppressive therapy and autologous HCT for relapsing-remitting MS. *Neurology* 2017; 88: 842–852.

37. Moore JJ, Massey JC, Ford CD, *et al.* Prospective phase II clinical trial of autologous haematopoietic stem cell transplant for treatment refractory multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2019; 90: 514–521.

38. Sormani MP, Muraro PA, Saccardi R, *et al.* NEDA status in highly active MS can be more easily obtained with autologous hematopoietic stem cell transplantation than other drugs. *Mult Scler* 2017; 23: 201–204.

39. Muraro PA, Martin R, Mancardi GL, *et al.* Autologous haematopoietic stem cell transplantation for treatment of multiple sclerosis. *Nat Rev Neurol* 2017; 13: 391–405.

40. Krasulova E, Trmeny M, Kozak T, *et al.* High-dose immunoablation with autologous haematopoietic stem cell transplantation in aggressive multiple sclerosis: a single centre 10-year experience. *Mult Scler* 2010; 16: 685–693.

41. Lee H, Nakamura K, Narayanan S, *et al.* Impact of immunoablation and autologous hematopoietic stem cell transplantation on gray and white matter atrophy in multiple sclerosis. *Mult Scler* 2018; 24: 1055–1066.

42. Burman J, Tolf A, Hagglund H, *et al.* Autologous haematopoietic stem cell transplantation for neurological diseases. *J Neurol Neurosurg Psychiatry* 2018; 89: 147–155.
43. Dubinsky AN, Burt RK, Martin R, et al. T-cell clones persisting in the circulation after autologous hematopoietic SCT are undetectable in the peripheral CD34+ selected graft. Bone Marrow Transplant 2010; 45: 325–331.

44. Snowden JA, Saccardi R, Allez M, et al. Haematopoietic SCT in severe autoimmune diseases: updated guidelines of the European group for blood and marrow transplantation. Bone Marrow Transplant 2012; 47: 770–790.

45. Moore J, Brooks P, Milliken S, et al. A pilot randomized trial comparing CD34-selected versus unmanipulated hematopoietic stem cell transplantation for severe, refractory rheumatoid arthritis. Arthritis Rheum 2002; 46: 2301–2309.

46. Mancardi G and Saccardi R. Autologous haematopoietic stem-cell transplantation in multiple sclerosis. Lancet Neurol 2008; 7: 626–636.

47. Farge D, Labopin M, Tyndall A, et al. Autologous hematopoietic stem cell transplantation for autoimmune diseases: an observational study on 12 years’ experience from the European group for blood and marrow transplantation working party on autoimmune diseases. Haematologica 2010; 95: 284–292.

48. Gratwohl A, Passweg J, Bocelli-Tyndall C, et al. Autologous hematopoietic stem cell transplantation for autoimmune diseases. Bone Marrow Transplant 2005; 35: 869–879.

49. Saiz A, Blanco Y, Carreras E, et al. Clinical and MRI outcome after autologous hematopoietic stem cell transplantation in MS. Neurology 2004; 62: 282–284.

50. Reston JT, Uhl S, Treadwell JR, et al. Autologous hematopoietic cell transplantation for multiple sclerosis: a systematic review. Mult Scler 2011; 17: 204–213.

51. Mariottini A, Innocenti C, Forci B, et al. Safety and efficacy of autologous hematopoietic stem-cell transplantation following natalizumab discontinuation in aggressive multiple sclerosis. Eur J Neurol 2019; 26: 624–630.

52. Fassas A, Passweg JR, Anagnostopoulos A, et al. Hematopoietic stem cell transplantation for multiple sclerosis. A retrospective multicenter study. J Neurol 2002; 249: 1088–1097.

53. Saccardi R, Tyndall A, Coghlan G, et al. Consensus statement concerning cardiotoxicity occurring during haematopoietic stem cell transplantation in the treatment of autoimmune diseases, with special reference to systemic sclerosis and multiple sclerosis. Bone Marrow Transplant 2004; 34: 877–881.

54. Morandi P, Ruffini PA, Benvenuto GM, et al. Cardiac toxicity of high-dose chemotherapy. Bone Marrow Transplant 2005; 35: 323–334.

55. Hartung HP, Gonssette R, Konig N, et al. Mitoxantrone in progressive multiple sclerosis: a placebo-controlled, double-blind, randomised, multicentre trial. Lancet 2002; 360: 2018–2025.

56. Tuohy O, Costelloe L, Hill-Cawthorne G, et al. Alemtuzumab treatment of multiple sclerosis: long-term safety and efficacy. J Neurol Neurosurg Psychiatry 2015; 86: 208–215.

57. Daikeler T, Labopin M, Di Gioia M, et al. Secondary autoimmune diseases occurring after HSCT for an autoimmune disease: a retrospective study of the EBMT autoimmune disease working party. Blood 2011; 118: 1693–1698.

58. Loh Y, Oyama Y, Statkute L, et al. Development of a secondary autoimmune disorder after hematopoietic stem cell transplantation for autoimmune diseases: role of conditioning regimen used. Blood 2007; 109: 2643–2548.

59. Weimer R, Staak A, Susal C, et al. ATG induction therapy: long-term effects on Th1 but not on Th2 responses. Transpl Int 2005; 18: 226–236.

60. Jansen RP. Fertility in older women. IPPF Med Bull 1984; 18: 4–6.

61. Nybo Andersen AM, Wohlfiart J, Christens P, et al. Maternal age and fetal loss: population based register linkage study. BMJ 2000; 320: 1708–1712.

62. Lasica M, Taylor E, Bhattacharyya P, et al. Fertility in premenopausal women post autologous stem cell transplant with BEAM conditioning. Eur J Haematol 2016; 97: 348–352.

63. Maciejewska M, Snarski E and Wiktor-Jedrzejczak W. A preliminary online study on menstruation recovery in women after autologous hematopoietic stem cell transplant for autoimmune diseases. Exp Clin Transplant 2016; 14: 665–669.

64. Glazer CH, Tottenborg SS, Giwercman A, et al. Male factor infertility and risk of multiple sclerosis: a register-based cohort study. Mult Scler. Epub ahead of print 1 October 2017. DOI: 10.1177/1352458517734069.

65. Thone J, Kollar S, Nousse D, et al. Serum anti-Mullerian hormone levels in reproductive-age women with relapsing-remitting multiple sclerosis. Mult Scler 2015; 21: 41–47.

66. Cohen JA, Coles AJ, Arnold DL, et al. Alemtuzumab versus interferon beta 1a as
first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet* 2012; 380: 1819–1828.

67. Coles AJ, Twyman CL, Arnold DL, et al. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet* 2012; 380: 1829–1839.

68. Giovannoni G, Comi G, Cook S, et al. A placebo-controlled trial of oral cladribine for relapsing multiple sclerosis. *N Engl J Med* 2010; 362: 416–426.

69. Arruda LCM, de Azevedo JTC, de Oliveira GLV, et al. Immunological correlates of favorable long-term clinical outcome in multiple sclerosis patients after autologous hematopoietic stem cell transplantation. *Clin Immunol* 2016; 169: 47–57.

70. Muraro PA, Douek DC, Packer A, et al. Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. *J Exp Med* 2005; 201: 805–816.

71. Mackall CL, Fleisher TA, Brown MR, et al. Distinctions between CD8+ and CD4+ T-cell regenerative pathways result in prolonged T-cell subset imbalance after intensive chemotherapy. *Blood* 1997; 89: 3700–3707.

72. Muraro PA, Robins H, Malhotra S, et al. T cell repertoire following autologous stem cell transplantation for multiple sclerosis. *J Clin Invest* 2014; 124: 1168–1172.

73. Darlington PJ, Touil T, Doucet JS, et al. Diminished Th17 (not Th1) responses underlie multiple sclerosis disease abrogation after hematopoietic stem cell transplantation. *Ann Neurol* 2013; 73: 341–354.

74. Darlington PJ, Stopnicki B, Touil T, et al. Natural killer cells regulate Th17 cells after autologous hematopoietic stem cell transplantation for relapsing remitting multiple sclerosis. *Front Immunol* 2018; 9: 834.

75. European Medicines Agency. Measures to minimise risk of serious side effects of multiple sclerosis medicine Lemtrada, https://www.ema.europa.eu/en/documents/referral/lemtrada-article-20-procedure-measures-minimise-risk-serious-side-effects-multiple-sclerosis_en-0.pdf (accessed 16 January 2020).

76. Comi G, Alroughani R, Boster AL, et al. Efficacy of alemtuzumab in relapsing-remitting MS patients who received additional courses after the initial two courses: pooled analysis of the CARE-MS, extension, and TOPAZ studies. *Mult Scler*. Epub ahead of print 25 November 2019. DOI: 10.1177/1352458519888610.

77. Hale G. The CD52 antigen and development of the CAMPATH antibodies. *Cytotherapy* 2001; 3: 137–143.

78. Rao SP, Sancho J, Campos-Rivera J, et al. Human peripheral blood mononuclear cells exhibit heterogeneous CD52 expression levels and show differential sensitivity to alemtuzumab mediated cytolysis. *PLoS One* 2012; 7: e39416.

79. Thompson SA, Jones JL, Cox AL, et al. B-cell reconstitution and BAFF after alemtuzumab (Campath-1H) treatment of multiple sclerosis. *J Clin Immunol* 2010; 30: 99–105.

80. Freedman MS, Kaplan JM and Markovic-Plese S. Insights into the mechanisms of the therapeutic efficacy of alemtuzumab in multiple sclerosis. *J Clin Cell Immunol* 2013; 4: 1000152.

81. Ceronie B, Jacobs BM, Baker D, et al. Cladribine treatment of multiple sclerosis is associated with depletion of memory B cells. *J Neurol* 2018; 265: 1199–1209.

82. Jones JL, Thompson SA, Loh P, et al. Human autoimmunity after lymphocyte depletion is caused by homeostatic T-cell proliferation. *Proc Natl Acad Sci U S A* 2013; 110: 20200–20205.

83. Zhang X, Tao Y, Chopra M, et al. Differential reconstitution of T cell subsets following immunodepleting treatment with alemtuzumab (anti-CD52 monoclonal antibody) in patients with relapsing-remitting multiple sclerosis. *J Immunol* 2013; 191: 5867–5874.

84. Giovannoni G, Soelberg Sorensen P, Cook S, et al. Safety and efficacy of cladribine tablets in patients with relapsing-remitting multiple sclerosis: results from the randomized extension trial of the CLARITY study. *Mult Scler* 2018; 24: 1594–1604.