Intense immunosuppression followed by autologous haematopoietic stem cell transplantation as a therapeutic strategy in aggressive forms of Multiple Sclerosis

Gianluigi Mancardi[1], Maria Pia Sormani[2], Paolo A. Muraro[3], Giacomo Boffa[1] and Riccardo Saccardi[4]

Gianluigi Mancardi

Neurological Clinic, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genova and Ospedale Policlinico San Martino, Largo Paolo Daneo 3, 16132 Genova, Italy

Maria Pia Sormani

Biostastic Unit , Department of Health Sciences (DISSAL), University of Genoa, via Pastore 1, 16132 Genova, Italy

Paolo Muraro

Division of Brain Sciences, Imperial College London, Burlington Danes Building, 192 Du Cane Road, London W 12 ONN, UK

Giacomo Boffa

Neurological Clinic, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genova e Ospedale Policlinico San Martino, Largo Paolo Daneo 3, 16132 Genova, Italy

Riccardo Saccardi

Cell Therapy and Transfusion Medicine Unit, Careggi University Hospital, Largo Brambilla, 3-50134, Firenze, Italy.

Correspondence to : Gianluigi Mancardi, Neurological Clinic, Largo Paolo Daneo 3 , 16132 Genova, Italy. E-mail: glmancardi@neurologia.unige.it

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Abstract: In the majority of relapsing multiple sclerosis patients the disease can be quite easily controlled by the already available and approved therapies. There are, however, some aggressive cases that continue to have clinical and MRI activity in spite of the treatment. These are the cases that may now receive benefit from intense immunosuppression followed by autologous haematopoietic stem cell transplantation (aHSCT). In this review we describe the method and the rationale of aHSCT, the more recently published studies that demonstrate its efficacy in selected multiple sclerosis cases, the problems related to the safety, and transplant related mortality risk of the procedure. Description of the ideal patient that can take advantage from aHSCT is outlined and, finally, the ongoing studies that are near to completition or are close to starting are briefly reported.

Introduction

Intense immunosuppression followed by autologous haematopoietic stem cell transplantation (aHSCT) is a treatment now considered by the neurological community as a possible strategy for aggressive forms of multiple sclerosis (MS). In past years it was barely taken into account by the majority of MS experts, on account of its poor safety profile and the absence of prospective randomized controlled studies. More recently, while randomized controlled trials are still lacking, numerous studies have been published which convincingly demonstrate its undeniable efficacy in the more aggressive forms of MS. These publications comprise a multicentre retrospective study[1], a large data base retrospective analysis[2], single arm prospective trials[3,4,5,6], a small phase II randomized study[7], a meta-analysis of all the published trials in any form of MS from 1995 to 2016[8] and, finally, a complete review of the rationale and the experience on aHSCT in MS[9]. There are, however, still many problems and doubts not completely solved. In particular, a prospective randomized comparative trial with a long term disability endpoint is still lacking. In this brief review, we discuss the lights and shadows of aHSCT in MS, and its actual place in the numerous treatment options that can be offered to MS patients, with the aim of contributing to the further development of this procedure in MS.

The procedure of aHSCT and how it works

The use of aHSCT in a clinical setting was preceded by convincing proof of efficacy carried out in preclinical experiments in animal models of adjuvant arthritis and experimental allergic encephalomyelitis[10,11]. In animals that spontaneously develop autoimmune disorders or in animals where the disease is caused by immunization with specific antigens, the disorder can be cured or successfully treated with either pseudo-autologous or autologous bone marrow transplantation[12,13]. Better results are obtained if the disease is still in the inflammatory stage, if high, rather than low, dose conditioning regimes are used, and if autoreactive T cells are eradicated from the graft[13]. Not surprisingly, all these considerations, that are derived from experiments in animals in the preclinical setting, can be now also be applied to humans with severe autoimmune disorders refractory to conventional therapies.

The procedure: Peripheral blood haematopietic stem cells (HSCs) are mobilized with cyclophosphamide (CY, 1.5-4 gr/m^2 over 1-2 days), followed by daily granulocyte colony-stimulating factor (G-CSF, 5-12 μg/kg/day). HSCs can be mobilized with G-CSF or GM-CSF alone but a previous treatment with CY is preferred because a flare of disease can appear if only G-CSF is used and also because CY, at this dosage, has a potent immunosuppressive function, depleting autoreactive lymphocytes which could otherwise be collected in the graft. Not rarely the patient starts already to improve after the mobilization treatment with CY. HSCs express the surface antigen CD34, which allows for their reliable detection both in the blood and in the graft, and are collected with a leukoapheresis procedure and cryopreserved. The graft can be manipulated in order to remove autoreactive clones (ex vivo T cell depletion), although it has not been demonstrated that this procedure improves the clinical outcome of treated patients[14]. A minimum of 4-5x10^6 CD34+ cells/kg body weight is usually recommended. After 30-60 days the patient is treated with a conditioning regimen which has the aim of eliminating the autoreactive clones in the immune system. Different conditioning protocols have been proposed and classified according to the intensity of the hemato-lymphopoietic system ablation. In the guidelines[15] of the European Blood and Marrow Transplantation Society (EBMT, www.ebmt.org) for HCST in autoimmune diseases, conditioning regimens are divided in to: 1. *high intensity regimens*, including either full dose of busulfan combined with both ex vivo and in vivo T cell depletion using rabbit anti-thymocte globulin (ATG), or total body irradiation (TBI - though this is no longer used); 2. *intermediate intensity regimens* such as BEAM, consisting of BCNU (carmustine), 300mg/m^2 at day -7; cytosine-arabinoside, 200 mg/m^2 and etoposide, 200 mg/m^2 from day -5 to day -2; and melphalan 140 mg/m^2 at day -2 plus ATG; 3. *low intensity regimens* such as CY (200mg/kg in 4 days) and ATG. The best conditioning regimen is still a matter of debate among the various groups that utilize aHSCT in MS. High intensity regimens have certainly a high capacity to eliminate autoreactive clones, but safety issues were raised. Low intensity conditioning regimen are almost immunoablative and therefore could appear to be more appropriate in an autoimmune setting. Low intensity regimens are likely to have a better safety profile although relapses after the procedure occurs in almost 25% of treated cases[16] and MRI activity is not completely eradicated[17] which is the case with high and intermediate intensity protocols. After the conditioning regimen, the cryopreserved graft is thawed and cells infused through a central venous catheter. ATG (3.75mg/kg at day +1 and+2 or 1,25 mg/kg per day for 4 days according to the brand) is administered before or after stem cell reinfusion

primarily with the intention of eradicating of autoreactive T cells which have escaped the conditioning regimen or have been reinfused with the graft (in vivo T cell depletion). After conditioning, an aplastic phase occurs , characterized by extremely low level of blood cells , that requires a prophylactic administration of haemoderivatives and antibiotics. Recovery of cell counts occurs usually in 10-15 days. After 3-4 weeks from conditioning the patient is usually discharged to home, but antiviral prophylaxis has to be continued for at least 6-12 months from HSCT.

How it works: what makes aHSCT of particular interest is not only that it destroys the autoreactive cells but that it is followed by an extensive renewal of the immune system. In the last 20 years numerous studies have evaluated the immunological reconstitution after aHSCT and there is a general consensus on the positive profound modifications that follow the procedure[18,19]. The mobilization regimen and, more importantly, the conditioning regimen and ATG destroy the pathogenic autoreactive clones, predominantly Th17 cells[20]. Mucosal associated invariant T cells (MAIT) characterized by CD8 phenotype, high expression of CD161 and secretion of IL-17 and IFNγ are also depleted[21] . In the first months after the procedure, homeostatic expansion of CD8 and, to a lesser degree CD4 T cells, occurs. After 1-2 years, there is an expansion of naïve CD4 and CD8 T cells that have a thymic origin with a broader clonal diversity and clonal specificity. There is also an increase of regulatory CD4+, $CD24^{high}$, $CD127^{-}$, $Fox3^{+}$ T cells[22] . Similar changes occur in the B repertoire. These modifications can induce a restoration of immune tolerance that, eventually, may persist for years. The different steps of aHSCT and of the immunological reconstitution are described in *Figure 1.*

Clinical outcome

The neurological outcome after aHSCT is only partially related to the intensity of the conditioning regimen but is mainly dependent on the clinical status of the patient. aHSCT has a profound anti-inflammatory effect and, therefore, aggressive MS cases with a short duration of disease, relapses and MRI signs of activity (new T2-FLAIR areas or gadolinium enhancing lesions) are certainly the cases that usually have a favorable outcome after transplantation.

The first period from 1995 to 2006

The first pilot trial of aHSCT in MS was published 20 years ago[23] and, in the following years, only small phase I studies carried out by single centers or by collaborative efforts of various teams of the same country were carried out. All these early studies were focused not only on the clinical outcome but also on the feasibility of the procedure and on its side effects and mortality risk. Recently, a retrospective study describing the long term outcome after aHSCT was published[2], evaluating all the MS cases treated between January 1995 and December 2006 and reported to the database of the Center for International Blood and Marrow Transplant Research (CIBMTR, Wisconsin, USA), and EBMT. Data were obtained from 281 patients: 218 (78%) were already in the progressive phase of the disease, with a median EDSS before mobilization of 6.5 and a median follow up of 6.6 years. Progression-free survival at 5 years was 46% and overall survival was 93%. Transplant related mortality occurring in the first 100 days after transplant was 2.8%. Factors associated with progression were progressive vs relapsing-remitting (RR) form of disease, older age and more than 2 previous disease modifying therapies. The obtained results were promising, taking into account that, after 5 years, almost 50% of treated patients did not progress, an encouraging finding, given that the majority of the treated population was composed of progressive MS cases who had had a clinical deterioration of at least 1 EDSS point in the previous year.

It had been, however, demonstrated already in 2001 that aHSCT had the capacity to completely suppress Gd-enhancing activity as evaluated with MRI[24], had a profound effect on the occurrence of relapses[25], and was particularly effective in severe MS cases with a rapidly evolving clinical course (malignant forms)[26], where aHSCT was often followed by a dramatic clinical improvement[27,28,29]. On the contrary, even high intensity conditioning regimens, including TBI, were not able to prevent disease progression in patients with a high disability score and a long-lasting progressive clinical course[30]. Therefore it was already apparent, ten years after the first studies, that aHSCT was more effective in active MS cases with a relapsing-remitting (RR) clinical course.

The second period from 2005 to 2017

After the first years, numerous papers published after 2005 showed that RR MS cases had a better outcome than progressive MS cases[16,31,32,33,34]. Moreover, in these cases a significant population of patients experienced a clinical improvement, with a decrease higher than 1 point at EDSS[27,29] , while this event was rarely observed in progressive cases. It was also reported that patients with Gd-enhancing activity at baseline fared significantly better than patients without Gd-enhancing lesions[1,33], pointing out that the clinical effect of aHSCT is mainly due to the capacity to suppress the inflammatory activity. Recently, a series of papers has unequivocally demonstrated the effect of aHSCT on MRI signs of activity and on the clinical outcome in severe forms of MS. A comparative randomized phase II trial evaluating aHSCT vs mitoxantrone – the ASTIMS trial- demonstrated that the number of new T2 lesions in a 4 year period was decreased of 79% in patients treated with aHSCT vs the cases that received mitoxantrone[7]. In the observational study reporting the Swedish experience[1] and in the prospective HALT study in USA[4,5], patients with a RR form of the disease who experienced relapses and progression of the disease before treatment were followed for a median follow up of 5 years, demonstrating a sustained remission of disease without progression of disability in 77% and 80% of cases respectively. Atkins et al[6] treated 24 MS cases with a high intensity regimen (busulfan and CY with ex vivo and in vivo T cell depletion), obtaining complete cessation of relapses and MRI activity for a median follow up of 6.7 years, demonstrating that aHSCT has the capacity to suppress the inflammatory process for a very long period of time. In the Canadian study[6], both RR and progressive MS cases were enrolled and indeed 25% of cases continued to progress in spite of absence of inflammation. . If we consider the NEDA (No Evidence of Disease Activity) status, that is the absence of relapses, progression of disability and MRI activity, as the ideal target of a therapy in MS, this target was reached after 3 years by 70-80% of cases treated with aHSCT[1,4,5,6], as compared to 30-60% of cases treated with alemtuzumab, ocrelizumab or daclizumab[35,36,37.]. NEDA status is, however, related to the frequency of scanning and to the time each treatment needs to reach its full effect: a re-baseline 6 months after the start of ocrelizumab demonstrated a proportion of NEDA patients of 75.5% at year 2[38]; the optimal re-baseline time depends on the treatment time of action, and could be different for each drug[35].

We recently evaluated all the cases treated in the most active centers of Italy in the period between 1996 and 2016, i.e. 122 consecutive cases that received aHSCT with BEAM ATG as the conditioning regimen (59% with a RR clinical course). The 3 years probability of progression free survival was 91% for RRMS and 62% for progressive MS cases ($p<0.001$). NEDA status at 5 years was maintained by 72% of RRMS patients and by 55% of SPMS ($p= 0.07$). Therefore, our experience (unpublished data), is completely in agreement with the more recent studies that demonstrate that aHSCT is extremely effective in reducing long term disease activity in aggressive MS patients. Recently a meta-analysis evaluating all the published studies from 1995 to 2016 was published, including 764 patients with a severe treatment refractory MS form of disease[39]. Pooled rate of progression was 17.1% at 2 years and 23.3% at 5 years but a lower progression rate was significantly associated with a high proportion of RR MS patients. Interestingly, in this large meta-analysis, no significant association was seen between the progression rate and the conditioning regimen utilized (high, intermediate or low intensity). In conclusion, in the last years numerous papers have been published which demonstrate that aHSCT is effective on clinical and MRI outcomes, especially in aggressive forms of MS with MRI and clinical signs of activity.

Is aHSCT really not effective in progressive forms of MS?

Beyond the profound effect on relapses and MRI inflammatory activity, some studies have demonstrated efficacy of aHSCT in preventing long-term neurological deterioration even in progressive MS patients[40,41]. Of interest, Schevchenko and collegues [41] reported that 93% (28 out of 30) of progressive MS patients had no neurological progression over a 5 year follow-up after aHSCT. In our cohort (unpublished data), 43% of 122 patients treated with aHSCT had a progressive course of the disease and subgroup analyses for progressive MS demonstrated that progression-free survival was almost 60% at 5 year. Similarly, the recent retrospective long-term analysis[2] of aHSCT in a cohort of 282 patients, mainly composed of progressive MS (78%), showed a progression-free survival of 46% at 5 years. Even if these results appear less significant compared to the much higher proportion of RRMS patients without neurological deterioration after aHSCT[1,3,4,5], they demand some consideration. Indeed, the majority of the treated population in these studies was composed of progressive MS cases who had a clinical deterioration of at least 1 point at the EDSS in the previous year. Because long-term stabilization of neurological disability is not an expected feature of aggressive forms of progressive MS, these data suggest that aHSCT may have reduced the risk of neurological deterioration over time even

in established progressive MS. In line with this hypothesis, recent data indicate that neurological deterioration in the progressive phase might be driven by compartmentalized inflammation behind the blood-brain barrier, opening the possibility that targeting inflammation might reduce accrual of neurological disability, as suggested by recent trials of B-cells targeted therapies[42,43.] However, controlled evidence of the efficacy of aHSCT for progressive MS is still lacking and more data from randomized clinical trial are needed. Moreover, data from published studies[8] and EBMT and CIBMTR registries[2], clearly indicate that a progressive course of the disease and a higher EDSS score are associated with an increased transplant mortality risk, raising concerns about the safety profile of AHSCT in progressive MS patients.

Safety of aHSCT

aHSCT is a demanding treatment that has safety problems related to the intensity of the utilized procedure and also to the clinical condition of the patient and the presence of comorbidities. The majority of toxic effects are related to the duration of bone marrow aplasia and the overall intensity of the induced immunosuppression. In general, high intensity regimens (such as TBI or busulfan with both ex vivo and in vivo T cell depletion) have more adverse effects[44] than intermediate (BEAM-ATG) and low intensity regimens (CY-ATG). However, adverse effects are common to all the transplantation procedures: they are defined as "early" when they appear within the first 100 days after transplantation and "late" thereafter. Early adverse events are neutropenic fever, sepsis, urinary infections, mucositis, gastrointestinal toxicities and viral reactivation (such as cytomegalovirus and Epstein Barr virus). Transient deterioration of a patient's neurological status can occur in the first days after aHSCT such as fatigue, headache, ataxia and are generally due to the presence of fever or to allergic reactions to G-CSF or ATG. Alopecia occurs in almost all the cases; interruption of the menstrual cycle is nearly constant and is usually followed by recovery of menstruation in the younger females or by a permanent amenorrhea in older women[45]. As such, the possibility of an enduring infertility is a real risk and should be carefully discussed between the patient and experts in the field before proceeding to aHSCT. Possible solutions to mitigate this exist, including gamete preservation, before the initiation of the chemotherapy and pharmacological ovarian protection during the conditioning regimen. Pregnancies with a favorable outcome after aHSCT have been, however, reported by

the autoimmune disease working party of EBMT[46]. Late toxic effects are due to varicella-zoster - primary infections or recurrence - and also to secondary autoimmune disorder such as thyroiditis, anti-factor VIII inhibitors or autoimmune cytopenias. These autoimmune disorders occur in 6.4% of treated cases[47] and appear more frequent in cases who have received a high intensity conditioning regimen (26% in the Atkins et al series[6]). Therefore, the most important safety problems related to aHSCT occur early in the first period (100 days) after transplantation, as opposed to the other therapies utilized in MS where safety problems occurs later[48]. The main problem of aHSCT is the risk of transplant related mortality (TRM). Deaths are mostly related to infections, although cardiotoxicity, veno-occlusive disease, neurological deterioration, massive hepatic necrosis are sporadically causes of TRM, which was reported to be 6% in the first EBMT data analysis[49] and 5.3% in the second EBMT study[50]. These first data were clearly unacceptable to the neurological community and profoundly limited, at that time, the development of aHSCT in severe MS cases. The mortality dropped to 1.3% in the period from 2001 to 2007[51] , further decreasing to 0,7% during 2008-2016 and to 0.2% in the last 5 years 2012-2016 [2,9]. In the recent metanalysis[8] that evaluated 764 transplanted patients reported in 15 studies published from 1995 to 2016, TRM was demonstrated to be significantly related to the year of transplant (3.6% before 2005 and 0.3% after 2005), to the baseline EDSS (3.2% if EDSS was > 5.5 and 0.3% if EDSS was ≤5.5) and to the proportion of RR MS cases present in the different studies (3.4% if RR cases were < to 44% of all the treated patients and 1% if RR cases were more than 44%). Interestingly, in this metanalysis TRM does not appear to be related to the intensity (high, intermediate, low) of the conditioning regimen. Therefore in recent years, mortality risk has significantly dropped due to a better selection of patients (younger cases with a RR clinical course and not severely disabled) and also to the improved experience of the hemato-neurological centers. It is of main relevance that aHSCT should be carried out in hematological centers with proven experience with allogenic transplants and autologous transplantation in autoimmune disorders. In our opinion a mortality risk of 0.3% can be accepted only in aggressive forms of MS[52] that do not respond to the current available therapies and have a high chance of otherwise irreversible disability.

aHSCT as a possible therapeutic strategy in MS

aHSCT is not intended to be a therapy for all MS patients but it could be a valuable therapeutic option for severe aggressive forms of MS that continue to deteriorate in spite of the approved available therapies. aHSCT was first used in MS more than 20 years ago and from that time its use continued to increase in Europe as well as in America and Asia. More than 1000 patients have been treated after 1997 and reported to the EBMT Registry up to June 2017 *(Figure 2)*. From these data it appears that in the first years the majority of treated patients were in the progressive phase of the disease but now aggressive forms of MS still in the RR phase of the disease are the main target of aHSCT . In Europe the more active countries are Italy, Sweden, United Kingdom and Poland (EBMT data, June 2017). It is interesting to note that while BEAM was the most used conditioning regimen, in later years in Europe the scheme of CY and ATG (low intensity regimen) appears to be now the most utilized regimen of therapy (*Figure 3*), especially in countries such as Sweden and United Kingdom where some centers are participating to the phase 3 randomized study organized by the Northwestern University of USA, where the conditioning regimen is a low intensity regimen (CY and ATG, MIST trial, see *ongoing study section*). At present , we do not have objective convincing data on the conditioning regimen with the best balance between efficacy and safety. The opinion of the authors of the present review is that intermediate intensity regimens such as BEAM+ATG are still the best choice, owing to their large use and experience demonstrating its efficacy and an acceptable safety profile. The EBMT Working Party for Autoimmune Diseases is currently launching a retrospective analysis aimed at comparing the clinical outcome in patients treated with either BEAM/ATG or CY/ATG regimens.

Selection of patients

The patient who is most likely to benefit from aHSCT (*Table 1*) is a relatively young person with MS, still ambulatory, with a RR clinical course, who has failed the approved therapies and with MRI signs of disease activity (new T2 lesions or Gd- enhancing lesions). aHSCT is probably the most effective anti-inflammatory therapy now available and therefore has to be used when there are clear clinical and radiological signs of inflammation. aHSCT can be considered after the failure of both first and second line therapy or after the failure of a single high efficacy therapy (such as alemtuzumab, natalizumab, ocrelizumab and daclizumab when available), when it is clear that the patient has an aggressive form of disease but before the patient become seriously disabled. It should not be used as a last rescue therapy after the failure of all available

treatments, because at that time the patient probably is already in the progressive phase of the disease and there is more degeneration rather than the inflammation, which is the target of aHSCT. Patients with a progressive clinical course without clinical relapses or MRI signs of activity should not be treated. However, if the patient has entered the progressive phase of the disease only a short period of time ago and has still clinical and MRI evidence of inflammatory activity, aHSCT can be considered, even if currently there are no definite data to support the use of aHSCT in active progressive MS. Finally, in our opinion, aHSCT is the best available therapy for the rare cases of highly aggressive MS patients that have a rapidly evolving clinical course with progression to a severe disability in a short period of time, previously called malignant forms of MS[27,28,29,53]. In these cases, aHSCT can be followed by a surprising and long-lasting clinical amelioration.

Ongoing studies

Only one comparative randomized phase II trial on aHSCT has been published[7]. In this study, however, aHSCT was compared to mitoxantrone, a therapy no longer used on account of safety problems. New prospective randomized controlled clinical trials in highly active RRMS and also in active progressive MS are eagerly waited. At the moment, a randomized trial comparing aHSCT (low intensity, CY and ATG) vs FDA approved standard of care is running (*MIST Trial*). The official title of the study is: "Hematopoietic stem cell therapy for patients with inflammatory multiple sclerosis failing alternative approved therapy: a randomized study", clinicaltrials.gov NCT 00273364. The estimated enrolment is of 110 patients with RRMS randomized in aHSCT vs interferon, GA, mitoxantrone, natalizumab, fingolimod or dimethyl fumarate, failing alternate approved therapy. The primary endpoint is an increase of 1 point at EDSS on consecutive evaluations 6 months apart. Patients will be followed for 5 years after randomization. The participating centers are the Northwestern University of Chicago (USA), the Uppsala University (Sweden) and the Sheffield Teaching Hospitals NHS Trust (UK). The study is ongoing but enrollment is closed and the estimated completion date is December 2018. Recently a randomized study comparing in RR MS aHSCT (BEAM - ATG conditioning regimen) vs the best available therapy is being developed in the USA with the support of the National Institute of Health (NIH). The *BE*st available therapy vs *A*utologous Hematopoietic Stem Cell *T*ransplantation for MS (BEAT-MS) protocol aims at recruiting RR MS patients with mild to moderate disability

and inflammatory activity who will be randomized to either the transplant arm or the best available approved treatment arm which includes high efficacy therapy such as natalizumab, alemtuzumab, ocrelizumab and may include new approved better or equivalent agents. A relapse-based primary endpoint is planned. A study with a similar treatment arms design, but different primary endpoint, is also organized in Italy[54] (*N*o *E*vidence of Disease Activity in aHSC*T* vs Best available therapy in aggressive forms of *MS*, *NET-MS*). The NET-MS Study will be a randomized clinical trial of aHSCT (investigative arm, BEAM-ATG) against approved highly efficacious DMT (natalizumab, alemtuzumab, daclizumab- or ocrelizumab and cladribine when approved and licensed). Enrolled RR MS patients will be randomized to one or other arm (1 vs 2 randomization scheme) and will be followed for 5 years, clinically and with MRI, and will be also asked to undergo extended collections of blood and CSF samples for research on the mechanism of the therapies. The primary endpoint will be the proportion of patients with absence of disease activity (NEDA). In Denmark, Sweden, Norway a study evaluating whether aHSCT is more efficacious at achieving NEDA than alemtuzumab in patients with highly active RRMS is also on the way to start (*RAM-MS*). This is a multicentre randomized controlled study that will enroll RR patients that will be treated with CY/ATG regimen (as used in the MIST Trial) as conditioning vs alemtuzumab, given and monitored per licence. Finally a study in the United Kingdom evaluating aHSCT vs alemtuzumab has been planned and financial support requested (*STAR-MS*).

Conclusion

The growing interest in aHSCT is demonstrated by the fact that recently some Health Regulatory Authorities such as the Swedish Board of Health and Welfare recognized aHSCT as a therapy in active MS[55]. The number of treated patients is increasing in recent years, in spite of the new therapies now available and the safety problems of the procedure. The authors of the present review are of the opinion that aHSCT already has a place among the various therapeutic strategies for MS. It is a reasonable option in aggressive MS cases which, despite treatment with approved therapies, are still clinically and MRI active and are at high risk of severe disability in a short period of time. Its use must be considered only before the patient is irreversibly compromised , when it is clear that the disease has a severe clinical course. It is of prime importance for neurological scientists, healthcare organizations, persons with MS and MS

Societies, that the ongoing prospective studies are organized and carried out, in order to finally demonstrate that aHSCT is an effective and feasible therapy for severe forms of MS

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Declaration of Conflicting Interests

GLM has received support from Biogen Idec, Merck Serono, Novartis, Teva, Genzyme and Sanofi Aventis for lecturing, travel expenses, attending meetings and research. MPS has received personal compensation for consulting services and for speaking activities from Biogen, Genzyme, Merck Serono, Novartis, Roche, and Teva. PM declares honoraria for speaking and travel support from Bayer, Biogen, Merck Serono and Novartis. GB has no conflict of interest. RS has received honoraria for lecturing from Sanofi.

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Legends

Figure 1. The aHSCT procedure and the immunological reconstitution.

aHSCT consists of 6 main steps: the procedure starts with mobilization of HSC from the bone marrow by injection of cyclophosphamide and granulocyte-colony stimulating factor (G-CSF). The autologous graft is harvested from the peripheral blood by leukoapheresis, (eventually undergoing CD34 selection for *ex vivo* T cell depletion). Ablation of the immune and, to a variable extent, myeloid system can be achieved by several conditioning protocols with different combination of cytotoxic drugs. The graft is then reinfused along with antithymocyte globulin (ATG) for *in vivo* T cell depletion. Different levels of supportive care are required during the aplastic phase, including antimicrobial prophylaxis, which will be continued for 6-12 months. Considering the strong induction property of aHSCT, most patients will not require additional therapy after the procedure. The bottom section depicts the proposed mechanisms of action of aHSCT: first, conditioning leads to depletion of pathogenic auto-immune cells, then homeostatic expansion of the T cell repertoire produces CD8+ and CD4+ T cells, followed (1-2 years from aHSCT) by the potentiation of immune regulation and in diversification of the T cell repertoire.

Figure 2. Number of aHSCT procedures per year, EBMT data.

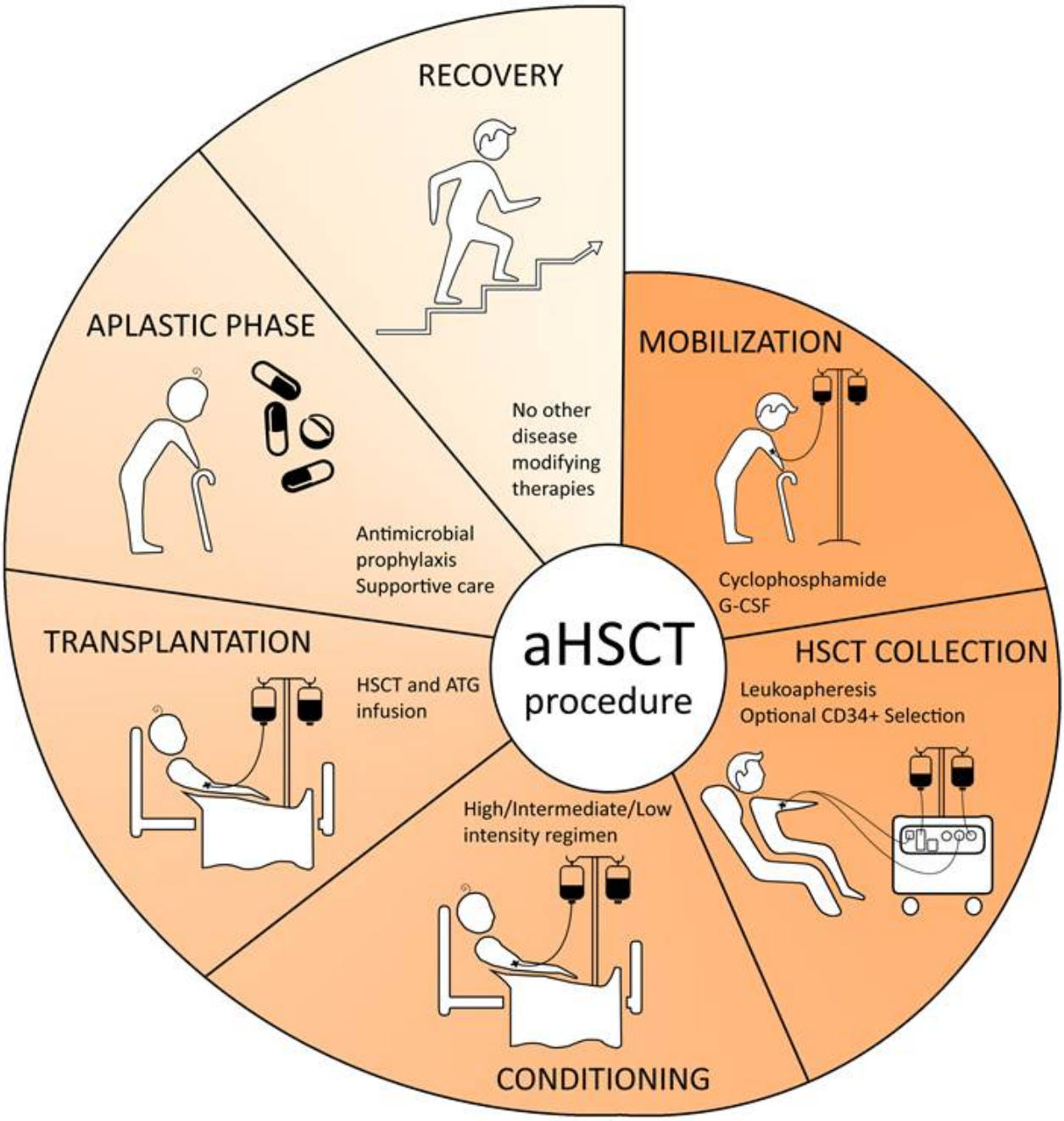
Total number of aHSCT to June 2017= 1004; *Partial data

Figure 3. Conditioning regimen per year, EBMT data.

Data available for 929 aHSCT; *Partial data

Table 1. The patient that can benefit from aHSCT

Figure 1


RECOVERY
APLASTIC PHASE
No other disease modifying therapies
Antimicrobial prophylaxis
Supportive care
MOBILIZATION
Cyclophosphamide
G-CSF
aHSCT procedure
TRANSPLANTATION
HSCT and ATG infusion
HSCT COLLECTION
Leukoapheresis
Optional CD34+ Selection
High/Intermediate/Low intensity regimen
CONDITIONING

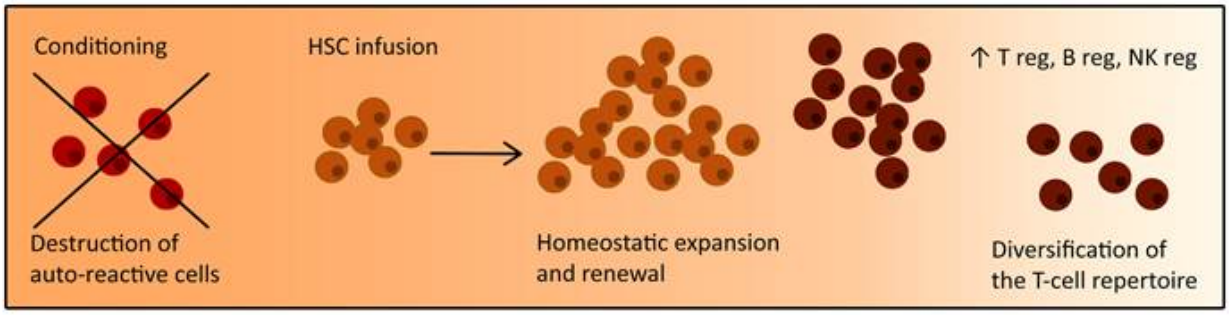

Conditioning
Destruction of auto-reactive cells
HSC infusion
Homeostatic expansion and renewal
↑ T reg, B reg, NK reg
Diversification of the T-cell repertoire

Figure 2

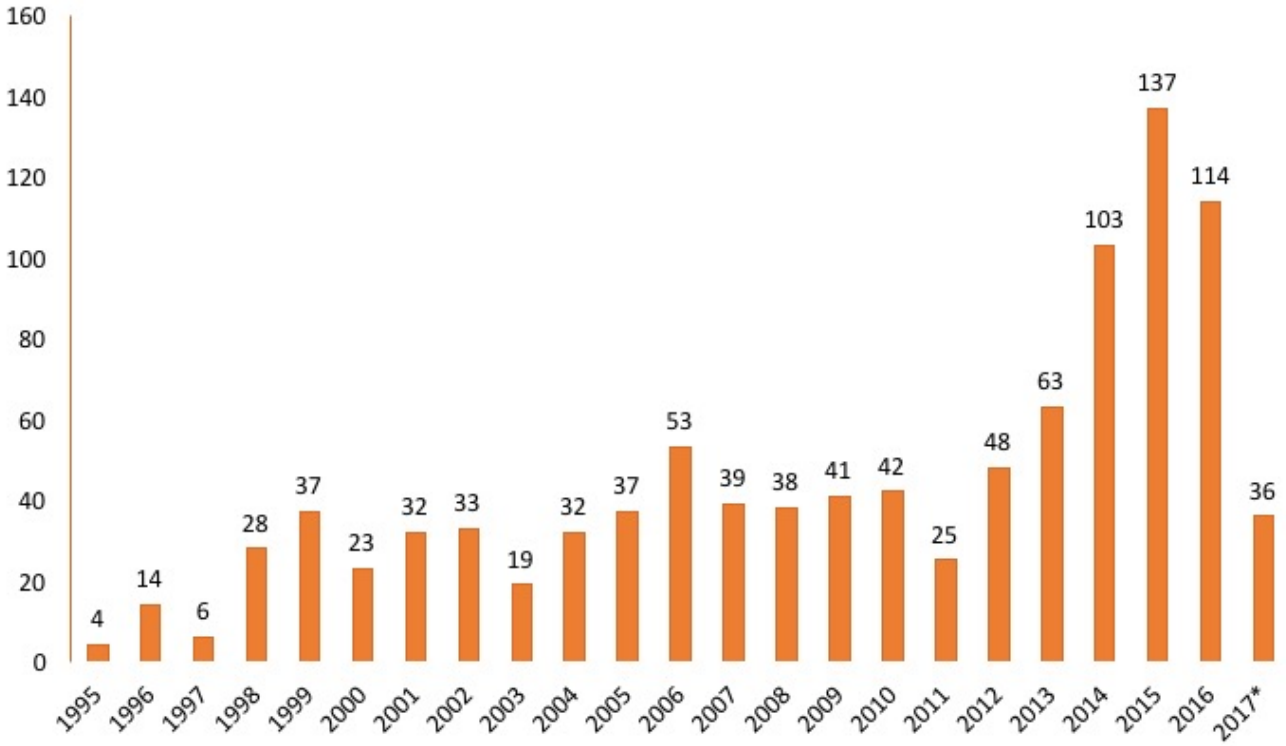

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Figure 3

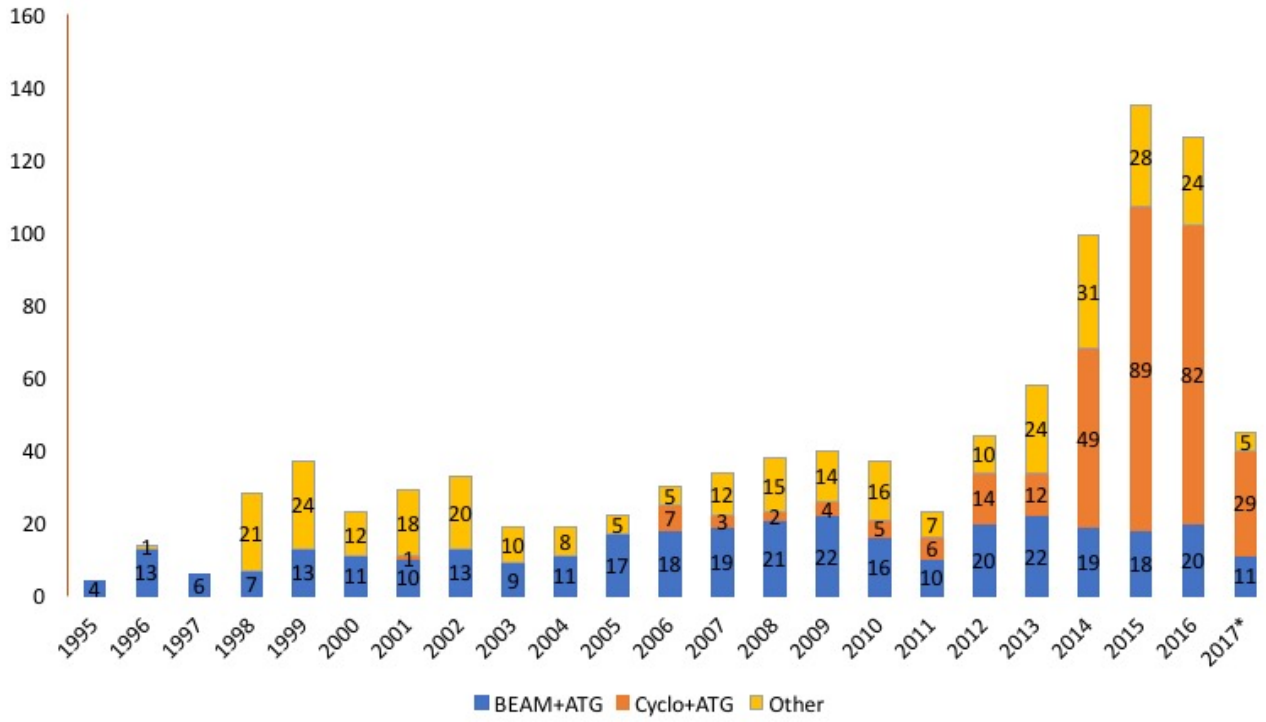

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BEAM+ATG
Cyclo+ATG
Other

Table 1

The patient that can benefit from aHSCT

An aggressive form of MS still in the RR phase of the disease

Failure of both first and a second line therapy or a second line therapy of high efficacy

Less than 45 years of age

Duration of disease under 10 years

Clinical signs of disease activity (relapses or disability progression)

MRI signs of activity (Gd enhancing areas or new T2 lesions)

EDSS not greater than 6.5 (but aHSCT can be considered if upper level of EDSS has only recently been reached and there are clinical and MRI signs of activity)

Absence of relevant comorbidities

Capacity to give a mindful informed consent

Progressive clinical course for a relatively short period of time with relapses or MRI signs of activity