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Autologous Haematopoietic Stem Cell Transplantation in Active Multiple Sclerosis: a Real-world Case Series

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Abstract

Objective: to examine outcomes in people with multiple sclerosis (PwMS) treated with autologous hematopoietic stem cell transplantation (AHSCT) in a real-world setting.
Methods: retrospective cohort study on PwMS treated with AHSCT at two centers in London, UK, consecutively between 2012 and 2019 who had ≥ 6 months of follow-up or died at any time. Primary outcomes were survival free of MS relapses, MRI new lesions and worsening of expanded disability status scale (EDSS). Adverse events rates were also examined.
Results: the cohort includes 120 PwMS; 52% had progressive MS (primary or secondary) and 48% had relapsing-remitting MS (RRMS). At baseline, the median expanded disability status scale (EDSS) was 6.0; 90% of the evaluable cases showed MRI activity in the 12 months preceding AHSCT. Median follow-up after AHSCT was 21 months (range 6–85). MS relapse-free survival was 93% at 2 years and 87% at 4 years after AHSCT. No new MRI lesions were detected in 90% of subjects at 2 years and 85% at 4 years. EDSS progression-free survival (PFS) was 75% at 2 years and 65% at 4 years. EBV reactivation and monoclonal paraproteinemia were associated with worse PFS. There were 3 transplant-related deaths within 100 days (2.5%), all following fluid overload and cardiac or respiratory failure.
Conclusions: efficacy outcomes of AHSCT in this real-world cohort are similar to those reported in more stringently selected clinical trial populations, although the risks may be higher.
Classification of evidence: this study is rated Class IV because of the uncontrolled, open-label design.

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Introduction

Autologous haematopoietic stem cell transplantation (AHSCT) has been increasingly used in recent years as treatment for multiple sclerosis (MS) and other severe autoimmune diseases, based on the hypothesis that the procedure may 'reset' the immune system and stop the inflammatory attack [1]. Clinical studies have demonstrated profound suppression of MS activity[2-4] and long-term clinical stabilisation[5]. Refinement of patient selection and treatment protocols has reduced treatment-related toxicity and mortality[6]. Clinically based-criteria have helped to define the optimal patient profile for whom AHSCT could be considered an appropriate treatment option[1]. A recent position statement from the American Society for Blood and Marrow Transplantation (ASBMT) and treatment guidelines from the European Bone Marrow Transplantation Society (EBMT) recommend considering AHSCT in selected people with (Pw)MS[7, 8]. Two small randomised controlled trials (RCTs) in relapsing MS provide proof of principle of higher efficacy of AHSCT compared to standard treatment on MRI [9] as well as clinical outcomes[10].

The objective of this observational study is to examine clinical efficacy outcomes and adverse events in a cohort of PwMS treated with AHSCT according to standard clinical practice at the two lead centers in London, UK. We also investigated the variables associated with the outcomes. Since symptomatic EBV reactivation increases risk of neurological sequelae, and the monitoring of Epstein-Barr Virus (EBV)-associated monoclonal paraprotein post-AHSCT has recently been recommended [11, 12], we also examined the relationship between EBV reactivation and monoclonal paraprotein formation in the AHSCT-treated MS cohort. Furthermore, we explored risk factors, including treatment-related morbidity, on efficacy outcomes.

Methods

Patient selection

Data was collected retrospectively on consecutive patients with (pw)MS who underwent AHSCT for treatment of MS between 15th February 2012 and January 2019 at Kings College Hospital (KCH) and from 12th April 2016 and January 2019 at Hammersmith Hospital (HH), London and had at least 6 months of follow-up or died at any time. All eligible patients were included in the retrospective analysis. Initially (2012-15) the indication for AHSCT was based on the agreement of at least two neurologists and one haematologist with expertise in

AHSCT that the treatment was in the patient's best interest in the absence of appropriate treatment alternatives. Eligibility criteria for treatment were formally defined in September 2015 to select patients with a profile consistent with inflammatory active MS, and, for relapsing-remitting MS (RRMS), treatment-refractory disease. The treatment inclusion and exclusion criteria for AHSCT that were implemented since September 2015 are summarized in Table 1. Eligibility had to be approved by a multi-disciplinary team (MDT) comprising neurologists and transplant haematologists from a number of independent centers in London and vicinity with established or developing expertise in AHSCT for MS. The MDT reviewed the clinical information collected in a referral form and discussed each case to assess eligibility by consensus. For some cases who did not strictly meet all the inclusion criteria but convincingly fulfilled the overall profile of eligibility (Table 1), the MDT made the clinical decision to offer AHSCT documenting the specific basis for the approval. For this study MRI reports were audited, and in some cases, scans were reviewed to examine any inconsistency. The patient flow, including the reasons for exclusion from treatment and from study analysis, is shown in the CONSORT diagram (Figure 1). The database was locked for analysis in July 2019 and statistical analysis was completed in December 2019.

Standard protocol approvals, registrations, and patient consents

All patients signed informed consent forms prior to initiating the treatment procedure and to give agreement to data collection and analysis. In line with standard practice all data was also reported to the European Blood and Marrow Transplant registry (EBMT) database.

Treatment procedure

Patients underwent AHSCT according to the approved protocols at the two centers. At Kings College Hospital (KCH) peripheral blood stem cells (PBSC) were mobilised following administration of cyclophosphamide (CY) 4g/m2 over 2 days (62 patients) or CY 2g/m2 over 1 day (3 patients) following a modification in the protocol in November 2018, with granulocyte colony-stimulating factor (G-CSF; 5 μg/kg subcutaneously) for 7 days until leukapheresis. Conditioning was performed using standard protocol of CY (50mg/kg for 4 days) and rabbit anti-thymocyte globulin (rATG, 2.5mg/kg/day for 3 days, total dose 7.5mg/kg) for *in-vivo* lymphodepletion followed by stem cell infusion. One patient, at the start of the KCH programme, was conditioned with a carmustine/ etoposide/ cytarabine/ melphalan regimen plus an equivalent dose of rATG (BEAM-ATG) prior to stem cell infusion. The median CD34 stem cell dose returned was 7.17 $x10^6$/kg (range 4.0-17.1

x10^6/kg). At Hammersmith Hospital (HH) peripheral blood stem cells were mobilized (52 patients) with CY 2g/m2 and daily GCSF (5 μg/kg subcutaneously) starting from day +3 from CY until leukapheresis. Transplant conditioning employed CY (50mg/kg for 4 days) and rATG (2mg/kg/day, total dose: 6mg/kg in 48 cases, or 2.5mg/kg/day, total dose: 7.5mg/kg in 4 cases following a protocol change in August 2018). One patient was mobilised with CY 1 g/m2 followed by G-CSF and plerixafor (2 doses) and conditioned with BEAM-ATG due to intolerance of cyclophosphamide. At both centers, the collected product was not CD34 selected or otherwise manipulated ex-vivo. The median CD34+ cell dose in the cryopreserved PBSC product was 7.75 x10^6/kg (range 2.2-24.3 x10^6/kg). After conditioning and reinfusion of the autologous PBSC product, G-CSF was administered starting from day +7 post AHCST until engraftment to half of the patients from KCH and to all the patients from HH.

Supportive medical treatments (including platelet and packed red cell transfusions, antimicrobial prophylaxis, dietetics and physiotherapy support) were provided during the inpatient period as per standard institutional protocols. Psychological support was available for all patients.

Clinical and MRI assessments

Clinical assessments were performed according to standard clinical practice at the centers. To qualify as events, relapses had to be confirmed by a clinician and recorded in the case notes. Changes in the EDSS scores in the short term before/after AHSCT were evaluated over the 24-month period comprising from -12 months pre-AHSCT to +12 months post-treatment. EDSS progression was defined as an increase in EDSS by 0.5 point if baseline EDSS was ≥ 6, and by 1 point if baseline EDSS < 6. MRI scans were performed as per local protocols. MRI analysis including new lesion counts was based on the neuroradiology clinical reports. Patient underwent the first post-transplant MRI scan on average 6 months (range 1-13) after AHSCT. For analyses requiring re-baselining of MRI, the first post-transplant MRI as described above was considered the new baseline scan and post-AHSCT MRI activity was evaluated by comparing with this one the subsequent scans obtained during follow-up.

Statistics

The database of transplants and outcomes was built in Microsoft Excel 2016 and statistical analyses were performed using IBM SPSS Statistics version 24.0. Patient characteristics are presented as medians (with inter-quartile ranges; IQR) for data with non-normal distribution.

Comparisons of baseline characteristics were performed using Mann-Whitney U test, Fisher's exact test, or Chi-squared test for trend as appropriate. The changes in EDSS scores between the 12 months preceding treatment and baseline (pre-mobilisation), and between baseline and 12 months post-AHSCT, were calculated. Pre- and post-transplant relapse rates were compared by a Wilcoxon test for paired data. Time to first relapse and to first MRI activity were studied by Kaplan Meier (KM) survival curves and compared between RR and progressive MS patients by the log-rank test. Confirmed disability worsening was defined when the EDSS changes defined above were confirmed at 6 months and was assessed by KM curves at univariate analysis and by a Cox model for multivariate analysis. The variables included in the analysis were age, gender, progressive vs relapsing MS subtype, total number of previous DMT, number high-efficacy DMT, EBV reactivation, time of EBV reactivation, highest EBV copy number, detection of serum paraprotein (paraproteinemia). The effect of baseline variables on the risk of developing paraproteinemia was studied by a multivariate Cox model.

Data availability

Any data not published within the article will be shared in anonymized form upon request from a qualified investigator.

Results

Pre-AHSCT MS disease characteristics

One hundred and twenty PwMS were included in the study and their demographic and clinical features at baseline are presented in Table 2. Sixty-two (52%) of cases had a progressive MS phenotype (primary or secondary progressive). At acceptance for AHSCT, 90% of the patients for whom data were available had evidence of MRI activity in the preceding 12 months, demonstrated by new T2 or gadolinium-enhancing lesions (Table 2); 85% of the evaluable patients had developed one or more T2 lesions and 59% had a gadolinium-enhancing lesion on MRI.

The cohort had mean age 42.3 years and median EDSS 6.0. There was no difference in the mean age or EDSS score of the PwMS treated at the two centers. RRMS patients had received an average of 2.3 previous disease-modifying treatments (SD 1.3) and SPMS 1.6 treatments (SD 1.1). Seventy (58%) patients had been treated prior to AHSCT with one or more DMT regarded as high efficacy (alemtuzumab, mitoxantrone, natalizumab,

ocrelizumab), amongst which 19 patients had alemtuzumab and 58 had natalizumab (7 had both). The median duration of follow-up after AHSCT, with the autologous graft infusion being day 0, was 21 months (range 6 – 85 months).

AHSCT admission and engraftment

The median duration of hospital inpatient admission was 22 days (mean 25.3±9.7 SD). Median time to neutrophil engraftment was 12 days (mean 12.3±9.2.55 SD). There were center differences: the duration of inpatient admission was longer at KCH (median 26 days; mean 27.5±9.7 days) than at HH (median 20 days, p=0.00097, Wilcoxon test [w=2786.5]; mean 21.7±8.0 days, p=0.00052, t-test). The mean time to neutrophil engraftment was also longer at KCH (median 13 days; mean 13.2±2.5 days; 1 missing) than at HH (median 11 days, p=0.00072, Wilcoxon test [w=2695.5]; mean 11.1±2.1 days, p=0.0064, t-test; 1 missing).

Neurological outcomes after AHSCT

Relapses. The relapse rate in the study population was compared before and after AHSCT. The overall annualised relapse rate dropped from 0.46 ± 0.57 in the 2 years prior to AHSCT to 0.08 ± 0.38 in the post-AHSCT follow-up at 4 years (Figure 2A; $p<0.001$, Wilcoxon test). Ninety-three percent of all cases were free from relapse at 2 years after AHSCT and 87% at 4 years after AHSCT. Relapses post-AHSCT occurred only in RRMS patients, and in that subgroup relapse-free survival was 87% at 2 years and 77% at 4 years.

MRI lesions. Annualised MRI new T2 lesion numbers without re-baselining were compared between the 12 months prior and the available follow-up of up to 4 years after AHSCT; the last MRI was performed after mean 22 months (SD 17.6). There was a significant reduction in new T2 lesions after AHSCT in the whole evaluable population ($p<0.0001$, chi-square test; Figure 2B). At survival analysis, 90% of subjects were free of new lesions at 2 years and 85% at 4 years. In contrast to relapses, there was no difference in the development of new T2 lesions between the RR and progressive MS subgroups (data not shown).

Neurological disability. To firstly evaluate the short-term evolution of neurological disability, the change in EDSS scores between -12 months prior to AHSCT and baseline (pre-mobilisation) was compared with the change between baseline and +12 months post-AHSCT.

In the whole population analysis, the average EDSS score change was +0.25 during the 12 months pre-AHSCT and +0.02 in the 12 months post-therapy (Figure 2C). In the subgroup analysis a clear difference emerged, with the RRMS subjects showing on average a small improvement 12 months post-AHSCT compared to baseline; and in contrast the progressive MS subgroup showing further deterioration (RRMS subgroup: EDSS score change +0.39 pre-AHSCT and -0.17 post-transplant; progressive subgroup: +0.11 pre-AHSCT and +0.24 post-AHSCT; $p<0.05$; Figure 2D).

We next examined the evolution of disability in the longer term, assessed as EDSS worsening confirmed at 6 months, or death during the entire follow-up. Seventy-five percent of the whole population did not have confirmed EDSS worsening at 2 years; the proportion decreased to 65% at 4 years' follow-up. There was no significant difference between the RRMS and progressive MS subgroups ($p=0.487$, log-rank; Figure 3A). In the RRMS subgroup, 13 subjects (including one death) had confirmed disability worsening. In the progressive MS subgroup, 15 subjects had confirmed disability worsening, which were 8 SPMS subjects and 7 (including 2 deaths) PPMS subjects. Using confirmed EDSS worsening as an outcome we explored factors that could predict failure of AHSCT. Demographic, disease-phenotype, number and type of previous treatments; and adverse events were included as variables in the analyses. Univariate and multivariate Cox analyses identified high (>5g/L) paraproteinemia as the only significant variable associated with confirmed EDSS progression over 4 years (odds ratio 1.07 [1.03, 1.10], $p<0.001$; KM plot in Figure 3B). Further modelling indicated that paraprotein levels were not predictive of relapses (odds ratio 0.96 [0.76, 1.20], $p=0.67$) or new T2 lesions on re-baselined MRI for up to 4 years (0.93 [0.72, 1.21]).

No evidence of disease activity (NEDA). NEDA has been increasingly used to demonstrate effects of treatment in MS. NEDA fulfilling the three endpoints, no relapses, no new MRI lesions and no worsening of neurological disability is denominated NEDA-3. In this study, data enabling calculation of NEDA-3 (without MRI re-baselining) were available for the majority of cases (107/120, including the 3 deaths, considered as events in the analysis). The survival analysis of NEDA-3 and its components in the whole cohort is shown in Figure 3C. The survival analysis of time to loss of NEDA-3 in the RRMS, SPMS and PPMS subgroups is presented in Figure 3D; the yearly NEDA-3 rates to year 4 post-AHSCT are supplied in Table 3.

Adverse events post-AHSCT

Early complications. Almost 90% of the treated patients experienced at least one early complication after AHSCT. There were differences in the rates of adverse events between the two centers, described in Table 4. Post mobilisation, fever/positive culture/neutropenia and readmission rates were higher in KCH cohort, possibly related to the higher CY dose (4g/m2 vs 2g/m2). For conditioning/HSCT the KCH cohort experienced more frequently fever, diarrhoea and EBV reactivation, whereas severe nausea and vomiting was higher in HH.

Transplant-related mortality (TRM). There were three deaths (2.5%) within 100 days from transplant. Two of the subjects had PPMS (one female and one male, age at death 58 and 42 years, respectively) and one had RRMS (female, age 51 years), and all three had EDSS 6.5 at baseline. Further clinical and treatment details are available from Dryad (Table e1): https://doi.org/10.5061/dryad.k0p2ngf82. Two deaths occurred the day prior to the planned autologous stem cell infusion; in both cases the primary cause was cardiac arrest, secondary causes were recent pulmonary oedema in one case and blood electrolytes abnormalities in the other. In the third case, death occurred 32 days after stem cells had been re-infused and was caused by acute respiratory distress syndrome secondary to chest infection and sepsis. To investigate the pathophysiological basis of these events, a detailed retrospective analysis was undertaken in the whole treated cohort. With potential relevance to the mortality events, fluid overload (defined by >5% weight gain +/- peripheral or central oedema and need for additional diuretics), was recorded in 78/118 patients (66%) and presented at a mean of 3 days (+/- 2.2 days SD) post first dose of ATG. Further data on fluid overload are available from Dryad (Table e2): https://doi.org/10.5061/dryad.k0p2ngf82.

Viral reactivations and paraprotein formation in the patient cohort. CMV reactivation was detected in 26 cases and pre-emptive treatment with valganciclovir or ganciclovir was required in 12/26 (46%) cases with no CMV disease observed. EBV serological status prior to receiving AHSCT was assessed in a subset of 66/120 patients (55%), mainly due to different testing policies in KCH, and HH and was positive in all cases apart from 1 indeterminate and 1 negative subject. In a subset of 85 subjects EBV DNA copy numbers in blood and paraprotein were measured regularly by standardised lab techniques at both sites. EBV reactivation (defined by viraemia >10 DNA copies/ml consecutively, as previously described [11]) was demonstrated in 87/109 (80%; 11 missing/not tested) of subjects post AHSCT. Of the 87 EBV reactivation cases, 20 (23%) cases were treated with Rituximab,

receiving a median of 4 courses (range 2–4). Hypogammaglobulinemia was detected in 7/20 Rituximab-treated cases. Using a stepwise multivariate Cox analysis, the following variables: lower baseline EDSS (p=0.018), symptoms consistent with viral reactivation(p=0.001), lower EBV DNAemia at date of 1st reactivation (<500k copies/mL; p=0.036), peak EBV DNAemia (>500k copies/mL; p=0.017) were associated with risk of developing paraprotein. The multivariate analysis is available on Dryad (Table e3): https://doi.org/10.5061/dryad.k0p2ngf82).

Late adverse events. Seven patients (5.8%) developed secondary autoimmune diseases (6 thyroiditis and one case of autoimmune thrombocytopenia) after a median of 17.5 months post-transplant (range 6 – 36). One of these patients had previously been treated with Alemtuzumab. One patient was diagnosed with melanoma 16 months after AHSCT; this patient had previously received 40 four-weekly doses of Natalizumab. Apart from these individual cases, there was no association of prior DMT with adverse events.

Discussion

Increasing evidence supports considering AHSCT as a treatment for patients with aggressive, inflammatory forms of MS[7, 8]. Studies in RRMS have demonstrated that AHSCT markedly reduces relapse rates, lesion development and improves disability[1]. Two RCTs have been reported with encouraging results[9, 10] and more definitive RCTs comparing AHSCT with contemporary therapies including high-efficacy biologicals are underway. The ASBMT and the EBMT recommend AHSCT as a clinical option for treatment of patients with active relapsing MS, particularly when standard therapy has failed [7, 8]. The role of AHSCT is less clear in progressive MS, with poor outcomes in subjects with advanced disease [13, 14] though some evidence led to asking the question whether amongst patients with earlier progressive disease and ongoing inflammatory MS, the rate of progression of disability might be attenuated post-therapy[5]. Regarding the safety of AHSCT, current data suggest that the treatment-related risk is higher than standard disease modifying therapy but is largely front-loaded as opposed to the poorly understood long-term risk of chronic immune suppression

induced by biological therapies; and the risk may be partly offset by higher efficacy against neuro-inflammation [15].

In this study we report the results of a retrospective analysis of data from 120 patients treated with AHSCT as part of standard care. Importantly and different from recent and ongoing clinical trials, our cohort included a substantial proportion (~50%) of patients with progressive MS, and any type and number of prior treatments was allowed, with the exclusion of total lymphoid irradiation and autologous or allogeneic haematopoietic stem cell transplantation. Selection of patients who could be offered AHSCT evolved during the survey period and became more restrictive with the introduction in September 2015 of an eligibility profile that included upper limits to age (65 years), disease duration (15 years) and neurological disability (EDSS 6.5). The criteria also required evidence of inflammatory disease activity by MRI demonstrating new T2 lesions and/or the presence of gadolinium enhancement. Even with these refinements, the patient cohort was less stringently selected than in most trials of treatments in MS.

As efficacy outcomes we examined MS relapses, MRI and EDSS evolution. Relapses were significantly suppressed after AHSCT compared to pre-transplant. Of interest, in this real-world treated cohort, the relapse-free proportion of RRMS subjects (~80% for up to 4 years post-HSCT) was not substantially different than those achieved in clinical trials[4, 10, 16]. In addition, MRI demonstrated almost complete suppression of new lesion development post-AHSCT in both the RRMS and progressive MS subgroups. Because persistence or reactivation of MRI would be expected more frequently in patients with RRMS, the results are consistent with a 'flooring' effect in both subgroups, reflecting high efficacy of AHSCT against MRI-detectable inflammation in the CNS, as previously demonstrated [17]. In regard to neurological disability, the clinical relevance of ongoing inflammation in RRMS patients and its radical suppression after AHSCT are the most plausible reasons for the improvement of neurological function we observe in the RRMS subgroup after AHSCT, consistent with previous reports [2, 10].

In the longer term, freedom from EDSS worsening (75% at 2 years and 65% at 4 years) was encouraging and rates were similar in the RRMS and progressive MS subgroups. This observation does not demonstrate a benefit of AHSCT in patients with progressive MS, but it does suggest a question. Following the licensing of DMTs for progressive MS, including ocrelizumab for PPMS and Siponimod for SPMS, RCT could be designed to compare

efficacy, safety and cost-effectiveness of AHSCT with approved therapy in subjects with inflammatory-active, progressive MS forms.

We investigated factors associated with progression of EDSS and the analysis revealed that high paraproteinemia (≥5 g/L) was a significant factor for EDSS progression together with symptomatic EBV reactivation. Particularly in light of the putative association of EBV in the pathogenesis of MS[18], we speculate that in some subjects the reactivation of EBV with high viral loads post-AHSCT may predispose and contribute, together with other as yet unknown susceptibility factors, to continued worsening post-treatment[11]. Development of monoclonal paraprotein could be of interest as a marker of immune dysregulation post EBV reactivation and its potential impact on neurological disability post AHSCT, as also observed in our cohort, and monitoring is now recommended[11, 12].

We examined NEDA and the rates of 65% at 2 years and 53% at 4 years after AHSCT are slightly below the ranges reported in a pooled analysis of AHSCT trials, where the proportion of NEDA subjects was 83.4% (range 70%–92%) at 2 years after AHSCT and 67% (range 59%–70%) at 5 years [19]. Even in our cohort, of which half were RRMS patients who had failed previous treatments including high efficacy biologics, the year 2 NEDA rate of 65% was better than any other DMT, among which even the most effective did not exceed 50%[1]. Of course these are indirect comparisons and should be used only to generate hypotheses.

In the study cohort 3 deaths were recorded, which were treatment-related and constitute a higher TRM than reported in recent cases series and in any published report that included only patients with RRMS. Two of the patients who died had PPMS; all three were at the upper limit of allowed disability, with EDSS 6.5, were middle aged (42, 51 and 58 years) and had comorbidities, though minor. All 3 received the same cyclophosphamide conditioning regimen, with some variation of ATG dosage; data available from Dryad (Table e1): https://doi.org/10.5061/dryad.k0p2ngf82. Higher baseline EDSS levels and a lower proportion of cases with RRMS have previously been reported as two factors associated with TRM [6]. In the same meta-analysis, older age and the conditioning regimen intensity were also considered and were not confirmed as significant in the multi-variate analysis [19]. In a multi-center cohort study of long-term outcomes, higher baseline EDSS was found to be independently associated with worse overall survival[20]. These associations support the notion that patients with higher EDSS and progressive MS forms are at higher risk of mortality during/after AHSCT.

In the evaluation of the causes of the 3 deaths in our cohort, cardiac adverse events and fluid overload were identified as factors, even though none of these patients was shown to have any impaired cardio-respiratory function at baseline pre-AHSCT. Fluid overload is an important side effect of conditioning that has not emerged clearly from earlier studies of AHSCT for MS but has recently been identified in a cancer population as a factor contributing to HSCT outcome [21]. In our cohort of PwMS a high incidence of clinically significant fluid overload was seen, likely related to conditioning regimen used with ATG; and to put in context, this was significantly higher incidence when we compared to a cohort of acquired Aplastic Anaemia (AA) patients (n=40) at KCH, which is predominantly an autoimmune disorder and in which, after treatment with horse ATG 40mg/kg/day for 4 days and cyclosporin A (CsA; 5mg/kg daily dose), only 22% developed significant fluid overload post ATG ($p<0.001$) despite the equivalent immunosuppressive and fluid retention properties of treatment with ATG and CsA and the higher median age (52 years) of the AA patients. The reason for the higher incidence of fluid overload in the MS cohort is unclear. We speculate that previous cardiotoxic previous DMT (mitoxantrone and cyclophosphamide), use of rATG formulation in the MS AHSCT procedures with added high dose steroids to reduce risk of ATG reaction; and potentially a sub-clinical form of neuro-autonomic dysfunction in MS patients might be risk factors. On the latter hypothesis, because no significant cardiac comorbidities were identified in pre-treatment standard organ assessments in this cohort, we suggest that a more detailed cardiac (e.g. cardiac injury biomarkers monitoring, stress ECHO and/or cardiac MRI for detailed structural and functional assessments) and autonomic evaluations (R-R and tilt test ECG) could help identify PwMS at excess risk. Compared to cancer, any TRM of AHSCT in MS is regarded as less acceptable because in the majority of patients, untreated or ineffectively treated MS is not immediately life-threatening. At the population level, in PwMS survival is reduced on average by 7 years[22]. However, at an individual level the reduction in life expectancy could be considerably more severe particularly in subjects with aggressive forms of MS, such as those included in this cohort. In addition, standard therapy is not free from risks and although the risk in the short-term is almost certainly lower, safety concerns have emerged from longer term follow-up prompting withdrawal and limitations of use of licensed MS therapies[23, 24]. Early non-TRM complications, such as fever, neutropenia, diarrhoea were common in this AHSCT cohort, as expected. Some of the differences observed in adverse event rates between the two centers could be related to the higher ATG dose at KCH (7.5 mg/Kg vs 6 mg/Kg at HH) where higher incidence of fever, diarrhoea and EBV reactivation were observed. Among late

adverse events, secondary autoimmune disease was observed at a rate similar to the ~5% reported in a larger multicenter cohort study [20].

This study has several limitations, including its retrospective design, some variation in treatment protocols, a relatively short follow-up and the lack of a treatment control arm. The heterogeneity of clinical phenotypes, age, disease duration and EDSS level is also a challenge in the analysis of outcome data, although such heterogeneity also provides the opportunity to explore AHSCT outcomes in a broader patient population and examine factors potentially associated with the outcomes, which could not be revealed in the selected populations usually enrolled in clinical trials.

Against the limitations, some important conclusions can be made. The results demonstrate the feasibility of this treatment strategy and provide new information on the potential benefits and risks in a real-world social healthcare setting. Efficacy outcomes similar to clinical trials can also be achieved in real-life patient populations, although risks can be higher especially in patients with more advanced disease. Furthermore, our study exemplifies a model of service development in the NHS where innovation can be initiated via multidisciplinary team collaboration even with minimal funding. Scaling up, long-term sustainability and optimisation of patient pathways, however, require adequate resources.

Appendix 1: Authors

Name	Location	Contribution
Richard Nicholas, PhD	Imperial College London, London, UK	Designed and conceptualized study; analyzed the data; Interpreted the data; drafted the manuscript
Elijah Rhone, PhD	King's College Hospital, London, UK	Major role in the acquisition of data; analyzed the data; Interpreted the data; revised the manuscript for intellectual content
Alice Mariottini, MD	University of Florence, Florence, Italy	Major role in the acquisition of data Interpreted the data; revised the manuscript for intellectual content
Eli Silber, MD	King's College Hospital, London, UK	Acquisition of funding for research. Major role in acquisition of data Interpreted the data; revised the manuscript for intellectual content
Omar Malik, PhD	Imperial College London, London, UK	Major role in the acquisition of data; revised the manuscript for intellectual content
Victoria Singh-Curry, PhD	Imperial College London, London, UK	Major role in the acquisition of data Interpreted the data; revised the manuscript for intellectual content
Ben Turner, PhD	Barts Health NHS Trust, London, UK	Major role in the acquisition of data Interpreted the data; revised the manuscript for intellectual content

Antonio Scalfari, PhD[1]	Imperial College London, London, UK	Interpreted the data; revised the manuscript for intellectual content
Olga Ciccarelli, PhD	UCL Queen Square Institute of Neurology, London, United Kingdom	Major role in the acquisition of data Interpreted the data; revised the manuscript for intellectual content
Maria Pia Sormani, PhD	University of Genova, Genova, Italy	Analyzed the data; Interpreted the data; revised the manuscript for intellectual content
Eduardo Olavarria, PhD	Imperial College London, London, UK	Major role in the acquisition of data Interpreted the data; revised the manuscript for intellectual content
Varun Mehra, MD	King's College Hospital NHS Foundation Trust, London, UK	Major role in the acquisition of data; analysed the data; Interpreted the data; revised the manuscript for intellectual content
Ian Gabriel, PhD	Imperial College London, London, UK	Major role in the acquisition of data Interpreted the data; revised the manuscript for intellectual content
Majid A. Kazmi, PhD	King's College Hospital NHS Foundation Trust, London, UK	Major role in the acquisition of data Interpreted the data; revised the manuscript for intellectual content

Paolo A Muraro, PhD	Imperial College London, London, UK	Designed and conceptualized the study; Interpreted the data; drafted the manuscript

Appendix 2: Coinvestigators

Name	Location	Role	Contribution
Victoria Williams, PhD	Kings Health NHS Trust London, UK	Neurologist	Referred patients, contributed to clinical decisions, reviewed patient pathway documents
Leonora Fisniku, PhD	BRIGHTON AND SUSSEX UNIVERSITY HOSPITALS NHS TRUST	Neurologist	Referred patients, contributed to clinical decisions, reviewed patient pathway documents
Ashwini Nandoskar, MD	Imperial NHS Trust, London, UK	Neurologist	Referred patients, contributed to clinical decisions, reviewed patient pathway documents
Peter Brex, PhD	Kings Health NHS Trust London, UK	Neurologist	Reviewed patient pathway documents
Wallace Brownlee, PhD	University College Hospitals NHS Trust, London, UK	Neurologist	Referred patients, contributed to clinical decisions, reviewed patient pathway documents

Waqar Rashid, PhD	St George's University Hospital Foundation Trust, London UK	Neurologist	Referred patients, contributed to clinical decisions, reviewed patient pathway documents
Khaled Abdel-Aziz PhD	St George's University Hospital Foundation Trust, London UK	Neurologist	Referred patients, contributed to clinical decisions, reviewed patient pathway documents
Victoria Potter, PhD	Kings Health NHS Trust London, UK	Haematologist	Contributed to clinical decisions, treatment protocols, patient pathway documents and organisation of transplant service
Jeff Davies, PhD	Barts Health and QMUL, London, UK	Haematologist	Contributed to clinical decisions, treatment protocols, patient pathway documents
Matthias Klammer, PhD	St. Georges NHS Trust, London, UK	Haematologist	Contributed to clinical decisions, treatment protocols, patient pathway documents
Heather Oakervee, PhD	Barts Health and QMUL, London, UK	Haematologist	Contributed to clinical decisions, treatment protocols, patient pathway documents
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Table 1. Profile of eligibility for treatment with AHSCT[1]

Inclusion criteria
1) Diagnosis of MS according to McDonald Criteria (REF[25-27]); a) for primary progressive MS the presence of oligoclonal bands in the cerebrospinal fluid was required. 2) Age 18-65 years; 3) Disease duration from diagnosis of ≤15 years; 4) Expanded disability status scale (EDSS) score between 0 and 6.5; 5) 'Inflammatory active MS' as defined by ≥1 gadolinium enhancing (Gd+) (>3mm) lesion (off steroids for one month) or ≥2 new T2 lesions on MRI within the last 12 months; a) subjects with RRMS had to experience treatment failure to at least one licensed DMT of high efficacy[2], defined as evidence of relapse, MRI activity, or EDSS increase after being on high efficacy DMT for at least 6 months.
Exclusion criteria
1. Eligibility for an ethically approved clinical trial where AHSCT is offered as one of the treatment arms; 2. Inability to adequately understand risk and benefits of AHSCT and give written informed consent; 3. Prior treatment with total lymphoid irradiation and autologous or allogeneic haematopoietic stem cell transplantation.

[1] Abbreviations: AHSCT, autologous hematopoietic stem cell transplantation; DMT, disease-modifying treatment; EDSS, expanded disability status scale; MS, multiple sclerosis; RRMS, relapsing remitting multiple sclerosis.

[2] High efficacy DMT: alemtuzumab, mitoxantrone, natalizumab, ocrelizumab.

Table 2. Demographics and disease characteristics at baseline

Patient and disease characteristics	Evaluable number	Total cohort	Relapsing Remitting MS	Secondary Progressive	Primary Progressive
Disease type: n (%)	120	120 (100%)	58 (48%)	40 (33%)	22 (18%)
Gender female: n (%)	120	58 (48%)	33 (57%)	19 (47%)	6 (27%)
Age (years): mean ±SD	120	42.3 ± 8.8	40.2 ± 8.7	43.6 ± 8.4	45.8 ± 8.7
Disease duration from diagnosis (years): mean ±SD	118	8.9 ± 5.3	9.2 ± 6.0	9.8 ± 4.5	6.6 ± 4.1
Baseline EDSS: median (IQR)	120	6.0 (5.5 – 6.5)	6.0 (4.0 – 6.0)	6.5 (6.0 - 6.5)	6.0 (4.87 – 6.5)
In the 2 years preceding HSCT: relapse rate, total number of relapses	116	0.48, 111	0.71, 80	0.36, 29	0.05, 2
Number of previous treatments: mean ±SD	120	1.7 ± 1.4	2.3 ± 1.3	1.6 ± 1.1	0.14 ± 0.3
Number of patients who tried high efficacy DMT [1]	120	70 (58%)	51 (88%)	18 (45%)	1 (4%)
Patients with new T2 lesions, n (%)	103	88 (85%)	41 (77%)	30 (91%)	17 (100%)
In the 12 months preceding HSCT: new T2 lesions: n of patients	103	0 lesion: 15 1 lesion: 25 ≥2 lesions: 63	0 lesion: 12 1 lesion: 12 ≥2 lesions: 29	0 lesion: 3 1 lesion: 5 ≥2 lesions: 25	0 lesion: 0 1 lesion: 8 ≥2 lesions: 9
Patients with GAD+ lesions in the preceding year, n (%)	95	56 (59%)	26 (58%)	16 (53%)	14 (70%)
Patients with new T2 and/or GAD+ lesions in the preceding year, n (%)	113	102 (90%)	46 (84%)	34 (94%)	22 (100%)

[1] High efficacy DMT: alemtuzumab, mitoxantrone, natalizumab, ocrelizumab.

Table 3. NEDA-3 rates

	Year 1	Year 2	Year 3	Year 4
Whole cohort	77%	65%	59%	53%
n at risk	107	67	30	12
RRMS[1]	79%	63%	58%	48%
n at risk	52	34	18	7
SPMS	82%	72%	72%	72%
n at risk	35	24	10	5
PPMS	61%	61%	20%	0%
n at risk	20	9	2	0

[1] Abbreviations: n, number; RRMS, relapsing remitting multiple sclerosis; PPMS, primary progressive multiple sclerosis; SPMS, secondary progressive multiple sclerosis. NEDA-3, no evidence of disease activity-3

Table 4. Side effects of mobilisation and conditioning treatments at the two study centers

n (%) /mean ± SD[1]	Kings (n=65)	Hammersmith (n=53)	P[2] [3]
Mobilisation			
Fever / Positive culture	3 (4.6) / 5 (7.7)	1 (1.9)/ 0	0.390
Fever OR +ve culture OR neutropenia	9 (13.8) 0	1 (1.9) 0	0.019*
Diarrhoea and vomit	3 (4.6)	4 (7.5)	0.387
Fluid Overload	1 (1.5)	0	0.551
Neurological worsening[4]	1 (1.5)	2 (3.8)	0.423
Thromboembolism	0	0	-
Readmission / LOS in days	25 (38) / 5.4±2.6	10 (19) / 3±1.9	0.016*
Conditioning/HSCT			
Fever / Positive culture conditioning[5]	62 (95.4) / 17(26.2)	42 (79.2) / 6 (11.3)	0.008* / 0.074
Fever / Positive culture HSCT[6]	48 (73.8) / 31 (47.7)	45 (84.9) / 15 (28.3)	0.216 / 0.050
Positive culture HSCT Gram +ve/Gram-ve (both)	10/23 (2)	5/11 (1)	0.493 / 0.124 (0.577)
Fever & +ve culture & neutropenia conditioning	2 (3)	1 (1.8)	0.577
Fever & +ve culture & neutropenia HSCT	30 (46.1)	15 (28.3)	0.073
Diarrhoea	52 (80)	31 (58.5)	0.019*
Severe nausea/vomiting	9 (13.8)	18 (34)	0.018*
Fluid Overload	51 (78.5)	41 (77.4)	0.920
Mucositis	21 (32.3)	8 (15.1)	0.052
Rash	21 (32.3)	11 (20.8)	0.256
Skin	12 (18.5)	8 (15)	0.807
Deranged LFTs	5 (7.7)	10 (18.9)	0.124
Neurological worsening[&]	22 (33.8)	20 (37.7)	0.806
Thromboembolism	1 (1.5)	4 (7.5)	0.125
ITU admission / LOS days	6(9) / 8.2±8.4	2(3.7) / 11 ± 7.1	0.213
EBV reactivation / not tested	58 (89.2) / 5	29 (54.7) / 4	<0.0001**

[1] Of the 120 cases in the cohort, 2 patients were treated at a different unit within KCH initially and since no data was available on their inpatient course, they were excluded from this analysis (n=118)

[2]

[3] P values were calculated with Chi square/Fisher Exact test; *signifies P<0.05 ; ** signifies P<0.01

[4] Neurological worsening was transient and related to fever or sepsis in most cases; Delirium was reported in two patients. In one patient the worsening was severe and a brain MRI was undertaken (uncertain small new lesion).

[5] "conditioning" indicates adverse event reported during administration of the conditioning chemotherapy

[6] "HSCT" indicates adverse event reported during or after infusion of the autologous PBSC graft (day 0)

Figure Legends

Figure 1. CONSORT diagram of subject enrolment in the study. The patient disposition is shown in the flow chart with information available about the reasons for exclusion from treatment with AHSCT or from the cohort analysis**.**

Figure 2. MS disease outcomes: relapse rate, MRI new lesions and change in EDSS score. A. Annualized relapse rate over two years prior to AHSCT and over up to 4 years after AHSCT demonstrates a significant reduction (mean±95 CI, $p<0.001$). B. New MRI T2 lesion development over one year before and over up to 4 years were categorized and the comparison demonstrated a significant reduction (chi square, $p<0.001$). C. Change in EDSS score before and after AHSCT over -12 months pre-transplant / +12 months post-transplant compared to treatment baseline (day 0 being transplant day), examined in the total population. D. Subgroup analysis of change in EDSS shows a difference in the relapsing vs progressive MS subgroups ($p<0.05$, Mann-Whitney test).

Figure 3. Survival analyses of MS outcomes in the longer term. A. Kaplan-Meier (KM) analysis of time to confirmed EDSS worsening in the RRMS, SPMS and PPMS subtypes; B. KM of time to confirmed EDSS worsening according to paraproteinemia (red line: none, green line: <5 g/L, blue line: ≥5 g/L) illustrates the association detected by multivariate analysis (reported in Results). **C.** KM of NEDA-3 and its components: time to relapse, to new T2 MRI lesion and of time to confirmed EDSS progression in the whole population**. D.** KM of NEDA-3 in the RRMS, SPMS and PPMS subgroups.