Autologous hematopoietic stem cell transplantation with reduced-intensity conditioning in multiple sclerosis


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High-dose immunosuppressive therapy with autologous hematopoietic stem cell transplantation (AHSCT) is a new and promising approach to multiple sclerosis (MS) treatment. In this article, we present the results of a prospective phase II open-label single-center study with the analysis of the safety and efficacy of high-dose immunosuppressive therapy + AHSCT with reduced-intensity conditioning regimen in 95 patients with different types of MS. The patients underwent early, conventional, and salvage/late transplantation. Efficacy was evaluated based on clinical and quality of life outcomes. No transplantation-related deaths were observed. The mobilization and transplantation procedures were well tolerated. All the patients, except one, responded to the treatment. At long-term follow-up (mean 46 months), the overall clinical response in terms of disease improvement or stabilization was 80%. The estimated progression-free survival at 5 years was 92% in the group after early AHSCT vs 73% in the group after conventional/salvage AHSCT. Statistically significant difference between the survival probabilities of two groups was determined (p = 0.01). No active, new, or enlarging lesions in magnetic resonance imaging were registered in patients without disease progression. All patients who did not have disease progression were off therapy throughout the post-transplantation period. AHSCT was accompanied by a significant improvement in patient’s quality of life with statistically significant changes in the majority of quality of life parameters (p < 0.05). The results of our study support the feasibility of AHSCT with reduced-intensity conditioning in MS patients. Multicenter cooperative studies are needed for better assessment of treatment results and optimization of the treatment protocol of AHSCT with reduced-intensity conditioning regimens in MS. © 2012 ISEH - Society for Hematology and Stem Cells. Published by Elsevier Inc.
cells may alter the characteristics of the T-cell responses and other immunological properties that might improve the clinical course of MS. At the same time, despite the promising clinical results, there are still several questions to be clarified before recommending HDIT+AHSCT as a treatment choice for MS patients.

One of the key issues is the selection of a conditioning regimen. In the neurological community worldwide, there are concerns that HDIT+AHSCT is accompanied by an increased risk of mortality and adverse effects. Major results of clinical studies with different intensity conditioning used in MS patients are [9,14–20] shown in Table 1.

In the recent Guidelines of European Group for Blood and Marrow Transplantation for HSCT in severe autoimmune diseases the risk-to-benefit ratio is defined as a major issue for such a treatment [21]. Transplantation-related mortality during the recent years is slightly above 1% [22]. The analysis of the data in the Autoimmune Disease Working Party registry of the European Group for Blood and Marrow Transplantation (EBMT) has shown that intensive HDIT regimens have been associated with increased toxicity, including transplant-related mortality [23,24]. Therefore, the rationale of evolution from myeloablative to nonmyeloablative transplant regimens has been discussed recently [25]. At present, BEAM as the conditioning regimen is the most frequently used in MS patients [7,12,13,17,26–30]. There are also low-intensity regimens: cyclophosphamide (CY) alone, melphalan alone, and fludarabine-based regimens [31]. It was shown that the CY/rabbit anti-thymocyte globulin (ATG) regimen is associated with similar outcome results, but presented less toxicity when compared with the BEAM/horse ATG regimen [19]. The rational to use less intensive conditioning regimens is supported by the suggestion that AHSCT is not only an immunosuppressive therapy, but also could have an immunomodulatory component [32]. Taking into account that a moderate intensity and less toxic regimen could induce durable long-term remission, comparable with the high-intensity regimens, but without being associated with the higher transplant-related mortality characteristic of high-intensity regimens, we aimed to study if the reduced-intensity regimens based on BEAM are safe and effective in MS patients. At the same time, there is evidence that the intensity of conditioning may be associated with a sustained long-term response and control of disease activity [33]. Thus, we provided a long-term post-transplant follow-up of our population of MS patients.

In addition, comprehensive evaluation of treatment outcomes after HDIT+AHSCT is very important. For MS patients, both disease-free period and improvement of patient’s QoL are recognized as important outcome parameters. With this in mind, in our study we aimed to evaluate both clinical and patient-reported outcomes after HDIT+AHSCT.

We report the results of a prospective phase II open-label single-center study with the analysis of the safety and efficacy of HDIT+AHSCT with reduced-intensity conditioning in 95 patients with different types and stages of MS.

### Patients and methods

Ninety-five patients with MS: secondary progressive MS (SPMS), 35 patients; primary progressive MS (PPMS), 15 patients; progressive-relapsing MS (PRMS), 3 patients; and relapsing-remitting (RRMS), 42 patients (mean age 34.5 years; male/female, 36/59) underwent HDIT+AHSCT in the Transplantation Unit, Department of Haematology and Cellular Therapy, National Medical Surgical Centre in Moscow from July 2006 to January 2011. The study was conducted according to the

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**Table 1. Clinical studies of multiple sclerosis treatment with different intensity conditioning followed by AHSCT**

<table>
<thead>
<tr>
<th>First author of study</th>
<th>No. of patients</th>
<th>Conditioning</th>
<th>Conditioning intensity grade</th>
<th>Progression-free survival</th>
<th>Treatment-related mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nash [14]</td>
<td>26</td>
<td>CY 120 mg/kg/TBI 800 cGy/ATG</td>
<td>High</td>
<td>76% at 40 months</td>
<td>3.8</td>
</tr>
<tr>
<td>Burt [9]</td>
<td>21</td>
<td>CY 120 mg/kg/TBI 1200 cGy</td>
<td>High</td>
<td>62% at 24 months</td>
<td>9.5</td>
</tr>
<tr>
<td>Freedman [15]</td>
<td>15</td>
<td>BU 9-16 mg/kg/CY 200 mg/kg/ATG</td>
<td>High</td>
<td>60% at 60 months</td>
<td>6.6</td>
</tr>
<tr>
<td>Saccardi [16]</td>
<td>178</td>
<td>BEAM/ATG (41%) BEAM (17%) BCNU/CY/ATG (11%) CY/TBI/ATG (9%) BU/ATG (6%) Others (11%) Unknown (5%)</td>
<td>High/intermediate</td>
<td>63% at 42 months</td>
<td>5.3</td>
</tr>
<tr>
<td>Ni [17]</td>
<td>21</td>
<td>CY 120 mg/kg/TBI 1000 cGy/ATG (5%) BEAM/ATG (95%)</td>
<td>High/intermediate</td>
<td>75% at 42 months</td>
<td>9.5</td>
</tr>
<tr>
<td>Krasulova [18]</td>
<td>33</td>
<td>BEAM/ATG or in vitro purged graft</td>
<td>Intermediate</td>
<td>64.3% at 5 years</td>
<td>0</td>
</tr>
<tr>
<td>Hamerschlak [19]</td>
<td>21</td>
<td>BEAM/ATG</td>
<td>Intermediate</td>
<td>47.6% at 3 years</td>
<td>7.5</td>
</tr>
<tr>
<td>Burt [20]</td>
<td>21</td>
<td>CY 200 mg/kg/ATG</td>
<td>Low</td>
<td>100% at 37 months</td>
<td>0</td>
</tr>
<tr>
<td>Hamerschlak [19]</td>
<td>20</td>
<td>CY 200 mg/kg/ATG</td>
<td>Low</td>
<td>70% at 2 years</td>
<td>0</td>
</tr>
</tbody>
</table>

TBI = total body irradiation.
principles of the Helsinki Declaration, and was approved by the Institute Research Board and local Ethics Committee before initiation. All patients gave written informed consent. Patients were eligible if they were aged between 18 and 55 years and met the McDonald criteria for clinically definite MS [34]. Other criteria for patient selection were Expanded Disability Status Scale (EDSS) score 1.5 to 8.0 (median EDSS at baseline was 3.5), normal mental status, and absence of severe concomitant diseases. The vast majority of patients were refractory to conventional therapy, which included interferon-beta, copaxone, and mitoxantrone, as well as steroids, azathioprine, intravenous immunoglobulin, and plasmapheresis in some patients. Forty-two patients underwent early (EDSS 1.5–3.0, patients soon after diagnosis in case of primary refractory disease or poor prognosis), 50 patients underwent conventional (EDSS 3.5–6.5, patients with secondary refractory disease), and 3 patients underwent salvage (EDSS 7.0–8.0, patients with high disease activity and rapid neurological deterioration in late stages of the disease) transplantation in accordance with the concept of HDIT+ASCT in MS [29]. The mean follow-up was 46 (range, 10–66) months.

Neurological assessment using EDSS was performed at baseline, at discharge, at 3, 6, 9, and 12 months after transplantation, every 6 months thereafter up to 48 months, and then at yearly intervals. Magnetic resonance imaging scans of the brain and cervical spinal cord with gadolinium enhancement were performed at baseline, at 3, 6, 9, and 12 months after transplantation, every 6 months thereafter up to 48 months, and then at yearly intervals. QoL was evaluated using RAND SF-36 questionnaire [35].

Hematopoietic stem cells were mobilized with granulocyte colony-stimulating factor at 10 μg/kg according to EBMT/European League Against Rheumatism guidelines. The mobilized cells were collected by apheresis, until a yield of at least 2 × 10⁶ per kg CD34⁺ cells was obtained. The grafts were not manipulated. Reduced-intensity conditioning regimen based on BEAM, i.e., low-intensity conditioning [31] was used. It included BCNU/CCNU 300 mg/m² and melphalan 50–100 mg/m² (BM) or BCNU/CCNU 300 mg/m², etoposide 75–100 mg/m², Ara-C 75–100 mg/m² and melphalan 50–100 mg/m² (mini BEAM-like). Sixty patients were conditioned with BM, others with mini BEAM-like. Conditioning was followed by AH SCT (day 0) ± horse ATG (ATGAM, Pharmacia & Upjohn Company, Peapack, NJ, USA) in a dose of 30 mg/kg on days 1 and 2 for in vivo T cell-depletion. Five micrograms per kilogram subcutaneous granulocyte colony-stimulating factor were administered from day 5 post-infusion until granulocyte recovery. For infection prophylaxis, oral ciprofloxacin and fluconazole were used.

Toxicity was evaluated in accordance with the National Cancer Institute Common Toxicity Criteria, version 2. Neutrophil engraftment was defined as the first day after transplantation when the absolute neutrophil count was >500 cells/mL. Platelet engraftment was defined as the first day after transplantation when the platelet count was >20,000 platelets/mL without platelet transfusion.

According to the EBMT criteria of response, patients with either steady EDSS scores representing a halt of disease progression or with improved EDSS scores, representing cessation of inflammation in the CNS, were regarded as responding to treatment [7]. Improvement in neurological function was defined as a decrease in the EDSS score of at least 0.5 points on two consecutive visits 3 months apart as compared with the baseline. For RRMS, a decrease in the number of relapses per year was defined as a clinical improvement. Disease progression was defined as an increase in the EDSS score of 0.5 points or more on a minimum of two occasions that were at least 3 months apart. For RRMS disease progression was defined as an increase in number of relapses per year. A relapse of MS was defined as an acute deterioration in neurological function that lasted for more than 24 hours without intercurrent illness or another cause for neurological impairment and with objective changes on neurological examination.

Transplantation-related mortality definition included every death occurring within 100 days of transplantation [12,13]. Progression-free survival was calculated using the Kaplan-Meier method. QoL data were analyzed using the Friedman repeated measure analysis of variance on ranks.

Treatment outcomes are reported as of November 2011, based on the last follow-up of each patient.

Results

Safety

No transplantation-related deaths were reported among the 95 MS patients, irrespective of their clinical condition at the time of transplantation. In addition, there were no deaths in the study throughout the follow-up period. The mobilization and transplantation procedures were well tolerated. Mobilization was successful in all cases. There were no nonhematological toxicities of grade III severity or greater during transplantation. Common adverse effects after the HDIT+AH SCT were thrombocytopenia (100%), neutropenia (100%), fatigue (100%), anemia (80%), alopecia (80%), neutropenic fever (31.6%), hepatic toxicity grade I and II (42.1%), transient neurological dysfunction (27.4%), enteropathy (7.4%), skin allergy (8.4%), pneumonia in 2 patients (2.1%), oral candidiasis in 1 patient (1.05%), nasal hemorrhage in 1 patient (1.05%), uterine bleeding in 2 patients (2.1%), oral herpes in 1 patient (1.05%), and genital herpes in 1 patient (1.05%). Documented sepsis was registered in three patients (3.2%) on day 7 and day 10 after transplantation. The first patient had severe sepsis and septic shock caused by Enterococcus faecium associated with pneumonia and enteropathy on day 7 after transplantation. Sepsis was successfully treated in the intensive care unit. The second patient had enteropathy, sepsis, and cytomegalovirus colitis with recurrent rectal bleeding on day 7 after transplantation. Sepsis and rectal bleeding were successfully treated in the intensive care unit. The third patient had systemic inflammatory response syndrome on day 10 after transplantation with febrile fever, tachycardia, and procalcitonin test >2, without positive blood culture. The patient was successfully treated by meropenem and vancomycin for 10 days.
Efficacy

Ninety patients with the follow-up period of at least 12 months or longer were included in the clinical outcome analysis. At 6 months post-transplantation all the patients except one responded to treatment: 37 patients (41%) achieved an objective improvement of neurological symptoms and 52 patients (58%) had disease stabilization. In one patient with PPMS disease, progression was registered: EDSS increased from 6.0 at baseline to 6.5 at 6 months post-transplantation. At 12 months after AHSCT, 53% of patients were stable and 43% demonstrated improvement in neurological function. Two more patients progressed: EDSS increase was registered after disease stabilization at 9 and 12 months post-transplantation, respectively. Both patients had SPMS and underwent conventional AHSCT.

At long-term follow-up (mean 46 months), the clinical response in 28 patients was classified as an improvement; 31 patients remained stable. At long-term follow-up, progression was found in 11 patients. Three patients progressed after 18 months of stabilization or improvement (two patients with SPMS and one patient with RRMS; all patients underwent conventional AHSCT); three more patients progressed after 24 months of stabilization or improvement (one patient with PPMS and one patient with RRMS; all patients underwent conventional AHSCT). In the group after early AHSCT disease progression was registered in only 2 (5%) of 39 patients during the whole period of the follow-up, at 24 and 43 months post-transplantation, respectively. In the group after conventional/salvage AHSCT, 12 (24%) patients progressed at different time points after transplantation. The estimated 5-year progression-free rates were 92% (95% CI, 73–98%) in early AHSCT group and 73% (95% CI, 58–84%) in conventional/salvage AHSCT group. Statistically significant difference between the survival probabilities of two groups was determined (log-rank test, p = 0.01, 95% CI; Fig. 3).

QoL monitoring during the entire study period was performed in 61 patients. The results showed remarkable improvement of QoL parameters (Fig. 4). QoL profiles demonstrate dramatic positive changes in patient’s QoL after treatment. We found a significant increase of five of eight SF-36 scales (except physical functioning, bodily pain and role-emotional functioning) already at 6 months after ASCT as compared with baseline (p < 0.05). Further QoL improvement was registered at 9 and 12 months post-transplantation. QoL parameters at baseline as compared with those at 12 months after AHSCT are presented in Table 2: at 12 months post-transplantation, a statistically
significant increase of all SF-36 scales, except bodily pain and role-emotional functioning, was registered as compared with baseline \((p < 0.05)\). Improved QoL parameters were preserved over the entire study period in all the patients who did not have disease progression.

**Discussion**

The results of a number of studies demonstrated promising results of MS treatment using HDIT+ASCT \([7, 9, 13, 14, 30, 31]\). At the same time HDIT+ASCT is known to be associated with a number of side effects and of major concern is the transplant-related mortality. By now, the most promising results of HDIT+ASCT have been obtained in MS patients with BEAM as a conditioning regimen \([16, 26]\). BEAM is an intermediate-intensity conditioning regimen, pioneered by Fassas et al. \([36]\). Taking into account serious concerns of neurological community that HDIT+ASCT is associated with the risk of mortality and adverse effects, as well as published EBMT data about the cases of mortality in MS patients treated with BEAM conditioning regimens, a new reduced-intensity conditioning regimen based on BEAM was proposed, and HDIT+ASCT with reduced-intensity conditioning was used. We report the results of HDIT+ASCT with reduced-intensity conditioning for 95 patients with different types and stages of MS.

The results of safety of ASCT obtained in our study are encouraging. Among 95 patients, there were no transplantation-related deaths. In addition, there were no deaths in our study within the overall follow-up period. As for the adverse effects, the majority of them were limited to the post-transplantation period and were short-lived. There were no severe neurological complications related to the transplantation. Moreover, all adverse effects could be controlled by the transplantation team and were reversible.
The efficacy of AHSCT was shown using both clinical outcomes and patient-reported outcomes. The analysis of clinical outcomes demonstrated remarkable results. All of the patients except one responded to the treatment. At long-term follow-up, overall clinical response was 80%. Similar data were found for MS patients treated with BEAM conditioning regimen [16]. Magnetic resonance imaging lesions are a major marker of inflammatory activity. In our group of patients, no active, new, or enlarging lesions were registered in patients without disease progression. Notably, all patients who did not have disease progression were off immunosuppressive or immunomodulatory therapy throughout the post-transplantation period.

In our study, progression-free survival after AHSCT was 82% at 5 years, which is consistent with the results of studies with intermediate and high-intensity conditioning regimens [6,10,16]. The analysis of QoL also demonstrated benefits of AHSCT in this patient population. QoL is an important outcome of MS treatment and its assessment provides the patient’s perspective on the overall effect of treatment and allows evaluating patient benefits. Our results definitely show that AHSCT resulted in significant improvement of patient’s QoL.

One of the advantages of our study is the performance of transplantation in patients with different stages of MS, including early stages, while most patients in the previous studies had late stages of MS. Our data support the idea that AHSCT is more effective in patients with early stages of active disease. In these patients, autoreactive T cells play a pivotal role in MS pathogenesis. High-dose immunosuppression eradicates autoimmune T cells. It is followed by AHSCT to restore the immune system, which is expected to become tolerant to autoantigens. Such a "resetting" of the immune system is only effective in the early stages of MS, particularly in relapsing-remitting MS. Later in the clinical course of the disease, processes of axonal degeneration prevail, and the damage to CNS tissue is too severe to expect a neurological recovery after HDIT+AHSCT. In our study, the estimated progression-free survival at 5 years was 92% in the group after early AHSCT vs 73% in the group after conventional/salvage AHSCT.

Thus, the risk-to-benefit ratio of HDIT+AHSCT with reduced-intensity conditioning in our population of MS patients is very favorable. The consistency of our clinical and QoL results, together with the persistence of improvement, is in favor of the efficacy of this treatment modality in MS patients. Overall, the results of our study support the feasibility of HDIT+AHSCT in MS patients. Multicenter cooperative studies are needed for better assessment of treatment results and optimization of the treatment protocol of AHSCT with reduced-intensity conditioning regimens in MS.

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Conflict of interest disclosure
No financial interest/relationships with financial interest relating to the topic of this article have been declared.

References

### Table 2. QoL parameters before and at 12 months after ASCT in MS patients (SF-36 questionnaire)

<table>
<thead>
<tr>
<th>Scale</th>
<th>Baseline, mean (SD)</th>
<th>12 Months after transplantation, mean (SD)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical functioning</td>
<td>59.7 (30.4)</td>
<td>72.2 (32.4)</td>
<td>0.003*</td>
</tr>
<tr>
<td>Role-physical functioning</td>
<td>38.9 (39.9)</td>
<td>56.7 (44.5)</td>
<td>0.03†</td>
</tr>
<tr>
<td>Bodily pain</td>
<td>73.2 (24.0)</td>
<td>79.5 (26.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>General health</td>
<td>49.4 (22.8)</td>
<td>62.5 (23.3)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Vitality</td>
<td>48.8 (21.9)</td>
<td>64.3 (25.3)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Social functioning</td>
<td>56.9 (29.4)</td>
<td>82.1 (24.5)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Role-emotional functioning</td>
<td>66.7 (41.4)</td>
<td>68.9 (41.0)</td>
<td>0.4</td>
</tr>
<tr>
<td>Mental health</td>
<td>60.0 (23.9)</td>
<td>71.5 (21.8)</td>
<td>0.004*</td>
</tr>
</tbody>
</table>

SD = standard deviation.
*Significant at 99% level CI, p < 0.01.
†Significant at 95% level CI, p < 0.05.


