Published in final edited form as:

Mult Scler. 2012 February ; 18(2): 202–209. doi:10.1177/1352458511419701.

Treatment of relapsing–remitting multiple sclerosis with high-dose cyclophosphamide induction followed by glatiramer acetate maintenance

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Abstract

Background—Previous studies have described stabilization of aggressive multiple sclerosis (MS) with one-time induction therapy with high-dose cyclophosphamide (HiCy). The long-term benefit of this stabilization followed by conventional therapy has not been explored.

Objective—The objective of this study was to evaluate the safety and clinical outcomes following treatment of relapsing–remitting MS with HiCy induction therapy followed by glatiramer acetate maintenance.

Methods—A retrospective review of a closely followed population of thirty two MS patients treated with HiCy (200mg/kg intravenous infusion over 4 days) followed by maintenance with glatiramer acetate was performed.

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Conflict of interest statement

Dr Harrison has received honoraria from the American College of Physicians for a textbook manuscript, and currently receives research support from Bayer Schering Pharma.

Dr Gladstone has no disclosures.

Dr Hammond has no disclosures.

Mr Cheng has no disclosures.

Dr Brodsky and Dr Jones are entitled to a share of royalties received by The Johns Hopkins University from Accentia Pharmaceuticals under a licensing agreement for sales of a method of administering high-dose cyclophosphamide. The study reported in this article could affect the value of this method of administering the drug. The terms of this arrangement are being managed by The Johns Hopkins University in accordance with its conflict of interest policies.

Dr Kerr is no longer employed by Johns Hopkins University, and is now a full time employee of Biogen Idec.

Dr Kaplin is a member of the Altimmune Board of Directors. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

Results—Annualized relapse rate was reduced from 1.37 in the 2 years prior to treatment to 0.27 over a mean post-treatment follow-up period of 14 months (range 0.5–33.8). The projected probability of relapse-free survival at 2 years was 0.64 (95% CI 0.37–0.82). The projected probability of Expanded Disability Status Scale (EDSS) progression-free survival at 2 years was 0.77 (95% CI 0.43–0.92). The mean number of gadolinium-enhanced lesions was reduced from 0.86 (SD 1.6) at baseline to 0 at 12 months and 0.08 (SD 0.28) at 15–24 months. A total of 55% of patients had no evidence of disease activity in follow-up. Infectious complications occurred in 47% with no long-term morbidity and no deaths.

Conclusions—Induction therapy with HiCy followed by long-term maintenance with glatiramer acetate is well tolerated in patients with MS, and appears to be efficacious in reducing the risk of relapse, disability progression, and new MRI lesions.

Keywords

cyclophosphamide; glatiramer acetate; immunosuppression; multiple sclerosis

Introduction

Despite recent advances in the treatment of multiple sclerosis (MS), including the addition of natalizumab¹ and fingolimod² to the therapeutic armamentarium, some patients have continued disease activity. Accordingly, alternative approaches are necessary, especially for those with an aggressive inflammatory clinical course. One approach being considered is near total immune suppression with high-dose chemotherapy, followed by stem cell rescue through bone marrow transplantation.^{3,4} While some studies have reported efficacy, significant safety concerns remain, including transplant mortality rates as high as 5.3%.⁵ Although this approach has the potential to induce long-term disease remissions, high rates of morbidity and mortality are unacceptable to most patients.

A similar, less-toxic approach is high-dose cyclophosphamide (HiCy) without bone marrow transplantation.⁶⁻⁸ Originally used as an alternative to bone marrow transplantation for severe aplastic anemia,^{9,10} HiCy has successfully been applied to a number of different autoimmune syndromes.¹¹⁻¹⁵ A recent comprehensive review of its use in many autoimmune syndromes described few long-term adverse effects.¹⁶ Three small open-label studies using HiCy in MS have thus far been published,^{6-8, 17} all using a regimen of 200 mg/kg of cyclophosphamide infused over 4 days that induces profound leucopenia, followed by temporary use of filgrastim (granulocyte-colony stimulating factor [G-CSF]) to hasten endogenous granulocyte recovery. A combined total of 47 patients were treated in these 3 studies, with a beneficial response in most. Although some infectious complications were noted in these studies, there was no significant morbidity or mortality.

While preliminary results demonstrate efficacy for HiCy as an induction therapy, its long-term benefit is not yet known. Because of the unknown duration of benefit, many patients who have received this therapy for treatment-refractory MS at Johns Hopkins were placed on maintenance therapy with glatiramer acetate (GA), which has clearly established long-term efficacy,^{18,19} including use in post-chemotherapy maintenance.^{20,21} We performed a retrospective case review of all patients prescribed initial induction therapy with HiCy followed by GA maintenance. The goals of this study were to expand on previously described safety data of HiCy¹⁶ in a large cohort of MS patients, and also to evaluate the safety and efficacy of the combination of HiCy and GA for relapsing–remitting MS.

Methods

Population

Permission was obtained from the Johns Hopkins Institutional Review Board for a retrospective medical records review. Patients receiving off-label treatment with HiCy (50 mg/kg intravenous infusion each day for 4 days, as described previously⁶) after our previous publication were identified. A total of 40 candidates were identified. Medical records were reviewed and demographic, clinical, laboratory, and radiologic data were recorded through the last clinical visit as of the end of September 2010. Three subjects were removed from further analysis because of a diagnosis of progressive MS. Another five subjects were removed because they had elected to not receive GA maintenance. The remaining 32 subjects thus had relapsing–remitting MS, were treated with HiCy, and were placed on GA 20 mg subcutaneous injection daily approximately 30 days after HiCy.

Recording of data

All data was derived from a detailed review of the medical records. As per departmental protocol, all patients undergoing HiCy treatment had a rigorous recommended follow-up schedule, with clinical visits (with Expanded Disability Status Score [EDSS]²² examinations), laboratory studies, and magnetic resonance imaging (MRI) examinations every 3 months for up to 2 years, all of which were documented in the patients' electronic medical record. Since EDSS exams were documented in the medical record, these were scored based on the treating physician's assessment. Clinical relapses were identified by description of the acute or subacute onset of new or worsening neurological symptoms, associated with an increase in disability, not otherwise attributable to an underlying infection or metabolic change. Sustained disability progression was defined as an increase in EDSS of 1.0 for patients with baseline EDSS <5.0, or 0.5 if baseline EDSS ≥ 5.0, which was not reversed on subsequent visits (3 months apart). The presence of gadolinium-enhancing lesions on brain MRI were ascertained from radiology reports and confirmed on visual inspection of the images by DH when available. More detailed image analysis was not possible given the retrospective nature of the review and the lack of uniformity of imaging protocols.

Statistical analysis

Statistical analysis was performed using the Stata IC 10.1 software package (College Station, TX). Standard descriptive statistics are reported for demographic and clinical data. Differences between mean values and proportions were analyzed by two-sided Student's *t*-test with statistical significance set as $p < 0.05$. Differences between median EDSS scores at differing time points were analyzed by Wilcoxon rank sum test. Annualized relapse rate during the observation period was determined by dividing the number of recorded clinical relapses by the total number of months of follow-up, normalized to 1 year. Survival analysis was used to examine the probability of being relapse or sustained progression-free during the observation period. Censoring occurred at the time of last known follow-up and survival failures occurred at the time of relapse (for relapse-free survival analysis) or the time of sustained disability progression (for progression-free survival analysis). Kaplan–Meier curves were used for visual inspection of this data and the probability of relapse or progression-free survival at 2 years was taken from the associated survival tables. Log-rank tests were used for assessment of differences in survival by dichotomous variables.

Results

Study population

Baseline characteristics can be found in Table 1. This population had active disease, with a median of 2 relapses (range 0–9) in the prior 2 years and had previously used a median of 2.5 (range 0–8) disease-modifying treatments. The treatment used immediately before HiCy was interferon-beta for seven patients, GA for seven patients, natalizumab for nine patients, mitoxantrone for four patients, other immunosuppressant agents for four patients, and one treatment-naïve patient. Patients also had moderate disability, with median baseline EDSS of 3.75 (range 1.5–6.5). Mean time of follow-up after treatment with HiCy was 14.0 months (range 0.5–33.8). Twenty-five patients reported complete adherence to GA. Of the 7 patients who did stop GA, this was reported to have occurred at a median of 12 months (range 1–24) after HiCy.

Hematologic response to HiCy

The hematologic response to HiCy is demonstrated graphically in Figure 1. The mean baseline white blood cell (WBC) count was 7389 cells/mm³ (range 4200–16,500). All patients experienced profound leucopenia with absolute neutropenia (ANC < 500) in response to HiCy, with a mean nadir WBC of 15.6 cells/mm³ (SD 25.9). Patients spent a mean of 10.0 (range 6–15) days with WBC below 1000 cells/mm³.

Mean baseline platelet count was 243,406 cells/mm³ (SD 71,899). As expected, platelet counts dropped in response to HiCy, with mean value at nadir of 18.4 cells/mm³ (range 5–139). Patients spent a mean of 8.3 (range 0–15) days with platelet counts below 50,000 cells/mm³ before returning to normal. By protocol, 11 (34%) patients required platelet transfusions. There were no bleeding complications.

Adverse events

HiCy treatment followed 30 days later by initiation of GA was tolerated with minimal morbidity and no mortality in this population. Two patients received infusions as outpatients, the remainder as inpatients. The median total days of hospitalization at the time of HiCy treatment (administration plus re-admissions for complications) was 4 (range 0–13). A list of the adverse events occurring at the time of HiCy treatment or during the follow-up period is recorded in Table 2. The most common side effect was nausea/vomiting-, occurring in 27 (84%) patients. Drug rash (presumably antimicrobial- or cyclophosphamide-related) occurred in 15 (47%) patients.

Fifteen (47%) patients had infectious complications. Four (13%) patients experienced catheter-based infections: bacteremia due to *Enterobacter* (one patient), coagulase-negative *Staphylococcus* (one patient), and *Pseudomonas* (one patient) species in three patients and one with significant erythema at the catheter site without bacteremia. One patient required transient admission to the ICU for treatment of sepsis with rapid response to antibiotics. Non-catheter-related infections occurred in 11 (34%) patients. There were three cases each of fungal skin infections and pneumonia. BK virus was found in the urine of two subjects experiencing dysuria and another patient had a bacterial urinary tract infection. One patient was treated for cholecystitis at an outside hospital 1 month after HiCy treatment. The remaining infections were diverticulitis of unclear source (one patient), *C. difficile* colitis (one patient), folliculitis (one patient), sinusitis (one patient), and oral herpes simplex virus I. All of the above infections responded to treatment and no long-term adverse effects occurred. Febrile neutropenia occurred in 11 (34%) of patients, all of whom received broad spectrum antibiotics until resolution of neutropenia.

Eleven (34%) patients experienced transient neurologic deterioration during or immediately after HiCy treatment. These patients described a worsening of baseline deficits or the recrudescence of prior symptoms and most occurred in the setting of concomitant fever. These were not felt to be clinical relapses and were not treated with intravenous steroids. All 11 patients had rapid return to baseline within a few days after reporting these symptoms.

Cardiac complications were rare, with one patient experiencing atrial fibrillation with rapid ventricular response requiring amiodarone treatment and one patient with transient supraventricular tachycardia. One patient experienced bilateral leg edema, but no cardiac abnormality was found. There were no documented cases of congestive heart failure.

There were four (13%) reports of hematuria, all of which were mild and transient, and no cases of hemorrhagic cystitis. Renal insufficiency due to acute interstitial nephritis (presumed to be related to antibiotic use) occurred in one patient and resolved without lasting complications.

Three patients experienced events in follow-up that persist. One patient was diagnosed with adrenal insufficiency 1 month after HiCy treatment. It should be noted that she was found to have a pituitary microadenoma and also had received multiple courses of intravenous corticosteroids in the 6 months before HiCy. This patient required long-term glucocorticoid supplementation and the pituitary lesion was stable at last follow-up. One patient had persistent leucopenia with WBC between 2000 and 3000 cells/mm³ despite having returned to normal WBC counts after HiCy treatment. This occurred 9 months after HiCy and persists 2 years after treatment. This patient had WBC in the normal range prior to treatment (~4800 cells/mm³) and has not experienced any infectious complications. Lastly, one 32-year-old female experienced symptomatic menopause after treatment with HiCy, which was attributed to premature ovarian failure.

MRI

MRI data is summarized in Table 3. Four patients (12.5%) did not have baseline scans available for review. Twenty-three (72%) had available scans at 3 and 6 months and 12 (38%) had available scans at 12 months. Of the available scans, 10 of 28 (36%) had at least one gadolinium-enhanced lesion on their baseline scan, 4 of whom had 3 lesions. This proportion was significantly reduced 3 and 12 months after HiCy (9% and 0% respectively). Of 13 scans performed in the 15–24-month time period, only one patient had one contrast enhancing lesion and only 7 (23%) had a new lesion on any follow-up scan ($p = 0.31$ compared with baseline). Mean gadolinium enhanced lesion count was reduced from 0.86 (SD 1.6) at baseline to 0.13 (SD 0.46) at 3 months ($p = 0.04$). Non-significant reductions in mean lesion count were also seen at the 6-, 12-, and 15–24-month periods.

Clinical response

Twenty-four patients (75%) remained relapse-free during the period of review (see Table 4). The annualized relapse rate was reduced from 1.37 in the prior 2 years to 0.27 during the study period, with a projected probability of relapse-free survival of 0.64 (95% CI 0.37–0.82) at 2 years (Figure 2). Five relapses occurred within the first 6 months after HiCy and three occurred after 1 year (median 6 months [range 1–34 months]). Of the eight patients that relapsed, only two patients had more than one relapse. The chance of relapse was not different in those who either had prior exposure to GA ($p = 0.82$) or in those who were not adherent to GA ($p = 0.33$). Both patients who experienced two relapses altered therapy after the second attack; one switching to interferon-beta and the other receiving a second HiCy treatment. Neither patient had subsequent attacks.

Median EDSS was 3.75 (range 1.5–6.5) at baseline, and was significantly reduced at 3 months (3.5, range 0–6.5, $p < 0.001$) and 6 months (3.5, range 1.5–6.5, $p = 0.02$). Although the mean improvement in EDSS from baseline to 1 year was 18% (range –33%–66%), this reduction was not statistically significant ($p = 0.07$).

Six patients (20%) had sustained progression of disability as measured by EDSS, four of whom had no documented relapses during the study period. The projected probability of remaining free of EDSS progression at 2 years was 0.77 (95% CI 0.43–0.92) by survival analysis (Figure 2). The probability of progression was not altered by prior GA use ($p = 0.69$) or GA adherence ($p = 0.18$). Eleven (37%) patients had sustained improvement in disability and 13 (43%) had no sustained EDSS changes (two patients did not have follow-up EDSS scores). The probability of EDSS improvement was not altered by prior GA exposure or adherence during follow-up.

Sixteen of 29 (55%) patients with available relapse, EDSS, and MRI data showed no evidence of disease activity in follow up, which was defined as no relapses, disability progression, or new MRI lesions.

Discussion

In this retrospective case review, we provide details on the largest cohort to date of patients with MS treated with HiCy. In this cohort, we found improvements in the clinical status of patients treated with HiCy followed by GA. Relapse rates were significantly reduced from pre-treatment baseline, 75% of patients remained relapse-free, only 20% of patients had sustained disability progression, and the majority of patients had little to no gadolinium-enhanced lesion activity on follow-up MRI.

Cyclophosphamide has a long history in the treatment of MS, having been utilized in some of the first published treatment trials in this condition,^{23,24} although at much lower doses than in this study. Cyclophosphamide effectively reduces the number of circulating T and B cells,^{25,26} in addition to creating a more favorable cytokine balance, shifting away from autoimmune Th1 responses and reductions in interleukin 12 (IL-12) and interferon gamma (IFN- γ).²⁷ Cyclophosphamide and its metabolites are also capable of penetrating the blood–brain barrier and can exert direct intrathecal immunologic effects.^{28–30} Despite these potentially beneficial biologic effects for MS, numerous clinical trials of varying protocols of administration have shown mixed results.^{31,32} These studies involved using doses of cyclophosphamide up to a maximum dose of 1600 mg/m² and were often designed to produce moderate leucopenia. Although some of these failures may have been due to the selection of patients (often studied in progressive patients), the lack of convincing clinical efficacy data has led to cyclophosphamide not being utilized as a standard therapy in MS.

The approach used in treatment of patients in this case review stands apart from many prior studies of cyclophosphamide, as the near total immunoablation achieved by the high doses used has been suggested to possibly ‘reboot’ the circulating immune system from surviving antigen-naïve hematopoietic stem cells.¹¹ It is hoped that this approach can lead to disease quiescence in those with aggressive relapsing MS. Further, with the addition of maintenance therapy with a standard disease-modifying agent it is possible that the duration of this benefit can be extended. This approach has been successfully utilized before, as evidenced by prior reports of mitoxantrone induction followed by GA maintenance.^{20,21} Although it is difficult to directly compare this study to our prior report using HiCy alone in relapsing–remitting MS,⁶ the addition of GA resulted in no additional toxicity and similar, if not superior efficacy. Whereas 55% of patients in this study had no evidence of follow-up disease activity, 33% had no disease activity by the same definition in the study of Krishnan

et al.⁶ It should be noted that the patients in the study of Krishnan et al. had more gadolinium-enhanced lesions at baseline and more robust follow-up. Any conclusions about superiority to HiCy alone, or in combination with GA compared with standard MS therapy would require a direct comparator group, the lack of which is a limitation of this study.

Until longer-term follow-up can be recorded, the duration of benefit of this approach remains unknown. Long-term data has been reported previously for HiCy treatment in myasthenia gravis, with treatment responders found to have long-term benefit (most with the use of an additional maintenance immunotherapy), lasting between 3 and 7 years.³³ Based on the substantial proportion of patients in this study projected to be relapse or EDSS progression-free at 2 years by survival analysis, it is possible that many of these patients will similarly experience years of beneficial response, perhaps extended by the use of maintenance immunotherapy with GA.

In addition, the open-label nature of this cohort also lends to influence from the phenomenon of 'regression to the mean', in which there is a statistical tendency for patients with MS enrolled in studies at the time of disease activity to have a reduction in relapse rates and regression of disability even with placebo treatment.³⁴ Regardless, the stabilization of disease seen in this cohort plus previous studies of HiCy in MS lends credence to the idea that HiCy, either alone or with GA maintenance, should be evaluated in the setting of a larger, randomized clinical trial.

This large cohort also adds significantly to the existing safety data for this treatment in MS, which is important in considering the future application of this approach in the setting of a larger clinical trial. In this review, there were no cases of mortality and minimal morbidity. This is similar to prior reports on the use of HiCy in MS.^{6-8,17} As expected, infections were common, occurring in 47% of patients. However, there was only one case of sepsis, and no long-term morbidity from these infections was noted. In addition, while febrile neutropenia was common (34%), all cases resolved with appropriate antibiotic coverage. Although rare cardiotoxicity can be seen,³⁵⁻³⁷ there were only two noted cardiac complications: one case of atrial fibrillation and one case of supraventricular tachycardia. Both responded to treatment and no patients developed congestive heart failure. In combination with similar safety data from the previous studies of HiCy in MS, it appears that administration of this treatment can be performed with relative safety, especially in light of the serious side effects occurring in US Food and Drug Administration (FDA)-approved and emerging second-line therapies in MS.³⁸⁻⁴⁰ Knowledge of this data will be critical for development of potential future randomized trials using this treatment approach.

The retrospective nature of this study is an important limitation. We were limited to only the information made available by the medical record, and thus any events not documented properly, or which occurred in patients lost to follow-up are not included. It should be noted, however, that because of the experimental nature of this approach, all patients and treating physicians involved in this treatment follow a rigorous institutional clinical monitoring protocol, involving assessments (MRI and EDSS exams included) for every 3 months for up to 2 years. The uniform nature of this protocol allowed for more cohesive retrospective data collection and analysis. Despite this plan, however, not all data was available at all potential time points, which did hamper more detailed disability assessments and MRI analyses than what is reported here.

Despite these limitations, this report represents a significant addition to the available data on the safety and efficacy of HiCy-based treatment strategies in MS. It is hoped that the availability of this information will increase interest in this potentially promising treatment and be informative as to the design of future randomized clinical trials. Based on this data, a

larger randomized study should be done to determine the true relative efficacy of this approach.

Acknowledgments

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Dr McArthur has been supported by grants NS44807, NS35609, and NS49465, is currently supported by 1P30MH075673.

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Figure 1. Hematologic response to high-dose cyclophosphamide (HiCy)

Graphs demonstrating trends in white blood cell count (A) and platelets (B) after administration of HiCy. Cyclophosphamide was administered for 4 days beginning on day -4. All patients received granulocyte colony stimulating factor (G-CSF) beginning on day 6 and until the resolution of neutropenia. Mean time from completion of HiCy to white blood cell nadir was 7.2 (SD 1.9) days and 9.4 (SD 1.7) for platelet nadir.

Figure 2. Relapse and progression-free survival

Kaplan–Meier curves for relapse (left) and progression-free (right) survival. For relapse-free survival, a survival failure was recorded at the time of a clinical relapse. For progression-free survival, a survival failure was recorded at the time of a sustained increase in Expanded Disability Status Scale (EDSS) ≥ 1.0 for patients with baseline EDSS < 5.0 or ≥ 0.5 for patients with baseline EDSS ≥ 5.0 .

Table 1 Baseline characteristics of treated patients ($n = 32$).

Characteristic	
Women, N (%)	16 (50%)
Age, mean (SD), years	41.7 (10.7)
Disease duration, mean (SD), years	10.2 (7.1)
Number of relapses in prior 2 years, median (range)	2 (0–9)
Number of prior failed disease-modifying therapies, median (range)	2.5 (0–8)
Baseline EDSS, median (range)	3.75 (1.5–6.5)
Baseline number of gadolinium-enhanced lesions on MRI, mean (SD)	0.86 (1.6)

EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; SD, standard deviation.

Table 2

Adverse events/side effects.

Adverse event/side effect	Number (%)
Nausea/Vomiting	27 (84)
Infections	15 (47)
Catheter infections	4 (13)
Non-catheter infections	11 (34)
Rash	15 (47)
Transient neurologic deterioration	11 (34)
Neutropenic fever	10 (31)
Hematuria	4 (13)
Liver function abnormality	3 (9)
Cessation of menses	1 (3)
Supraventricular tachycardia	1 (3)
Atrial fibrillation	1 (3)
Peripheral edema	1 (3)
Persistent lymphopenia	1 (3)
Pleural effusion	1 (3)
Transient intractable diarrhea	1 (3)
Adrenal insufficiency	1 (3)
Acute interstitial nephritis	1 (3)

Table 3

Gadolinium enhancement on brain magnetic resonance imaging.

Time point	0 lesions	1 lesion	2 lesions	3 lesions	<i>p</i> -value [*]	Mean # of lesions (SD)	<i>p</i> -value ^{**}
Baseline	18	4	2	4	–	0.86 (1.6)	–
3 months	21	1	1	0	0.02	0.13 (0.46)	0.04
6 months	20	2	1	0	0.07	0.67 (0.58)	0.05
12 months	12	0	0	0	0.02	0 (0)	0.07
15–24 months [†]	12	1	0	0	0.06	0.08 (0.28)	0.09

p-values attaining statistical significance at $p < 0.05$ are highlighted in bold.

^{*} *p*-value for Student's *t*-test comparing proportion of patients with gadolinium-enhanced lesions between baseline and each time point.

^{**} *p*-value for Student's *t*-test comparing mean number of gadolinium-enhanced lesions at baseline to each time point.

[†] Last follow-up scan in 15–24-month range was used as the representative sample.

Table 4

Clinical outcomes.

	Pre-treatment Status	Post-treatment Status	Percentage without event	Probability of event-free survival at 2 years
Relapses	<i>Annualized relapse rate:</i> * 1.37	<i>Annualized relapse rate:</i> † 0.27	75% relapse-free	0.64 (95% CI 0.37–0.82)
Disability progression	<i>Median EDSS at baseline:</i> 3.75 (range 1.5–6.5)	<i>Median EDSS at 1 year:</i> 3.5 (range 1.0–6.5)	80% without sustained disability progression	0.77 (95% CI 0.43–0.92)

Event-free survival calculated by survival analysis, with 'event' being a documented clinical relapse in the relapse analysis or sustained disability progression in the disability progression analysis.

EDSS, Expanded Disability Status Scale; HiCy, high-dose cyclophosphamide.

* Annualized relapse rate in the 2 years prior to HiCy therapy.

† Annualized relapse rate during the study period.