Neurol Sci
DOI 10.1007/s10072-011-0848-1

ORIGINAL ARTICLE

Natalizumab in aggressive multiple sclerosis after haematopoietic stem cell transplantation

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Received: 28 December 2010 / Accepted: 28 October 2011
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Abstract High-dose cyclophosphamide followed by autologous haematopoietic stem cell transplantation (HDC-AHSCT) is a treatment option for aggressive and refractory multiple sclerosis (MS). Natalizumab is a monoclonal antibody approved for relapsing-remitting (RR) MS unresponsive to immunomodulating drugs. Nothing is known about the use of natalizumab in patients after HDC-AHSCT. We describe five female RR-MS patients with incomplete response to HDC-AHSCT. Natalizumab was then administered with abolition of both MRI and clinical activity. No severe adverse events, in particular opportunistic infections such as Progressive Multifocal Leukoencephalopathy (PML), were observed. Our results suggest that the use of natalizumab in aggressive RR-MS after HDC-AHSCT could be effective and safe. The very long-term risk of adverse events due to sequential aggressive immunosuppression has to be established.

Keywords Multiple sclerosis · Autologous haematopoietic stem cell transplantation · Natalizumab · Immunosuppression · Cyclophosphamide

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease mediated by autoreactive immune cells that affect CNS causing oligodendrocyte damage, demyelination, and axonal loss. The early phase of the disease is usually characterised by a relapsing course with further progressive accumulation of neurological disability, but in some cases aggressive and refractory disease course in spite of conventional treatment could lead to high disability in a very short period of time.

The currently available treatments include steroids for the acute phase of the relapse, immunomodulating agents (e.g., interferon beta and glatiramer acetate), immunosuppressive therapies (e.g., mitoxantrone, cyclophosphamide) and, more recently, a humanised monoclonal antibody, natalizumab.

Although many patients respond fairly well to the approved treatments, some patients continue to rapidly deteriorate and accumulate significant motor and cognitive disabilities [1].

Intense immunosuppression followed by autologous haematopoietic stem cell transplantation (AHSCT) has been assessed as a possible new therapeutic strategy in severe autoimmune disorders, particularly as it has been shown to be efficacious in animal models of immune-mediated diseases [2, 3].

The rationale relies on the eradication of the self-reactive immune cells by intense immunosuppression and the achievement of a full immune reconstitution on the

Published online: 25 November 2011

engraftment of autologous haemato-lymphopoietic stem cells.

Now more than 400 MS people have been treated with AHSCT and reported so far in the European Bone Marrow Transplantation database; despite the concerns regarding different protocols and disease forms treated, the probability of remaining alive without confirmed disability progression on EDSS (PFS) after 3 years is 60–70% and 50–60% after 6–8 years [4].

The main point of AHSCT is the conditioning regimen used to eradicate the autoreactive clones in the target organ and in the peripheral blood, bone marrow, and lymphoid tissue: in fact both PFS and toxicity depend upon the regimen used.

According to the degree of lymphoablative and myeloablative activity conditioning regimens could be classified as high-, intermediate- and low-intensity regimens [5].

Currently, a small number of conditioning protocols have been proposed and used in MS: the most protocol used in Europe is BEAM. This regimen includes 300 mg/m^2 carmustine (1,3-bis[2-chloroethyl]-1-nitrosourea) at day −7,200 mg/m^2 etoposide and 200 mg/m^2 cytarabine (arabinosylcytosine) from day −6 to day −3, and 140 mg/m^2 melphalan at day −2. BEAM is considered as an intermediate-intensity regimen. However, although effective on MRI and clinical grounds it has some drawbacks [6, 7]. In particular, there is a long-term risk of cancer associated with the treatment especially related to drugs such as etoposide and melphalan [8]. BEAM is a myeloablative regimen, inducing a profound decrease of WBC and platelets, which prolongs for 8–14 days. In this period, early toxicity is common, with fever in almost all the cases, sepsis in the majority of patients, frequent Cytomegalovirus reactivation and occurrence of other infections. Moreover, in an autoimmune disorder such as MS, a lymphoablative regimen is probably more appropriate than a deeply myeloablative therapy such as BEAM. Therefore, while at the moment BEAM plus anti-thymocytes globulin (ATG) has to be considered the best treatment schedule in the AHSCT context, the search of other conditioning regimens with a better safety profile, with more lymphoablative than myeloablative properties and a similar efficacy has to be pursued. Currently in Italy a trial with a new low-intensity conditioning regimen that aims to evaluate the safety profile, the activity on MRI and on clinical progression is ongoing: this new "Light" regimen includes cyclophosphamide 120 mg/kg followed by ATG at 3.75 mg/kg/day for 2 days [9].

More recently the new monoclonal antibody natalizumab has been approved for relapsing forms of severe MS refractory to conventional immunomodulating or immunosuppressant therapies. In addition, a retrospective post hoc analysis of data from AFFIRM study showed a significant proportion of patients (37% vs. 7% in the placebo group) completely free from disease activity (both clinically and radiologically) over 2 years irrespective to disease activity at baseline, indicating that disease remission could be a viable treatment goal in MS [10].

The aim of our article is to discuss the efficacy and safety of natalizumab in a group of aggressive MS patients in whom AHSCT failed to achieve a satisfactory control of the disease, as a recent report by Mouzaki et al. [11] described the immune response in term of T subset population in a single patient who has been treated with natalizumab after failure of AHSCT.

Methods

This is a prospective observational clinical study on a group of aggressive MS patients treated with natalizumab after failure of AHSCT.

All patients signed written informed consent and local ethic committee approved the use of AHSCT for the treatment of aggressive MS patients according to the Italian Government rules on compassionate use of therapeutics. Natalizumab has been approved later after the time of AHSCT use in these patients.

AHSCT protocol: mobilization was performed with cyclophosphamide (CY) 4 g/m^2 and granulocyte colony-stimulating factor 5 μg/kg/day. The conditioning therapy consisted of CY 120 mg/kg in 2 days and ATG 3.75 mg/kg/day in 2 days after cell reinfusion.

Natalizumab was administered according to standard approved protocol.

Clinical assessment of patients has been performed every 3 months over the study period.

After AHSCT patients performed triple-dose-gadolinium (Gd) brain MRI monthly for 6 months and then every 3 months. During natalizumab, single-dose-Gd brain MRI was performed every 6 months.

Routine blood analyses and lymphocyte subpopulation FACS analysis were performed before and during natalizumab treatment till the end of follow-up (up to 40 months). Natalizumab treatment was started exclusively after normalization of CD4, CD8 and CD4/CD8 ratio [12].

Results

Five consecutive females with aggressive relapsing-remitting MS were studied (Table 1; Fig. 1).

Patient 1, a 27-year-old woman, was diagnosed with relapsing-remitting MS in 1999 and received IFNb1a IM (for 14 months), mitoxantrone (for 16 months), glatiramer

Table 1 Demographical and clinical data of the five female RR-MS patients

Age at time of AHSCT	Months of disease duration at the time of AHSCT	No. of relapses 1 year before AHSCT	No. of months between AHSCT and natalizumab	Therapy eventually used after AHSCT and before natalizumab	No. of months without Gd-enhanced lesions after AHSCT	No. of months without relapses after AHSCT	Months of follow-up during natalizumab
24.6 (18–28)	49 (12–76)	3.2 (0[a]–8)	22.4 (13–29)	Pt 1, 4 and 5 no therapy Pt 2 IFNb1b Pt 3 IFNb1a IM	11.2 (5–19)	12.2 (6–28)	31.2 (18–40)

Data represent mean and range (in brackets)

AHSCT autologous haematopoietic stem cell transplantation, *EDSS* expanded disability status scale, *Pt* patient, *IM* intramuscular

[a] This patient was treated with AHSCT for mitoxantrone-induced leukaemia

acetate (for 6 months) and IFNb1b (for 1 month) as disease-modifying agents. In Feb 2006 she underwent AHSCT because of of mitoxantrone-related acute myeloid leukaemia [13]. 7 months after AHSCT she developed optic neuritis and six new Gd-enhanced lesions on brain MRI after 19 months. At the time of AHSCT her Expanded Disability Status Scale score (EDSS) was 6.5 with further improvement after AHSCT to 6.0. Natalizumab was prescribed 25 months after AHSCT with no evidence of clinical or radiological activity till the end of follow-up (34 months).

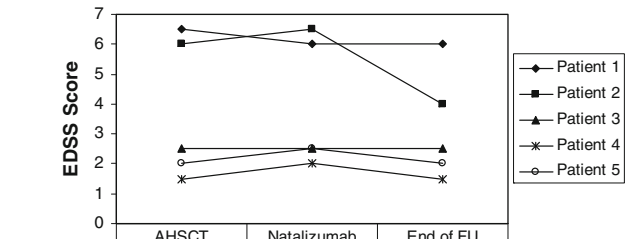
Patient 2, a 24-year-old woman with 5-year history of relapsing-remitting MS, was treated with mitoxantrone (14 months), IFNb1b (16 months), and cyclophosphamide (3 months). In January 2006 she underwent AHSCT because of continuing relapses (8/year; EDSS score of 6.0). 6 months after AHSCT she developed new Gd-enhanced lesions and clinical relapse. She was treated with IFNb1b for 3 months with neurological deterioration (EDSS score of 6.5) followed by natalizumab administration 21 months after AHSCT. A clinical relapse occurred after the first natalizumab infusion, but no other clinical relapses or Gd-enhanced lesions were detected till the end of follow-up (40 months). During natalizumab treatment she showed the reduction of neurological disability to 4.0 in EDSS score.

Patient 3, a 28-year-old woman with 4-year history of relapsing-remitting MS, previously treated with IFNb1a and mitoxantrone (30 and 18 months respectively), also suffered from type-I diabetes. Three relapses occurred during 1 year; for this reason, in December 2006 she underwent AHSCT (EDSS score of 2.5). Gd-enhanced lesions and clinical relapse developed 5 months after AHSCT; thereafter IFNb1a IM was administered for 6 months and then natalizumab was prescribed. Gd-enhanced lesions were detected 2 months after starting natalizumab treatment; further follow-up (36 months) did not show any clinical (EDSS score of 2.5) or MRI activity.

Patient 4, a 18-year-old woman with one-year history of relapsing-remitting MS was previously treated with IFNb1a SC for 12 months. Despite the treatment she developed three relapses during 1 year and underwent AHSCT in July 2006 (EDSS score of 1.5). After AHSCT she showed Gd-enhanced lesions and clinical relapse 14 months after AHSCT (EDSS score of 2.0). No new clinical relapses or Gd-enhanced lesions were detected during 28 months of natalizumab treatment (EDSS score of 1.5).

Patient 5, a 26-year-old woman with 3-year history of relapsing-remitting MS, previously treated with IFNb1b and mitoxantrone (8 and 4 months respectively), showed significant clinical (8 relapses during the last 2 years) and MRI disease activity. AHSCT was performed in Jan 2007 with MS course stabilization for 28 months. One year after AHSCT a single Gd-enhanced lesion was observed on brain MRI with clinical activity. In May 2009 (28 months after transplantation) she developed clinical relapse with multiple Gd-enhanced lesions on MRI. At that time natalizumab was prescribed with no evidence of clinical or radiological activity till the end of follow-up (18 months).

Anti-natalizumab antibodies tested negative in all patients. Persistent increase of CD19+ B-cells was



	AHSCT	Natalizumab	End of FU
Patient 1	6,5	6	6
Patient 2	6	6,5	4
Patient 3	2,5	2,5	2,5
Patient 4	1,5	2	1,5
Patient 5	2	2,5	2

Fig. 1 EDSS score at the time of AHSCT, natalizumab onset and at the end of follow-up

detected during natalizumab in all patients. No severe adverse events occurred during natalizumab treatment.

Discussion

Intense immunosuppression followed by AHSCT in aggressive MS patients refractory to immunomodulatory treatments showed progression-free survival proportion between 50 and 77% in the short term [5]. In particular, use of HDC followed by AHSCT in patients with aggressive MS can stop the disease for a limited period without long-term effect, as showed by our five prospective cases and other recent reports [14].

Taking into consideration the conditioning regimen used in our patients, it has to be debated whether this kind of "Light" non-myeloablative protocol could achieve profound immunosuppression: in fact, other regimen based on higher dosage of cyclophosphamide failed to achieve a complete control of the disease. Nevertheless, to some extent immunosuppression has been reached demonstrated by the fact that both MRI and clinical activity occurred after more than half a year from transplantation (mean 11.2 and 12.2 months, respectively).

More importantly, no data are available on possible therapeutic options after intense immunosuppression followed by AHSCT taking into consideration the increased risk of infections and malignancies.

Although not a form of immunosuppression, natalizumab is considered for patients with very active MS on the basis of data from clinical trials [10]. In our patients we tried to use natalizumab as rescue therapy as soon as it became available in Italy.

Our results show that natalizumab could be effective in patients after HDC followed by AHSCT, for at least 31.2 months (range 18–40 months): the appearance of clinical relapse after the first injection of natalizumab in patient 2 is consistent with previous data of early relapses after the first infusion [15].

However, it is still unknown how long immunosuppression persists after AHSCT and to what extent it enhances the risk of opportunistic infections. The risk of PML JCV-related or other opportunistic infections in these patients because of prolonged immunosuppression after AHSCT must be considered: recent data in fact report a higher risk of PML in natalizumab-treated patients who previously underwent immunosuppressive therapies (Biogen data on file reported at ECTRIMS 2010). Nevertheless, none of the patients who developed PML after natalizumab was previously treated with AHSCT.

Taking into consideration that there is now evidence that AHSCT in humans triggers a reconstitution programme that leads to the comprehensive renewal of the T-cell repertoire [16], it can be debated if it could also restore the protection against infection and in particular against opportunistic infection as PML.

In fact also the reappearance of MS disease activity demonstrates the immune system reconstitution, and so it can be argued that the renewal of immune system by CD34+ cells' infusion and bone marrow colonization completely restore the immunocompetence and that if this is the case no prolonged effect of immunosuppression exists. That was proved by the normal values of lymphocyte subpopulation and serum immunoglobulin concentration before starting natalizumab in our patients (mean 22.4 months after AHSCT, range 13–29 months).

No severe adverse events occurred during natalizumab. Immunological findings were similar to other natalizumab-treated patients. In fact, the increased number of CD19+ B-cells has already been described [17].

Whether the effect of natalizumab therapy is strengthened by previous immunosuppression or is independent is as yet unknown: it should be taken into consideration that renewal of the immune system could modify the response to further disease-modifying therapy [16].

In conclusion, our experience suggests that the use of natalizumab in aggressive MS patients after HDC followed by AHSCT could be effective and safe, as also described by Mouzaki et al. [11].

The very long-term risk of adverse events due to sequential aggressive immunosuppression has to be established in larger multicentre studies.

Conflict of interest This study was not sponsored. M. Capobianco received grants from Biogen-Idec, Biogen-Dompè, Sanofi-Aventis, Schering-Bayer, Merck-Serono and Teva. A. Bertolotto has served as a consultant and received research grants from Biogen-Idec, Biogen-Dompè, Sanofi-Aventis, Schering-Bayer, Merck-Serono and Teva. M. G. Marrosu has served as a consultant and received research grants from Biogen-Idec, Biogen-Dompè, Sanofi-Aventis, Schering-Bayer, Merck-Serono and Teva. J. Frau received grants from Biogen-Idec, Biogen-Dompè, Sanofi-Aventis, Schering-Bayer, Merck-Serono and Teva. E. Cocco received grants from Biogen-Idec, Biogen-Dompè, Sanofi-Aventis, Schering-Bayer, Merck-Serono and Teva. E. Mamusa received grants from Biogen-Idec, Biogen-Dompè, Sanofi-Aventis, Schering-Bayer, Merck-Serono and Teva.

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