Natalizumab in the treatment of multiple sclerosis

Therapeutic Advances in Neurological Disorders
(2009) 2(2) 115–128
DOI: 10.1177/1756285608101861

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***Abstract*:** Natalizumab reduced the rate of clinical relapse at one year by 68% and the risk of sustained progression of disability by 42–54% over 2 years in its pivotal phase III trial (AFFIRM) in relapsing–remitting multiple sclerosis (RRMS). Natalizumab is generally well tolerated, but due to rare and potentially fatal side-effects, it was approved with a restricted-distribution format in 2006. Expert statements and the European Medical Agency recommend the use of natalizumab after failure of first-line disease-modifying therapies in patients with relapsing forms of MS. As part of the risk management plan, worldwide extensive safety programmes aim to provide more data on natalizumab safety in clinical practice. At the end of September 2008, 48 000 patients have received natalizumab and 18 000 patients are on treatment for at least 1 year. The assessment of risk and benefit is still ongoing.

***Keywords*:** natalizumab, multiple sclerosis, disease-modifying therapy

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Introduction

Multiple sclerosis (MS) is regarded as a chronic inflammatory autoimmune disease of the central nervous systems (CNS) that affects more than 2.5 million people worldwide [Compston, 2006]. Most patients suffer from a relapsing–remitting course that is usually characterised by between one and two episodes of neurological deficits per year that often tend to resolve at least partly after days to months. However, single relapses can cause permanent disability. While severe disability is infrequent in the early years after diagnosis, patients experience relevant disability after a median time of about 8 years (Expanded Disability Status Scale (EDSS) 4) and may become wheelchair-bound after about 30 years on average [Confavreux and Vukusic, 2006]. The overall healthcare costs of MS in the United States and Europe are similar and at a mean about $50 000 per patient per year and rise tremendously with increasing disability. The proportion of cost of disease-modifying therapy (DMT) depends on disability status and range from 50% (EDSS < 4) to about 23% (EDSS > 6) [Kobelt *et al.* 2006].

The aetiology and pathogenesis of MS are not fully understood. Autoreactive immune cells (auto-aggressive T-cells), targeted to myelin constituents, migrate across the blood–brain barrier and initiate inflammatory processes within the CNS. Transmigration is regulated by chemo-attractant cytokines and adhesions molecules. Natalizumab is the first licensed monoclonal antibody directed to one of these adhesion molecules [Leger *et al.* 1997]. It was derived from experimental data in the 1990s [Yednock *et al.* 1992] with a mere 13 years between proof of concept and clinical licensing.

Mechanism of action of natalizumab

Natalizumab is an immunoglobulin G4 (IgG4) kappa monoclonal antibody produced in murine myeloma cells. It contains a human IgG4 framework region and complementarity determining regions of a murine antibody. It does not activate complement and persists longer in the blood than other immunoglobulins [Mountain and Adair, 1992]. Natalizumab is directed against α4-beta-1-integrin (very late activation antigen-4, VLA-4), a surface molecule found on all leukocytes with exception of neutrophils [Stüve *et al.* 2007; Leger *et al.* 1997]. Thus, natalizumab inhibits the interaction between VLA-4 and vascular cell adhesion molecule-1 (VCAM-1)

expressed on endothelial cells. It is thought that this interaction controls leukocyte adhesion, attachment and migration across the blood–brain barrier into CNS [Sandborn and Yednock, 2003; Lobb and Hemler, 1994; Yednock *et al.* 1992; Burkly *et al.* 1991; Damle and Aruffo, 1991]. Natalizumab may also alleviate ongoing CNS inflammation, mediated by leukocytes already present in the CNS by blockade the interactions between VLA-4 and extracellular matrix proteins such as osteopontin and fibronectin [Bayless *et al.* 1998; Lobb and Hemler, 1994]. Moreover, natalizumab can inhibit the interaction of leukocytes with the mucosal adressin cell adhesion molecule-1 in the small intestine. Thus, it can block the transendothelial migration of mononuclear cells into inflammatory tissue in patients with Crohn's disease [von Andrian and Engelhardt, 2003].

Pharmacokinetics and pharmacodynamics

The pharmacokinetic of natalizumab was systematically investigated in three single infusion studies with volunteers, in nine target-population studies with MS patients and two pharmacokinetic studies with patients with Crohn's disease [European Medicine Agency, 2006; Vollmer *et al.* 2004; Rudick and Sandrock, 2004; Sheremata *et al.* 1999]. These data provided evidence that the pharmacokinetic of natalizumab is nonlinear, resulting in a fixed dose recommendation of 300 mg. Patients who received 3 mg of natalizumab per kg body-weight had more than 80% VLA-4 saturation on peripheral blood leukocytes [Miller *et al.* 2003] and detectable concentrations for 8 weeks [Sheremata *et al.* 1999]. After multiple doses, natalizumab has a mean half-life of 16 ± 4 days with a clearance of 13.1 ± 5 ml/hour. The clearance was only weakly correlated with body weight over the range 40–100 kg. The biological effects persist for about 12 weeks and changes in the distribution of cerebrospinal fluid (CSF) cells for about 6 months after cessation were found [Hauser and Weiner, 2006; Niino *et al.* 2006; Stüve *et al.* 2006a]. Higher doses of natalizumab resulted in longer mean half-lives and slower mean total body clearance despite the use of weight-based dosing [Sheremata *et al.* 1999]. Pharmacokinetics of natalizumab in paediatric MS patients, elderly patients or patients with renal or hepatic insufficiency have not been studied.

Efficacy in clinical studies

MRI efficacy of natalizumab in MS patients were first reported in 1999 [Tubridy *et al.* 1999]. In this randomized, double-blind, placebo-controlled trial, 72 patients received either two infusions of natalizumab (3 mg/kg) with an interval of 4 weeks or placebo. At week 12 the number of new active lesions on MRI was significantly lower in the treatment arm.

In 2003 the results of a major phase II trial were published [Miller *et al.* 2003]. Sixty-eight patients received 3 mg/kg of natalizumab, 74 patients 6 mg/kg of natalizumab, and 71 patients placebo intravenously every 28 days for 6 months. Natalizumab suppressed the formation of contrast-enhancing lesions by about 90% (primary endpoint). This effect was already manifest 1 month after the first dose and sustained over the whole treatment phase. Based on these positive preliminary results, two large phase III studies (AFFIRM and SENTINEL) were conducted [Polman *et al.* 2006; Rudick *et al.* 2006]. Both were multicenter, double-blind, randomized and placebo-controlled trials. Study characteristics of AFFIRM are summarized in Table 1.

AFFIRM investigated 942 patients randomly assigned to receive natalizumab at a dose of 300 mg (627 patients) or placebo (315 patients) by intravenous infusion every 4 weeks for 2 years. After 1 year of treatment the annualized relapse rate in the natalizumab group was significantly lower (0.26) compared with the placebo group (0.81) (relative risk reduction of 68%; $p < 0.001$). The effect was maintained at 2 years. At 2 years, the cumulative probability of disability progression was 17% in patients receiving natalizumab compared to 29% in the placebo group (relative risk reduction of 42%; $p < 0.001$). Regarding the secondary endpoints, natalizumab reduced both the mean number of new or enlarging hyper-intense T2 MRI lesions over two years from 11.0 to 1.9 (relative risk reduction 83%; $p < 0.001$) and the mean number of gadolinium-enhancing lesions from 1.2 to 0.1 (relative risk reduction of 92%; $p < 0.001$).

In the SENTINEL study ($n = 1171$, 589 randomised to natalizumab) [Rudick *et al.* 2006], natalizumab 300 mg or placebo was added to interferon-beta 1a once weekly intramuscular (Avonex®) for patients who had had at least one relapse during 12 months of previous treatment

Table 1. Results of the AFFIRM study [Polman *et al.* 2006].

AFFIRM			
Design	Randomized double-blind placebo-controlled parallel-group trial		
Inclusion criteria	RRMS, EDSS 0–5, relapse within 1 year before enrolment, age 18–50 years		
Exclusion criteria	PPMS, SPMS, PRMS, relapse within 50 days before first dose, cp or mitox within the previous year, immunomodulatories for more than 6 months		
Duration of the study	120 weeks		
	Natalizumab 300 mg i.v. monthly	Placebo	*p* value
Subjects			
- Randomised	627	315	
- Completing 1 year	609	296	
- Completing 2 years	589	285	
- Age, years, median (range)	36 (18–50)	37 (19–50)	
- Duration of MS disease, years, median (range)	5 (0–34)	6 (0–33)	
- Time since diagnosis, years, median (range)	2 (0–24)	2 (0–23)	
- Relapses in previous year, mean ± SD	1.53 ± 0.91	1.5 ± 0.77	
- EDSS baseline, median (range)	2 (0–6)	2 (0–6)	
Results			
Annual relapse rate			
- After 1 year (primary endpoint)	0.261	0.805	<0.001
- After 2 years	0.235	0.733	
- Odds ratio at 2 years	0.32 ($CI_{95\%}$0.26–0.4)		<0.001
Relapse free			
- After 1 year	77%	56%	<0.001
- After 2 years	67%	41%	<0.001
Disability			
- Cumulative probability of progression (primary outcome, 24-week confirmation)	11%	23%	<0.001
- Hazard ratio	0.46 ($CI_{95\%}$0.33–0.64)		<0.001
MRI			
- Median% change in T2 lesion volume	−9.4%	+8.8%	<0.001
- Mean number of new or new enlarging T2 lesions	1.9	11.0	<0.001
- Mean number of Gd-enhancing lesions	0.1	1.2	<0.001

Abbreviations: EDSS, Expanded Disability Status Scale; SD, standard deviation; Gd, gadolinium; $CI_{95\%}$, 95% confidence interval. RRMS, relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis; PRMS, progressive relapsing multiple sclerosis; cp, cyclophosphamide; mitox, mitoxantrone.

with interferon-beta 1a intramuscular (Avonex®). Overall efficacy parameter resembled results from AFFIRM study. The study ended a month earlier than planned, because of the occurrence of progressive multifocal leukoencephalopathy (PML) in two patients who received natalizumab in addition to interferon-beta 1a intramuscular.

Adverse events

Natalizumab was well tolerated in its pivotal trials. In AFFRIM, 6% of the patients receiving natalizumab and 4% of the patients in the placebo group discontinued the study. The most common adverse events (AEs) in the phase II trials were headache and infections. Two deaths

occurred during the AFFIRM study, both in the natalizumab group: one patient died due to malignant melanoma, which was pre-existing, a second patient died of alcohol intoxication after having received 25 doses of natalizumab. Other AEs in AFFIRM included headache (38% in the natalizumab group *versus* 33% in the placebo group), fatigue (27% in the natalizumab group *versus* 21% in the placebo group), arthralgia (19% in the natalizumab group *versus* 14% of the patients receiving placebo) and allergic reactions (9% in the natalizumab group *versus* 4% in the placebo group). The overall frequency of infections did not differ between natalizumab and placebo.

Acute hypersensitivity reactions

Acute hypersensitivity reactions usually occur within 2 hours after beginning of natalizumab infusions. In AFFIRM, 27 hypersensitivity reactions occurred in 25 patients receiving natalizumab (4%). Seventeen of these 25 patients (68%) were persistently anti-natalizumab antibody positive. Fifty-six per cent of the hypersensitivity reactions (mostly urticaria) occurred during the second infusion. Re-exposure was the major risk for developing acute hypersensitivity reactions and the risk of developing type I allergic reactions decreased with longer treatment duration. Severe hypersensitivity reactions were rare (0.8%) and patients recovered without sequelae [Polman *et al.* 2006].

Infusion-related symptoms

In AFFIRM, infusion-related symptoms occurred in 19% of patients receiving natalizumab compared with 14% of patients with placebo. The most common infusion related symptom was headache (5% in the natalizumab group *versus* 3% in the placebo group). Interestingly, serum sickness-like reactions (serum III allergic reaction) in antibody-negative patients were described recently [Hellwig *et al.* 2008]. Such patients with delayed infusion related discomfort better tolerated the natalizumab infusion by administration of 250 mg methyl-prednisolone intravenously the day before and after natalizumab infusions and decreasing the infusion rate from 100 ml/h to 50 ml/h.

Anti-natalizumab antibodies

Infusion-related AEs and anti-nataliazumab antibodies were associated. In the pivotal trials, infusion-related reactions occurred in 76% of persistently antibody positive patients compared with 20% of antibody-negative and 25% of transiently positive patients [Rudick *et al.* 2006; Polman *et al.* 2006]. Hypersensitivity reactions were experienced by 46% of the persistently anti-natalizumab antibody positive patients, 15% of the transiently positive patients and 0.7% of antibody-negative patients. Anti-natalizumab antibodies had the potential to neutralise natalizumab in vitro. About 9–11% of the patients receiving natalizumab developed antibodies at least once during treatment, 3–5% transiently and 6% persistently. The presence of antibodies was correlated not only with a higher incidence of infusion-related symptoms but also with a reduction in serum concentrations of natalizumab and reduced efficacy. In patients with persistent anti-natalizumab antibodies the level of natalizumab remains below the limit of quantification. Owing to high therapy costs and near loss of efficacy in persistently antibody-positive individuals [Calabresi *et al.* 2007], antibody testing is justified in every patient receiving natalizumab. The antibody testing should be repeated after 2–3 months. Persistent antibodies to natalizumab should result in treatment discontinuation [Krumbholz *et al.* 2007].

Progressive multifocal leukencephalopathy

Progressive multifocal leukencephalopathy (PML) is a demyelinating infectious CNS disease usually observed in immunodeficient patients, especially HIV-positive individuals, caused by the JC virus. JC are the initials of the first patient in whom the virus was isolated in 1971 [Padgett *et al.* 1971].

JCV is a double-stranded DNA virus. Depending on the region, up to 95% of the population have antibodies against JCV [Walker, 1983]. Following infection, the virus becomes latent in bone marrow, tonsils, spleen and kidney but it is unclear whether latency universally follows infection [Sabath and Major, 2002; Caldarelli-Stefano *et al.* 1999; Monaco *et al.* 1998, 1996]. JCV has been identified in circulating white blood cells, predominantly in B cells of healthy adults and AIDS patients [Gallia *et al.* 1997] and in almost 50% of brains from immunological normal individuals post mortem [Elsner and Dorries, 1992; Mori *et al.* 1992; White *et al.* 1992].

PML was first described in 1958 in chronic lymphatic leukaemia and Hodgkin disease [Astrom *et al.* 1958] and it remained a rare condition until the advent of AIDS in the 1980s. From 1958 to 1984 only 230 cases were described in English language literature [Brooks and Walker, 1984]. Since 1979 the incidence of PML has increased 50-fold. Eighty-five per cent of patients with PML are HIV-positive, but even in AIDS patients in the pre-high active antiretroviral therapy era the prevalence of PML was only 5% [Koralnik, 2006]. PML has been also described in patients with lymphoproliferative diseases, organ transplants, bone marrow transplantation and in patients receiving immunosuppressive drugs (including azathioprine and mitoxantrone) [Crowder *et al.* 2005; Shitrit *et al.* 2005; Cuevas and Fuchs, 2004; Warnatz *et al.* 2003; Daibata *et al.* 2001; Ouwens *et al.* 2000; Tubridy *et al.* 2000; Morgenstern and Pardo, 1995; Rankin and Scaravilli, 1995; Silver *et al.* 1995; White *et al.* 1992; Dawson, 1982; Nagashima *et al.* 1982; Peters *et al.* 1980; Weitzman *et al.* 1978; Malas and Weiss, 1977; Bleyer *et al.* 1973]. Until 2006 PML has not been described in MS patients.

Pathogenesis of PML in MS patients

The pathogenesis of PML in patients receiving natalizumab is complex and not fully understood. The issue of PML in patients with monoclonal antibodies is not restricted to natalizumab [Houff and Berger, 2008; Martin *et al.* 2006; Uppenkamp *et al.* 2002]. PML occurred in association with rituximab, a monoclonal antibody which induces B-cell depletion and efalizumab in a patient with psoriasis [Crowder *et al.* 2005; Shitrit *et al.* 2005]. Nevertheless PML in natalizumab-treated patients remains unique because the patients with PML treated with other monoclonal antibodies had conditions that were already associated with a higher risk for PML. Currently, it is unclear whether PML is an off-target AE of natalizumab or a direct consequence of VLA-4 blocking. Natalizumab was found not only to decrease the number of CD4+ T cells within the CSF up to 6 months after cessation of natalizumab treatment but also the number of antigen-presenting cells and the expression of MHC class II in the Virchow Robin spaces but the significance of this finding with regard to PML is uncertain. It is unclear whether the duration of natalizumab treatment predisposes to development of PML *per se*. Previous cases occurred after 8–37 doses and a pattern for PML development is not obvious. Also, there is no evidence that a 'drug holiday' could decrease the risk of PML [Martin *et al.* 2008 Stüve *et al.* 2006a, 2006b]. It is noteworthy that the patient with Crohn's disease and PML had a drug holiday [Van Assche *et al.* 2005]. He received three monthly infusions of natalizumab during the Evaluation of Natalizumab as Continuous Therapy 1 (ENACT-1) trial, followed by treatment with placebo for 9 months in the ENACT-2 trial. First symptoms of PML occurred after a further five infusions of natalizumab.

Houff and Berger [2008] postulated that by blocking VLA-4, natalizumab may prevent the entry of JCV-specific cytotoxic T cells into the brain, necessary for the control of latent JCV infection. Another possible pathogenetic mechanism includes the VLA-4 dependent homing and retention of lymphocytes in bone marrow and spleen [von Andrian and Engelhardt, 2003], both sites of viral latency. This mechanism leads to an increase of peripheral leukocytes and possibly also to an increase of JC viral load. Retrospective analyses of 214 serum samples collected during the treatment with natalizumab showed detectable JCV DNA in only 2.3% of patients, which is not outside of the expected range in healthy individuals [Major *et al.* 2005], but assaying blood plasma may underestimate the JCV viral load [Houff and Berger, 2008]. However, the presence of JCV DNA in the blood of MS patients on interferon-beta treatment did not seem to be a risk factor for PML [Delbue *et al.* 2007]. Plasma JCV DNA is neither predictive nor diagnostic for PML [Koralnik, 2006]. Thus, routine blood testing for plasma JCV is not helpful to improve drug safety [Gold *et al.* 2007]. Although the detection of JCV DNA in CSF is not a mere biomarker for PML, this test confirms the diagnosis in suspected cases. However in 10–40% of HIV patients with active PML, JCV DNA could not be detected in CSF [Marzocchetti *et al.* 2005; McGuire *et al.* 1995]. In natalizumab recipients, JCV DNA was detected in CSF only in patients with PML and not in 400 samples of CSF without PML [Yousry *et al.* 2006]. JCV DNA polymerase chain reaction (PCR) in CSF is recommended in all natalizumab recipients presenting with any clinical feature suggestive of PML.

PML in MS patients

During the pivotal trials with natalizumab, three PML cases occurred: one case in a Crohn's disease trial (ENACT) [Van Assche *et al.* 2005] and two cases in MS patients in SENTINEL [Kleinschmidt-DeMasters and Tyler, 2005; Langer-Gould *et al.* 2005]. PML in the patient with Crohn's disease was first histologically diagnosed as astrocytoma but post-mortem analysis revealed the diagnosis of PML. In July and October 2008, Biogen Idec and Elan distributed information about three further new PML cases. Among them is one patient who had never received any other DMT. These are the first cases of PML in an MS patient receiving natalizumab as monotherapy outside from clinical trials. Table 2 summarizes all cases of PML associated with natalizumab therapy thus far.

Management of new neurological symptoms in natalizumab patients

PML should be taken into account in any patient receiving natalizumab who presents with new neurological symptoms. Although there may be some typical differences between PML and MS relapses, differentiation can be difficult in individual cases. So far, optic neuritis has not been reported in PML and spinal manifestation is very rare [Bernal-Cano *et al.* 2008]. In contrast to patients with MS relapses, the development of subacute neuropsychological abnormalities are common in PML. In case of relapse, short courses of glucocorticosteroid therapy (3–5 days methyl-prednisolone 500–1000 mg/day) can be given during therapy with natalizumab. A long-lasting combination therapy with steroids should be avoided since it results in immunosuppression [Gold *et al.* 2007].

In case of uncertainty and PML suspicion, natalizumab treatment has to be stopped and MRI has to be performed. If the clinical presentation and/or the MRI raise suspicion to PML, CSF for JCV DNA testing by PCR has to be obtained. If PCR is negative but suspicion remains, repeated CSF analyses have to be performed until PCR is positive or PML can be ruled out. PML treatment may be initiated before PCR results are indicative in cases of strong clinical suspicion and/or typical MRI findings (see below). Physicians should be aware of the possibility that other opportunistic infections may occur during natalizumab treatment and should include them in the differential diagnosis.

Table 2. Overview of PML cases in patients receiving natalizumab.

n	Authors	Year	Gender	Age	Disease	Location	No. of doses	Study	Treatment regime	DMT before	Outcome
1	Van Asche *et al.*	2005	M	60	Crohńs	Europe	8	ENACT	Monotherapy	Aza, infliximab	Death
2	Langer-Gould *et al.*	2006	M	41	RRMS	USA	28	SENTINEL	Combination with Ifn-b	None	Severe Disability
3	Kleinschmidt *et al.*	2006	F	46	RRMS	USA	37	SENTINEL	Combination with Ifn-b	None	Death
4	Biogen data[1]	2008	M	37	RRMS	Europe	17	Post-Marketing	Monotherapy	None	Disabled*
5	Biogen data[1]	2008	M	52	RRMS	Europe	14	Post-Marketing	Monotherapy	Ifn-b, aza	Disabled*
6	Biogen data[2]	2008	n.k.	n.k.	RRMS	USA	14	Post-Marketing	Monotherapy	Ifn-b, ga, mtx	n.k.

Abbrevations: no. of doses, number of doses of natalizumab prior to first PML-symptoms; DMT, disease modifying therapy; RRMS, relapsing-remitting multiple sclerosis; aza, azathioprine; mtx, methotrexat; ga, glatiramer acetate; ifn-b, interferon-beta; ENACT, Evaluation of Natalizumab as Continuous Therapy in Crohn's disease. [1]Data presented by R. Gold at the World Congress on Therapy of Multiple Sclerosis in Montreal, Sep, 2008; [2] Press release from Biogen Idec on 29 oct 2008; * current status; n.k., currently not known.

Table 3. Therapeutic options in PML [Stüve *et al.* 2007].

Therapeutic options in natalizumab-associated PML	
Antiviral therapy	■ Cidofovir ■ Topotecan ■ Mefloquine ■ Cytosine arabinoside
Immunomodulatories	■ Interferon-beta ■ Interferon-alpha ■ Interleukin 2 ■ 5HT-2a receptor blockers ■ Intravenous immunoglobulins
Haematopoietic growth factors	■ Granulocyte colony stimulating factors
Other interventions	■ Plasmapheresis ■ Leukapheresis

Treatment options of PML in patients receiving natalizumab

There is no proven treatment for PML. Early PML diagnosis could be of crucial importance and immune reconstitution may result in improved outcome [Shitrit *et al.* 2005; Clifford *et al.* 1999]. Recently, Stüve and Bennett [2007] reviewed possible interventions in patients with PML. Potential therapeutic options are summarized in Table 3. The primary goals of PML treatment include immune reconstitution, antiviral therapy and elimination of natalizumab. However, it remains uncertain if any of these strategies will improve the outcome.

To eliminate free unbound natalizumab, plasma exchange is thought to be effective, safe and well tolerated [Khatri *et al.* 2008; Lehmann *et al.* 2006a, 2006b]. According to the natalizumab PLEX study, five plasma exchange sessions every other day reduces natalizumab concentrations to <1 mg/ml in over 95% of patients [Khatri *et al.* 2008; Lehmann *et al.* 2006a, 2006b]. Eighteen days after plasma exchange leukocytes transmigration significantly improved in an *in vitro* model of the blood–brain barrier [Fox *et al.* 2008].

Intravenous immunoglobulins may represent another therapeutic option. By binding to the antigen-binding fragment of natalizumab, immunoglobulins could block the binding of natalizumab to VLA-4. Currently, experimental approaches to PML treatment include the evolution of antisera from patients who have developed anti-natalizumab antibodies and proteins that mimic VLA-4 antigen. To date, there are no uniform guidelines for PML treatment [Marra *et al.* 2002; Hall *et al.* 1998].

Analogue to AIDS patients, the reconstitution of the immune system can be associated with increasing inflammation and clinical deterioration, so-called immune reconstitution inflammatory syndrome (IRIS) [Kappos *et al.* 2007]. In HIV-positive patients, IRIS is usually associated with an increasing CD4+ cell count in plasma and decrease of HIV RNA load [Venkataramana *et al.* 2006]. The management of IRIS is beyond the limits of this review.

Malignancy

The incidence of breast cancer and basal cell carcinoma seen in AFFIRM was within keeping of the rates commonly seen in the population at large [European Medicine Agency, 2006]. There is no evidence for mutagenic effects of natalizumab either clinically or *in vitro* (human chromosomal aberration assays). Natalizumab had no effect on tumour growth or metastasis in a xenograft model involving an α4-integrin–positive human melanoma and leukemia tumour line implanted in nude mice [European Medicine Agency, 2006]. In February 2008 two cases of melanoma in women with MS treated with natalizumab were published [Mullen *et al.* 2008]. The authors presented in one patient a 'rapidly changing mole' after a single dose of natalizumab and in another patient an ocular melanoma derived from a long-standing ocular nevus after several doses of natalizumab. Another case of metastatic melanoma occurred in the AFFIRM study [Polman *et al.* 2006]. There is controversy regarding the potential effect of natalizumab on melanoma cells. While the one postulate that the integrin-endothelium-contact could promote metastasis [Klemke *et al.* 2007; Garofalo *et al.* 1995], the others believe

in a protective effect of the integrins and harmful effect of natalizumab [Qian *et al.* 1994].

We have also observed a melanoma in situ (unpublished data) derived from an atypical mole in a 41-year-old woman after 12 doses of natalizumab. Treatment was continued after surgical excision and we could neither ascertain 'rapidly changing moles' nor 'dramatic increase in size' of atypical nevi. Currently, there is no evidence that melanoma is more common in patients treated with natalizumab than in the general population [Panzara *et al.* 2008]. Atypical moles should be closely monitored. Active malignancy except of basal cell carcinoma represents a contraindication for natalizumab treatment.

Increase of liver enzymes

Potential liver injury with natalizumab raised interest in February 2008. In AFFIRM, the incidence of hepatic events and an increase in liver enzymes were 4–5% and similar in the verum and placebo group [Polman *et al.* 2006]. Liver-related severe AEs in natalizumab-treated individuals were attributable to other causes such as cholelithiasis or drugs including interferon-beta. However, one patient in the phase I study in healthy volunteers did develop an unexplained hepatitis [European Medicine Agency, 2006]. As in November 2007, the total cumulative exposure to natalizumab was estimated to be 24 000 patients, there were eight serious cases of hepatic events where the association to natalizumab was probable. In two patients the elevated liver enzymes recurred upon re-challenge, providing evidence for natalizumab-induced injury. All patients had at least one confounding risk factor. No case resulted in liver transplant or death. Currently, liver enzyme examination is recommended before treatment initiation with natalizumab and in case of clinical signs of liver dysfunction.

Pregnancy

Immunohistological studies indicated that natalizumab binds to the placenta and foetal tissues, indicating a potential for teratogenic and/or abortifacient activity. A reproductive toxicology study evaluating the effects of natalizumab demonstrated no foetotoxicity or drug-related teratogenic effects.

Data on 95 pregnancies during clinical studies and three pregnancies post-marketing have shown that exposure to natalizumab during pregnancy had no negative effect on pregnancy outcomes [Bozic *et al.* 2007]. Since there is lack of data on natalizumab therapy in pregnant women, natalizumab is contraindicated during pregnancy. In case of pregnancy during natalizumab treatment, immediate discontinuation of natalizumab is necessary and close monitoring of the foetus should follow. Ideally, natalizumab should be discontinued at least 3 months before conception.

License

Natalizumab was first approved by the Food and Drug Administration (FDA) for treatment of RRMS in November 2004 on the basis of 1-year results of the pivotal trials AFFIRM and SENTINEL. Because of the two PML cases, distribution of natalizumab was suspended in March 2005. An extensive safety study on 3417 patients with MS, Crohn's disease or rheumatoid arthritis who had received natalizumab in clinical trials was undertaken. Additional cases of PML were not identified [Yousry *et al.* 2006]. In March 2006, the Advisory Panel of the FDA voted in favour of the return of natalizumab on the market as monotherapy in relapsing forms of MS with a black-box warning about PML. Treatment with natalizumab was restricted to physicians participating in a risk management program (Tysabri® Outreach: Unified Commitment to Health, TOUCH). In addition, the safety of natalizumab is being further assessed in a 5000-patient registry cohort (Tysabri Global Observation Program in Safety, TYGRIS) in the European Union and North America with 5-year follow-up for infections requiring hospitalization, cases of PML, malignancies, and all AEs that are serious or medically significant.

Current indication

Head-to-head studies to determine the relative efficacy of natalizumab in comparison with other DMTs were not undertaken. Results from phase III trials suggested superior efficacy compared to the corresponding trials for interferon-beta or glatiramer acetate, but across-trial comparisons can be misleading.

Because of safety concerns, EMEA has restricted the use of natalizumab to the following patients groups: (a) patients who have failed to respond

to a full and adequate course of interferon-beta. Patients should have had at least one relapse in the previous year while on therapy, and have at least 9 T2-hyperintense lesions in cranial MRI or at least one gadolinium-enhancing lesion; and (b) patients with rapidly evolving severe relapsing remitting MS, defined by two or more disabling relapses in one year, and with one or more gadolinium-enhancing lesions on brain MRI or a significant increase in T2 lesion load as compared with a previous recent MRI. [European Medicine Agency, 2006]. Phase II trials supported a benefit of natalizumab in patients with progressive forms who still have relapses.

Before switching from other DMT to natalizumab it is obligatory to evaluate possible reasons for lack of efficacy; for example, pseudo relapses (during fever, infections), neutralizing antibodies to interferon-beta or nonadherence. Currently, there are no data from phase III trials for patients below the age of 18, over 55 years or EDSS > 5.

Contraindications

Natalizumab is contraindicated in immunocompromised patients with increased risk of opportunistic infections. Immune competence is crucial before treatment initiation. Expert recommendations suggested that neutrophils should be more than 1500 cells/µl, lymphocytes more than 1000 cells/µl, CD4+ cells more than 500 cells/µl and CD8+ cells more than 250 cells/µl [Gold *et al.* 2007]. Also the CD4/CD8 ratio should be within normal limits. However, there is no evidence that CD4 T-cells play a critical role in the development of opportunistic infections during natalizumab treatment. Natalizumab is contraindicated in patients with PML. Concomitant immunomodulatory agents or immunosuppressive drugs are contraindicated.

Natalizumab treatment guidelines

Natalizumab should only be used by physicians with adequate facilities for the application of monoclonal antibodies, with experience in the diagnosis and treatment of MS, and with timely access to MRI (preferably within 24 hours). Natalizumab is administered at a dose of 300 mg intravenously over 1 hour. Patients have to be monitored one additional hour after infusion including clinical surveillance, intermittent measurement of pulse and blood pressure. A temporary suspension of the drug (i.e. for days up to a few weeks) is not expected to compromise its effectiveness [O'Connor *et al.* 2006]. Expert guidelines were issued to provide further guidance of patient selection and monitoring [Gold *et al.* 2007; Kappos *et al.* 2007].

Pre-treatment examinations and wash-out period

The pivotal studies do not provide guidance for the switching procedures in clinical practise. Expert statements were issued but one has to acknowledge that the underlying evidence is weak. Currently, for interferon-beta and glatiramer acetate it is unclear whether a wash-out is necessary and if such a procedure will have any impact on the risk of PML [Kappos *et al.* 2007]. We cessate interferon-beta or glatiramer acetate 2 weeks before natalizumab treatment. Recommendations on an empirical basis for azathioprine, methotrexate and mycophenolate mofetil suggest a wash-out period of 3 months and for mitoxantrone of 6 months [Kappos *et al.* 2007; Hartung *et al.* 2002]. Moreover, clinical and laboratory findings (see section on 'contraindications') should be taken into account [Gold *et al.* 2007]. Conditions which were considered to be associated with an immunocompromised status include history of invasive fungal infections, severe herpes infection, HIV infection, opportunistic infections and current active infections [Gold *et al.* 2007]. A pretreatment cranial MRI scan is obligatory and should be performed within 3 months of starting natalizumab therapy. This scan is needed for comparison with subsequent scans if patients experience worsening. Annual follow-up scans are necessary as a minimum.

Rebound effects after cessation of natalizumab treatment

Tubridy *et al.* [1999] were the first who reported possible rebound effects after cessation of natalizumab treatment. In this phase II trial of a single dose of natalizumab the authors observed an increased relapse rate compared to placebo in the second week after treatment without differences in MRI activity. In another phase II single-dose trial, O'Connor *et al.* [2004] could not observe such a rebound effect. Although neutralizing antibodies decreased the clinical and MRI benefit of natalizumab, there was no rebound phenomenon in the pivotal phase III trials [Calabresi *et al.* 2007]. Vellinga *et al.* [2008] found that lesion activity increased after cessation

of treatment and almost only in patients who received up to eight infusions. Patients who received 30 to 37 infusions had no MRI rebound effect suggesting that patients with short natalizumab treatment may be more likely to experience rebound effects [Fox and Kappos, 2008]. The overall data rather suggest a return to previous disease activity than an overshoot (rebound) after cessation of natalizumab in most conditions.

Conclusions

Clinical trial data demonstrated substantial efficacy and a favourable risk–benefit profile. Although across-study comparisons may be misleading for reasons of different patient populations and trial design, it appears that the efficacy of natalizumab is higher than what we can expect from interferon-beta and glatiramer acetate treatment. However, the pivotal trials were not designed to examine the efficacy of natalizumab in second-line therapy and some questions are yet unanswered. Post hoc subgroup analyses from AFFIRM in highly active patients (at least two relapses in 1 year prior to study inclusion and at least 1 Gd enhancing lesion at baseline revealed a comparable efficacy of the drug in this subgroup (Biogen Idec, data on file). Our own experiences with 31 patients who had treatment failure with other DMT underlines the potential of natalizumab in second-line therapy: annual relapse rates on interferon-beta or glatiramer acetate decreased from 2.3 to 0.2 after one year of natalizumab treatment and MRI efficacy resembled data from the pivotal trials [Putzki 2008]. Although such data is reassuring, only a randomised head-to-head trial could answer the question of the efficacy of natalizumab in comparison with other DMT. In times of limited resources for health care, it is important that the use of natalizumab was associated with favourable cost-effectiveness outcome [Gani *et al.* 2008].

At the end of September 2008, 48 000 patients received natalizumab in the combined clinical and post-marketing settings, 18 000 patients are on treatment for at least 1 year and 9500 have been exposed to natalizumab for at least 18 months. The pivotal trials suggested an incidence of PML of 1:1000 over 18 months [Yousry *et al.* 2006]. Current data have not indicated a higher incidence of PML but more definite conclusions can be drawn within the next 6–12 months.

Targeted biological therapies have introduced remarkable improvement for the treatment and for the life of hundreds of thousands of patients suffering from autoimmune disorders such as rheumatoid arthritis or systemic lupus erythematosus. In MS, natalizumab represents the first targeted therapy and has opened up a new era of antibody treatment. Other monoclonal antibodies like alemtuzumab, daclizumab or rituximab have already shown promising efficacy in the treatment of RRMS. Future challenges include the selection of the appropriate drug for individual conditions and careful evaluation of risks and benefits.

Conflict of interest statement

NP has received honoraria, travel grants, personal compensation and research grants from Bayer Schering, Biogen Idec, Merck Serono, Sanofi Aventis and TEVA.

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