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Pharmacological Properties, Toxicology and Scientific Rationale for the use of Natalizumab (Tysabri®) in Inflammatory Diseases

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ABSTRACT

Natalizumab (Tysabri®) was the first adhesion molecule antagonist to make it into clinical trial for patients with multiple sclerosis (MS) and other inflammatory disorders. Natalizumab is a humanized recombinant monoclonal antibody (MAb) that binds to the alpha $(\alpha)_4$ chain of the α_4 beta $(\beta)_1$ (very late activating antigen 4; VLA-4) and $\alpha_4\beta_7$ integrins. The scientific rationale for natalizumab therapy is the reduction of leukocyte extravasation into peripheral tissues. Natalizumab, like other VLA-4 antagonists, may also interfere with the activation of T lymphocytes in secondary lymphoid organs and their reactivation in the central nervous system (CNS).

Shortly after its approval for the treatment of relapsing-remitting MS (RR-MS), three patients who were treated with natalizumab in the setting of clinical trials developed progressive multifocal leukoencephalopathy (PML), an opportunistic infection of the brain with the polyoma virus JC. It remains to be elucidated why the use of this VLA-4 antagonist is associated with an increased incidence of PML. Natalizumab was recently reapproved for the treatment of relapsing forms of MS. In this review, we outline the scientific rationale for using natalizumab in MS and other inflammatory disorders. In addition, an overview

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of pharmacological properties, clinical efficacy, safety, and toxicology of natalizumab is provided.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system (CNS). Histopathologically, most acute active MS lesions are characterized by infiltration of monocytes and lymphocytes (Lucchinetti et al. 2000). Thus, the egress of inflammatory cells from the peripheral blood into the brain and spinal cord is considered a critical event in the pathogenesis of MS. Over the last 15 years, cell adhesion of leukocytes to the endothelial wall has been a major target of drug development (Yednock et al. 1992; Theien et al. 2001; Piraino et al. 2002; Theien et al. 2003; Piraino et al. 2005a; Piraino et al. 2005b). Natalizumab was the first pharmacological agent that was successfully brought to clinical trial and approved for the treatment of RR-MS (Miller et al. 2003; Polman et al. 2006; Rudick et al. 2006). Natalizumab is a humanized recombinant MAb that binds to the alpha $(\alpha)_4$ chain of the α_4 beta $(\beta)_1$ (very late activating antigen 4; VLA-4) and $\alpha_4\beta_7$ integrins. The scientific rationale for natalizumab therapy is the reduction of leukocyte extravasations into the peripheral tissues, including the brain and the spinal cord by interfering with the physical interaction of VLA-4 with its natural ligands, vascular cell adhesion molecule (VCAM)-1 and fibronectin (FN).

Recently, two patients with MS and one patient with Crohn disease who were treated with natalizumab in the setting of clinical trials developed progressive multifocal leukoencephalopathy (PML), an opportunistic infection of the brain with the polyoma virus JC (Kleinschmidt-Demasters and Tyler 2005; Langer-Gould et al. 2005; Van Assche et al. 2005). Two patients had a lethal outcome (Kleinschmidt-Demasters and Tyler 2005; Van Assche et al. 2005). Based on the medical history, physical examination, magnetic resonance imaging (MRI), and the incidence of seropositive testing for JC virus by polymerase chain reaction (PCR) in MS patients treated with natalizumab, the expected incidence of PML in MS patients treated with natalizumab was estimated to be 1 in a 1000 (95% confidence interval: 1:357 to 1:5000) (Yousry et al. 2006). The biological events leading to PML and potentially other infections of the CNS associated with natalizumab therapy are unknown.

In this review, we outline the pharmacological properties and the toxicology of natalizumab. In addition, we describe the scientific rationale for its use in inflammatory diseases of the CNS, as well as results of clinical studies that let to its approval for use in patients with RR-MS.

SCIENTIFIC RATIONALE

Very Late Activating Antigen in Leukocyte Adherence to Endothelial Walls

Migration of leukocytes from the blood into the CNS involves multiple events that occur in a defined chronological and spatial order, including rolling, chemoattraction, cell adhesion, and proteolytic degradation of biological membranes (Luster et al. 2005) (Fig. 1). Each step in this multi-step paradigm is conditional for the next (Luster et al. 2005). It is thought that slow rolling on endothelial walls allows leukocytes to identify proper arrays of chemoattractants and integrin ligands. Prolonged selectin-mediated rolling of neutrophils

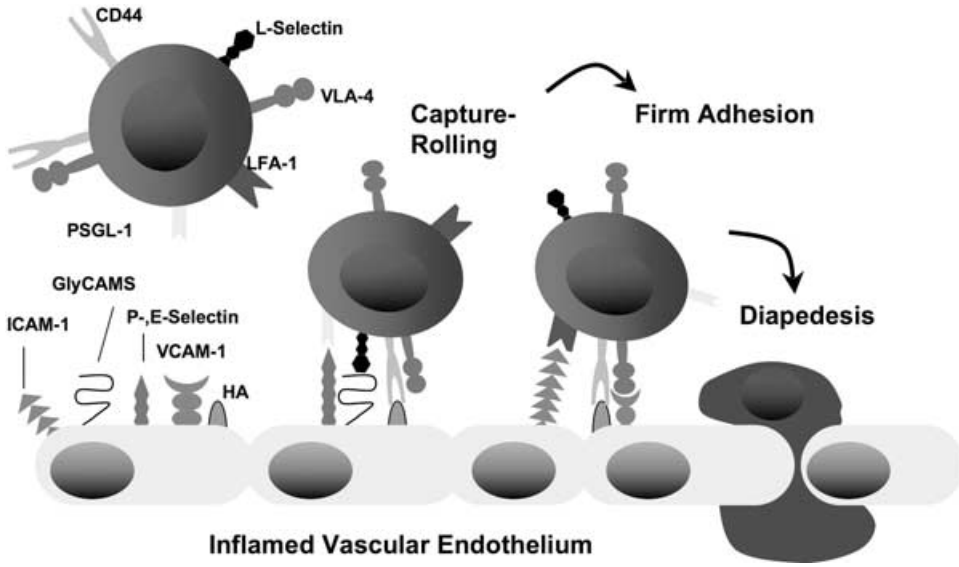

FIG. 1. Schematic of leukocyte migration into the central nervous system. Abbreviations: PSGL-1, P-selectin glycoprotein ligand-1; HA, hyaluronan; VLA-4, very late activating antigen-4; LFA-1, leukocyte function-associated antigen-1; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; GlyCAMS, glycosylated cell adhesion molecules. Modified with permission from Bennett (2006). http://www.ingentaconnect.com/content/maney/nres

and lymphocytes may also lead to integrin activation (Kunkel et al. 2000; Atarashi et al. 2005; Smith et al. 2004). Once firmly arrested, integrins serve for leukocytes to bind to other blood-borne leukocytes and platelets. Activated T-cells and B-cell blasts express highly adhesive integrins (Vajkoczy et al. 2001). All other circulating leukocytes maintain their integrins in mostly inactive states and must undergo *in situ* modulation to develop high avidity for their specific endothelial ligands (Carman and Springer 2003).

Following rolling, the firm adhesion of lymphocytes and myeloid cells in venules is mediated by the *in situ* activation of at least one of the four main integrins: $\alpha_4\beta_7$, LFA-1, Mac-1, and VLA-4 (Luster et al. 2005). Integrins of the β_1 subfamily, specifically VLA-1, VLA-2, VLA-4, VLA-5, and VLA-6, have been shown to facilitate leukocyte migration across the basement membrane of blood vessels, as well as across extracellular matrix (ECM). The interaction of these integrins with ECM may propagate leukocyte immobilization, or bridging between cells (Shimizu et al. 1990b). There is considerable redundancy amongst integrins and their ligands: multiple ligands have been identified for a single receptor (LFA-1/ICAM-1 and LFA-1/ICAM-2), and multiple receptors bind a single ligand (VLA-4/fibronectin [FN], and VLA-5/FN) (Shimizu et al. 1990b). The proadhesive properties of integrins are overlapping and additive, and depend on specific cytoskeletal and transmembrane associations with cytoskeletal adaptor molecules (Liu et al. 2000). Also, membrane effectors, including tetraspanins, CD47, CD98, and CD44, modulate adhesive properties of integrins (Nandi et al. 2004). These effector molecules regulate conformational switches of integrins, as well as the clustering of these molecules on the cell surface (Carman and Springer, 2003; Shamri et al. 2005). New data suggest that integrins are activated bidirectionally: (1) cytoplasmic rearrangements of their subunit tails, and (2) extracellular

binding by their ligands (Kim et al. 2004). A specific combination of chemokines and G protein–coupled receptors (GPCRs) is required for activation of integrin-dependent arrest under shear flow (Ley 2003). This process likely involves G_i protein signaling to two key GTPases, RhoA, and Rap-1 (Kinashi 2005; Laudanna et al. 2002).

Very Late Activating Antigen as a Costimulatory Molecule

Each of the integrin receptor/ligand interaction previously mentioned is capable of providing a potent costimulatory signal to CD3-mediated T-cell activation. Specifically, the VLA-4-mediated interaction of resting human $CD4^+$ T lymphocytes with FN has been shown to promote CD3-mediated T-cell proliferation (Shimizu et al. 1990a). Coimmobilization with MAbs to CD3 and FN consistently results in strong T-cell proliferation. Blocking experiments with MAbs showed that three VLA integrin receptor/ligand interactions mediate costimulation: VLA-4/FN, VLA-5/FN, and VLA-6/laminin (LN) (Shimizu et al. 1990a). The costimulation provided by FN and LN in the system used by Shimizu et al. was stronger than costimulatory signals provided by cytokines, including IL-1 beta, IL-6, and IL-7 (Shimizu et al. 1990a). Other investigators showed that immobilized FN enhances anti-CD3-induced proliferation of both $CD45RA^{dim}$ and $CD45RA^{bright}$ subsets of $CD4^+$ and $CD8^+$ T-cells. Enhancement of anti-CD3-induced proliferation by immobilized FN was completely inhibited by a MAb to CD29, the integrin beta 1-chain of VLA-4 (Davis et al. 1990). In addition, FN enhanced anti-CD3 responses when it was immobilized to a separate surface (Davis et al. 1990). Anti-CD3-stimulated proliferation in the presence of immobilized FN was also partially decreased by MAbs to either VLA-4 or VLA-5, and completely by a combination of both MAbs (Davis et al. 1990). Thus, at least two FN receptors are involved in FN-mediated costimulation of T-cell proliferation: VLA-4 and VLA-5 (Davis et al. 1990). Nojima et al. showed that the A and B epitopes of VLA-4 play a key role in VLA-4-mediated T-cell costimulation. Moreover, these investigators demonstrated that the solid phase cross-linking of VLA-4 using antibodies (against A and B) or the CS-1 region of FN, stimulates tyrosine phosphorylation of 140-, 120-, 80- to 70-, 60- to 55-, 50-, and 45-kDa proteins in addition to the 105-kDa protein (Nojima et al. 1992). In contrast, antibody ligation of the C epitope of VLA-4 mainly induces tyrosine phosphorylation of pp105, weakly induces other protein tyrosine phosphorylation, and additionally induces only minimal T-cell costimulation. Using immunoblotting, the same group of investigators identified some of the tyrosine-phosphorylated proteins to be phospholipase C gamma (pp140), pp125 focal adhesion kinase (pp120), paxillin (pp70 and pp50), p59fyn/p56lck (pp60-55), and mitogen-activated protein kinase (pp45) (Sato et al. 1995).

The Role of Very Late Activating Antigen-4 on the Homing of Hematopoetic Progenitor Cells

It has been recently shown by numerous investigators that $CD34^+$ hematopoietic precursor cells express high levels of VLA-4 on their surface (Steen et al. 1997; Bellucci et al. 1999; Yamaguchi et al. 1998). Specifically, the interaction of VLA-4 on hematopoietic precursor cells with VCAM-1 on bone marrow stromal cells appears to be a survival factor. Wang et al. (1998) demonstrated that the addition of a MAb against VLA-4 to co-cultures of stromal cells and CD34-selected cells induces apoptosis of CD34-selected cells as measured by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick

end-labeling method (Wang et al. 1998). In contrast, the level of adhesion between more differentiated hematopoietic cells and stromal cells was not significantly altered by addition of anti-VLA-4 antibodies (Wang et al. 1998). Similarly, the level of apoptosis of differentiated hematopoietic cells was not significantly altered by the addition of anti-VLA-4 MAbs (Wang et al. 1998). Because the expression level of VLA-4 is quantitatively similar between early and late myeloid cells, there may be a difference in the functional state of this integrin between myeloid cell populations.

Very Late Activating Antigen-Antagonists as Therapies for Animal Models of Multiple Sclerosis

Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS that has been widely used to study immunological mechanisms underlying inflammatory disorders of the CNS (Rivers et al. 1933; Rivers and Schwentker 1935; Zamvil and Steinman 1990). Similar to MS, EAE lesions in the brain and spinal cord show the inflammatory infiltrates of monocytes and lymphocytes (Swanborg 1995). The presentation of antigen (Ag) in secondary lymphoid organs is thought to be an early event in EAE pathogenesis. Activated leukocytes are capable of adhering to the endothelium of blood vessel walls and migrating into the CNS. There, antigen-specific T-cells are reactivated through the presentation of an identical or similar Ag by perivascular Ag-presenting cells (APCs), including hematopoetic macrophages (Hickey and Kimura 1988; Huitinga et al. 1990) and dendritic cells (DCs) (Greter et al. 2005). In 1992, Yednock and coworkers investigated the effects of MAbs against VLA-4 in the EAE model (Yednock et al. 1992). Using an *in vitro* adhesion assay on tissue sections, the authors found that lymphocytes and monocytes bound selectively to the endothelium of brain vessels in areas of EAE-associated inflammation (Yednock et al. 1992). Binding of leukocytes was inhibited by antibodies against the integrin $\alpha_4\beta_1$ (VLA-4) (Yednock et al. 1992). When tested *in vivo*, anti-α_4 integrin effectively prevented the development of EAE, and the accumulation of leukocytes in the CNS (Yednock et al. 1992).

To test the differential contribution of VLA-4 during disease onset and disease perpetuation in EAE, Theien et al. compared the ability of anti-VLA-4 to regulate proteolipid protein (PLP) peptide 139-151-induced relapsing of EAE when administered either before or after disease onset (Theien et al. 2001). MAbs to VLA-4 inhibited the onset and reduced the severity of clinical disease (Theien et al. 2001). In contrast, treatment either at the peak of acute disease or during remission exacerbated disease relapses and increased the accumulation of $CD4^+$ T-cells in the CNS. Interestingly, anti-VLA-4 treatment before or during clinically apparent EAE enhanced $CD4^+$ T_h1 T-cell responses to both the priming peptide and endogenous myelin epitopes, which presumably were released from the CNS secondary to acute tissue damage.

Theien and coworkers also investigated the ability of a small-molecule VLA-4 antagonist to regulate $PLP_{p139-151}$-induced R-EAE. Administration of this agent one week after peptide priming and before the onset of clinical EAE delayed the onset of clinical disease but led to severe disease exacerbation upon discontinuation of treatment (Theien et al. 2003). When therapy was initiated at the peak of acute disease, disease remission and inhibition of clinical relapses were observed; however, disease activity recurred following treatment removal (Theien et al. 2003). These data are supported by the results of additional studies utilizing a different small molecule VLA-4 inhibitor to ameliorate acute EAE (Piraino et al.

2005a). Initiation of therapy during disease remission worsened clinical disease, and discontinuation of therapy resulted in posttreatment exacerbations (Theien et al. 2003). Enhanced disease was caused by the release of encephalitogenic cells from the periphery, and the accumulation of T lymphocytes in the CNS. Yet another small molecule inhibitor of VLA-4 was effective in reversing clinical and histopathological signs of chronic EAE when treatment was started 40 days after active immunization (Piraino et al. 2002). The investigators reported extensive remyelination after 40 days of treatment with the same molecule (Piraino et al. 2005b). Half of the treated animals regained motor function, whereas there was no significant repair or gain of motor function in vehicle-treated animals (Piraino et al. 2005b).

Natalizumab was also used successfully in the EAE model of MS. Specifically, administration of natalizumab after the onset of EAE resulted in a reversal of clinical disease and reduced the accumulation of T lymphocytes and monocytes in the CNS (Kanwar et al. 2000). Because natalizumab is a humanized IgG_4 antibody, it has to be considered highly immunogenic when injected into a murine host. Therefore, the results of these EAE experiments have to be interpreted with some caution.

In summary, the results of these studies suggest that VLA-4-antagonism is an effective therapy in preventing CNS inflammatory disease. Controversy, however, remains regarding the reported outcomes of anti-VLA-4 therapy at disease onset and during the disease course of EAE.

PHARMACOLOGICAL PROPERTIES OF NATALIZUMAB

Natalizumab is a humanized recombinant MAb. Humanization of a murine MAb (AN100226m) was initiated for two reasons: (1) to reduce immunogenicity, and (2) to allow repeated administration in human patients (Mountain and Adair 1992; Leger et al. 1997). Attachment of the complementarity-determining regions (CDRs) of AN100226m to a human immunoglobulin (Ig)-G_4 backbone generated an antibody that is approximately 99% human. Human IgG_4 does not readily bind and activate complement. Thus, natalizumab was not designed to lyse its target, but to physically interfere with the binding of VLA-4. In addition, IgG_4 antibodies have the advantage of a longer biological half-life than other human IgG (Mountain and Adair 1992). *In vitro* assays demonstrated that natalizumab had similar binding saturation to $\alpha_4\beta_1$ integrins and functional antagonism of VCAM-1 interaction as its murine predecessor.

Natalizumab is administered parenterally as an intravenous infusion in humans. The reported biological half-life of natalizumab at doses used in a large Phase II trial (Miller et al. 2003) and two Phase III trials (Polman et al. 2006; Rudick et al. 2006) is approximately 11 days (Sheremata et al. 1999). Specifically, individuals who received natalizumab at a dose of 3 mg/kg per month had detectable serum levels of antibody for 3 to 8 weeks (Sheremata et al. 1999). There was a differential mode of biodistribution, consisting of a rapid distribution phase and a relatively long terminal phase (Sheremata et al. 1999). In another study using a monthly dose of 3 mg/kg of natalizumab, mean serum concentrations were 69.7, 27.1, 15.9, 8.1, 4.9, and 2.2 μg/mL at day 1, day 3, and weeks 1, 2, 3, and 4 (O'Connor et al. 2004). At a dose of 6 mg/kg, natalizumab serum concentrations and corresponding PK values were elevated in the presence of IFNβ-1a (30 μg i.m. per week), when compared to the same dose administered as monotherapy (Vollmer et al. 2004).

The repeat administration of 300 mg of natalizumab, the dose that was used in two Phase III clinical trials in patients with multiple sclerosis (Polman et al. 2006; Rudick et al. 2006), led to a mean serum concentration of 110 ± 52 μg/mL. The mean average steady-state trough concentrations ranged from 23 μg/mL to 29 μg/mL, which was achieved approximately 24 weeks after monthly dosing. At this concentration, the mean half-life, distribution volume, and clearance rate of natalizumab were 11 ± 4 days, 5.7 ± 1.9 l, and 16 ± 5 mL/hour, respectively.

Several factors have an impact on the binding of natalizumab to VLA-4, including hydrogen bonds, hydrophobic interactions, van der Waals forces, and electrostatic forces (Wilson and Stanfield 1994; Andersson et al. 2001; Leckband et al. 2000). In general, the interaction of antibodies to a specific antigen is reversible. This is also true for the interaction between natalizumab and VLA-4, which follows the basic thermodynamic principles of reversible bimolecular interactions. The binding of antibody to its target results in an on/off binding equilibrium. This equilibrium may vary significantly for each antibody–antigen combination, and there are numerous determinants including the properties of the antibody, the antigen, and the rate of diffusion.

Based on our knowledge of the biophysical properties of integrins, the *in vivo* binding equilibrium between natalizumab and VLA-4 is unlikely to be constant. Specifically, it was demonstrated that integrins alter their adhesiveness to ligands by conformational change and modulation of cell surface expression (Hynes 2002; Hogg et al. 2003). Therefore, it is impossible to exactly quantify two commonly used measures that describe the strength of association between an antibody and antigen: "affinity", which defines the binding force between an antibody and a single antigen determinant, and "avidity", which describes the overall strength and stability of antibody–antigen interactions. In the context of a clinical trial, saturation of the α4 chain of VLA-4 at a dose of 1 mg/kg per month was present for approximately 1 week. At a dose of 3 mg/kg per month, α4 receptor saturation lasted 4 weeks (O'Connor et al. 2004).

With regard to the pharmacodynamic properties of natalizumab, several *in vitro* and *in vivo* studies were recently published. Niino et al. utilized an *in vitro* Boyden chamber migration assay to assess the effect of natalizumab on the migratory behavior of leukocyte subsets (Niino et al. 2006). These investigators showed by flow cytometry that the pre-infusion expression of VLA-4 differs among different leukocyte phenotypes (Niino et al. 2006). After natalizumab treatment, the migratory capacity of white blood cells was significantly decreased, correlating with the expression of unbound VLA-4 (Niino et al. 2006). The authors showed that a single dose of natalizumab did not saturate unbound VLA-4. In addition, it was demonstrated that while the interpatient effects of natalizumab varied considerably, the intrapatient effects of natalizumab were stable over multiple infusions (Niino et al. 2006).

Stüve et al. recently showed that natalizumab significantly decreases the cells of $CD4^+$ and $CD8^+$ T lymphocytes, $CD19^+$ B cells, and $CD138^+$ plasma cells in the cerebrospinal fluid (CSF) of patients on natalizumab therapy (Stüve et al. 2006b). In addition, it was shown that the cell numbers remained unchanged 6 months after cessation of natalizumab therapy (Stüve et al. 2006b). There was a differential effect of natalizumab on lymphocyte subsets, with numbers of $CD4^+$ T-cells and $CD19^+$ B cells in the CSF being predominantly affected (Stüve et al. 2006a). The $CD4^+/CD8^+$ T-cell ratio in the peripheral blood also decreased with increasing numbers of natalizumab treatments (Stüve et al. 2006a). Both, the Boyden chamber assay utilized by Niino et al. as well as the measurement of cell numbers in the CSF after natalizumab therapy, have certain limitations with regard to assessing the effect

of this agent on the migration of leukocytes into the brain and spinal cord parenchyma. The Boyden chamber assay poorly reflects the properties of a cellular blood–brain barrier, and in particular the interaction of blood leukocytes with endothelial cells and antigen-presenting cells in perivascular spaces. The CSF as a tissue compartment may also not accurately reflect the ability of the blood–brain barrier to filter the egress of cells from the blood into the CNS parenchyma.

CLINICAL STUDIES

Natalizumab as a Therapy for Multiple Sclerosis

Natalizumab has demonstrated robust efficacy in Phase II and III clinical trials (Tables 1 and 2). The effects on clinical endpoints (relapses, disability) and MRI (T2 lesions, gadolinium-positive (Gd+) lesions were rapid and significant, mirroring the beneficial effects observed in the EAE model. Indeed, the excellent risk–benefit profile at the conclusion of the first year of the Phase III trial prompted early approval of the agent for use in relapsing forms of MS.

Phase II Studies

Three Phase II studies of natalizumab were conducted in MS patients (Tubridy et al. 1999; Miller et al. 2003; O'Connor et al. 2004). The first study was a randomized, double-blind, placebo-controlled trial that examined MRI outcomes in 72 patients with either RR-MS or secondary progressive multiple sclerosis (SP-MS). Patients received consecutive monthly infusions of either natalizumab (3 mg/kg) ($n = 37$) or placebo ($n = 35$) and were monitored for a total of 24 weeks. Clinical requirements included an EDSS between 2.0 and 7.0, and two or more clinical exacerbations 1 to 18 months prior to enrollment. The primary outcome measure was the cumulative number of new active T2 and Gd+ lesions. Active T2 lesions were defined as new or enlarging lesions on T2-weighted imaging. Secondary outcomes included additional MRI measures, number of clinical relapses, and neurologic disability (EDSS, Guys Neurological Disability Scale).

Natalizumab reduced by half the number of new active and new Gd+ lesions when compared to placebo (Table 2) (Tubridy et al. 1999). Alternative MRI measures, clinical relapses, and disability measures were not significantly different. Interestingly, clinical relapses were significantly higher in the natalizumab-treated group relative to placebo ($p = 0.005$) during the second 12 weeks of the study. This finding was thought to result from relative clinical inactivity amongst the placebo group or rebound inflammatory activity in the natalizumab-treated cohort. Indeed, such rebound inflammatory activity was observed after discontinuation of VLA-4 inhibition in the EAE model (Theien et al. 2001).

Miller et al. conducted a Phase II trial of natalizumab in a large cohort of RR-MS and SP-MS patients (Miller et al. 2003). Enrollment criteria included an EDSS score of 2.0–6.5, two or more relapses over the prior 2 years, and three or more T2 lesions on MRI. Patients were randomized to receive either intravenous natalizumab (3 mg/kg or 6 mg/kg) or placebo every 28 days for 6 months. The primary outcome measure was the number of new Gd+ lesions. Secondary and tertiary outcome measures included additional MRI metrics, relapse frequency, and EDSS change (Miller et al. 2003). Treatment with natalizumab significantly reduced the number of new Gd+ lesions, the total volume of Gd+ lesions, the number of T2 active lesions, and the number of persistent Gd+ lesions (Table 2) (Miller et al. 2003).

TABLE 1. Clinical outcomes in natalizumab Phase II and III trials.

Clinical trial	Clinical phase (duration)	Relapse rate	Proportion relapse-free	Disability
Tubridy et al. (1999)	II (12 weeks)	No difference (12 weeks)	No difference (12 weeks)	*EDSS improved ≥ 0.5* 10%-Placebo 31%-Natalizumab
Miller et al. (2003)	II (6 months)	Not evaluated	62%-Placebo 81%-Natalizumab (3 mg/kg) 81%-Natalizumab (6 mg/kg)	*Mean EDSS change* ↑ 0.03-Placebo ↓ 0.14-Natalizumab (3 mg/kg) ↓ 0.03-Natalizumab (6 mg/kg)
AFFIRM Polman et al. (2006)	III (2 years)	0.73-Placebo 0.23-Natalizumab ($\rho < 0.001$)	41%-Placebo 67%-Natalizumab ($\rho < 0.001$)	*Disability progression (%)* 29%-Placebo 17%-Natalizumab ($\rho < 0.001$)
SENTINEL Rudick et al. (2006)	III (2 years)	0.75-Placebo 0.34-Natalizumab ($\rho = 0.001$)	32%-Placebo 54%-Natalizumab ($\rho < 0.001$)	*Disability progression (%)* 29%-Placebo 23%-Natalizumab ($\rho = 0.02$)

TABLE 2. MRI outcomes in natalizumab Phase II and III trials.

Clinical trial	Clinical phase (duration)	New or enlarging T2 lesions* (mean)	Gadolinium-enhancing lesions* (mean)
Tubridy et al. (1999)	II (12 weeks)	1.8-Natalizumab 3.6-Placebo	1.2-Natalizumab 2.0-Placebo
Miller et al. (2003)	II (6 months)	9.7-Placebo 0.8-Natalizumab (3 mg/kg) 1.1-Natalizumab (6 mg/kg)	9.6-Placebo 0.7-Natalizumab (3 mg/kg) Natalizumab (6 mg/kg)
O'Connor et al. (2004)	II (1 dose)	Not evaluated	*Increase in lesion volume*# 42.4%-Placebo 14.4%-Natalizumab (1 mg/kg) 5.0%-Natalizumab (3 mg/kg)
AFFIRM Polman et al. (2006)	III (2 years)	1.9-Natalizumab 11.0-Placebo ($\rho < 0.001$)	0.1-Natalizumab 1.2-Placebo ($\rho < 0.001$)
SENTINEL Rudick et al. (2006)	III (2 years)	0.9-Natalizumab 5.4-Placebo ($\rho = 0.001$)	0.1-Natalizumab 0.9-Placebo ($\rho = 0.001$)

*Number of lesions.
#Lesion volume determined by semi-automated method (Grimaud et al. 1996).

The difference between the natalizumab doses was not statistically significant (Miller et al. 2003). In addition to the beneficial effects on MRI, natalizumab significantly improved clinical outcomes in treated patients (Miller et al. 2003). Natalizumab significantly reduced the number of patients experiencing a relapse during the 6-month study period relative to placebo (38% vs. 19%, $p = 0.02$) (Miller et al. 2003). No enhancement in relapse activity was observed in the 6-month follow-up period (Miller et al. 2003).

O'Connor and colleagues evaluated the effects of a single dose of natalizumab on functional recovery following an isolated relapse in RR-MS and SP-MS patients (O'Connor et al. 2004). Patients were required to have mild to moderate disease (EDSS $\leq$ 5.5) and stable functional subscales for $\geq$30 days prior to the qualifying relapse (O'Connor et al. 2004). A single infusion of natalizumab had no observable effect on the clinical course of recovery following a demyelinating relapse despite a statistically significant reduction in the volume of Gd+ lesions in the treated cohort (O'Connor et al. 2004). Although the short duration of natalizumab therapy may have prevented a salutary effect from being observed, the failure of natalizumab to have a salutary effect on relapse recovery suggests that the benefit of high-dose intravenous glucocorticoids on the rate of relapse recovery may be independent of its effects on the blood–brain barrier and Gd+ lesion activity.

Phase III Studies

Two Phase III trials of natalizumab have been conducted in patients with relapsing forms of MS. The AFFIRM (Natalizumab Safety and Efficacy in RR-MS) study evaluated the safety and efficacy of natalizumab as monotherapy in RR-MS patients (Polman et al. 2006). The SENTINEL (Safety and Efficacy of Natalizumab in Combination with Avonex

[IFNβ-1a] in Patients with RR-MS) study assessed the efficacy and safety of natalizumab in combination with IFNβ-1a (Rudick et al. 2006).

In the AFFIRM study, patients were randomized in a 2:1 ratio to receive intravenous natalizumab 300 mg or placebo every 4 weeks for up to 116 weeks. (Polman and Uitdehaag 2003; Polman et al. 2006). Inclusion criteria included a baseline EDSS score of 0.0–5.0; $\geq$1 relapse within the prior 12 months; and MRI lesions consistent with MS (Polman et al. 2006). The primary study endpoints were the rate of clinical relapses at 1 year and progression in disability (EDSS) at 2 years (Polman et al. 2006). Secondary endpoints included the number of Gd+ lesions, number of new or enlarging T2 lesions, T2 lesion volume, the number of T1-hypointense lesions, the proportion of relapse-free patients, the rate of clinical relapses, and disability progression as measured by the Multiple Sclerosis Functional Composite (Polman et al. 2006). In AFFIRM, 627 patients received natalizumab and 315 patients received placebo; the median time from MS diagnosis was 2 years (Polman et al. 2006). Natalizumab monotherapy had significant benefit on all primary and secondary endpoints. At 2 years, natalizumab significantly reduced the annualized relapse rate by 68% ($p < 0.001$) and the risk of sustained disability progression by 42% compared with placebo (hazard ratio [HR] = 0.58; $p < 0.001$) (Table 1) (Polman et al. 2006). The proportion of relapse-free patients was modestly increased with natalizumab monotherapy (67% vs. 41%, $p < 0.001$), likely a reflection of the low relapse activity across the study population.

All MRI endpoints demonstrated significant improvement in treated individuals. Over 2 years, natalizumab significantly reduced the mean number of new or enlarging T2-hyperintense lesions by 83% (1.9 vs. 11.0, $p < 0.001$) and the mean number of Gd+ lesions by 92% (0.1 vs. 1.2, $p < 0.001$) relative to placebo (Table 2) (Polman et al. 2006). Indeed, 57% of natalizumab-treated patients showed no new or enlargening T2-hyperintense lesions compared to 15% of placebo-treated individuals.

In the SENTINEL trial, patients on a standard regimen of IM IFNβ-1a who experienced breakthrough disease were randomized (1:1) to receive intravenous natalizumab 300 mg or placebo once monthly for up to 116 weeks (Rudick et al. 2006). Inclusion criteria included a baseline EDSS score of 0.0–5.0, treatment with IM IFNβ-1a for $\geq$12 months prior to randomization, $\geq$1 relapse within the 12 months prior to randomization, and MRI lesions consistent with MS. Primary and secondary endpoints were identical to those in the AFFIRM trial (Polman et al. 2006).

In SENTINEL, 589 patients received natalizumab and 582 received placebo; the average duration of disease was 5 years. At the 2-year endpoint, the addition of natalizumab to IM IFNβ-1a reduced the annualized relapse rate by 55% ($p < 0.001$) and the risk of sustained disability by 24% (HR = 0.76; $p = 0.02$) (Table 1) (Rudick et al. 2006). The addition of natalizumab to IM IFNβ-1a reduced the probability of relapse by 50% (HR = 0.50; $p < 0.001$). In parallel with its clinical benefit, the combination of natalizumab and IM IFNβ-1a significantly reduced the level of MRI activity when compared to treatment with IM IFNβ-1a alone. At 2 years, the addition of natalizumab to IM IFNβ-1a reduced new or enlargening T2 lesions by 83% (0.9 vs. 5.4; $p < 0.001$) and the number of Gd+ lesions by 89% (0.1 vs. 0.9; $p < 0.001$). Although the study was not designed to distinguish the individual contributions of natalizumab and IM IFNβ-1a to treatment response in the SENTINEL trial, the absence of effect of continued IM IFNβ-1a on T2 and Gd+ lesion activity suggests that the majority of benefit was due solely to the natalizumab.

The outstanding efficacy of natalizumab in treating relapsing forms of MS has prompted comparison to currently approved disease modifying agents (DMAs: IFNβ-1a, IFNβ-1b,

and glatiramer acetate) (Ropper 2006). Although tempting, such comparisons are inappropriate due to differences in the enrolled study populations and the disease activity within the placebo groups. The duration of disease in MS patients may make a significant difference in their responses to DMAs. Indeed, trials investigating the benefit of early IFNβ treatment in patients with clinically isolated demyelinating syndromes (Jacobs et al. 2000; Comi et al. 2001; Kappos et al. 2006) demonstrate improved clinical and MRI outcomes when compared to identical trials enrolling MS patients with established relapsing disease (Jacobs et al. 1996; The Once Weekly Interferon for MS Study Group 1999; The INFβ Multiple Sclerosis Study Group 1993). Additionally, differences in the clinical activity of the placebo arms of two clinical trials may over- or underemphasize relative risk reduction depending on the clinical outcome that is examined. For example, in AFFIRM, the relative reduction in relapse rate and the relative increase in the proportion of relapse-free patients at 2 years were 68% and 63%, respectively. In comparison, in the Prevention of Relapses and Disability by Interferon-1a Subcutaneously in Multiple Sclerosis (PRISMS) trial, the relative changes in the same outcomes were 32% and 100%, respectively, with SC IFN β-1a (44 μg three times weekly) treatment (1998). The differences in relapse activity between the placebo groups in the AFFIRM and PRISMS trials allow natalizumab to appear paradoxically both better and worse than SC IFNβ-1a. This should emphasize that until a head-to-head trial is performed, it is important to avoid superficial comparisons.

Immunogenicity of Natalizumab

Anti-natalizumab antibodies were detected during all phases of clinical development (Sheremata et al. 1999; Miller et al. 2003; Vollmer et al. 2004; Polman et al. 2006; Rudick et al. 2006). In the largest Phase II study, 11% of patients who received either 3 mg/kg or 6 mg/kg i.v. natalizumab developed anti-natalizumab antibodies. Similarly, in the Phase III AFFIRM and SENTINEL studies, anti-natalizumab antibodies developed in 9% and 12% of treated patients, respectively (Polman et al. 2006; Rudick et al. 2006). Persistent antibodies, defined as antibodies detected on two or more occasions at least 42 days apart, were observed in 6% of patients. The presence of anti-natalizumab antibodies was universally associated with loss of clinical efficacy and infusion-related adverse events (see below).

Adverse Events Associated with Natalizumab Therapy

Approximately 3000 patients were enrolled in clinical trials to evaluate the safety and clinical effectiveness of natalizumab. The trials were not limited to MS but included additional disorders such as Crohn disease and rheumatoid arthritis. In Phase II trials, adverse events were not elevated in natalizumab-treated patients relative to placebo (Miller et al. 2003; O'Connor et al. 2004). The most frequently reported problems were pharyngitis, headache, infection, and back pain. Two patients developed a serum-sickness reaction and one patient developed urticaria (Miller et al. 2003).

In the Phase III AFFIRM study, the safety profile of natalizumab was similar to that observed in the Phase II trials. Adverse events that were more common in the natalizumab-treated group included fatigue and allergic reaction. When MS relapses were disregarded, there were similar occurrences of serious adverse events in the natalizumab- and placebo-treated groups. The total number of infections (79% with both natalizumab and placebo) and number of serious infections (3.2% natalizumab group and 2.6% placebo) were not

significantly different between treatment groups (Polman et al. 2006). Infusion reactions were more common in the natalizumab-treated population (24% vs. 18%), but most were mild; characterized by urticaria, pruritus, headache, nausea, and rigors; and typically did not require discontinuation of therapy. Serious anaphylactoid reactions were reported in five individuals; there were no residual consequences.

In the SENTINEL trial, adverse events that were more frequent in the combination therapy group included anxiety, pharyngitis, sinus congestion, and peripheral edema. The most frequent serious adverse event was MS relapse, occurring in 5% of combination therapy patients and 9% of IM IFNβ-1a patients. Although the overall rate of both total and serious infections were similar between the treatment groups, a single patient was reported with progressive multifocal leukoencephalopathy (PML), a polyoma virus (JC virus) infection of the CNS, following 29 doses of natalizumab (Langer-Gould et al. 2005). An additional fatal case of PML developed in the open-label extension phase of the study (Kleinschmidt-Demasters and Tyler 2005). Similar to the results of the AFFIRM study, infusion reactions were more frequent in the natalizumab-treated group but were typically treated symptomatically; only two severe hypersensitivity reactions were reported (Rudick et al. 2006).

On February 28, 2005, Biogen Idec Inc. and Elan Corp., the manufacturers of natalizumab, announced the voluntary withdrawal of this agent and the use of natalizumab in clinical trials was discontinued (http://www.biogenidec.com/news/BiogenIDECPR˙069.htm). This decision was made after it was reported that two MS patients who had received 28 and 30 doses of natalizumab in the SENTINEL combination trial with interferon beta-1a (Avonex®) were diagnosed with PML (Kleinschmidt-Demasters and Tyler 2005; Langer-Gould et al. 2005). One patient had a fatal outcome in the open-label extension phase of the study (Kleinschmidt-Demasters and Tyler 2005). On March 30, 2005, Biogen Idec Inc. and Elan Corp. issued a press release in which it was announced that a patient who had received eight doses of natalizumab over an 18-month period in an open label Crohn disease clinical trial was diagnosed postmortem with PML (http://www.biogenidec.com/news/BiogenIDECPR_072.htm) (Van Assche et al. 2005). The patient's prior medication history included multiple courses of immunosuppressive agents. In July 2003, the patient had been misdiagnosed with an astrocytoma, and died later that year (Van Assche et al. 2005).

A recent study tested the risk of PML in 3116 patients exposed to natalizumab (Yousry et al. 2006). The investigators evaluated the medical history, physical examination, MRI (2917 patients), and the CSF JC virus polymerase chain reaction (PCR) (396 patients) of individuals exposed to this agent in the setting of clinical trials. The study concluded that the risk of developing PML under natalizumab therapy is currently estimated at 0.1% (95% confidence interval 0.02% to 0.3%) (Yousry et al. 2006). The risk of PML or other infections associated with longer treatment remains unknown. The potential etiology of PML in the setting of natalizumab therapy has been discussed (Ransohoff 2005; Bennett 2006; Berger and Houff 2006) and is beyond the scope of this review.

CONCLUSIONS

The release of natalizumab ushers in a new era in the treatment of MS. The clinical success of selective adhesion molecule inhibition in relapsing MS establishes a new avenue for

treating inflammatory disorders of the CNS. Given the multiple mechanisms through which VLA-4 may modulate immune function, it is incumbent upon future translational research to elucidate the pathways of natalizumab action that are relevant to the potential benefits and the risks observed during clinical development. Although the short-term risk–benefit ratio of natalizumab therapy appears favorable, the long-term risk–benefit ratio remains uncertain. As therapy with natalizumab resumes worldwide, the neurologic community will garner more information about the long-term risks and benefits of this powerful therapeutic modality. Despite all of these efforts, however, physicians may still remain in the dark regarding the long-term risks and benefits of natalizumab therapy. Future research efforts may allow for the design of novel adhesion molecule therapies, but the murine EAE model will always remain inadequate for revealing long-term or human-specific therapeutic risks. As a result, the medical community may need to alter the manner through which novel immunomodulatory therapies are developed, approved for clinical use, and monitored in treated populations.

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