Natalizumab has been available as a multiple sclerosis treatment for more than 5 years in Europe and the United States. Natalizumab was granted approval by the US Food and Drug Administration in 2004, only 12 years after its molecular target was cloned. Shortly after initial approval, natalizumab use was suspended pending a safety review when several natalizumab recipients were diagnosed as having progressive multifocal leukoencephalopathy. After the safety review, natalizumab was reintroduced to the market in 2006. Since then, more than 92,000 patients have been treated with the drug. Risk stratification algorithms and progressive multifocal leukoencephalopathy management strategies have been developed, which facilitate more personalized decision making and safer natalizumab use. This review article summarizes the evolution of natalizumab from target molecule discovery through regulatory approval, voluntary suspension, reapproval, and clinical use. The natalizumab story highlights both the opportunities and risks inherent in a novel biological therapy for a progressive neurologic disease.
molecule was first cloned by Lobb and colleagues at Biogen in the late 1980s. A study published by Lobb and colleagues in 1990 suggested that the VCAM1/VLA-4 ligand-receptor pair could play a key role in recruiting leukocytes into the central nervous system (Figure 2B). This interaction is mediated by T-cell clones reactive to myelin basic protein. When antibodies to α4β1 integrin were administered 48 hours after the injection of disease-causing T cells, paralysis was ameliorated and pathologic inflammatory infiltration of the brain was inhibited. In a comment published in Nature, the collaborators proposed that “[t]herapy based on inhibiting α4β1 integrin, or the ligand for this receptor on brain endothelium, may prove effective in treating inflammatory diseases of the central nervous system.” Using immunohistochemical techniques, neuropathologists identified adhesion molecules in MS brains in 1995. The ligand for α4β1 integrin, VCAM1, first cloned at Biogen in 1989, was identified in brain endothelium from patients with MS.

Figure 2 illustrates the interaction between α4 integrin on the surface of circulating leukocytes and its receptor, VCAM1, on the luminal surface of the brain endothelial cells in mediating transmigration across the blood-brain barrier (Figure 2A). This interaction is blocked by natalizumab, inhibiting entry of activated leukocytes into the central nervous system (Figure 2B). Alpha-4 integrin was subsequently found to be a key signal for entry into other anatomic compartments, including the inflamed bowel in Crohn disease and the pancreas in an animal model of type 1 diabetes mellitus.

DISCOVERY

In the 1980s, analysis of lymphocyte migration occurred in many centers. At Stanford and Harvard universities, Butcher and Pickeral and Springer were elucidating the molecular and biophysical basis of how lymphocytes moved from circulation to sites of inflammation. Notable molecules in the process of lymphocyte migration included integrins and selectins as well as members of the emerging family of immunoglobulin supergenes, exemplified by the molecules of the major histocompatibility complex and various proteins named with the root cellular adhesion molecule. Cellular adhesion molecules included intracellular adhesion molecule (ICAM; CD54) and vascular cell adhesion molecule (VCAM; CD106). The vascular cell adhesion molecule was first cloned by Lobb and colleagues at Biogen in the late 1980s. A study published by Lobb and colleagues in 1990 suggested that the VCAM1/VLA-4 ligand-receptor pair could play a key role in recruiting mononuclear leukocytes to areas of inflammation.

An attractive idea emerged, which predicted that specific molecules might act as guidance molecules enabling lymphocytes to recognize regions of inflammation in a specific organ. This idea was translated to the concept that, like postal zip codes that made mail delivery to specific addresses more efficient, there were similar molecular markers that provided cues for lymphocytes to home to organs such as the brain during infection and perhaps in the course of autoimmune diseases. The idea was branded the zip code hypothesis.

In the early 1990s, the Steinman Laboratory at Stanford University and Ted Yednock at Athena Neuroscience (later acquired by Elan Pharmaceuticals) collaborated to determine the molecules involved in lymphocyte homing to an inflamed brain. They studied an animal model of MS called experimental autoimmune encephalomyelitis (EAE). The Stanford and Athena team induced EAE and demonstrated antibodies to αβ integrin, which binds to ICAM1, had no effect on lymphocyte binding. Antibodies specific for the α chains were applied to the frozen section assay; an antibody to α4 integrin inhibited lymphocyte binding to the frozen brain sections. Antibodies to α3, α5, and α6 were ineffective, whether alone or in combination. Results were confirmed using freshly isolated rat and mouse lymphocytes, as well as fresh lymphocytes from human peripheral blood.

Many alternate adhesion molecules that were thought to play a role in the binding of lymphocytes to inflamed EAE blood vessels were shown to have no effect. For example, although ICAM1 expression on EAE vessels had been demonstrated, antibodies to α2 integrin, which binds to ICAM1, had no effect on lymphocyte binding.

Collaborators next determined that in vivo administration of antibodies to α4/β1 integrin could block paralysis induced by pathogenic T-cell clones that induce EAE. Paralysis of rats occurs within 5 days following administration of T-cell clones reactive to myelin basic protein. When antibodies to α4β1 integrin were administered 48 hours after the injection of disease-causing T cells, paralysis was ameliorated and pathologic inflammatory infiltration of the brain was inhibited. In a comment published in Nature, the collaborators proposed that “[t]herapy based on inhibiting α4β1 integrin, or the ligand for this receptor on brain endothelium, may prove effective in treating inflammatory diseases of the central nervous system.” Using immunohistochemical techniques, neuropathologists identified adhesion molecules in MS brains in 1995. The ligand for α4β1 integrin, VCAM1, first cloned at Biogen in 1989, was identified in brain endothelium from patients with MS.

Figure 1. Timeline of major natalizumab milestones. EMEA indicates European Medicines Agency; FDA, Food and Drug Administration; MS, multiple sclerosis; PML, progressive multifocal leukoencephalopathy; RRMS, relapsing-remitting MS.

α4 integrin was subsequently found to be a key signal for entry into other anatomic compartments, including the inflamed bowel in Crohn disease and the pancreas in an animal model of type 1 diabetes mellitus.
Figure 2. Schematic representations. A, Activated leukocytes expressing α4 integrin, which induces receptor-mediated adhesion to endothelial cells. This interaction is required for transmigration of activated leukocytes into the central nervous system. B, Natalizumab’s presumed primary mechanism of action—inhibiting the interaction between α4 integrin and its receptor. In this way, natalizumab inhibits trafficking of activated leukocytes into the central nervous system. VCAM1 indicates vascular cell adhesion molecule 1.

EARLY CLINICAL DEVELOPMENT

Operating independently at first but later acquired by Elan Corporation (in 1996)20 as a wholly owned subsidiary, Athena Neurosciences tested the humanized, anti-α4 integrin antibody natalizumab (then called Antegren) in a safety and pharmacokinetic study, publishing the results in 1999.21 In 2000, Biogen Idec and Elan agreed to collaborate on the development, manufacture, and commercialization of natalizumab.22 Sheremata and colleagues21 tested single intravenous infusions of natalizumab and reported that doses ranging from 0.03 mg/kg to 3.0 mg/kg were safe. Natalizumab was detectable in serum for 3 to 8 weeks after intravenous infusion.

The details of phase 1 and phase 2 clinical studies have previously been published,21,23-27 and the results are summarized in Table 1 and Table 2. Initial phase 1 study results provided pharmacokinetic, safety, and tolerability data that supported further development.21 Serial magnetic resonance imaging (MRI) studies during the late 1980s and early 1990s had shown that gadolinium enhancement, indicating breakdown of the blood-brain barrier with acute inflammation, was a consistent finding in new lesions in patients with relapsing MS.28,29 A high frequency of clinically silent new enhancing lesions was observed in natural history studies, and protocols were established for using this imaging activity measure to evaluate potential new therapies in proof-of-concept trials.30 The effect of new gadolinium-enhancing lesions was recognized as an appropriate outcome measure for testing natalizumab’s efficacy, given its known mechanism of action; it was investigated in a small, parallel-group, placebo-controlled trial in which subjects received 2 doses of placebo or natalizumab 1 month apart and were followed up with regular MRI scans for 6 months. The trial was smaller than desirable for phase 2 evaluation of efficacy by MRI, necessitated in part by a limited supply of drug. However, the study reached its primary end point: the adjusted mean cumulative number of new active lesions was lower in the natalizumab-treated group than in the placebo group (1.8 vs 3.6; P = .04, analysis of covariance).23 Most of the new active lesions were areas of new enhancement. In an accompanying editorial,31 the trial finding was described as a “near hit.” Had the study not reached its primary end point, one could speculate that it would have been a near miss and that the drug would not have been investigated further. Also, a possible increase in relapse rate following withdrawal of natalizumab therapy was noted in this trial; as in the second study half, there were significantly more acute clinical exacerbations in the natalizumab group.22 Another study failed to show more rapid or complete relapse recovery with natalizumab treatment, although a significant decrease in gadolinium-enhancing lesion volume was noted for both active treatment groups early in the study. There was no evidence of increased relapses following natalizumab withdrawal.20

An important study by Miller et al27 tested 2 doses of natalizumab in patients with RRMS or secondary progressive MS (SPMS) to determine the effectiveness in suppressing gadolinium-enhancing brain lesions. While this study was proceeding and before results were unblinded, Biogen licensed the rights to develop natalizumab for MS from Elan Pharmaceuticals. The decision was based on results from the Sheremata et al,21 Tubridy et al,22 and O’Connor et al23 studies, which demonstrated safety and were thought to suggest efficacy. The corporate decision to acquire rights to develop natalizumab was based on the hope that natalizumab would be effective both as monotherapy and as an add-on to intramuscular interferon β-1a (IFNβ-1a) (A. Sandrock, oral communication, January 2012).

The Miller et al study27 tested placebo vs natalizumab, 3 mg/kg and 6 mg/kg, given intravenously on a monthly basis for 6 months, followed by a 6-month observation, and it was robustly powered to detect an effect on MRI lesion activity. This study demonstrated profound suppression of new gadolinium-enhancing lesions during a 6-month treatment phase. Magnetic resonance imaging activity returned to baseline levels during the 6-month observation period. Figure 3 shows the effect of natalizumab on new gadolinium-enhancing lesions. By month 2, natalizumab was associated with a greater than 90% reduction compared with placebo. Natalizumab-treated patients also had significantly fewer clinical relapses. A subgroup analysis of data from both dose groups found reduced conversion of gadolinium-enhancing lesions to T1-hypointense lesions.32 Early research also found that there were no significant interactions between IFNβ-1a and natalizumab.21 The...
Glatiramer Acetate and Natalizumab Combination Evaluation study\(^\text{24}\) found no loss of efficacy resulting from combination therapy and confirmed a strong effect of natalizumab on disease activity in RRMS.

**PIVOTAL STUDIES**

At the time pivotal studies of natalizumab were being designed, several IFN\(\beta\) products and glatiramer acetate were firmly established as first-line disease-modifying therapies. Combination therapy was an attractive approach to augment the effect of IFN\(\beta\) or glatiramer acetate because it was recognized that the treatment effects of these drugs were modest. For this reason, the Safety and Efficacy of Natalizumab in Combination with IFN\(\beta\)-1a in Patients with Relapsing-Remitting Multiple Sclerosis (SENTINEL) study was designed to determine whether natalizumab could augment the effectiveness of intramuscular IFN\(\beta\)-1a. The need to determine whether natalizumab was effective as monotherapy was also recognized, so the placebo-controlled Natalizumab Safety and Efficacy in Relapsing-Remitting Multiple Sclerosis (AFFIRM) study was planned and conducted simultaneously. Detailed results of these 2 large, pivotal, double-blind, randomized, multicenter, phase 3 efficacy and safety studies were published together in *The New England Journal of Medicine*.\(^33,34\)

*Table 3* includes additional (post hoc) analyses of re-

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**Table 1. Phase 1 Prespecified End Points Publications**

<table>
<thead>
<tr>
<th>Trial Description</th>
<th>Patients, No.</th>
<th>Key Result/Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized, controlled trial; 5-level dose escalation(^\text{21})</td>
<td>28</td>
<td>All doses safe and well tolerated; serum concentration of natalizumab detected 3 to 8 wk after 1-mg/kg and 3-mg/kg intravenous doses</td>
</tr>
<tr>
<td>Open label; natalizumab and interferon (\beta)-1a(^\text{23})</td>
<td>38</td>
<td>Combination of natalizumab and interferon (\beta)-1a safe and well tolerated</td>
</tr>
</tbody>
</table>

**Table 2. Phase 2 Prespecified End Points Publications**

<table>
<thead>
<tr>
<th>Trial Description</th>
<th>Patients, No.</th>
<th>Key Result/Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCT; double blind; natalizumab plus glatiramer acetate combination vs glatiramer acetate alone(^\text{24})</td>
<td>110</td>
<td>New active lesion rate, 0.03 vs 0.11 (combination vs glatiramer acetate alone; (P = .03)); mean new gadolinium-enhancing lesions, 0.6 vs 2.3 (combination vs glatiramer acetate alone; (P = .02)); mean newly enlarging T2-hyperintense lesions, 0.5 vs 1.3 (combination vs glatiramer acetate alone; (P = .03)); combination safe, well tolerated</td>
</tr>
<tr>
<td>RCT; double blind(^\text{25})</td>
<td>72</td>
<td>First 12 wk: mean active lesions, 1.8 vs 3.6 per patient (natalizumab vs placebo; (P = .04)); mean new gadolinium-enhancing lesions, 1.6 vs 3.3 per patient (natalizumab vs placebo; (P = .02)). Second 12 wk: no difference between groups in lesions; acute MS exacerbations, 14 vs 3 patients (natalizumab vs placebo; (P = .005))</td>
</tr>
<tr>
<td>RCT; double blind(^\text{26})</td>
<td>180</td>
<td>Natalizumab (single dose) did not hasten clinical recovery after relapse. Mean lesion volume increase in wk 3: 14.4 and 5.0 vs 42.4 voxel increase (1 mg/kg and 3 mg/kg doses vs placebo, (P = .049) and (P = .052), respectively; (P = .02) for doses combined vs placebo). However, no differences seen by wk 14</td>
</tr>
<tr>
<td>RCT; double blind(^\text{27})</td>
<td>213</td>
<td>Inflammatory brain lesions, 0.7 and 1.1 vs 9.6 (natalizumab, 3 mg/kg and 6 mg/kg vs placebo; (P &lt; .001)). Relapses, 19% and 19% vs 38% of patients (natalizumab, 1 mg/kg and 3 mg/kg vs placebo; (P = .02))</td>
</tr>
</tbody>
</table>

**Figure 3.** Effect of natalizumab on gadolinium-enhancing lesions from a phase 2 trial. Note the rapid onset and near complete inhibition of gadolinium-enhancing lesions associated with natalizumab.\(^\text{27}\) \(*P < .01\) different from placebo; \(\dagger P < .001\) different from placebo. 

Abbreviations: MS, multiple sclerosis; RCT, randomized controlled trial.

Glatiramer Acetate and Natalizumab Combination Evaluation study\(^\text{24}\) found no loss of efficacy resulting from combination therapy and confirmed a strong effect of natalizumab on disease activity in RRMS.
Table 3. Phase 3 Prespecified End Points Publications

<table>
<thead>
<tr>
<th>Patients, No.</th>
<th>Trial: Key Result/Conclusion²⁹</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td>942</td>
<td>AFFIRM²⁴: ARR reduced by 68% by natalizumab; disability progression reduced by 42% compared with placebo (P &lt; .001 both parameters). New/enlarging T2-hyperintense lesions reduced by 83% by natalizumab; gadolinium-enhancing lesions reduced by 92% (P &lt; .001, both parameters)</td>
<td></td>
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<tr>
<td>1171</td>
<td>SENTINEL²⁴: ARR reduced by 55% by natalizumab/IFNβ-1a combination vs IFNβ-1a alone; new/enlarging T2-hyperintense lesions reduced by 83%; mean number of gadolinium-enhancing lesions reduced by 89% (P &lt; .001, all comparisons shown)</td>
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<tr>
<td>1171</td>
<td>Mean T2-lesion volume decreased by 11.8% in natalizumab and IFNβ-1a groups and increased by 27.8% in IFNβ-1a group (P &lt; .001). Median T1-hypointense lesion volume increased by 3.2% in natalizumab and IFNβ-1a groups, and by 11.0% in IFNβ-1a group (P &lt; .001). BPF reduced by 0.31% in natalizumab and IFNβ-1a groups vs 0.40% in IFNβ-1a group (P = .02)</td>
<td></td>
</tr>
<tr>
<td>856/997</td>
<td>AFFIRM/SENTINEL²⁶: AFFIRM: mean change in EDSS score, 0.04 vs 0.41 (natalizumab vs placebo; P &lt; .001); natalizumab reduced the proportion of patients with EDSS progression from a score of ≤3.0 to a score of &gt;4.0 by 67% (P &lt; .001), reduced progression to cognitive deficit by 43% (P = .01), and reduced MS hospitalizations by 39% (P = .005) and relapses requiring steroids by 69% (P &lt; .001). SENTINEL: mean change in EDSS score, 0.15 vs 0.30 (natalizumab plus IFNβ-1a vs IFNβ-1a; P &lt; .01); natalizumab reduced the proportion of patients with EDSS progression from a score of ≤3.0 to a score of &gt;4.0 by 29% (P = .19) and reduced MS hospitalizations by 31% (P = .02) and relapses requiring steroids by 61% (P &lt; .001)</td>
<td></td>
</tr>
<tr>
<td>942</td>
<td>AFFIRM²⁵: Natalizumab reduced gadolinium-enhancing lesions by 92%, new/enlarging T2-hyperintense lesions by 83%, and new T1-hypointense lesions by 76% (P &lt; .001 for all comparisons)</td>
<td></td>
</tr>
<tr>
<td>942/1171</td>
<td>AFFIRM/SENTINEL²⁶: Risk for significant visual loss reduced by 35% in AFFIRM, 28% in SENTINEL; nonnatalizumab groups worsened</td>
<td></td>
</tr>
<tr>
<td>942/1171</td>
<td>AFFIRM/SENTINEL²⁶: AFFIRM: mean Physical and Mental Component Summary scores significantly improved from baseline to wk 104 (P &lt; .001 compared with placebo) in treatment-naïve patients with highly active disease. SENTINEL: mean Physical and Mental Component Summary scores significantly improved from baseline to wk 104 (P &lt; .001); improvement in 5 of 8 individual SF-36 scales with natalizumab plus IFNβ-1a vs placebo. SENTINEL: mean Physical and Mental Component Summary scores significantly improved from baseline to wk 104 (P &lt; .001); improvement in 5 of 8 individual SF-36 scales with natalizumab plus IFNβ-1a vs IFNβ-1a alone</td>
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</tbody>
</table>

Abbreviations: AFFIRM, Natalizumab Safety and Efficacy in Relapsing-Remitting Multiple Sclerosis study; ARR, annualized relapse rate; BPF, brain parenchymal fraction; EDSS, Expanded Disability Status Scale; IFN, interferon; MS, multiple sclerosis; SENTINEL, Safety and Efficacy of Natalizumab in Combination with IFNβ-1a in Patients with Relapsing-Remitting Multiple Sclerosis study; SF-36, Short Form-36.

²AFFIRM and SENTINEL trials described in detail in published papers.²³⁴ Analyses of these trials reflect 2-year data.

Table 4. Post Hoc Analyses of Phases 2 and 3 Trial Data

<table>
<thead>
<tr>
<th>Phase</th>
<th>Patients, No.</th>
<th>Trial Description or Data Source: Key Result/Conclusion²⁹</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>213</td>
<td>Subgroup analysis²³ of Miller study²⁵: Patients with new gadolinium-enhancing lesions that evolved to T1-hypointense lesions: 26% vs 68% (pooled natalizumab group vs placebo; P &lt; .01)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>213</td>
<td>Subgroup analysis²⁶ of Miller study²⁵: Lower-level on-study relapse with natalizumab, particularly in patients with active disease. Relapses by baseline number of relapses in 2-y prestudy, natalizumab vs placebo: 2 relapses prestudy, 16% vs 24%; 3 relapses prestudy, 22% vs 35%; ≥3 relapses prestudy, 23% vs 71%. Relapses by baseline number of gadolinium-enhancing lesions at baseline, natalizumab vs placebo: 0 lesions, 21% vs 33%; 1-2 lesions, 24% vs 42%; ≥2 lesions, 4% vs 60%</td>
<td></td>
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<tr>
<td>3</td>
<td>40</td>
<td>AFFIRM/SENTINEL²⁶ (data combined for analysis): Relapse rate reduced by 60% (P = .02); mean number of gadolinium-enhancing lesions reduced by 79% (P = .03); mean number of new or enlarged T2-weighted lesions reduced by 90% (P = .008) by natalizumab in patients of African descent</td>
<td></td>
</tr>
<tr>
<td>942/1171</td>
<td>AFFIRM/SENTINEL²⁶: AFFIRM: Natalizumab reduced risk for disability progression by 53% (P = .03) and relapse rate by 81% (P &lt; .001 compared with placebo) in treatment-naïve patients with highly active disease. SENTINEL: Natalizumab plus IFNβ-1a reduced risk for disability progression by 58% (P = .04) and relapse rate by 76% (P &lt; .001 compared with IFNβ-1a alone) in patients with highly active disease despite IFNβ-1a treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>713</td>
<td>AFFIRM²⁶: Natalizumab vs placebo: 64% vs 39% free of clinical disease activity, 58% vs 14% free of radiologic disease activity, 37% vs 7% free of combined clinical and radiologic disease activity (P &lt; .001 for all comparisons). Consistent across subgroups of highly active or nonhighly active baseline disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>620</td>
<td>AFFIRM²⁶: In patients with baseline EDSS scores ≥2.0, a 69% improvement in disability was found with natalizumab vs placebo (P = .006)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>942/1171</td>
<td>AFFIRM/SENTINEL²⁶: AFFIRM: Natalizumab (compared with placebo) reduced ARR by 58% (P = .004) in patients with ≥1 gadolinium-enhancing lesions and by 70% (P &lt; .001) in patients with ≥1 new/enlarging T2-hypointense lesions. SENTINEL: Natalizumab plus IFNβ-1a (compared with IFNβ-1a alone) decreased ARR by 45% in patients with ≥1 gadolinium-enhancing lesions (P = .008) and by 60% in patients with ≥1 new/enlarging T2-hyperintense lesions (P &lt; .001)</td>
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</tbody>
</table>

Abbreviations: AFFIRM, Natalizumab Safety and Efficacy in Relapsing-Remitting Multiple Sclerosis study; ARR, annualized relapse rate; EDSS, Expanded Disability Status Scale; IFN, interferon; SENTINEL, Safety and Efficacy of Natalizumab in Combination with IFNβ-1a in Patients with Relapsing-Remitting Multiple Sclerosis study.

²AFFIRM and SENTINEL trials described in detail in published papers.²³⁴ Analyses of these trials reflect 2-year data.

sulting data²⁵-²⁹. Table 4 summarizes the results for presupervised end points of these studies.²⁷,³²,⁶⁹-⁷⁵

In the 2-year AFFIRM study, natalizumab significantly reduced the annualized relapse rate (ARR), as well as the likelihood of confirmed Expanded Disability Status Scale (EDSS) score worsening, the number of new or enlarging T2-hyperintense lesions,³³ gadolinium-enhancing lesions, new nonenhancing T1-hypointense lesions, and brain atrophy in year 2 (measured with brain parenchymal fraction software) compared with placebo.³⁷,³⁸ In the 2-year SENTINEL study, the combination of natalizumab and IFNβ-1a significantly reduced
the ARR compared with IFNβ-1a monotherapy, as well as the accumulation of new or enlarging T2-hyperintense lesions and T1-hypointense lesions and brain atrophy in year 2 compared with IFNβ-1a alone.\(^{35}\)

In both AFFIRM and SENTINEL, 9% to 12% of the patients’ results were positive for antinatalizumab antibodies at some point during the studies, and 6% of the patients’ results had persistently positive antinatalizumab antibody titers. Patients with persistent antinatalizumab antibodies had reduced trough serum concentrations and significantly reduced efficacy with natalizumab in the progression of disability, relapse rate, and disease activity on MRI. While patients with transiently positive antibody titers experienced a delay in the full therapeutic effect of natalizumab, efficacy was restored when antibodies became undetectable after 6 months of treatment. Persistently antibody-positive patients also had a higher incidence of infusion-related AEs and hypersensitivity reactions. These results suggested that clinicians should test for antinatalizumab antibodies in patients exhibiting a suboptimal clinical response or persistent infusion-related AEs.\(^{47}\)

On the basis of year 1 results from AFFIRM and SENTINEL, the FDA conducted an accelerated review and approved natalizumab for the treatment of relapsing forms of MS in November 2004, 12 years after the discovery of the target molecule\(^{44,45}\) and 7 years after the start of clinical testing. Approval based on accelerated review signified a view by the FDA that natalizumab offered significant advantages over existing drugs in an area of high unmet medical need. Approval was contingent on completion of phase 3 studies.\(^{7}\)

The AFFIRM and SENTINEL studies led to additional publications reporting results from protocol-specified end points and post hoc analyses (Table 3 and Table 4). Analyses of imaging end points from SENTINEL showed that natalizumab added to IFNβ-1a reduced gadolinium-enhancing, T1-hypointense, and T2 lesion activity, as well as brain atrophy progression.\(^{39}\) Another analysis of AFFIRM and SENTINEL prespecified end points determined that natalizumab reduced the risk for significant visual loss in both studies.\(^{30}\) An analysis of health-related quality of life end points from AFFIRM and SENTINEL showed that health-related quality of life, measured by components of Short Form–36 and visual analog scale results, improved significantly with natalizumab treatment.\(^{30}\) A post hoc analysis of AFFIRM data showed that natalizumab was associated with a higher proportion of patients who remained free of disease activity by clinical and radiologic criteria.\(^{43}\)

### SERIOUS ADVERSE EVENT

In February 2005, when AFFIRM was complete, SENTINEL was nearing completion, and just months after FDA approval of natalizumab, PML was recognized in 2 patients from SENTINEL. Natalizumab marketing and clinical study dosing was abruptly halted after approximately 5000 patients with MS had initiated natalizumab therapy or were about to do so. One of the patients with PML presented with fulminant disease and died within 2 months after symptoms started. Both patients with PML had received natalizumab in combination with intramuscular IFNβ-1a.\(^{5,9}\) Because there had been no reported cases of PML after many years of IFN use in MS, it was apparent that a serious special risk for PML must be associated with natalizumab therapy. It was unclear whether IFNβ-1a treatment was a necessary cofactor for the risk for natalizumab-associated PML.

Close review of AEs during natalizumab treatment soon revealed a third patient who was treated for inflammatory bowel disease but who had died of a brain lesion diagnosed as an astrocytoma. On further review, it was evident this patient had died of PML.\(^{10}\)

Marketing was immediately suspended and physicians worldwide were notified.\(^{2}\) Biogen Idec organized screening of all patients exposed to the drug during clinical studies, supervised by a 3-member independent adjudication committee. In addition to suspending natalizumab dosing, all patients were to be examined for new or progressive neurologic disease and a brain MRI scan was to be performed. It was recommended that cerebrospinal fluid also be collected. Scans were reviewed by a central reading center, and cases with suspicious lesions or history were reviewed by the adjudication committee.\(^{48}\) No further cases were found, leading to a risk estimate of 1:1000 exposed patients (95% CI, 0.2-2.8) with a median exposure of approximately 18 months of therapy. During the year following discovery of PML, no further cases emerged from the clinical study experience. One of the patients with MS in whom therapy was withdrawn survived with serious disability after discontinuing natalizumab therapy and undergoing a variety of purported treatments for PML. Interestingly, immune reconstitution inflammatory syndrome (IRIS) was noted several months after natalizumab discontinuation, consistent with active immune response following the biologic decay of blockade of α4 integrin.\(^{8}\)

A review of exposed patients yielded an estimated risk that many believed was acceptable given the demonstrated therapeutic benefits of natalizumab for MS lesions, relapses, and disability. Expert US and European panels supported reapproval of natalizumab marketing, albeit with enhanced safety programs seeking to fully ascertain and mitigate PML risk. In the United States, while not restricted to second-line treatment, natalizumab was generally recommended as therapy for patients with breakthrough disease despite first-line therapy or those who were unable to tolerate 1 of the first-line therapies.\(^{50}\) Infusion sites prepared with safety training were established and monthly symptom questionnaires were used to identify patients with symptoms consistent with PML. When disease activity was detected, MRI and, if appropriate, cerebrospinal fluid monitoring for JCV were recommended. In Europe, the drug was reinstated for patients with high disease activity despite treatment with first-line drugs or those with rapidly evolving severe RRMS.\(^{31}\)

### POSTMARKETING EXPERIENCE

#### Progressive Multifocal Leukoencephalopathy

Two years passed before additional PML cases surfaced, suggesting that duration of therapy might be a risk fac-
 Thus, PML with notable IRIS characteristics typifies PML in this setting. Diagnosis continues to be enhanced by detection of JCV in the cerebrospinal fluid.\textsuperscript{32} JC viral loads have been variable but often are quite low, requiring a sensitive and specific polymerase chain reaction assay. JC virus DNA loads have not had much prognostic value and indeed often transiently increase even as PML is controlled by the inflammatory response. Seizures have also been more commonly associated with PML in this setting, perhaps correlating with inflammatory characteristics. While PML in this setting remains a very dangerous and aggressive brain infection, approximately 80% of current patients with PML survive with variable degrees of brain injury ranging from mild (one-third of patients) to highly disabling (one-third of patients), in contrast to the generally fatal prognosis in patients with advanced AIDS prior to successful highly active antiretroviral treatment.\textsuperscript{33} Careful monitoring, early detection of new lesions, removal of natalizumab via plasma exchange, and active treatment of IRIS all appear to contribute to more favorable survival rates, although controlled studies of these interventions have not been possible to date. eTable 1 (http://www.jamaneuro.com) summarizes PML-related publications.

**Risk Mitigation**

In response to the identification of PML risk associated with natalizumab, a global pharmacovigilance plan was instituted. Components of the plan include the mandatory (in the United States) Tysabri Outreach: Unified Commitment to Health Prescribing Program; the Tysabri Global Observational Program in Safety cohort study; an ongoing, open-label, multinational study that includes participants from previous AFFIRM, SENTINEL, and Glatiramer Acetate and Natalizumab Combination Evaluation studies; and the Tysabri Observational Program. In addition, a Tysabri Pregnancy Exposure Registry evaluates outcomes of pregnancies in natalizumab-treated patients.\textsuperscript{37} These programs are ongoing; their final results have not yet been published.

Differences in risk between Europe (where risk has apparently been higher) and the United States have suggested that prior immunosuppression, including treatment with mitoxantrone hydrochloride, cyclophosphamide, methotrexate, and similar agents, may augment PML risk approximately 4-fold.\textsuperscript{38} A more powerful predictor appears to be prior JCV infection, as reflected by the presence of anti-JCV antibodies. It is generally accepted that a primary JCV infection in childhood is followed by a clinical latent state, and then immunosuppression later in life permits viral reactivation, leading to PML.\textsuperscript{39} The current European-approved and United States-approved labels for natalizumab reflect 3 established risk factors for PML: positive anti–JCV antibody serostatus, prior or current immunosuppressant therapy, and natalizumab treatment duration (especially duration longer than 2 years).\textsuperscript{21,34} Thus far, treatment duration beyond 3 years has not been associated with increasing risk as might be predicted if risk was compounded over time.\textsuperscript{56,60} A risk stratification algorithm that estimates risk for 5 subsets of patients was recently published.\textsuperscript{59} Patients with nega-
tive results for anti-JCV antibodies had the lowest risk for PML (0.09 cases or fewer per 1000 patients), while patients with all 3 risk factors had an estimated incidence of approximately 11.1 cases per 1000 patients, a more than 120-fold difference.

STRATIFY JCV (2-step anti-JCV antibody assay; Focus Diagnostics; now commercially offered) uses a 2-stage process to classify patients’ results as anti-JCV antibody positive or negative. Using this assay, all PML cases to date with antecedent specimens have had prior positive assays. A negative assay result appears to be associated with substantially lower risk, suggesting this may be a practical means of risk stratification. In contrast, detection of JCV DNA in blood and urine has not proved to be predictive in the largest experiences reported to date. Importantly, the results for almost half of patients with MS tested with STRATIFY JCV are reported to be negative for anti-JCV antibodies. This suggests that approximately half of patients with MS have a much lower level risk for PML with natalizumab. In those patients, the threshold for using this drug might be significantly reduced.

The entire experience of this serious, unexpected complication has introduced a cautionary note for drug development and regulation. There is an active ongoing discussion among regulators, researchers, and patient advocates seeking successful ways to continue development of promising drugs while limiting the hazard to patients who take these medications. Experience with natalizumab suggests that responsible drug development and monitoring, using carefully designed risk mitigation, can continue even in the face of very serious complications when use is justified by benefits to patients.

### Withdrawing Therapy

The need to discontinue natalizumab dosing in February 2005 resulted in an opportunity to study the effects of natalizumab withdrawal on MS disease activity. Several reports have appeared describing the course of MS after withdrawing natalizumab. Table 5 summarizes these reports. In the largest study to date, O’Connor and colleagues reported the return to pretreatment levels of MRI and clinical activity in more than 1800 natalizumab-treated patients within 3 to 6 months of discontinuing treatment. The return of disease activity occurred in patients who instituted alternative disease-modifying therapies and patients without further disease therapy. Similar effects were observed in patients with highly active pretreatment disease and those with lower levels of disease activity.

A smaller study of 21 patients who electively interrupted natalizumab treatment confirmed a return of disease activity after discontinuation; relapse was experienced by 19% of patients and contrast-enhancing lesions were noted in 47% of patients at 104 and 79 days, respectively, since the last infusion. A chart review study of 48 cases found that ARRs were significantly greater 12 months before and 3 to 24 months after natalizumab treatment. An observational study found that 7 of 10 patients had a combination of relapse and new enhancing lesions 6 months after discontinuing natalizumab. Overall, evidence from available studies suggests that there is a return to baseline levels of disease activity, rather than a return to higher levels of disease activity, in patients who stop natalizumab treatment.

### Additional Studies

Preliminary results of some global pharmacovigilance studies have been published; further results are pending. As of June 2011, a preliminary analysis of Tysabri Observational Program data (n = 3638) found that patients taking natalizumab who were therapy naïve had a significantly greater decrease in ARR compared with patients previously treated with immunosuppressants (0.19 vs 0.37; P = .001). Patients with mild disability (EDSS score, <2.0) also had a greater decrease in ARR while taking natalizumab compared with patients who had moderate (EDSS score, 2.5–4.0) to severe (EDSS score, 4.5–9.5) disability at baseline (ARR: 0.17, 0.25, and 0.27, respectively; P = .002). Preliminary findings from the Tysabri Pregnancy Exposure Registry in 77 pregnant women indicated that there were no AEs of natalizumab on pregnancy outcomes. Final results are pending publication.

There have been several reports from various countries/regions on the postmarketing experience with natalizumab, as described in eTable 2. Several studies noted...
The effects of natalizumab in progressive forms of MS are under investigation. In an early clinical study, patients with SPMS who were treated with natalizumab had fewer new enhancing lesions than did those treated with placebo, and the difference was statistically significant for patients treated with either 3 mg/kg or 6 mg/kg. A retrospective observational study of natalizumab-treated patients with MS in Toronto, Canada, found a significant reduction in the ARR (68%) in patients with SPMS, although no change in EDSS score was noted. In a Swedish postmarketing surveillance study, no significant changes in disability, disease severity, or patient-reported impact of MS were noted during 24 months in patients with SPMS (n = 68) compared with improvements in all of these areas in patients with RRMS. A study of natalizumab in SPMS and primary progressive MS was completed in a small number of patients in early 2012, and a large, phase 3 study of the effect of natalizumab on reducing disability progression in patients with SPMS is under way. At this point, natalizumab has not been found effective in slowing or stopping disability progression in SPMS or primary progressive MS. It is clear that the anti-inflammatory effects of natalizumab result in strong clinical benefit during the RRMS stage of disease by inhibiting brain inflammation, inflammation-mediated tissue injury, and relapses. The effects of natalizumab in later stages of MS, where inflammation-independent neurodegeneration underlies neurologic worsening, are not yet clear.

Natalizumab could also be tested at the clinically isolated syndrome stage for patients who are anti-JCV antibody negative. The underlying scientific rationale for those studies would be to determine whether highly effective anti-inflammatory therapy at the earliest opportunity would normalize brain atrophy. Finally, the impact of disease activity–free status on long-term disability will become clear only with long-term follow-up studies.

Natalizumab treatment has not been approved in the pediatric population. To our knowledge, natalizumab treatment in children with MS has been studied in a limited number of cases. In case reports on 4 children who had high levels of disease activity or tolerated first-line drugs poorly, clinical and MRI disease activity ceased for periods ranging from 12 to 24 months when patients were treated with natalizumab. Two small, open-label, uncontrolled, nonrandomized studies have been conducted in children. A study of 19 children (mean age, 14.6 years) with active MS in Italy observed a dramatic decrease in disease activity during a mean follow-up of 15 months, as evidence by a significantly diminished median EDSS score and no new gadolinium-enhancing activity. In a study of 24 children with MS (mean age, 14 years) in the United States, 83% of patients remained stable with respect to MRI findings and clinical parameters during a mean 1.5-year follow-up. In both the case studies and the studies of larger numbers of children, patients tolerated natalizumab relatively well. In the study of 24 children, 4 patients discontinued therapy because of poor tolerance and/or hypersensitivity reaction. Optimal natalizumab dosing and duration have not been established in the pediatric population. To our knowledge, there have been no cases of natalizumab-associated PML reported in the pediatric population to date.

**CONCLUSIONS**

Looking back from the vantage point of more than 5 years since natalizumab became available in the United States and Europe, the story of this drug’s journey from bench to bedside is a unique one that is instructive in both the opportunities and risks that can accompany the advent of each novel biological therapy. Several lessons have been learned from the development, approval, and ongoing monitoring of natalizumab treatment, and these lessons can be applied, with a likely outcome of increased clinical success and safety, to future therapies. In summary: (1) improved understanding of basic biology can lead directly to new, highly specific therapeutic targets. (2) Even with an aggressive and highly effective clinical development program, the lag time between molecular target discovery and natalizumab approval was 12 years. (3) This development program could have easily gone in a different direction had the Tubridy et al study been a clear miss (ie, failed to reach the expected primary end point) instead of a “near hit,” highlighting the fine line between success and failure in early clinical development, as well as the importance of careful study design. (4) Clinicians and researchers must remain vigilant for unexpected AEs. (5) Benefits can and must be balanced against risks, and efforts are needed to increase benefits through appropriate patient selection and decrease risks through risk mitigation and stratification. (6) Finally, once a highly effective treatment is identified, it provides many opportunities for further study of disease mechanisms.
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4. US Food and Drug Administration. Nata


