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## A Randomized, Placebo-Controlled Trial of Natalizumab for Relapsing Multiple Sclerosis

Chris H. Polman, M.D., Paul W. O'Connor, M.D., Eva Havrdova, M.D., Michael Hutchinson, M.D., Ludwig Kappos, M.D., David H. Miller, M.D., J. Theodore Phillips, M.D., Ph.D., Fred D. Lublin, M.D., Gavin Giovannoni, M.D., Andrzej Wajgt, M.D., Martin Toal, M.B., M.F.P.M., Frances Lynn, M.Sc., Michael A. Panzara, M.D., M.P.H., and Alfred W. Sandrock, M.D., Ph.D., for the AFFIRM Investigators\*

### ABSTRACT

#### BACKGROUND

Natalizumab is the first  $\alpha_4$  integrin antagonist in a new class of selective adhesion-molecule inhibitors. We report the results of a two-year phase 3 trial of natalizumab in patients with relapsing multiple sclerosis.

#### METHODS

Of a total of 942 patients, 627 were randomly assigned to receive natalizumab (at a dose of 300 mg) and 315 to receive placebo by intravenous infusion every four weeks for more than two years. The primary end points were the rate of clinical relapse at one year and the rate of sustained progression of disability, as measured by the Expanded Disability Status Scale, at two years.

#### RESULTS

Natalizumab reduced the risk of sustained progression of disability by 42 percent over two years (hazard ratio, 0.58; 95 percent confidence interval, 0.43 to 0.77;  $P < 0.001$ ). The cumulative probability of progression (on the basis of Kaplan–Meier analysis) was 17 percent in the natalizumab group and 29 percent in the placebo group. Natalizumab reduced the rate of clinical relapse at one year by 68 percent ( $P < 0.001$ ) and led to an 83 percent reduction in the accumulation of new or enlarging hyperintense lesions, as detected by  $T_2$ -weighted magnetic resonance imaging (MRI), over two years (mean numbers of lesions, 1.9 with natalizumab and 11.0 with placebo;  $P < 0.001$ ). There were 92 percent fewer lesions (as detected by gadolinium-enhanced MRI) in the natalizumab group than in the placebo group at both one and two years ( $P < 0.001$ ). The adverse events that were significantly more frequent in the natalizumab group than in the placebo group were fatigue (27 percent vs. 21 percent,  $P = 0.048$ ) and allergic reaction (9 percent vs. 4 percent,  $P = 0.012$ ). Hypersensitivity reactions of any kind occurred in 25 patients receiving natalizumab (4 percent), and serious hypersensitivity reactions occurred in 8 patients (1 percent).

#### CONCLUSIONS

Natalizumab reduced the risk of the sustained progression of disability and the rate of clinical relapse in patients with relapsing multiple sclerosis. Adhesion-molecule inhibitors hold promise as an effective treatment for relapsing multiple sclerosis. (ClinicalTrials.gov number, NCT00027300.)

From the Vrije Universiteit Medical Center, Amsterdam (C.H.P.); St. Michael's Hospital, Toronto (P.W.O.); General Teaching Hospital, Prague, Czech Republic (E.H.); St. Vincent's University Hospital, Dublin, Ireland (M.H.); University Hospital Basel, Basel, Switzerland (L.K.); Institute of Neurology, London (D.H.M., G.G.); Texas Neurology, Dallas (J.T.P.); Mt. Sinai School of Medicine, New York (F.D.L.); Silesian Medical University, Katowice, Poland (A.W.); and Biogen Idec, Cambridge, Mass. (M.T., F.L., M.A.P., A.W.S.). Address reprint requests to Dr. Polman at the VU Medical Center, P.O. Box 7057, Amsterdam 1007 MB, the Netherlands, or at [ch.polman@vumc.nl](mailto:ch.polman@vumc.nl).

\*Additional members of the Natalizumab Safety and Efficacy in Relapsing Remitting Multiple Sclerosis (AFFIRM) study group are listed in the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org).

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RELAPSING MULTIPLE SCLEROSIS IS CHARACTERIZED by the intermittent development of inflammatory lesions in the brain and spinal cord, resulting in plaques of demyelination and axonal loss. Lymphocyte migration across the blood-brain barrier is thought to be an important early step in the formation of lesions.<sup>1</sup> The interaction of  $\alpha_4\beta_1$  integrin, a protein on the surface of lymphocytes, with vascular-cell adhesion molecule 1 (VCAM-1), which is expressed on the surface of vascular endothelial cells in brain and spinal cord blood vessels, mediates the adhesion and migration of lymphocytes in areas of inflammation.<sup>2-6</sup> Furthermore, the interaction of  $\alpha_4\beta_1$  integrin with ligands such as fibronectin<sup>3</sup> and osteopontin<sup>7</sup> may modulate the survival, priming, and activation of leukocytes that have gained access to the parenchyma of the central nervous system.<sup>8-11</sup> Natalizumab (Tysabri, Biogen Idec and Elan Pharmaceuticals), which belongs to a new class of selective adhesion-molecule inhibitors, binds to the  $\alpha_4$  subunit of  $\alpha_4\beta_1$  and  $\alpha_4\beta_7$  integrins and blocks binding to their endothelial receptors (VCAM-1 and mucosal addressin-cell adhesion molecule 1, respectively), thereby attenuating inflammation.<sup>12</sup> Natalizumab may also modulate ongoing inflammatory reactions by inhibiting the binding of  $\alpha_4$ -positive leukocytes with fibronectin and osteopontin.

Current therapies for multiple sclerosis, including interferon beta and glatiramer acetate, are only moderately effective, reducing the annualized rate of relapse by about one third.<sup>13-16</sup> On the basis of the favorable results of a phase 2 trial of natalizumab in patients with relapsing multiple sclerosis,<sup>17</sup> a two-year phase 3 clinical trial, the Natalizumab Safety and Efficacy in Relapsing Remitting Multiple Sclerosis (AFFIRM) study, was initiated to confirm the efficacy of natalizumab in relapsing multiple sclerosis and to evaluate the safety of long-term treatment.

## METHODS

### PATIENTS

Ninety-nine clinical centers in Europe, North America, Australia, and New Zealand enrolled 942 patients beginning on November 6, 2001. All patients gave written informed consent. The study protocol was developed by the investigator advisory committee and sponsors, was approved by central and local ethics committees, and was

overseen by an independent safety-monitoring committee. Study data were collected by the investigators and an independent organization (PPD International) and were held and analyzed by Biogen Idec and Elan Pharmaceuticals. All members of the publication committee had full access to the data. During the study, the investigator advisory committee and sponsor representatives met at least monthly to discuss study progress. The manuscript was written by Drs. Polman and Panzara, with input from all coauthors. All authors vouch for the veracity and completeness of the data and data analysis.

Enrollment was limited to men and women who were between the ages of 18 and 50 years and had a diagnosis of relapsing multiple sclerosis<sup>18</sup>; who had a score of 0 to 5.0 on the Expanded Disability Status Scale (EDSS), a rating that ranges from 0 to 10, with higher scores indicating more severe disease<sup>19</sup>; who had undergone magnetic resonance imaging (MRI) showing lesions consistent with multiple sclerosis; and who had had at least one medically documented relapse within the 12 months before the study began. Patients with disease that was categorized as primary progressive, secondary progressive, or progressive relapsing were excluded.<sup>20</sup> Additional exclusion criteria included the following: a relapse within 50 days before the administration of the first dose of the study drug, treatment with cyclophosphamide or mitoxantrone within the previous year, or treatment with interferon beta, glatiramer acetate, cyclosporine, azathioprine, methotrexate, or intravenous immune globulin within the previous 6 months. Patients who had received treatment with interferon beta, glatiramer acetate, or both for more than six months were also excluded.

### STUDY DESIGN AND RANDOMIZATION

Patients were randomly assigned in a 2:1 ratio to receive either natalizumab (at a dose of 300 mg) or placebo by intravenous infusion every 4 weeks for up to 116 weeks. Patients were randomly assigned to treatment that was stratified according to study site in blocks of three (two active, one placebo) with the use of a computer-generated block randomization schedule and a multidigit identification number, implemented by an interactive voice-response system. All study personnel, patients, sponsor personnel involved in the conduct of the study, and the investigator advisory

committee were unaware of treatment assignments throughout the study.

#### STUDY PROCEDURES AND END POINTS

At each study site, primary and backup examining neurologists and primary and backup treating neurologists were designated. Treating neurologists were responsible for all aspects of patient care, including the management of adverse events and the treatment of relapsing disease. Examining neurologists performed objective evaluation with use of the EDSS and neurologic examination during all study visits; they were not in contact with patients in any other capacity, so as to reduce the possibility of being unblinded by side effects or laboratory assessments.

Patients visited the clinic every 12 weeks for scoring on the EDSS, blood chemical and hematologic analyses, evaluation of adverse events, and testing for anti-natalizumab antibodies. Patients were also seen by the treating neurologist at unscheduled visits within 72 hours after the onset of new neurologic symptoms. If a relapse was suspected, the patient was referred to the examining neurologist, who evaluated the patient within five days after the event. Relapses were defined as new or recurrent neurologic symptoms not associated with fever or infection that lasted for at least 24 hours and were accompanied by new neurologic signs found by the examining neurologist. At the discretion of the treating neurologist, relapses were treated with intravenous methylprednisolone at a dose of 1000 mg per day for three or five days. Patients whose disability progression was sustained for 12 weeks were allowed to continue participation in the study and were given the option of adding an available treatment for multiple sclerosis as rescue medication per protocol while continuing to receive the study drug. Patients were strongly encouraged to remain in the study for follow-up assessments even if they had discontinued the study drug.

Proton-density-weighted or T<sub>2</sub>-weighted and gadolinium-enhanced T<sub>1</sub>-weighted MRI scans of the brain were obtained at baseline, at week 52, and at week 104. Contiguous, 3-mm-thick axial slices through the whole brain were acquired. MRI analysis was performed at the Central Reading Center at the Institute of Neurology, University College London, by experienced raters who were unaware of treatment assignment.

The study had primary and secondary end

points at two prespecified times. An assessment of the inflammatory characteristics of the disease was performed at one year and of the progression of the irreversible destructive process at two years. At one year, the primary end point was the rate of clinical relapse, and secondary efficacy end points were the number of new or enlarging hyperintense lesions as detected by T<sub>2</sub>-weighted MRI, the number of lesions as detected by gadolinium-enhanced MRI, and the proportion of relapse-free patients. At 2 years, the primary end point was the cumulative probability of sustained progression of disability, which was defined as an increase of 1.0 or more on the EDSS from a baseline score of 1.0 or more or an increase of 1.5 or more from a baseline score of 0 that was sustained for 12 weeks (progression could not be confirmed during a relapse). Secondary efficacy end points at two years were the rate of clinical relapse, the volume of lesions as detected by T<sub>2</sub>-weighted MRI, the number of new hypointense lesions as detected by unenhanced T<sub>1</sub>-weighted MRI, and the progression of disability as measured by the Multiple Sclerosis Functional Composite. This report presents data on the one-year and two-year primary end points and the one-year secondary end points for which data were also available at two years.

Binding antibodies against natalizumab were assessed with the use of an enzyme-linked immunosorbent assay. Samples that were positive for binding antibodies (0.5 µg per milliliter) were further tested by flow cytometry to assess the ability of the antibodies to interfere with the binding of natalizumab to α<sub>4</sub> integrin.

#### STATISTICAL ANALYSIS

The estimate of sample size was based on data from previous trials of natalizumab<sup>17</sup> and of interferon beta-1a<sup>13</sup> with the use of two-sided tests, with an alpha level of 0.05. The annualized rate of relapse at one year was predicted to be 0.6 with natalizumab and 0.9 with placebo. For an annualized relapse rate, a likelihood-ratio test was used to determine the sample size required for 90 percent power (n=765), with a 2:1 ratio of natalizumab to placebo. With an assumed dropout rate of 15 percent and rounding, the number of patients needed was estimated to be 900. In order to power the study for the two-year end point of disability progression, progression rates at the end of two years were assumed to be 34.9

percent for the placebo group and 22.7 percent for the natalizumab group. Simulations of the log-rank test for survival were run with 60 percent of the accrual in the first 24 weeks and the remainder in the next 24 weeks, assuming a 20 percent dropout rate over the 2-year study. The sample size of 900 provided 90 percent power with the use of a Bonferroni adjustment for multiple end points, maintaining the type 1 error rate of 0.05.

P values that are reported for most baseline demographic and disease characteristics were calculated with the use of a t-test to compare differences in means. The exceptions were sex, race, and diagnosis of multiple sclerosis by the McDonald criteria,<sup>18</sup> for which a chi-square statistic was used to compare treatment groups.

The primary end point at two years was the cumulative probability of sustained progression of disability. This was assessed by an analysis of the time until the onset of the progression of disability that was sustained over 12 weeks with the use of the Cox proportional-hazards model. The annualized rate of relapse (the primary end point at one year) was calculated by Poisson regression; relapses that occurred after rescue treatment was initiated for patients who had had a sustained progression of disability (per protocol) were censored. The predefined statistical models included baseline scores on the EDSS for sustained progression of disability and the number of relapses in the previous year for the relapse rate. Additional baseline factors were tested for inclusion in each of the models, including the EDSS score ( $\leq 3.5$  or  $> 3.5$ ), the presence or absence of lesions as detected by gadolinium-enhanced MRI, the number of hyperintense lesions as detected by T<sub>2</sub>-weighted MRI ( $< 9$  or  $\geq 9$ ), and age ( $< 40$  or  $\geq 40$  years).<sup>21-23</sup> Each covariate was tested in the model for statistical significance with the use of a backward-selection procedure, and only statistically significant covariates ( $P \leq 0.10$ ) were included in the model. Only age was included in the final model for disability progression; the EDSS score, the presence or absence of lesions as detected by gadolinium-enhanced MRI, and age were included for the rate of relapse.

For the progression of disability, a sensitivity analysis was conducted on the change in EDSS scores that was sustained for 24 weeks. For the annualized relapse rate, sensitivity analyses were performed with and without censoring, as well

as with and without adjustment for significant covariates. The unadjusted relapse rate was calculated as the total number of relapses divided by the total number of patient-years followed for each treatment group. The Hochberg procedure<sup>24</sup> for multiple comparisons was used for the analysis of the two primary end points (the annualized relapse rate and the time to sustained progression of disability). Hence, the significance level was set so that if the higher of the P values for the analyses of these end points was  $\leq 0.05$ , then both end points were considered to be statistically significant; otherwise, the lower of the P values was tested at a significance level of 0.025.

Secondary efficacy end points were rank-ordered, and a closed testing procedure was used, so that if statistical significance was not achieved for an end point, end points of a lower rank were not considered to be statistically significant. Secondary efficacy end points were analyzed by logistic regression that included a term for the treatment group and the respective baseline measure as a covariate. In the analyses of secondary end points, missing values were imputed using the mean for the respective measures in the study population.

Differences between treatment groups with regard to adverse events were analyzed by the chi-square test, and serious adverse events were analyzed by Fisher's exact test. Poisson regression was used to calculate the difference between the rates of infection in each treatment group.

All analyses followed the intention-to-treat principle. All reported P values are two-tailed. The one-year analyses occurred when 900 patient-years of data had been collected. The date on which the database was locked for the two-year analyses was January 31, 2005, which resulted in 2076 patient-years of observation and 1338 patient-years of exposure to natalizumab.

## RESULTS

### STUDY POPULATION

Among the 942 patients, 627 were assigned to receive natalizumab and 315 to receive placebo. There were no significant differences in baseline characteristics between the treatment groups (Table 1). A total of 856 patients (91 percent) completed the 120-week study (Fig. 1), and 83 patients (a total of 9 percent, including 8 percent of patients in the natalizumab group and 10 percent



of those in the placebo group) withdrew from the study. Thirty-nine patients discontinued the study drug but completed follow-up (a total of 4 percent, including 4 percent of patients in the natalizumab group and 5 percent of those in the placebo group). Three patients who were assigned to receive placebo were never treated; these patients were included in the intention-to-treat efficacy analyses but were excluded from the safety analyses.

#### EFFICACY

A sustained progression of disability over two years (the two-year primary end point) was significantly less likely in the natalizumab group than in the placebo group (Fig. 2). At two years, the cumulative probability of progression (on the basis of Kaplan–Meier analysis) was 17 percent in the natalizumab group and 29 percent in the placebo group (hazard ratio, 0.58; 95 percent confidence interval, 0.43 to 0.77;  $P < 0.001$ ), which represents a decrease of 12 percentage points or a relative 42 percent decrease in the risk of a sustained progression of disability with natalizumab (Table 2). The sensitivity analysis of progression of disability that was sustained for 24 weeks yielded a 54 percent risk reduction in the natalizumab group (hazard ratio, 0.46; 95 percent confidence interval, 0.33 to 0.64;  $P < 0.001$ ).

After one year of treatment, natalizumab reduced the annualized rate of relapse to 0.26 relapse per year, as compared with 0.81 relapse per year in the placebo group ( $P < 0.001$ ) (Table 2). The 68 percent relative reduction in the annualized rate of relapse produced by natalizumab was maintained at two years ( $P < 0.001$ ). Subgroup and sensitivity analyses showed results consistent with the primary analysis. The proportion of relapse-free patients was significantly higher in the natalizumab group than in the placebo group at one year (77 percent vs. 56 percent,  $P < 0.001$ ) and at two years (67 percent vs. 41 percent,  $P < 0.001$ ). Natalizumab reduced the risk of relapse over two years by 59 percent (hazard ratio, 0.41; 95 percent confidence interval, 0.34 to 0.51;  $P < 0.001$ ). An analysis of relapse in 51 patients in the natalizumab group and 27 patients in the placebo group who discontinued the study drug showed a return to baseline disease activity when natalizumab therapy was stopped but no evidence of rebound; 25 relapses were reported by 15 patients in the natalizumab group (29 percent) after discontinuation of the study

medication, as compared with 13 relapses reported by 8 patients in the placebo group (30 percent), giving an annualized relapse rate of 0.495 for patients receiving natalizumab, as compared with a rate of 0.608 for those receiving placebo (data not shown).

Natalizumab reduced the mean number of new or enlarging hyperintense lesions detected by  $T_2$ -weighted MRI over two years by 83 percent, as compared with placebo ( $P < 0.001$ ) (Table 2). Over two years, no new or enlarging hyperintense lesions developed in 57 percent of patients in the natalizumab group, as compared with 15 percent of patients in the placebo group. In contrast, 68 percent of patients in the placebo group had at least three new or enlarging hyperintense lesions, as compared with only 18 percent of patients in the natalizumab group. Natalizumab reduced the mean number of lesions as detected by gadolinium-enhanced MRI by 92 percent as compared with placebo at both one year and two years ( $P < 0.001$ ) (Table 2). In addition, lesions detected by gadolinium-enhanced MRI were absent in 97 percent of patients in the natalizumab group as compared with 72 percent of patients in the placebo group on MRI scanning at two years.

#### SAFETY

Over the course of the two-year study, 596 patients receiving natalizumab (95 percent) and 300 of the 312 patients receiving placebo (96 percent) reported at least one adverse event. As shown in Table 3, adverse events that were significantly more common in the natalizumab group were fatigue and allergic reaction. The most severe adverse events reported by patients were mild in 17 percent, moderate in 55 percent, and severe in 23 percent of patients in the natalizumab group and mild in 13 percent, moderate in 56 percent, and severe in 27 percent in the placebo group. Serious adverse events were observed in 19 percent of patients receiving natalizumab and in 24 percent of patients receiving placebo ( $P = 0.06$ ); the most common serious adverse events were relapsing multiple sclerosis (6 percent with natalizumab and 13 percent with placebo;  $P < 0.001$ ), cholelithiasis (<1 percent with natalizumab and <1 percent with placebo), and the need for rehabilitation therapy (<1 percent with natalizumab and <1 percent with placebo). Two deaths occurred during the study, both in the natalizumab group. One patient, who died of malignant melanoma,

**Table 1. Baseline Characteristics of the Patients.\***

Characteristic	Natalizumab (N = 627)	Placebo (N = 315)	Total (N = 942)	P Value
Age — yr				
Mean	35.6±8.5	36.7±7.8	36.0±8.3	0.056
Range	18–50	19–50	18–50	
Sex — no. of patients (%)				
Male	178 (28)	104 (33)	282 (30)	0.144
Female	449 (72)	211 (67)	660 (70)	
Race — no. of patients (%)†				
White	603 (96)	296 (94)	899 (95)	0.126
Other	24 (4)	19 (6)	43 (5)	
McDonald criteria — no. of patients (%)‡				
1 (≥2 attacks, ≥2 lesions)	528 (84)	261 (83)	789 (84)	0.938
2 (≥2 attacks, 1 lesion)	72 (11)	40 (13)	112 (12)	
3 (1 attack, ≥2 lesions)	18 (3)	10 (3)	28 (3)	
4 (1 attack, 1 lesion)	9 (1)	4 (1)	13 (1)	
Disease duration — yr				
Median	5.0	6.0	5.0	0.511
Range	0–34	0–33	0–34	
No. of relapses in past yr — no. of patients (%)				
0	6 (<1)	6 (2)	12 (1)	
1	368 (59)	180 (57)	548 (58)	
2	197 (31)	102 (32)	299 (32)	
≥3	56 (9)	27 (9)	83 (9)	
Mean	1.53±0.91	1.50±0.77	1.52±0.86	0.640
Range	0–12	0–5	0–12	
EDSS score — no. of patients (%)				
0	31 (5)	18 (6)	49 (5)	
1.0–1.5	179 (29)	94 (30)	273 (29)	
2.0–2.5	208 (33)	103 (33)	311 (33)	
3.0–3.5	130 (21)	63 (20)	193 (20)	
4.0–4.5	60 (10)	28 (9)	88 (9)	
5.0	17 (3)	7 (2)	24 (3)	
≥5.5	2 (<1)	2 (<1)	4 (<1)	
Mean	2.3±1.2	2.3±1.2	2.3±1.2	0.784
Range	0–6	0–6	0–6	

had a history of malignant melanoma and had noted a new lesion at the time of receiving the first dose of natalizumab; he had received a total of five doses of natalizumab before receiving a confirmed diagnosis. A second patient died of alcohol intoxication after having received 25 doses of natalizumab.

Infections were generally mild to moderate in severity and did not lead to drug discontinuation. The overall incidence of infection was 79 percent in each treatment group and occurred at a rate of 1 per patient-year in each group. When the rate was reanalyzed to include multiple occurrences of infection, it went up in each group,

**Table 1. (Continued.)**

Characteristic	Natalizumab (N=627)	Placebo (N=315)	Total (N=942)	P Value
No. of lesions on gadolinium-enhanced MRI — no. of patients (%)				
0	307 (49)	170 (54)	477 (51)	
1	115 (18)	55 (17)	170 (18)	
2	66 (11)	24 (8)	90 (10)	
3	38 (6)	18 (6)	56 (6)	
≥4	100 (16)	46 (15)	146 (15)	
Missing data	1 (<1)	2 (<1)	3 (<1)	
Mean	2.2±4.7	2.0±4.8	2.2±4.7	0.511
Range	0–36	0–39	0–39	
No. of lesions on T <sub>2</sub> -weighted MRI — no. of patients (%)				
<9	29 (5)	15 (5)	44 (5)	0.921
≥9	597 (95)	299 (95)	896 (95)	
Missing data	1 (<1)	1 (<1)	2 (<1)	

\* Plus-minus values are means ±SD. EDSS denotes Expanded Disability Status Scale (range of scores, 0 to 10, with higher scores indicating more severe disease), and MRI magnetic resonance imaging. Percentages may not sum to 100, because of rounding.

† Race was determined at the time of enrollment by the treating investigator.

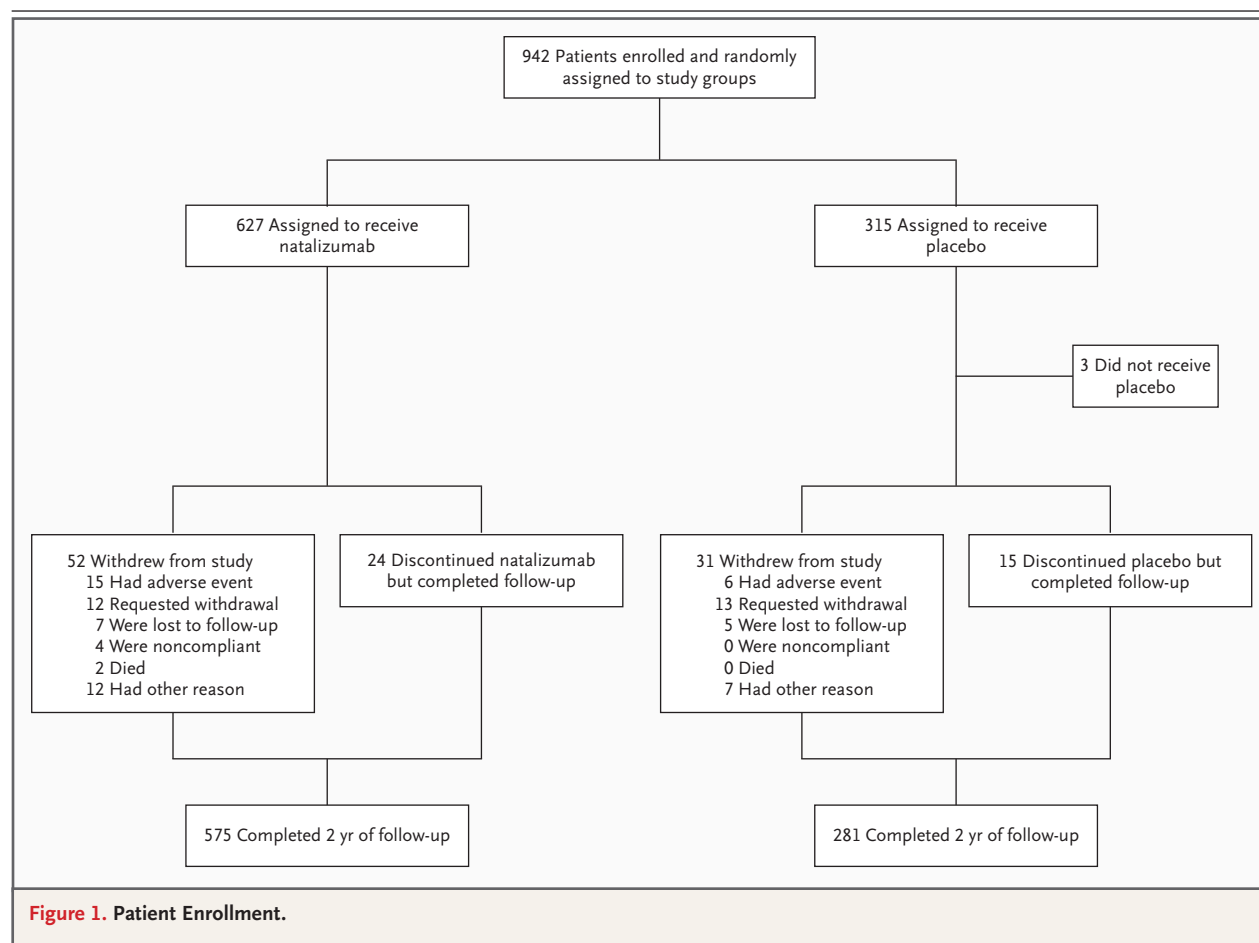
‡ Criteria are from McDonald et al.<sup>18</sup>

as expected. However, there remained no significant differences between the groups, with infections occurring at a rate of 1.52 per patient-year in the natalizumab group and 1.42 per patient-year in the placebo group ( $P=0.32$ ). Common infections were nasopharyngitis (32 percent of patients receiving natalizumab and 33 percent of patients receiving placebo), influenza (17 percent and 16 percent, respectively), upper respiratory tract viral infection (13 percent and 15 percent), urinary tract infection not otherwise specified (13 percent and 12 percent), upper respiratory tract infection not otherwise specified (13 percent and 11 percent), and pharyngitis (12 percent and 10 percent). Serious infections occurred in 3.2 percent of patients receiving natalizumab and in 2.6 percent of patients receiving placebo. In the natalizumab group, the serious infections included four cases of pneumonia and five cases of urinary tract infection or urosepsis; the remaining infections that were reported as serious were of various causes and included pilonidal cyst infection, cellulitis, febrile infection, gastroenteritis, cryptosporidial diarrhea, mononucleosis, osteomyelitis, sinusitis, tonsillitis, viral infec-

tion, appendicitis, and an infection of unclear cause. In the placebo group, serious infections included two cases of appendicitis, two cases of gastroenteritis, and one case each of infection not otherwise specified, bladder infection, cystitis, and influenza.

A total of six cases of cancer were reported — one case (<1 percent) in the placebo group and five cases (<1 percent) in the natalizumab group. The five cases of cancer that occurred in natalizumab-treated patients included three cases of breast cancer, one case of stage 0 cervical cancer, and one case of newly diagnosed metastatic melanoma. There was one case of basal-cell carcinoma in the placebo group.

Infusion reactions were defined as any event that occurred within two hours after the start of the one-hour infusion; they were reported in 148 patients receiving natalizumab (24 percent) and in 55 patients receiving placebo (18 percent) ( $P=0.04$ ). The most common infusion reaction was headache (5 percent with natalizumab and 3 percent with placebo). Most reactions were treated symptomatically and did not result in discontinuation of the study drug. Hypersensitivity reactions were



defined as reports of hypersensitivity, allergic reaction, or anaphylactic or anaphylactoid reaction by the investigator, as well as any report of urticaria, allergic dermatitis, or hives. The category was determined by the investigator on the basis of clinical judgment and severity. Twenty-five patients receiving natalizumab (4 percent) had 27 hypersensitivity reactions: 12 cases of urticaria or generalized urticaria, 1 of allergic dermatitis, 8 of a reaction called hypersensitivity, and 5 of anaphylactic or anaphylactoid reactions (urticaria plus other signs). One patient with a hypersensitivity reaction during the 7th infusion received other doses according to schedule and had an anaphylactic or anaphylactoid reaction during the 13th infusion. Fifteen reactions occurred on the second infusion. Eight hypersensitivity reactions (1.3 percent) were reported as serious adverse events among all patients receiving natalizumab, of which 5 reactions (0.8 percent) were considered serious systemic reactions (i.e., anaphylactic or anaphylactoid reactions).

Per protocol, the study drug was to be discontinued in all patients who had hypersensitivity reactions. Five of the eight patients with serious adverse events had respiratory or chest symptoms, but only one patient required supplemental oxygen. No cardiovascular compromise was associated with any of these events, although one patient did receive epinephrine. All patients recovered without sequelae.

Because of adverse effects, 6 percent of the patients receiving natalizumab and 4 percent of those receiving placebo discontinued the study drug, and 3 percent of patients receiving natalizumab and 2 percent receiving placebo withdrew from the study. There were no significant differences between treatment groups in the proportions of patients with clinically notable changes in laboratory values. Increases in the number of lymphocytes, monocytes, eosinophils, and basophils were seen in natalizumab-treated patients without elevations in the number of neutrophils.



These increases are consistent with expression of  $\alpha_4\beta_1$  on these white-cell subgroups and are a known pharmacodynamic effect of natalizumab. Increases in nucleated red cells were also seen transiently in a small number of patients. All changes were reversible, were without clinical effects, and returned to baseline levels, usually within 16 weeks after the last dose was administered.

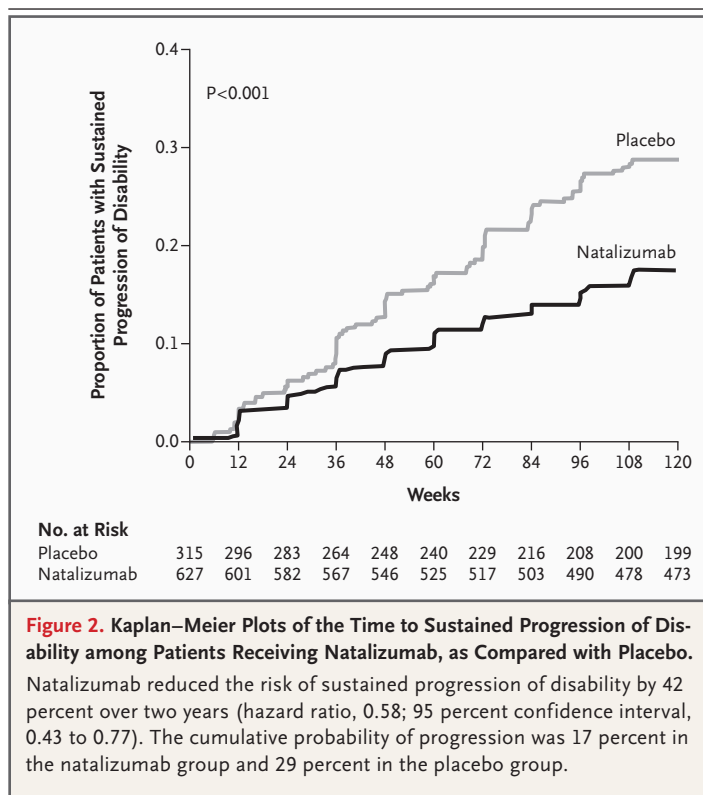
#### IMMUNOGENICITY

Fifty-seven patients receiving natalizumab (9 percent) had detectable antibodies at some time during the study. Of these 57 patients, persistent antibodies to natalizumab (antibodies detected at  $\geq 2$  times that were  $\geq 42$  days apart) developed in 37 patients (6 percent), who also had an increase in infusion-related adverse events and a loss of efficacy of natalizumab.

#### DISCUSSION

The results of AFFIRM support the hypothesis that the interaction between  $\alpha_4\beta_1$  integrin and its targets is an important component of inflammation of the central nervous system in multiple sclerosis and that the disruption of these interactions and the resultant attenuation of inflammation are beneficial to patients. In patients with relapsing multiple sclerosis, natalizumab significantly reduced the risk of progression of disability and the annualized rate of relapse over two years of treatment. The effect of natalizumab was rapid in onset and was sustained. In addition, efficacy was observed in terms of all secondary end points (an 83 percent reduction in the number of lesions as detected by T<sub>2</sub>-weighted MRI and a 92 percent reduction in the number of lesions as detected by gadolinium-enhanced MRI) and all sensitivity analyses of the primary end points, indicating the robustness of the result.

Disease-modifying therapies have become the cornerstone of treatment for patients with relapsing multiple sclerosis. The two-year registration trials of the therapies that are currently available (interferon beta products and glatiramer acetate) have shown that these agents reduce the annualized rate of relapse by about one third.<sup>13-16</sup> In addition, neither interferon beta-1b nor glatiramer acetate had statistically significant effects on the progression of disability in patients with relapsing disease.<sup>14,15</sup> Hence, there remains a need



for more effective treatments for relapsing multiple sclerosis. The results of this study suggest that natalizumab may offer greater benefit to patients with relapsing multiple sclerosis than the other therapies.

The data presented here represent 30 percent of the placebo-controlled experience (patient-years of exposure) with natalizumab in patients with multiple sclerosis or Crohn's disease and 48 percent of the experience with natalizumab in patients with multiple sclerosis. In our study, natalizumab was safe as monotherapy over two years. In February 2005, all administration of natalizumab was voluntarily suspended by the manufacturers when they were notified of two cases of progressive multifocal leukoencephalopathy (PML). Both patients had received more than two years of natalizumab in combination with interferon beta-1a in a separate trial. Later, an additional case of PML was identified in a patient with Crohn's disease who had previously received a mistaken diagnosis of astrocytoma. The patient had received eight infusions of natalizumab. Detailed case histories of these three patients have been published elsewhere.<sup>25-27</sup> An extensive safety evaluation of patients who received natalizumab

**Table 2.** End Points as Determined by Clinical Results and MRI Evaluation.\*

End Point	1 Year			2 Years		
	Natalizumab (N=627)	Placebo (N=315)	P Value	Natalizumab (N=627)	Placebo (N=315)	P Value
<b>Clinical</b>						
Primary end point at 2 years: cumulative probability of sustained disability progression — %†	—	—		17	29	<0.001‡
Primary end point at 1 year: annualized relapse rate — mean (95% CI)§						
Preplanned interim analysis (after 900 patient-years)	0.26 (0.21–0.32)	0.81 (0.67–0.97)	<0.001	—	—	
Final analysis	0.27 (0.21–0.33)	0.78 (0.64–0.94)	<0.001	0.23 (0.19–0.28)	0.73 (0.62–0.87)	<0.001
Number of relapses — no. of patients (%)						
0	501 (80)	189 (60)		454 (72)	146 (46)	
1	106 (17)	76 (24)		123 (20)	65 (21)	
2	17 (3)	36 (11)		36 (6)	63 (20)	
≥3	3 (<1)	14 (4)		14 (2)	41 (13)	
Sensitivity analysis¶						
Adjusted annualized relapse rate	—	—		0.24	0.75	<0.001
Unadjusted annualized relapse rate	—	—		0.22	0.64	<0.001
Mean relapse rate per patient	—	—		0.22	0.67	<0.001
<b>MRI</b>						
	0–1 Year			0–2 Years		
Number of new or enlarging T <sub>2</sub> -hyperintense lesions — no. of patients (%)			<0.001			<0.001
0	382 (61)	72 (23)		360 (57)	46 (15)	
1	112 (18)	41 (13)		106 (17)	32 (10)	
2	40 (6)	23 (7)		48 (8)	24 (8)	
≥3	93 (15)	179 (57)		113 (18)	213 (68)	
Number of new or enlarging T <sub>2</sub> -hyperintense lesions						
Mean	1.2±4.7	6.1±9.0		1.9±9.2	11.0±15.7	
Median	0	3.0		0	5.0	
Minimum, maximum	0, 98	0, 77		0, 196	0, 91	
	At 1 Year			At 2 Years		
Number of gadolinium-enhancing lesions — no. of patients (%)			<0.001			<0.001
0	605 (96)	213 (68)		608 (97)	227 (72)	
1	17 (3)	42 (13)		12 (2)	39 (12)	
2	3 (<1)	15 (5)		1 (<1)	9 (3)	
≥3	2 (<1)	45 (14)		6 (<1)	40 (13)	
Number of gadolinium-enhancing lesions						
Mean	0.1±1.3	1.3±3.2		0.1±1.4	1.2±3.9	
Median	0	0		0	0	
Minimum, maximum	0, 32	0, 33		0, 32	0, 48	

\* Plus-minus values are means ±SD. CI denotes confidence interval.

† Sustained disability progression was defined as an increase of 1.0 point or more in scores on the Expanded Disability Status Scale from a baseline score of 1.0 or more or an increase of 1.5 points or more from a baseline score of 0 that was sustained for 12 weeks.

‡ The hazard ratio for sustained progression of disability in the natalizumab group as compared with the placebo group was 0.58 (95 percent confidence interval, 0.43 to 0.77).

§ Relapses that occurred after sustained progression of disability was reached and rescue treatment was initiated (per protocol) were censored.

¶ Analysis includes relapses that occurred after sustained progression was reached and rescue treatment was initiated (per protocol).

|| The mean relapse rate per patient is the number of relapses for each patient divided by the total number of years of follow-up.

**Table 3. Adverse Events.\***

Adverse event	Natalizumab (N = 627) % of patients	Placebo (N = 312) % of patients	P Value	Adverse event	Natalizumab (N = 627) % of patients	Placebo (N = 312) % of patients	P Value
General condition				Menstrual disorder†			
Headache	38	33	0.137	Irregular menstruation or dysmenorrhea	7	4	0.102
Fatigue	27	21	0.048	Amenorrhea	2	1	0.405
Arthralgia	19	14	0.106	Neurologic condition			
Urinary urgency or frequency	9	7	0.365	Vertigo	6	5	0.779
Allergic reaction	9	4	0.012	Tremor	3	3	0.566
Chest discomfort	5	3	0.169	<b>Serious adverse event‡</b>			
Local bleeding	3	2	0.386	Relapse of multiple sclerosis	6	13	<0.001
Rigors	3	1	0.080	Cholelithiasis	<1	<1	0.435
Syncope	3	3	0.895	Need for rehabilitation therapy	<1	<1	0.999
Infection				Urinary tract infection, NOS	<1	0	0.308
Urinary tract	20	17	0.257	Depression	<1	<1	0.669
Lower respiratory tract	17	16	0.644	Anaphylactic reaction	<1	0	0.555
Gastroenteritis	11	9	0.328	Hypersensitivity reaction	<1	0	0.555
Vaginitis†	10	6	0.133	Fall	<1	<1	0.999
Tonsillitis	7	5	0.291	Breast cancer, NOS	<1	0	0.999
Psychiatric condition (depression)	19	16	0.197	Anaphylactoid reaction	<1	0	0.999
Gastrointestinal condition				Convulsion, NOS	<1	<1	0.604
Abdominal discomfort	11	10	0.561	Gastritis, NOS	<1	0	0.999
Abnormal liver-function results	5	4	0.406	Cervical dysplasia	<1	0	0.999
Skin				Alcohol poisoning	<1	<1	0.999
Rash	11	9	0.301	Head injury	<1	<1	0.999
Dermatitis	7	4	0.053	Thermal burn	<1	0	0.999
Pruritus	4	2	0.090				

\* NOS denotes not otherwise specified.

† The percentage and P value were calculated on the basis of the number of female patients.

‡ Serious adverse events are listed only if they occurred in two or more patients in the natalizumab group.

in a clinical trial, also reported in this issue of the *Journal*, found no new confirmed cases of PML in patients treated with natalizumab.<sup>28</sup> (The results of another clinical trial of natalizumab — in this case, administered with interferon beta-1a — also appear in this issue.<sup>29</sup>)

In conclusion, our study provides evidence that natalizumab significantly reduces the progression of disability and the occurrence of clinical relapse and suppresses the formation of lesions as visualized by MRI in patients with relapsing multiple sclerosis. Moreover, our data indicate that efficacy is realized early and persists throughout the treatment period. Within the 30-month evaluation period of this trial, natalizumab monotherapy had an excellent safety and tolerability profile. Continued assessments of long-term treatment with natalizumab will better define the safety

profile of this effective therapy and establish its place in the arsenal of treatments for relapsing multiple sclerosis.

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