ORIGINAL ARTICLE

Natalizumab plus Interferon Beta-1a for Relapsing Multiple Sclerosis

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ABSTRACT

BACKGROUND

Interferon beta is used to modify the course of relapsing multiple sclerosis. Despite interferon beta therapy, many patients have relapses. Natalizumab, an α_4 integrin antagonist, appeared to be safe and effective alone and when added to interferon beta-1a in preliminary studies.

METHODS

We randomly assigned 1171 patients who, despite interferon beta-1a therapy, had had at least one relapse during the 12-month period before randomization to receive continued interferon beta-1a in combination with 300 mg of natalizumab (589 patients) or placebo (582 patients) intravenously every 4 weeks for up to 116 weeks. The primary end points were the rate of clinical relapse at 1 year and the cumulative probability of disability progression sustained for 12 weeks, as measured by the Expanded Disability Status Scale, at 2 years.

RESULTS

Combination therapy resulted in a 24 percent reduction in the relative risk of sustained disability progression (hazard ratio, 0.76; 95 percent confidence interval, 0.61 to 0.96; P=0.02). Kaplan–Meier estimates of the cumulative probability of progression at two years were 23 percent with combination therapy and 29 percent with interferon beta-1a alone. Combination therapy was associated with a lower annualized rate of relapse over a two-year period than was interferon beta-1a alone (0.34 vs. 0.75, P<0.001) and with fewer new or enlarging lesions on T_2-weighted magnetic resonance imaging (0.9 vs. 5.4, P<0.001). Adverse events associated with combination therapy were anxiety, pharyngitis, sinus congestion, and peripheral edema. Two cases of progressive multifocal leukoencephalopathy, one of which was fatal, were diagnosed in natalizumab-treated patients.

CONCLUSIONS

Natalizumab added to interferon beta-1a was significantly more effective than interferon beta-1a alone in patients with relapsing multiple sclerosis. Additional research is needed to elucidate the benefits and risks of this combination treatment. (ClinicalTrials.gov number, NCT00030966.)

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*The Safety and Efficacy of Natalizumab in Combination with Interferon Beta-1a in Patients with Relapsing Remitting Multiple Sclerosis (SENTINEL) Investigators are listed in the Supplementary Appendix, available with the full text of this article at www.nejm.org.

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THE ADHESION MOLECULE $\alpha_4\beta_1$ INTEGRIN is a key initiator of the inflammatory cascade involved in the pathogenesis of multiple sclerosis.[1-4] Natalizumab (Tysabri, Biogen Idec and Elan Pharmaceuticals) is the first α_4 integrin antagonist in a new class of selective adhesion-molecule inhibitors for the treatment of multiple sclerosis. Natalizumab binds to α_4 integrin on the surface of leukocytes, inhibiting their migration into the brain and thereby reducing inflammation.

Current disease-modifying therapies for relapsing–remitting multiple sclerosis (interferon beta and glatiramer acetate) are only partially effective,[5-8] and most patients with multiple sclerosis have breakthrough disease activity despite therapy with these agents. Hence, there is a need for additional treatment options in multiple sclerosis. Natalizumab is an attractive therapy to add to current disease-modifying therapies in patients with breakthrough disease because preliminary efficacy[9] and safety[10] data have been favorable and because the mechanism of action of natalizumab may complement those of other disease-modifying therapies.[11-17]

The Safety and Efficacy of Natalizumab in Combination with Interferon Beta-1a in Patients with Relapsing Remitting Multiple Sclerosis (SENTINEL) study was a two-year, phase 3 clinical trial designed to determine whether natalizumab, when added to interferon beta-1a, has efficacy in addition to that associated with interferon beta-1a alone. The trial was also designed to confirm the safety of natalizumab when added to interferon beta-1a.

METHODS

PATIENTS

One hundred twenty-four clinical centers in Europe and the United States enrolled 1196 patients beginning on January 14, 2002. All patients gave written informed consent. The study protocol was developed by the investigator advisory committee and the sponsors and was approved by central and local ethics committees, and the study was overseen by an independent safety-monitoring committee. Data were collected by the investigators and an independent organization (PPD International) and were held and analyzed by Biogen Idec and Elan Pharmaceuticals. During the study, the investigator advisory committee and representatives of Biogen Idec met at least monthly to review and manage the study. The manuscript was written by Drs. Rudick and Panzara, with input from each of the other authors; all the authors vouch for the veracity and completeness of the data and analyses.

Eligible patients were 18 to 55 years of age; had a diagnosis of relapsing–remitting multiple sclerosis,[18] a score on the Expanded Disability Status Scale (EDSS) (possible scores range from 0 to 10, with higher scores indicating more severe disease) between 0 and 5.0,[19] and a magnetic resonance imaging (MRI) scan revealing lesions consistent with a diagnosis of multiple sclerosis; had received treatment with interferon beta-1a for at least 12 months before randomization; and had had at least one relapse during the 12-month period before randomization. Patients were ineligible if they had primary progressive, secondary progressive, or progressive relapsing multiple sclerosis[20]; if they had had a relapse within 50 days before randomization; or if they had been treated with an approved disease-modifying therapy other than interferon beta-1a intramuscularly once weekly within the 12-month period before randomization.

STUDY DESIGN AND RANDOMIZATION

This study was a randomized, double-blind, placebo-controlled, parallel-group, phase 3 clinical trial. Data from 1171 of the 1196 patients enrolled were analyzed, because a single center with 25 patients was excluded before unblinding owing to irregularities in data. Patients were randomly assigned, in a 1:1 ratio, to receive 300 mg of natalizumab (589 patients) or placebo (582 patients) intravenously every 4 weeks in addition to interferon beta-1a (Avonex, Biogen Idec) at a dose of 30 μg intramuscularly once weekly for up to 116 weeks. Randomization was stratified according to study site in blocks of four (two active and two placebo) with the use of a computer-generated schedule and a multidigit identification number, implemented by way of an interactive voice-response system. All study personnel, patients, sponsor personnel involved in the conduct of the study, and members of the investigator advisory committee were blinded to the treatment assignments throughout the study.

STUDY PROCEDURE AND END POINTS

Each site designated primary and backup examining neurologists and treating neurologists. The

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examining neurologists performed the EDSS and neurologic examinations but were otherwise not involved in the patients' medical care. The treating neurologists were responsible for all patient care, including the management of adverse events and relapses of multiple sclerosis.

Clinical visits every 12 weeks included assessment of relapses, EDSS evaluation, blood chemical and hematologic tests, assessment of any adverse events, and immunogenicity studies. Patients were also seen by a treating neurologist during unscheduled visits within 72 hours after the development of new symptoms so that they could be assessed for possible relapses or adverse events. If a relapse was suspected, the patient was evaluated by the examining neurologist. Relapses were defined as the development of new or recurrent neurologic symptoms not associated with fever or infection, lasting at least 24 hours, and accompanied by new, objective neurologic findings. 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 who had disability progression that was sustained for 12 weeks were asked to provide consent to continue study participation and were given the option of adding an available multiple sclerosis treatment as rescue medication, according to protocol, while continuing to receive the study drug. Patients who discontinued the study drug were strongly encouraged to remain in the study for follow-up assessments, and all patients who continued to participate in the study were evaluated (according to the intention-to-treat principle).

Proton-density, T_2-weighted MRI scans and gadolinium-enhanced T_1-weighted MRI scans of the brain were obtained at baseline and at weeks 52 and 104. Forty contiguous, 3-mm-thick axial slices were acquired. MRI analyses were performed centrally at the MS-MRI Evaluation Center (Basel, Switzerland) by blinded raters. The scans were checked for artifacts, compliance with scanning requirements, and repositioning.

The primary efficacy end point was the rate of clinical relapse at one year. Secondary end points at one year were the number of new or enlarging T_2-hyperintense lesions, the number of gadolinium-enhancing lesions, and the proportion of patients free of relapse. The primary efficacy end point at two years was the cumulative probability of sustained disability progression, defined as an increase by at least 1.0 point in the EDSS score from a baseline score of at least 1.0 or an increase by at least 1.5 points in the EDSS score from a baseline score of 0, sustained for 12 weeks; progression could not be confirmed during a relapse. Secondary end points at two years were the rate of clinical relapse, the volume of T_2-hyperintense lesions, the number of new T_1-hypointense lesions, and disability as measured by the Multiple Sclerosis Functional Composite.[21] This report presents data pertaining to primary end points and key secondary efficacy end points, as well as safety data. Results pertaining to additional secondary end points and tertiary end points are not included in this report.

Binding antibodies against natalizumab were assessed with use of an enzyme-linked immunosorbent assay. Positive samples (0.5 μg per milliliter) were further tested in a flow-cytometry assay to determine whether these antibodies interfered with the binding of natalizumab to α_4 integrin.

STATISTICAL ANALYSIS

The sample size was estimated, on the basis of data from previous trials of natalizumab[9] and interferon beta-1a,[6] with the use of two-sided tests with an experiment-wise alpha of 0.05. The annualized rate of relapse among patients receiving combination therapy at one year was predicted to be 0.6, as compared with 0.9 among patients receiving interferon beta-1a alone. For the annualized relapse rate, the likelihood-ratio test was used to determine the sample size with half the patients receiving active drug and half receiving placebo. With an assumed dropout rate of 17 percent, rounding, a type I error rate of 2.5 percent, and a type II error rate of 90 percent, the number of patients needed was estimated to be 1200. To power the study for the two-year end point of disability progression, we assumed a progression rate of 34.9 percent at the end of two years in the group assigned to interferon beta-1a alone and a progression rate of 22.7 percent at the end of two years (a 35 percent improvement) in the combination-therapy group. Simulations of the log-rank test were run with 60 percent of the accrual in the first 24 weeks and the remainder in the next 24 weeks. With an assumed dropout rate of 20 percent, the sample size of 1200 provided at least 92 percent power with a Bonferroni adjustment for multiple end points and with the type I error rate maintained at 5 percent.

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The baseline characteristics of the patients were analyzed with the use of a t-test, with the exceptions of sex, race, and diagnosis of multiple sclerosis (based on the McDonald criteria[18]), which were analyzed with the use of a chi-square test. The time to the onset of disability progression sustained for 12 weeks was used to determine the cumulative probability of disability progression estimated by the Kaplan–Meier method. The Cox proportional-hazards model, adjusted for the baseline EDSS score, was used to compare the Kaplan–Meier curves. The annualized relapse rate was calculated by Poisson regression and adjusted for the number of relapses in the year before randomization; data pertaining to relapses that occurred after rescue treatment was initiated (per protocol) were censored. Additional baseline factors were tested for inclusion in each of the models: EDSS score (≤3.5 or >3.5), gadolinium-enhancing lesions (present or absent), the number of T_2-hyperintense lesions (<9 or ≥9), and age (<40 or ≥40 years).[22-24] Each covariate was tested in the model for statistical significance by a backward-selection procedure, and only statistically significant covariates (P≤0.10) were included in the final models. No additional covariates were included in the analysis of disability progression. Three additional covariates (baseline EDSS score, the presence or absence of gadolinium-enhancing lesions at baseline, and age) were included in the analysis of relapse rate.

A sensitivity analysis of disability progression (based on the change in EDSS score) sustained for 24 weeks was also conducted. For the annualized rate of relapse, sensitivity analyses were performed with and without censoring, as well as with and without adjustment for significant covariates. The unadjusted rate of relapse was calculated as the total number of relapses divided by the total number of subject-years of follow-up in each treatment group. The Hochberg procedure[25] for multiple comparisons was used in the analysis of the two primary end points; hence, the significance level was set such that if the higher of the P values for the analyses of these end points was less than or equal to 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 ranked-ordered, and a closed testing procedure was used such that if statistical significance was not achieved for a given end point, then end points of a lower rank were considered not statistically significant. Secondary efficacy end points were analyzed by logistic regression with a term for treatment group and with their respective baseline values as covariates; missing values were imputed by using the mean in the study population.

Adverse events were analyzed with use of the chi-square test, and serious adverse events were analyzed with use of 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 date on which the database was locked for the two-year analyses was May 31, 2005, and as a result there were 2528 patient-years of observation and 1222 patient-years of exposure to natalizumab.

RESULTS

PATIENTS

SENTINEL was stopped approximately one month early, on February 28, 2005, because of two reports of progressive multifocal leukoencephalopathy (PML). Of the 1171 patients, a total of 1003 (86 percent) completed the 120-week study; 168 patients (14 percent overall; 12 percent of the group assigned to interferon beta-1a plus natalizumab and 16 percent of the group assigned to interferon beta-1a alone) withdrew from the study (Fig. 1). Sixty-four patients discontinued the study drug but completed follow-up (5 percent overall; 5 percent of the combination-therapy group and 6 percent of the group assigned to interferon beta-1a alone). There were no significant differences in demographic or disease-related characteristics at baseline between the two treatment groups, with the exception of the duration of disease (median, seven years in the combination-therapy group and eight years in the group assigned to interferon beta-1a alone; P=0.02) (Table 1).

The SENTINEL data represent 28 percent of the placebo-controlled experience with natalizumab (in terms of patient-years of exposure) in both multiple sclerosis and Crohn's disease and 44 percent of the overall experience in multiple sclerosis.

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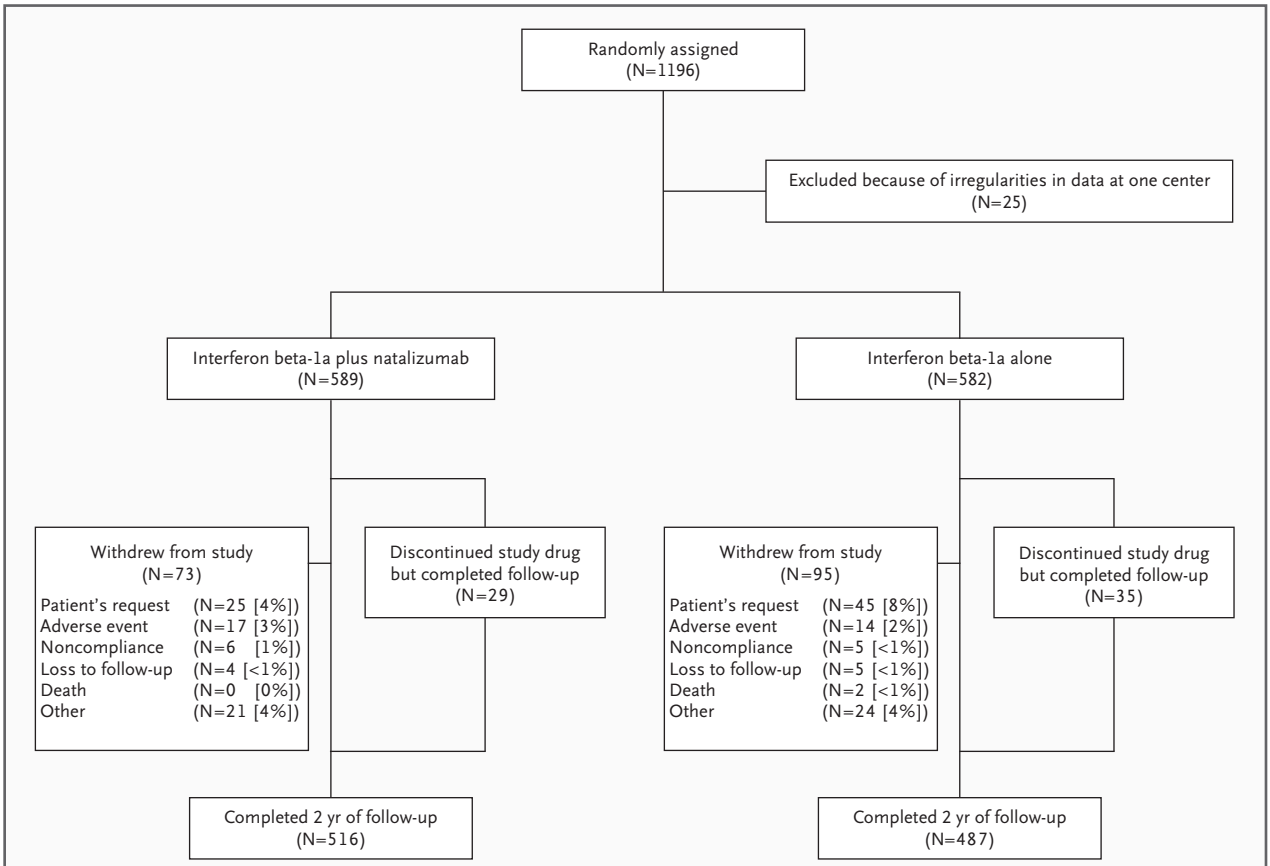

Figure 1. Disposition of Patients during the Two-Year SENTINEL Study.

EFFICACY

Kaplan–Meier estimates of the cumulative probability of sustained disability progression at 2 years were 23 percent with combination therapy and 29 percent with interferon beta-1a alone (Fig. 2 and Table 2). Combination therapy resulted in a 24 percent decrease in the risk of sustained disability progression (hazard ratio, 0.76; 95 percent confidence interval, 0.61 to 0.96; P=0.02). In the sensitivity analysis of the risk of disability progression sustained for 24 weeks, estimates of the cumulative probability of progression by 2 years were 15 percent for combination therapy and 18 percent for interferon beta-1a alone (representing an 18 percent reduction with combination therapy); however, this difference was not statistically significant (P=0.17).

Combination therapy reduced the annualized rate of relapse at one year, which was 0.82 with interferon beta-1a alone, to 0.38 (P<0.001) — a 54 percent reduction (Table 2). This difference was maintained at two years, at which time the rate was 0.75 with interferon beta-1a alone and 0.34 with combination therapy (a 55 percent reduction with combination therapy, P<0.001). Subgroup analyses (according to relapse history, EDSS score, age, sex, the presence or absence of gadolinium-enhancing lesions, and the number of T_2-hyperintense lesions) and a sensitivity analysis of relapse rate showed consistent results. The proportion of patients who were relapse-free at two years was 54 percent in the combination-therapy group, as compared with 32 percent in the group assigned to interferon beta-1a alone (P<0.001). The risk of relapse was 50 percent lower with combination therapy (hazard ratio, 0.50; 95 percent confidence interval, 0.43 to 0.59; P<0.001).

The number of new or enlarging T_2-hyperintense lesions over the two-year period was re-

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Table 1. Baseline Characteristics of the Patients.*

Characteristic	Interferon Beta-1a plus Natalizumab (N=589)	Interferon Beta-1a Alone (N=582)	Total (N=1171)
Age — yr			
Mean ±SD	38.8±7.7	39.1±7.6	38.9±7.7
Range	18–55	19–55	18–55
Sex — no. of patients (%)			
Male	147 (25)	162 (28)	309 (26)
Female	442 (75)	420 (72)	862 (74)
Race — no. of patients (%)†			
White	550 (93)	542 (93)	1092 (93)
Other	39 (7)	40 (7)	79 (7)
Duration of disease — yr			
Median	7.0‡	8.0	7.0
Range	1–34	1–34	1–34
No. of relapses in previous 1 yr — no. of patients (%)			
0	0	1 (<1)	1 (<1)
1	390 (66)	357 (61)	747 (64)
2	153 (26)	174 (30)	327 (28)
≥3	44 (7)	50 (9)	94 (8)
Missing data	2 (<1)	0	2 (<1)
No. of relapses in previous 1 yr			
Mean ±SD	1.44±0.75	1.49±0.72	1.47±0.73
Range	1–7	0–5	0–7
EDSS score — no. of patients (%)§			
0	24 (4)	19 (3)	43 (4)
1.0–1.5	145 (25)	143 (25)	288 (25)
2.0–2.5	214 (36)	203 (35)	417 (36)
3.0–3.5	125 (21)	126 (22)	251 (21)
4.0–4.5	68 (12)	72 (12)	140 (12)
5.0	12 (2)	16 (3)	28 (2)
≥5.5	1 (<1)	3 (<1)	4 (<1)
EDSS score			
Mean ±SD	2.4±1.1	2.5±1.1	2.4±1.1
Range	0–6.0	0–5.5	0–6.0

duced from 5.4 with interferon beta-1a alone to 0.9 with combination therapy (P<0.001), representing an 83 percent reduction with combination therapy (Table 2). The mean number of gadolinium-enhancing lesions at two years was 0.9 with interferon beta-1a alone and 0.1 with combination therapy, representing an 89 percent reduction (P<0.001).

SAFETY

At least one adverse event was reported by 584 patients assigned to receive interferon beta-1a plus natalizumab (>99 percent) and 578 assigned to receive interferon beta-1a alone (>99 percent). Adverse events significantly associated with combination therapy were anxiety, pharyngitis, sinus congestion, and peripheral edema (Table 3). The

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Table 1. (Continued.)

Characteristic	Interferon Beta-1a plus Natalizumab (N=589)	Interferon Beta-1a Alone (N=582)	Total (N=1171)
No. of gadolinium-enhancing lesions — no. of patients (%)			
0	392 (67)	374 (64)	766 (65)
1	98 (17)	105 (18)	203 (17)
2	31 (5)	32 (5)	63 (5)
3	20 (3)	26 (4)	46 (4)
≥4	43 (7)	42 (7)	85 (7)
Missing data	5 (<1)	3 (<1)	8 (<1)
No. of gadolinium-enhancing lesions			
Mean ±SD	0.9±2.5	0.9±1.9	0.9±2.2
Range	0–24	0–16	0–24
No. of T_2-hyperintense lesions — no. (%)			
<9	67 (11)	52 (9)	119 (10)
≥9	519 (88)	528 (91)	1047 (89)
Missing data	3 (<1)	2 (<1)	5 (<1)
Duration of interferon beta-1a therapy before study — mo			
Mean ±SE	33.6±0.7	35.4±0.7	34.5±0.5
Median	29.0	32.0	31.0
Range	10–88	11–99	10–99

* Percentages may not total 100, because of rounding.
† Race was determined at the time of enrollment by the treating investigator.
‡ P≤0.02 for the comparison with the group assigned to interferon beta-1a alone.
§ EDSS denotes the Expanded Disability Status Scale (possible scores range from 0 to 10, with higher scores indicating more severe disease).

worst adverse events associated with combination therapy were mild in 10 percent of the patients, moderate in 54 percent, and severe in 35 percent; the respective percentages for interferon beta-1a alone were 5 percent, 57 percent, and 37 percent. Serious adverse events were observed in 18 percent of the patients assigned to combination therapy and 21 percent of those assigned to interferon beta-1a alone (P=0.23). The most common serious adverse event was a relapse of multiple sclerosis, which occurred in 5 percent of the patients in the combination-therapy group and 9 percent of those in the interferon beta-1a group (P=0.002). One of the serious adverse events reported was PML, which occurred in a patient who had received 29 doses of natalizumab. A second patient received a diagnosis of PML after her completion of the two-year study and after she had received 37 doses of natalizumab. The details of these cases of PML have been reported previously.[26,27] Two of the patients assigned to interferon beta-1a alone died: one was a 47-year-old woman with a history of sinus arrhythmia and heart murmur, and the other was a 23-year-old woman with a history of headache, pain, and use of prescribed methadone who died during sleep.

Depression was assessed every six months with use of the Beck Depression Inventory II.[28] There were no differences between the treatment groups in Beck Depression Inventory II scores during the study (data not shown).

The incidence of infection was 83 percent in the combination-therapy group and 81 percent in the group assigned to interferon beta-1a alone; infections occurred at a rate of 1 per patient-year in each group. When the data pertaining to infection were reanalyzed to include multiple occurrences, the rate increased in each group, as expected. However, there remained no significant difference between the groups, with infection rates of 1.54 per patient-year with combination therapy and 1.53 per patient-year with interferon

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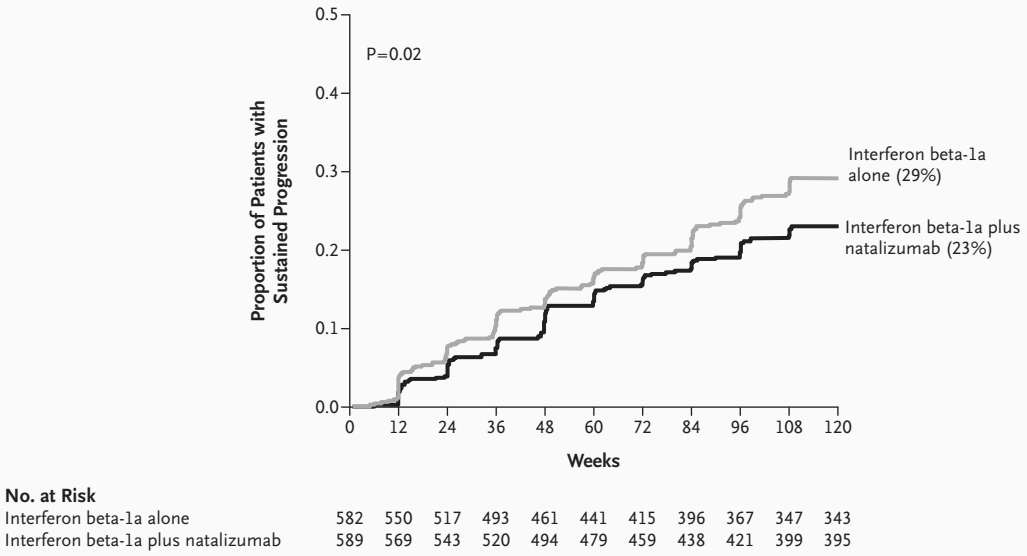

Figure 2. Kaplan–Meier Plots of the Time to Sustained Disability Progression.

The hazard ratio for sustained progression in the combination-therapy group as compared with the group given interferon beta-1a alone was 0.76 (95 percent confidence interval, 0.61 to 0.96).

beta-1a alone (P=0.95). Common infections were nasopharyngitis (39 percent vs. 35 percent); urinary tract infection, not otherwise specified (18 percent vs. 19 percent); sinusitis, not otherwise specified (18 percent vs. 15 percent); upper respiratory tract infection, not otherwise specified (17 percent vs. 18 percent); and influenza (17 percent vs. 15 percent). Serious infections occurred in 2.7 percent and 2.9 percent of the patients assigned to combination therapy and interferon beta-1a alone, respectively. There were no cases of tuberculosis. The incidence of cancer was 1 percent in the combination-therapy group and 2 percent in the group assigned to interferon beta-1a alone.

Infusion reactions, defined as any event occurring within two hours after the start of an infusion, occurred in 24 percent of the patients in the combination-therapy group and 20 percent of those in the group assigned to interferon beta-1a alone (P=0.11). The most common infusion reaction was headache. Most reactions were treated symptomatically and did not result in discontinuation of the study drug. Hypersensitivity reactions included all events reported on the basis of clinical judgment as hypersensitivity, an allergic reaction, an anaphylactic or anaphylactoid reaction, urticaria, or hives by the investigator and were categorized according to severity. Eleven patients assigned to combination therapy (1.9 percent) had a hypersensitivity reaction; 8 of the 11 hypersensitivity reactions were isolated cases of urticaria (2 of which were severe). In addition, two patients assigned to interferon beta-1a alone (0.3 percent) had hypersensitivity reactions associated with the infusion of placebo; both were cases of mild urticaria. There was no cardiopulmonary compromise associated with any event. Natalizumab was discontinued, and the episodes resolved without sequelae.

Eight percent of the patients in the combination-therapy group and 7 percent of those in the group assigned to interferon beta-1a alone discontinued the study drug because of an adverse event. Three percent and 2 percent, respectively, withdrew from the study because of an adverse event.

Natalizumab-treated patients had increases in lymphocytes, monocytes, eosinophils, and basophils — changes consistent with the drug's known pharmacodynamic effects and the presence of α_4 integrins on these cell types. Increases in nucleated red cells also were seen transiently in a small number of patients. These laboratory changes were not associated with any clinical manifestations and were reversible, with values returning to baseline within 16 weeks after the last dose. Elevations in neutrophils were not observed. No

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Table 2. Clinical and Magnetic Resonance Imaging End Points.*

End Point	1 Yr			2 Yr		
	Interferon Beta-1a plus Natalizumab (N=589)	Interferon Beta-1a Alone (N=582)	P Value	Interferon Beta-1a plus Natalizumab (N=589)	Interferon Beta-1a Alone (N=582)	P Value
Clinical						
Primary end point at 2 yr: cumulative probability of sustained disability progression — %†	—	—		23	29	0.02‡
Primary end point at 1 yr: annualized relapse rate — mean (95% CI)§						
Preplanned interim analysis (after 1200 patient-yr)	0.38 (0.33–0.45)	0.82 (0.72–0.92)	<0.001			
Final analysis	0.38 (0.32–0.45)	0.81 (0.72–0.92)	<0.001	0.34 (0.29–0.39)	0.75 (0.67–0.84)	0.001
No. of relapses — no. of patients (%)						
0	424 (72)	296 (51)		359 (61)	217 (37)	
1	132 (22)	183 (31)		158 (27)	164 (28)	
2	30 (5)	77 (13)		41 (7)	105 (18)	
≥3	3 (<1)	26 (4)		31 (5)	96 (16)	
Sensitivity analysis¶						
Adjusted annualized rate of relapse	—	—		0.34 (0.29–0.39)	0.75 (0.67–0.84)	0.001
Unadjusted annualized rate of relapse	—	—		0.31	0.70	0.001
Per-subject mean relapse rate‖	—	—		0.33	0.75	0.001
Magnetic resonance imaging						
No. of new or enlarging T_2-hyperintense lesions — no. of patients (%)			<0.001			0.001
0	422 (72)	248 (43)		394 (67)	176 (30)	
1	108 (18)	114 (20)		76 (13)	55 (9)	
2	32 (5)	66 (11)		39 (7)	59 (10)	
≥3	27 (5)	154 (26)		80 (14)	292 (50)	
No. of new or enlarging T_2-hyperintense lesions						
Mean ±SD	0.5±1.2	2.4±4.1		0.9±2.1	5.4±8.7	
Median	0	1.0		0	3	
Minimum, maximum	0, 14	0, 28		0, 27	0, 64	
No. of gadolinium-enhancing lesions — no. of patients (%)			<0.001			0.001
0	563 (96)	436 (75)		568 (96)	435 (75)	
1	19 (3)	73 (13)		13 (2)	67 (12)	
2	3 (<1)	28 (5)		4 (<1)	33 (6)	
≥3	4 (<1)	45 (8)		4 (<1)	47 (8)	
No. of gadolinium-enhancing lesions						
Mean ±SD	0.1±0.4	0.8±2.5		0.1±0.6	0.9±3.2	
Median	0	0		0	0	
Minimum, maximum	0, 4	0, 43		0, 12	0, 43	

* P values are for the comparison between the combination-therapy group and the group assigned to interferon beta-1a alone. CI denotes confidence interval.
† Sustained disability progression was defined as an increase by at least 1.0 point in the Expanded Disability Status Scale (EDSS) score from a baseline score of at least 1.0 or an increase by at least 1.5 points in the EDSS score from a baseline score of 0, sustained for 12 weeks. Progression could not be confirmed during a relapse.
‡ The hazard ratio for sustained disability progression in the combination-therapy group as compared with the group assigned to interferon beta-1a alone was 0.76 (95 percent confidence interval, 0.61 to 0.96).
§ Data pertaining to relapses that occurred after sustained progression was reached and rescue treatment was initiated (per protocol) were censored.
¶ The sensitivity analysis included relapses that occurred after sustained progression was reached and rescue treatment was initiated (per protocol).
‖ The per-subject mean relapse rate is the number of relapses for each patient divided by the total number of years of follow-up in the study for that patient.

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Table 3. Adverse Events.*

Event	Interferon Beta-1a plus Natalizumab (N=589)	Interferon Beta-1a Alone (N=582)
	percent of patients	
Adverse events†		
Headache	46	44
Nasopharyngitis	39	35
Pain in arms or legs	22	21
Depression	21	18
Influenza-like illness	20	19
Diarrhea, NOS	19	16
Insomnia	18	17
Sinusitis, NOS	18	15
Influenza	17	15
Nausea	17	15
Myalgia	13	10
Anxiety	12‡	8
Cough	11	9
Viral upper respiratory tract infection, NOS	8	7
Pharyngitis	7§	4
Vomiting, NOS	7	5
Muscle cramp	6	5
Abdominal pain, NOS	6	5
Sinus congestion	6‡	3
Seasonal allergy	6	4
Peripheral edema	5¶	1
Tremor	5	3
Sinus headache	5	3

Event	Interferon Beta-1a plus Natalizumab (N=589)	Interferon Beta-1a Alone (N=582)
	percent of patients	
Serious adverse events‖		
Relapse of multiple sclerosis	5‡	9
Appendicitis	<1	<1
Abdominal pain, NOS	<1	0
Basal-cell carcinoma	<1	<1
Ovarian cyst	<1	0
Chest pain	<1	<1
Cholelithiasis	<1	<1
Depression	<1	<1
Intervertebral disk herniation	<1	<1
Multiple sclerosis	<1	<1
Perforating appendicitis	<1	0
Colitis, NOS	<1	0
Dehydration	<1	0
Fall	<1	0
Abnormal liver-function values	<1	<1
Traffic accident	<1	0
Urinary tract infection, NOS	<1	<1
Uterine fibroids	<1	<1
Viral infection, NOS	<1	0
PML**	<1	0

* NOS denotes not otherwise specified, and PML progressive multifocal leukoencephalopathy.
† The adverse events listed are those that occurred at an incidence of at least 5 percent in the combination-therapy group and at an incidence of at least 1 percent in the combination-therapy group as compared with the group assigned to interferon beta-1a alone.
‡ P≤0.01 for the comparison with the group assigned to interferon beta-1a alone.
§ P≤0.05 for the comparison with the group assigned to interferon beta-1a alone.
¶ P≤0.001 for the comparison with the group assigned to interferon beta-1a alone.
‖ Serious adverse events were those that occurred in at least two patients (0.01 percent) in the combination-therapy group.
** Although there was only one case of PML during the Safety and Efficacy of Natalizumab in Combination with Interferon Beta-1a in Patients with Relapsing Remitting Multiple Sclerosis study, it is included in this table because of its importance and severity; a second, fatal case of PML was identified after the end of the two-year study.

increase in the incidence of chemical abnormalities, including the results of liver-function tests, was observed with combination therapy.

IMMUNOGENICITY

Seventy patients (12 percent of the combination-therapy group) had antibodies to natalizumab. Persistent antinatalizumab antibodies (detectable on at least two occasions 42 or more days apart) developed in 38 patients (6 percent), resulting in a loss of efficacy and an increase in infusion-related adverse events. The incidence of new neutralizing antibodies to interferon beta-1a was 1 percent among patients assigned to combination therapy and less than 1 percent among those assigned to interferon beta-1a alone.

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DISCUSSION

Phase 3 trials have shown that over a two-year period, 62 to 75 percent of patients have clinical relapses while receiving interferon beta therapy.[5,6,8] For patients who have breakthrough disease while receiving disease-modifying therapies, clinical practice includes the addition of a second partially effective agent; however, there is no class I evidence to support this treatment strategy. The primary objective of SENTINEL was to address this common clinical scenario — specifically, to determine whether the addition of natalizumab to interferon beta-1a would reduce breakthrough disease activity in patients already receiving interferon beta-1a therapy. This approach has been used effectively in the development of combination therapy for rheumatoid arthritis.[29-32]

The addition of natalizumab to interferon beta-1a reduced the risk of disability progression by 24 percent over a two-year period as compared with interferon beta-1a alone (P=0.02). The sensitivity analysis of disability sustained for 24 weeks did not reach statistical significance (P=0.17); however, that analysis was exploratory, and the study was not adequately powered to assess the treatment effect on the basis of this definition. We also found that combination therapy reduced the annualized rate of relapse by 55 percent over a two-year period as compared with interferon beta-1a alone (P<0.001).

Accumulation of T_2-hyperintense MRI lesions has been linked to future progression of brain atrophy[33] and long-term disability[34,35] in relapsing multiple sclerosis. The number of new or enlarging T_2-hyperintense lesions in patients receiving interferon beta-1a alone was similar to the findings of another study of interferon beta-1a in relapsing multiple sclerosis that used the same imaging methods.[36] The addition of natalizumab to interferon beta-1a further reduced the number of new or enlarging T_2-hyperintense lesions by 83 percent, and approximately two thirds of the patients assigned to combination therapy remained free of new lesions for two years.

Natalizumab interferes with the activity of α_4 integrin, altering cell migration into the central nervous system and possibly blocking interactions between α_4 integrin and its ligands within the central nervous system itself.[1-4] Interferon beta has pleiotropic effects on cellular functions that are relevant to efficacy in multiple sclerosis and distinct from those of natalizumab.[11-14] In addition, studies have shown that, like natalizumab, interferon beta may prevent leukocyte migration across the blood–brain barrier by altering the expression of adhesion molecules.[15-17] The additional efficacy of the combination over that conferred by interferon beta alone suggests that the interaction between $\alpha_4\beta_1$ integrin and its targets is a key mediator of inflammation and subsequent demyelination in multiple sclerosis.

In February 2005, administration of natalizumab was suspended when two cases of PML were identified. In one of the cases, PML was diagnosed during SENTINEL, and in the other it was diagnosed after the patient had completed SENTINEL and had begun participating in an open-label safety study of natalizumab and interferon beta-1a. Later, an additional case of PML was identified post mortem in a patient with Crohn's disease who had previously received a diagnosis of astrocytoma. Details of these three cases have recently been published.[26,27,37] An extensive safety evaluation of patients in clinical trials who were receiving natalizumab at the time of the drug suspension did not identify additional cases of PML (see the article by Yousry et al. in this issue of the *Journal*[38]). The mechanisms by which natalizumab may increase the risk of PML are unknown, but they may involve altered trafficking of lymphoid cells harboring latent JC virus, decreased immune surveillance, or a combination of these processes.[39] The role of interferon beta in combination with natalizumab is also not clear, given that PML has never been associated with interferon beta alone.

SENTINEL was designed to determine whether natalizumab added to interferon beta-1a is better than interferon beta-1a alone. The results of all prespecified analyses of primary and secondary end points were positive and statistically significant. A natalizumab-monotherapy group was not included in the trial because this design would have required withdrawal of an approved therapy in order to switch to an experimental one at a time (in 2001) when the long-term safety and efficacy of natalizumab were unknown. This approach was believed to be unacceptable by the investigator advisory committee. Hence, additional studies would be required to determine whether combination therapy with natalizumab

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and interferon beta-1a is more efficacious than natalizumab alone and to define further the role of natalizumab combination therapy in clinical practice. The results of another trial of natalizumab, administered without interferon beta-1a, also appear in this issue of the *Journal*.[40]

SENTINEL systematically evaluated combination therapy as compared with standard interferon beta therapy in relapsing multiple sclerosis. The study showed that in patients with multiple sclerosis who have breakthrough disease during interferon beta treatment, combination therapy has significant benefits when compared with interferon beta-1a alone. Additional studies will be required for further assessment of the long-term safety of combination therapy with natalizumab and for assessment of its efficacy relative to that of natalizumab alone.

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