ORIGINAL ARTICLE

B-Cell Depletion with Rituximab in Relapsing–Remitting Multiple Sclerosis

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ABSTRACT

BACKGROUND

There is increasing evidence that B lymphocytes are involved in the pathogenesis of multiple sclerosis, and they may be a therapeutic target. Rituximab, a monoclonal antibody, selectively targets and depletes CD20+ B lymphocytes.

METHODS

In a phase 2, double-blind, 48-week trial involving 104 patients with relapsing–remitting multiple sclerosis, we assigned 69 patients to receive 1000 mg of intravenous rituximab and 35 patients to receive placebo on days 1 and 15. The primary end point was the total count of gadolinium-enhancing lesions detected on magnetic resonance imaging scans of the brain at weeks 12, 16, 20, and 24. Clinical outcomes included safety, the proportion of patients who had relapses, and the annualized rate of relapse.

RESULTS

As compared with patients who received placebo, patients who received rituximab had reduced counts of total gadolinium-enhancing lesions at weeks 12, 16, 20, and 24 ($P<0.001$) and of total new gadolinium-enhancing lesions over the same period ($P<0.001$); these results were sustained for 48 weeks ($P<0.001$). As compared with patients in the placebo group, the proportion of patients in the rituximab group with relapses was significantly reduced at week 24 (14.5% vs. 34.3%, $P=0.02$) and week 48 (20.3% vs. 40.0%, $P=0.04$). More patients in the rituximab group than in the placebo group had adverse events within 24 hours after the first infusion, most of which were mild-to-moderate events; after the second infusion, the numbers of events were similar in the two groups.

CONCLUSIONS

A single course of rituximab reduced inflammatory brain lesions and clinical relapses for 48 weeks. This trial was not designed to assess long-term safety or to detect uncommon adverse events. The data provide evidence of B-cell involvement in the pathophysiology of relapsing–remitting multiple sclerosis. (ClinicalTrials.gov number, NCT00097188.)

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Multiple sclerosis, the prototypical inflammatory demyelinating disease of the central nervous system, is second only to trauma as a cause of acquired neurologic disability in young adults.[1] Multiple sclerosis usually begins as a relapsing, episodic disorder (relapsing–remitting multiple sclerosis), evolving into a chronic neurodegenerative condition characterized by progressive neurologic disability.[2]

The traditional view of the pathophysiology of multiple sclerosis has held that inflammation is principally mediated by CD4+ type 1 helper T cells. Therapies (e.g., interferon beta and glatiramer acetate) developed on the basis of this theory decrease the relapse rate by approximately one third[3,4] but do not fully prevent the occurrence of exacerbations or accumulation of disabilities, and they are largely ineffective against purely progressive forms of multiple sclerosis.[5] The α_4 integrin inhibitor natalizumab, a monoclonal antibody that disrupts lymphocyte movement into the central nervous system, is effective in preventing relapses and also reduces the risk of sustained progression of disability in relapsing–remitting multiple sclerosis.[6] The prevention of focal inflammatory lesions early in the disease may delay the development of progressive multiple sclerosis[7]; this underscores the importance of developing more effective therapies for relapsing–remitting multiple sclerosis.

In contrast to earlier concepts of disease suggesting that pathogenic T cells are sufficient for full expression of multiple sclerosis, it is now evident that autoimmune B cells and humoral immune mechanisms also play key roles.[8] The existence of a humoral component in multiple sclerosis has been implicitly recognized for decades, evidenced by the inclusion of cerebrospinal fluid oligoclonal bands and increased intrathecal IgG synthesis in the diagnostic criteria for multiple sclerosis.[9-11] The deposition of antibody and activation of complement associated with vesicular disintegration of the myelin membrane are present in most lesions in multiple sclerosis,[12-14] and autoantibodies directed against diverse antigens can also be detected in the cerebrospinal fluid of many patients with multiple sclerosis.[15] Memory B cells, which cross the blood–brain barrier, are believed to undergo restimulation, antigen-driven affinity maturation, clonal expansion, and differentiation into antibody-secreting plasma cells within the highly supportive central nervous system environment.[16] Clonally expanded populations of memory B cells

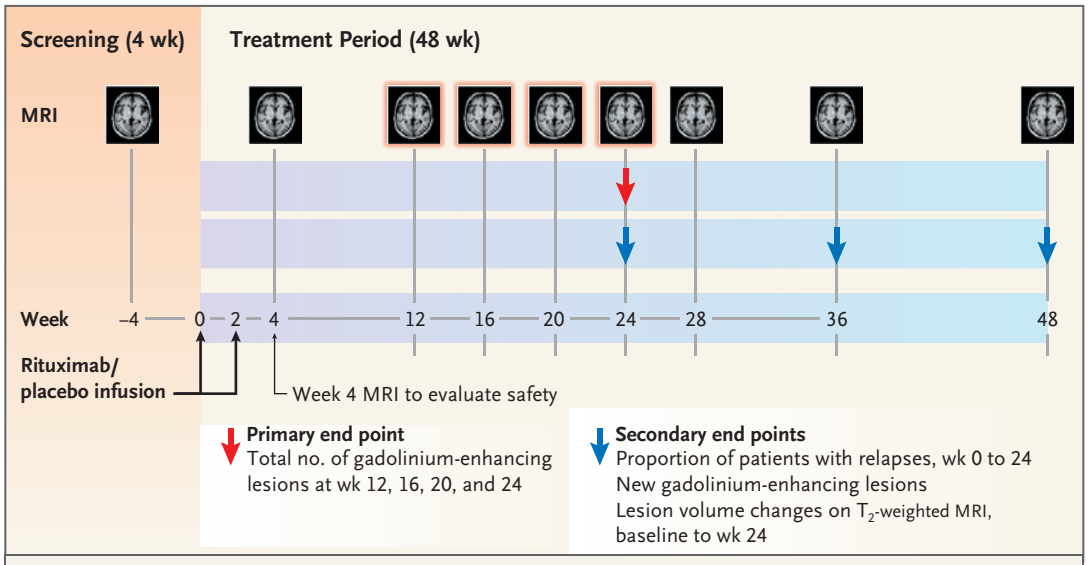

Figure 1. Study Design.

Patients were randomly assigned in a 2:1 ratio to receive rituximab or placebo. They were hierarchically stratified according to study site, status with respect to previous treatment with interferon beta or glatiramer acetate (either no treatment or discontinuation of medication >6 months previously vs. treatment within the previous 6 months), and baseline disease severity according to the Expanded Disability Status Scale (EDSS) score (≤2.5 vs. >2.5). The EDSS is an ordinal scale ranging from 0 (normal neurologic examination) to 10.0 (death) in 0.5-step intervals.

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Table 1. Baseline Characteristics of the Patients.*

Variable	Placebo (N=35)	Rituximab (N=69)
Age — yr	41.5±8.5	39.6±8.7
Sex — no. (%)		
Female	29 (82.9)	52 (75.4)
Male	6 (17.1)	17 (24.6)
Disease duration — yr		
Since onset	9.6±7.1	9.6±6.4
Since diagnosis	6.9±6.2	6.2±5.2
Relapses in past year		
Relapses — no. of patients (%)		
0 relapses	2 (5.7)†	4 (5.8)†
1 relapse	27 (77.1)	52 (75.4)
2 relapses	5 (14.3)	8 (11.6)
3 relapses	0	4 (5.8)
≥4 relapses	1 (2.9)	1 (1.4)
Median (range)	1.0 (0–5)	1.0 (0–4)
EDSS score		
Score — no. of patients (%)		
0	1 (2.9)	2 (2.9)
1.0–1.5	8 (22.9)	9 (13.0)
2.0–2.5	10 (28.6)	24 (34.8)
3.0–3.5	7 (20.0)	20 (29.0)
4.0–4.5	7 (20.0)	11 (15.9)
5.0	2 (5.7)	3 (4.3)
Median (range)	2.5 (0–5)	2.5 (0–5)
Previous treatment with interferon beta or glatiramer acetate — no. of patients (%)		
None or discontinued >6 mo before study entry	21 (60.0)	44 (63.8)
Treatment within 6 mo before study entry	14 (40.0)	25 (36.2)
Any key therapy for MS in previous 2 yr — no. of patients (%)‡		
Glatiramer acetate	8 (22.9)	18 (26.1)
Interferon beta-1a	16 (45.7)	24 (34.8)
Interferon beta-1b	5 (14.3)	13 (18.8)
Methylprednisolone or methylprednisolone sodium succinate	8 (22.9)	19 (27.5)
Natalizumab	2 (5.7)	5 (7.2)
Any key therapy for MS — no. of patients (%)	27 (77.1)	54 (78.3)
Gadolinium-enhancing lesions		
No. of lesions — no. of patients (%)		
0 lesions§	30 (85.7)	44 (63.8)
1 lesion	2 (5.7)	7 (10.1)
2 lesions	2 (5.7)	4 (5.8)
3 lesions	0	2 (2.9)
≥4 lesions	1 (2.9)	11 (15.9)
Mean no. of lesions	0.3±0.8	2.1±5.6
Median no. of lesions (range)	0 (0–4)	0 (0–36)

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Table 1. (Continued.)

Variable	Placebo (N=35)	Rituximab (N=69)
Volume of lesions detected on MRI — mm^3		
On gadolinium-enhanced MRI		
Mean	29.2±127.5	211.6±702.2
Median	0	0
On T_2-weighted MRI		
Mean	5723.1±5514.8	6452.2±8022.2
Median	4032.0	2878.5
On T_1-weighted MRI		
Mean	717.6±1025.0	784.2±1206.4
Median	369.0	211.0

* Plus–minus values are means ±SD. EDSS denotes Expanded Disability Status Scale (range of scores, 0 to 10.0, with higher scores indicating more severe disease), MRI magnetic resonance imaging, and MS multiple sclerosis.
† These patients had relapses more than 1 year before study entry.
‡ Any key therapy for multiple sclerosis was defined as natalizumab, interferon beta, interferon beta-1a, interferon beta-1b, glatiramer acetate, azathioprine, immune globulin, methylprednisolone, methylprednisolone sodium succinate, or more than 20 mg of prednisone.
§ The proportion of patients without gadolinium-enhancing lesions was greater in the placebo group than in the rituximab group (85.7% vs. 63.8%, P=0.02); P values were calculated with the use of the chi-square test.

and plasma cells are found in lesions and cerebrospinal fluid from patients with multiple sclerosis,[17-21] and they can be detected even at the onset of clinical symptoms of the disease.[22] Abnormalities in B-cell cytokine responses have also been reported in patients with multiple sclerosis.[23]

Rituximab (Rituxan, Genentech and Biogen Idec) is a genetically engineered chimeric monoclonal antibody that depletes CD20+ B cells through a combination of cell-mediated and complement-dependent cytotoxic effects and the promotion of apoptosis.[24-26] B-cell depletion affects antibody production, cytokine networks, and B-cell–mediated antigen presentation and activation of T cells and macrophages.[27] On the basis of the effects of rituximab and the known immunopathology of multiple sclerosis, we performed a phase 2 trial of the agent in patients with relapsing–remitting multiple sclerosis.

METHODS

STUDY DESIGN AND RANDOMIZATION

The trial was a randomized, double-blind, placebo-controlled study conducted at 32 centers in the United States and Canada. The protocol was approved by the institutional review board and the ethics committee of each institution. Written informed consent was obtained from each patient or the patient's legal guardian. Patients were randomly assigned in a 2:1 ratio to receive rituximab or placebo (Fig. 1), and they were hierarchically stratified according to study site, status with respect to previous treatment with interferon beta or glatiramer acetate (either no treatment or discontinuation of medication ≥6 months previously vs. treatment within the previous 6 months), and baseline disease severity according to the Expanded Disability Status Scale (EDSS) score (≤2.5 vs. >2.5). The EDSS is an ordinal scale ranging from 0 (normal neurologic examination) to 10.0 (death) in 0.5-step intervals.[28] Patients received 1000-mg intravenous infusions of rituximab or placebo on study days 1 and 15 (Fig. 1).

The study was designed jointly by Genentech and the investigators. Data were collected by the investigators and held and analyzed by Genentech. All members of the publication committee had full access to the data. All the authors vouch for the veracity and completeness of the data and data analysis.

PATIENTS

Enrollment was limited to patients 18 to 55 years of age with a diagnosis of relapsing–remitting multiple sclerosis,[29] at least one relapse during

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the preceding year, and an entry score of 0 to 5.0 on the EDSS. Exclusion criteria included disease categorized as secondary progressive, primary progressive, or progressive relapsing disease; relapse within 30 days; cyclophosphamide or mitoxantrone treatment within 12 months; systemic corticosteroid therapy within 30 days; treatment with interferon beta, glatiramer acetate, natalizumab, plasmapheresis, or intravenous immune globulin within 60 days; or non–lymphocyte-depleting immunosuppressive therapies within 90 days.

STUDY PROCEDURES AND END POINTS

To prevent potential breaks in blinding because of observed efficacy, adverse events, or changes in laboratory values, each site had both a treating investigator and an examining investigator. The treating investigator was the safety assessor and made all treatment decisions based on the patient's clinical response and laboratory findings. The examining investigator was the efficacy assessor, who administered the EDSS and Multiple Sclerosis Functional Composite Scale with access only to those data. Staff members from a central magnetic resonance imaging (MRI) reading center (NeuroRx, Montreal) who were unaware of the data evaluated all scans. Each site was instructed not to obtain MRI scans within 30 days after the last dose of corticosteroids prescribed for relapse, except for safety reasons.

At regularly scheduled visits over a period of 48 weeks, neurologic and physical examinations, MRI, and routine laboratory tests were performed and adverse events were recorded. After week 48, patients who remained peripherally B-cell–depleted continued in safety follow-up until their B-cell counts returned to the lower limit of the normal range or the baseline value, whichever was lower. Brain MRI scans with and without the administration of gadolinium were obtained at baseline and at weeks 4, 12, 16, 20, 24, 28, 36, and 48. Patients were evaluated for relapses at unscheduled visits if a clinically significant change in their condition occurred. Relapse was defined as new or recurrent neurologic symptoms that were consistent with multiple sclerosis, lasted for at least 48 hours, and were preceded by a relatively stable or improving neurologic state for at least 30 days. The treating investigator could treat relapses with systemic corticosteroids. In addition to routine laboratory tests, levels of CD19+ B cells, immunoglobulins (IgG, IgA, and IgM), and human antichimeric antibodies were measured. Because rituximab interferes with flow-cytometric analysis of CD20, CD19, which has a similar expression profile, was used as a surrogate marker. The Common Toxicity Criteria, version 3.0,[30] were used to grade adverse events.

On days 1 and 15, acetaminophen (at a dose of 1 g) and diphenhydramine hydrochloride (at a dose of 50 mg) were administered orally 30 to 60 minutes before each infusion. Infusion-related reactions were to be treated with acetaminophen (paracetamol) plus intramuscular or slow intravenous administration of an antihistamine (diphenhydramine hydrochloride), a bronchodilator, or both, if indicated. If a severe infusion-related reaction occurred, the infusion was to be immediately interrupted, and symptomatic treatment initiated.

The primary efficacy end point was the sum of the number of gadolinium-enhancing lesions on serial T_1-weighted MRI brain scans at weeks 12, 16, 20, and 24. Thus, lesions that persisted for more than 4 weeks were counted more than once. Key secondary, exploratory efficacy outcome measures were the proportion of patients with relapses; the annualized rate of relapse; the total number of new gadolinium-enhancing lesions observed on serial T_1-weighted MRI brain scans at weeks 12, 16, 20, and 24 (i.e., lesions persisting for more than 4 weeks were counted only once); and the change from the baseline lesion volume on T_2-weighted MRI scans. Because a reference scan was needed to determine whether a lesion was new, there was no count of new gadolinium-enhancing lesions for the baseline scan.

STATISTICAL ANALYSIS

Eight new gadolinium-enhancing lesions detected on four T_1-weighted MRI scans between weeks 12 and 24 were expected in patients in the placebo group, and it was assumed that the data would follow a negative binomial distribution, resulting in an expected standard deviation of 11.7 for the placebo group. Assuming a 60% reduction in numbers of gadolinium-enhancing lesions detected on T_1-weighted MRI in the rituximab group as compared with the placebo group, it was expected that the mean number of lesions would be 3.2 and the standard deviation would be 4.87, with the standard deviation calculated with the use of the same method. Originally, the

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Table 2. Study Sample, Reasons for Study Discontinuation, and Safety Follow-up.

Variable	Placebo (N=35)	Rituximab (N=69)	Total (N=104)
		no. of patients (%)	
Study sample			
At 24 wk	30 (85.7)	66 (95.7)	96 (92.3)
At 28 wk	26 (74.3)	65 (94.2)	91 (87.5)
At 36 wk	26 (74.3)	63 (91.3)	89 (85.6)
At 40 wk	24 (68.6)	60 (87.0)	84 (80.8)
At 48 wk (completion of study)	21 (60.0)	58 (84.1)	79 (76.0)
Discontinuation before wk 48	14 (40.0)	11 (15.9)	25 (24.0)
Reason for discontinuation			
Death	0	1 (1.4)*	1 (1.0)*
Adverse events	0	1 (1.4)	1 (1.0)
Pregnancy	1 (2.9)	0	1 (1.0)
Lost to follow-up	2 (5.7)	2 (2.9)	4 (3.8)
Patient's decision	4 (11.4)	0	4 (3.8)
Physician's decision	3 (8.6)	3 (4.3)	6 (5.8)
Relapse	2 (5.7)	2 (2.9)	4 (3.8)
Initiation of excluded therapy	2 (5.7)	2 (2.9)	4 (3.8)
Safety follow-up			
Did not enter	8 (22.9)	4 (5.8)	12 (11.5)
Entered	6 (42.9)†	7 (10.1)	13 (12.5)
Completed	5 (14.3)	7 (10.1)	12 (11.5)

* This death was due to homicide.
† Six of the 14 patients who entered the safety follow-up received placebo.

trial was designed to enroll 280 patients, but before the primary end point at week 24, the sample-size target was reduced to 99 patients while the investigators were still unaware of the data. With the sample of 99 patients, the study would have 70% power at a two-sided significance level of 0.05 (PASS statistical software, version 6.0). No interim analysis was conducted.

Analyses were performed on an intention-to-treat basis. The van Elteren's test was applied to rank-transformed data to compare the study groups for the primary end point, with stratification according to baseline EDSS score (≤2.5 or >2.5), previous treatment with interferon beta or glatiramer acetate, and the absence or presence of baseline gadolinium-enhancing lesions. Missing values for gadolinium-enhancing lesions detected on T_1-weighted MRI were replaced by the average number of lesions detected on available scans obtained during the first 24 weeks. Similar analyses were performed for other lesion-count end points. The change in the total volume of lesions detected on T_2-weighted MRI brain scans from screening to week 24 and week 36 was compared between the study groups with the use of Friedman's analysis of covariance (ranked data) adjusted for the baseline volume of lesions detected on T_2-weighted MRI, study-group assignment (placebo or rituximab), baseline EDSS score, and status with respect to previous treatment with interferon beta or glatiramer acetate. The last observation was carried forward to impute missing values. The proportion of patients with relapses was analyzed with the use of a Cochran–Mantel–Haenszel chi-square test with stratification according to baseline EDSS score and status with respect to previous treatment with interferon beta or glatiramer acetate. Patients who discontinued treatment early without having a relapse were considered to be relapse-free. The annualized rate of relapse was analyzed with the use of Poisson regression, with adjustment for exposure time in years and for baseline EDSS score and status with respect to previous treatment with interferon beta or glatiramer acetate.

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Table 3. MRI and Clinical End Points.*

End Point	Placebo (N=35)	Rituximab (N=69)	P Value
MRI			
Gadolinium-enhancing lesions at wk 12, 16, 20, 24			
Total no. of lesions — no. of patients (%)			<0.001†
0 lesions	18 (51.4)	53 (80.3)	
>0–1 lesion	4 (11.4)	6 (9.1)	
>1–2 lesions	5 (14.3)	5 (7.6)	
>2–3 lesions	1 (2.9)	0	
>3 lesions	7 (20.0)	2 (3.0)	
Mean no. of lesions	5.5±15.0	0.5±2.0	
Median no. of lesions (range)	0 (0–80.3)	0 (0–14.0)	
No. of new lesions — no. of patients (%)			<0.001†
0 lesions	19 (54.3)	56 (84.8)	
>0–1 lesion	3 (8.6)	7 (10.6)	
1–2 lesions	5 (14.3)	3 (4.5)	
>2–3 lesions	1 (2.9)	0	
>3 lesions	7 (20.0)	0	
Mean no. of new lesions	4.5±12.6	0.2±0.4	
Median no. of new lesions (range)	0 (0–68.7)	0 (0–2.0)	
Changes in volume of lesions on T_2-weighted MRI from baseline — mm^3			
From baseline to wk 24			
Mean	436.3±1358.4	−163.1±1187.6	0.008‡
Median	17.0	0	
From baseline to wk 36			
Mean	417.8±1305.1	−175.4±1188.1	0.004‡
Median	123.0	−10.5	
Clinical			
Relapses at 24 wk			
Patients with relapse — no. (%)§	12 (34.3)	10 (14.5)	0.02¶
Relative risk (90% CI)‖	2.3 (1.3–4.3)	—	
Relapses between wk 0 and wk 24 — no. of patients (%)			
0 relapses	23 (65.7)	59 (85.5)	
1 relapse	11 (31.4)	9 (13.0)	
2 relapses	1 (2.9)	1 (1.4)	
≥3 relapses	0	0	
Mean no. of relapses (range)	0.37±0.55 (0–2)	0.16±0.41 (0–2)	
Relapses at 48 wk			
Patients with relapse — no. (%)‡	14 (40.0)	14 (20.3)	0.04¶
Relative risk (90% CI)‖	1.9 (1.1–3.2)	—	

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Table 3. (Continued.)

End Point	Placebo (N=35)	Rituximab (N=69)	P Value
Clinical			
Relapses between wk 0 and wk 48 — no. of patients (%)			
0 relapses	21 (60.0)	55 (79.7)	
1 relapse	11 (31.4)	8 (11.6)	
2 relapses	1 (2.9)	5 (7.2)	
≥3 relapses	2 (5.7)	1 (1.4)	
Mean no. of relapses (range)	0.54±0.82 (0–3)	0.30±0.67 (0–3)	
Annualized rate of relapse from wk 0 to wk 24			
Total no. of relapses	13	11	
Total subject-years of follow-up	15.9	31.3	
Unadjusted rate	0.8	0.4	
Adjusted rate (90% CI)**	0.8 (0.53–1.31)	0.4 (0.23–0.60)	0.04††
Mean‡‡	0.8±1.20	0.3±0.86	
Median	0	0	
Annualized rate of relapse from wk 0 to wk 48			0.08††
Total no. of relapses	19	21	
Total subject-years of follow-up	27.2	59.7	
Unadjusted rate	0.7	0.4	
Adjusted rate (90% CI)**	0.7 (0.46–1.12)	0.4 (0.24–0.57)	
Mean‡‡	0.7±1.05	0.4±0.81	
Median	0	0	

* Plus–minus values are means ±SD. Numbers of gadolinium-enhancing lesions were not whole numbers because of the inclusion of imputed data. Data on MRI findings were available for 66 patients in the rituximab group.
† The P value is based on van Elteren's test stratified according to the baseline Expanded Disability Status Scale (EDSS) score (range of scores, 0 to 10.0, with higher scores indicating more severe disease), status with respect to previous treatment, and baseline gadolinium-enhancing lesions in 100 patients.
‡ The P value is based on Friedman's analysis of covariance (ranked data), adjusted for the baseline total volume of lesions detected on T_2-weighted MRI and stratified according to the baseline EDSS score (≤2.5 or >2.5) and status with respect to previous treatment with interferon beta or glatiramer acetate; all tests were two-sided.
§ The patients who discontinued treatment before weeks 24 and 48 were considered to be relapse-free if they did not have any relapse during the study period.
¶ The P value is based on the Cochran–Mantel–Haenszel chi-square test stratified according to the baseline EDSS score (≤2.5 or >2.5) and status with respect to previous treatment with interferon beta or glatiramer acetate.
‖ The logit-estimate of the relative risk of relapse was adjusted according to the baseline EDSS score (≤2.5 or >2.5) and status with respect to previous treatment with interferon beta or glatiramer acetate.
** 90% CIs are based on the logit-adjusted method.
†† The P value is based on Poisson regression adjusted for the baseline EDSS score (≤2.5 or >2.5) and status with respect to previous treatment with interferon beta or glatiramer acetate.
‡‡ The mean annualized relapse rate per patient is the number of relapses for each patient divided by the total number of years of follow-up.

RESULTS

STUDY POPULATION

A total of 104 patients were enrolled in the study; 69 were randomly assigned to receive rituximab and 35 to receive placebo. Baseline characteristics were generally balanced between the two groups (Table 1). However, at baseline the proportion of patients without gadolinium-enhancing lesions was greater in the placebo group than in the rituximab group (85.7% vs. 63.8%, P=0.02). Of the 104 patients, 96 (92.3%) completed 24 weeks (Table 2), and 79 patients (76.0%) completed 48 weeks (84.1% in the rituximab group and 60.0% in the

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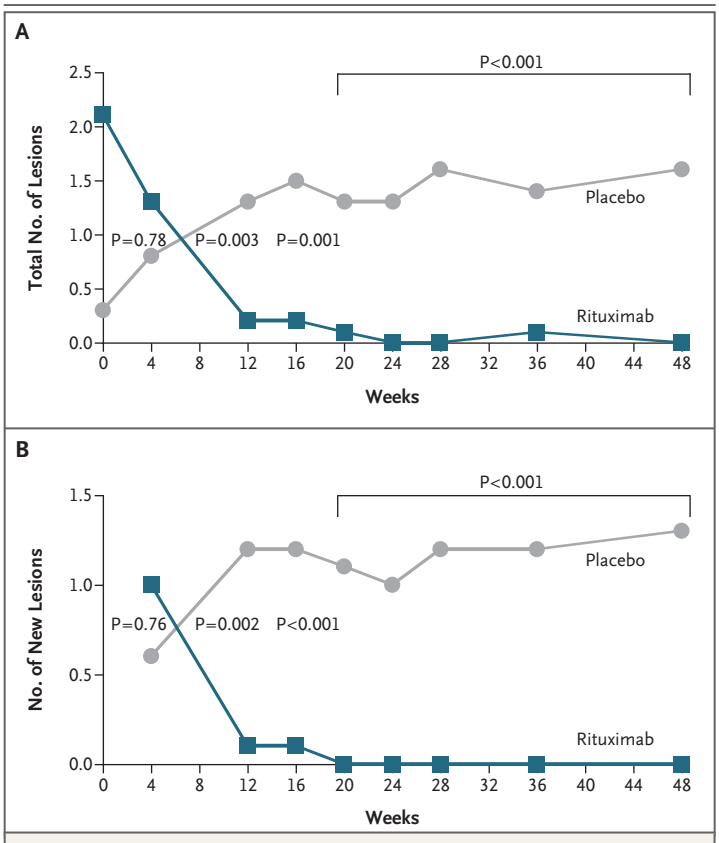

Figure 2. Gadolinium-Enhancing Lesions in Each Study Group from Baseline to Week 48.

Panel A shows the mean total number of gadolinium-enhancing lesions by week, and Panel B shows the mean number of new gadolinium-enhancing lesions by week. Missing values were imputed by averaging the available data. The baseline MRI was obtained at week −4.

placebo group). Twelve of the 25 patients who discontinued the study treatment early completed the safety follow-up (10.1% of patients in the rituximab group and 14.3% of patients in the placebo group).

EFFICACY

Primary End Point

Patients who received rituximab had a reduction in total gadolinium-enhancing lesion counts at weeks 12, 16, 20, and 24 as compared with patients who received placebo (P<0.001) (Table 3). Patients receiving rituximab had a mean of 0.5 gadolinium-enhancing lesion, as compared with 5.5 lesions in patients receiving placebo, a relative reduction of 91%. Beginning at week 12, as compared with placebo, rituximab reduced gadolinium-enhancing lesions at each study week (P=0.003 to P<0.001) (Fig. 2A).

Key Secondary End Points

The proportion of patients with relapses was reduced in the rituximab group at week 24 (14.5% vs. 34.3% in the placebo group; P=0.02) and week 48 (20.3% vs. 40.0%, P=0.04). Patients in the placebo group were more likely to have had a relapse at week 24 (relative risk, 2.3; 90% confidence interval [CI], 1.3 to 4.3) and at week 48 (relative risk, 1.9; 90% CI, 1.1 to 3.2) (Table 3). Patients in the rituximab group, as compared with those in the placebo group, had a lower annualized rate of relapse at 24 weeks (0.37 vs. 0.84, P=0.04) but not at 48 weeks (0.37 vs. 0.72, P=0.08).

Rituximab reduced new gadolinium-enhancing lesions at weeks 12, 16, 20, and 24, as compared with placebo (P<0.001) (Table 3 and Fig. 2B). The reduction in the volume of lesions detected on T_2-weighted MRI from baseline to week 24 and from baseline to week 36 was greater in patients who received rituximab than in patients who received placebo (P=0.008 and P=0.004, respectively).

PHARMACODYNAMICS AND IMMUNOGENICITY

Treatment with rituximab was associated with rapid and near-complete depletion (>95% reduction from baseline) of CD19+ peripheral B lymphocytes from 2 weeks after treatment until 24 weeks; by week 48, CD19+ cells had returned to 30.7% of baseline values. CD19+ peripheral B lymphocytes remained relatively stable in the placebo group for 48 weeks. CD3+ T lymphocytes were not appreciably altered by rituximab.

Immunoglobulin levels, assessed at baseline, at 24 and 48 weeks, and at the time of relapse, if one occurred, were below the lower limit of the normal range in 7.9% of patients who received rituximab and 3.0% of patients who received placebo. IgM levels were below the lower limit of the normal range in 22.4% of patients who received rituximab and 8.6% of patients who received placebo. Median IgM, IgG, and IgA levels remained above the lower limit of the normal range in both groups throughout the trial.

At screening and week 24, no patients in the rituximab group tested positive for human antichimeric antibodies to rituximab. At week 48, 14 of 58 patients who completed the study treatment (24.1%) tested positive for human antichimeric antibodies; no patient in the placebo group tested positive at any time (Table 4). Of seven patients in the rituximab group who discontin-

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ued the treatment early, entered the safety follow-up period, and underwent assessment for human antichimeric antibodies, two were positive for human antichimeric antibodies. Thus, 16 of 65 patients (24.6%) were positive for human antichimeric antibodies. There was no apparent association between positivity for human antichimeric antibodies and the type or severity of adverse events or the efficacy response at week 24, 36, or 48.

SAFETY

More patients in the rituximab group (78.3%) than in the placebo group (40.0%) had infusion-associated adverse events within 24 hours after the first infusion. Within 24 hours after the second infusion, fewer patients in the rituximab group (20.3%) than in the placebo group (40.0%) had adverse events (Table 4). A total of 50 of the 54 patients in the rituximab group (92.6%) reported infusion-associated adverse events that were mild to moderate (grade 1 or 2) in severity. The remaining four patients (7.4%) reported grade 3 events associated with infusion; these included headache, back pain, depression, limb pain, general pain, heat sensations, pruritus, and rash. No grade 4 events associated with infusion were reported.

Grade 4 events were reported in three patients who received rituximab: one patient had an ischemic coronary-artery syndrome, one patient had a malignant thyroid neoplasm, and one patient had symptoms of acute and progressive multiple sclerosis. Serious adverse events were reported in 14.3% of patients in the placebo group and 13% of patients in the rituximab group. A total of 5.7% of patients in the placebo group and 4.3% of patients in the rituximab group withdrew from the study because of adverse events. One death, which was due to homicide, occurred in the rituximab group.

The incidence of any infection was similar in the placebo group (71.4%) and the rituximab group (69.6%). The most common infections (occurring in ≥10% of patients) in the rituximab group were nasopharyngitis, upper respiratory tract infections, urinary tract infections, and sinusitis. Urinary tract infections were more common among patients who received rituximab than among patients who received placebo (14.5% of the rituximab group vs. 8.6% of the placebo group), as was sinusitis (13.0% vs. 8.6%). No clinically significant opportunistic infections were reported. Infection-associated serious adverse events were reported in 5.7% of patients in the placebo group and 2.9% of patients in the rituximab group. Of the two serious infection-related adverse events (gastroenteritis and bronchitis) in the rituximab group, both resolved without sequelae; the last recorded values for immunoglobulin levels before these infections were above the lower limit of the normal range in both patients.

DISCUSSION

The results of this phase 2 trial provide MRI and clinical evidence that selective CD20+ B-cell depletion is a potentially effective approach in the treatment of relapsing–remitting multiple sclerosis. As compared with placebo, rituximab significantly reduced the number of gadolinium-enhancing lesions and the number of relapses.

The predominant mechanism responsible for this improvement in disease activity associated with rituximab is unknown. The reduction in inflammatory lesions occurred at the earliest time point measured — within 4 weeks after the first dose — an effect also observed in an open-label, phase 1 trial of rituximab in patients with relapsing–remitting multiple sclerosis.[31] Plasma cells are not targeted by rituximab, and total antibody levels were not significantly reduced after a single treatment course; thus, the rapid onset of action is unlikely to be explained by a reduction in pathogenic autoantibodies. A more likely explanation is that the effects of rituximab on MRI and clinical outcomes seen in this trial resulted from lysis of memory B cells located in the peripheral blood and lymphoid tissues, or perhaps in the central nervous system. Interference with antigen presentation by B cells, or with activation of T cells or macrophages by pro-inflammatory B-cell cytokines such as interferon-γ and interleukin-12, may also play a role.[32]

Treatment with rituximab led to a rapid and complete depletion of CD20+ peripheral B cells (as measured by CD19 expression). Because CD20 is not expressed on stem cells or plasma cells,[33] median immunoglobulin levels remained above the normal range throughout the trial. It remains to be seen what effects long-term B-cell depletion will have on immunoglobulin levels and the long-term risk of infection. In patients with rheumatoid arthritis, B-cell levels have not been shown to

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Table 4. Adverse Events in the Safety Population.

Events	Placebo (N=35)	Rituximab (N=69)
Any event — no. of patients (%)*	35 (100)	68 (98.6)
Grade 1	8 (22.9)	15 (21.7)
Grade 2	18 (51.4)	28 (40.6)
Grade 3	9 (25.7)	21 (30.4)
Grade 4	0	3 (4.3)
Grade 5	0	1 (1.4)
Serious adverse event†	5 (14.3)	9 (13.0)
Event leading to withdrawal from study — no. of patients (%)	2 (5.7)	3 (4.3)
Drug-related events affecting ≥10% in either group — no. of patients (%)		
Chills	0	14 (20.3)
Headache	7 (20.0)	13 (18.8)
Nausea	4 (11.4)	12 (17.4)
Pruritus	0	10 (14.5)
Pyrexia	0	9 (13.0)
Fatigue	2 (5.7)	8 (11.6)
Throat irritation	0	8 (11.6)
Pharyngolaryngeal pain	0	7 (10.1)
Diarrhea	4 (11.4)	2 (2.9)
Infusion-associated event — no. of patients (%)‡		
First infusion at wk 0	14 (40.0)	54 (78.3)
Second infusion at wk 2	14 (40.0)	14 (20.3)
Patients positive for HACA 48 wk after randomization — no./total no. (%)	0/25 (0)	16/65 (24.6)
All infection-associated events and serious adverse events — no. of patients (%)†		
Any event	25 (71.4)	48 (69.6)
Serious adverse event§	2 (5.7)	2 (2.9)
Specific infection-associated events affecting ≥10% in either group — no. of patients (%)		
Nasopharyngitis	6 (17.1)	14 (20.3)
Upper respiratory tract infection	6 (17.1)	13 (18.8)
Urinary tract infection	3 (8.6)	10 (14.5)
Sinusitis	3 (8.6)	9 (13.0)
Influenza	4 (11.4)	4 (5.8)

* Grades correspond to the Common Toxicity Criteria, version 3.0.[30]
† Serious adverse events were defined as life threatening, resulting in death, requiring prolonged inpatient hospitalization, disabling, resulting in a congenital anomaly or malignant condition, or requiring surgical intervention to prevent one of these outcomes.
‡ Infusion-associated events included any adverse event occurring during or within 24 hours after rituximab infusion in 67 patients in the rituximab group at week 2.
§ Sialoadenitis and viral infection developed in patients in the placebo group, and gastroenteritis and bronchitis developed in patients in the rituximab group. HACA denotes human antichimeric antibodies.

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correlate with clinical response.[34] In our study, human antichimeric antibodies were detected in approximately one quarter of patients in the rituximab group who were tested. We did not observe associations between human antichimeric antibodies and adverse events or efficacy responses, but larger studies are needed to evaluate these potential associations.

Events within 24 hours after infusion occurred in a higher proportion of patients in the rituximab group than in the placebo group; patients did not receive treatment with glucocorticoids before infusion. As expected, the frequency of these events was greater during the first infusion than during the second. The events included fever, chills, rigors, nausea, pruritus, asthenia, and hypotension — events that are known to be associated with cytokine-release syndrome during B-cell lysis[35] and are consistent with those reported in patients with rheumatoid arthritis treated with rituximab.[36]

In conclusion, a single course of rituximab significantly reduced both MRI and clinical evidence of inflammatory activity for 48 weeks. The magnitude of this effect and the rapidity of its onset provide support for the theory of B-cell involvement in the immunopathologic process of multiple sclerosis, and they show that B-cell depletion has the potential to decrease disease activity in patients with the relapsing form of this disease. This small 48-week trial was not designed to assess long-term safety or to detect uncommon adverse events. Opportunistic infections, such as JC virus infection resulting in progressive multifocal leukoencephalopathy, have been reported in other patient populations treated with rituximab,[37] although a cause-and-effect relationship has not been established for this complication. The degree of response observed in this study suggests that anti-CD20 agents such as rituximab may be an option for treating relapsing–remitting multiple sclerosis, provided that the observed efficacy and safety profile are sustained in larger and longer-term controlled trials.

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APPENDIX

In addition to the authors, the following investigators participated in the study: Drs. Mark Agius, Bruce Berwald, Patrick J. Cahill, Peter Calabresi, S. Mitchell Freedman, Suzanne K. Gazda, Scott L. Gold, Barry A. Hendin, Stuart Hoffman, George J. Hutton, Francois H. Jacques, Lloyd Kasper, Bhupendra O. Khatri, Stephen Kirzinger, Mariko Kita, Robert Lada, Yves Lapierre, Clyde Markowitz, Richard Mesher, Hemanth Rao, Howard Rossman, Horea G. Rus, Stuart Shafer, James M. Smith, and Michael Stein.

REFERENCES

1. Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron 2006;52:61-76.
2. Weinshenker BG, Bass B, Rice GP, et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. Brain 1989;112:133-46.
3. Jacobs LD, Cookfair DL, Rudick RA, et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. Ann Neurol 1996;39:285-94. [Erratum, Ann Neurol 1996;40:480.]
4. Johnson KP, Brooks BR, Cohen JA, et al. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. Neurology 1995;45:1268-76.
5. Wolinsky JS, Narayana PA, O'Connor P, et al. Glatiramer acetate in primary progressive multiple sclerosis: results of a multinational, multicenter, double-blind, placebo-controlled trial. Ann Neurol 2007;61:14-24.
6. Polman CH, O'Connor PW, Havrdova E, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 2006;354:899-910.
7. Trojano M, Pellegrini F, Fuiani A, et al. New natural history of interferon-beta-treated relapsing multiple sclerosis. Ann Neurol 2007;61:300-6.
8. Owens GP, Bennett JL, Gilden DH,

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Burgoon MP. The B cell response in multiple sclerosis. Neurol Res 2006;28:236-44.
9. Andersson L, Bolling M, Wirestam R, Holtås S, Ståhlberg F. Combined diffusion weighting and CSF suppression in functional MRI. NMR Biomed 2002;15:235-40.
10. Link H, Huang Y-M. Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness. J Neuroimmunol 2007;180:17-28.
11. Sidén A. Isoelectric focusing and crossed immunoelectrofocusing of CSF immunoglobulins in MS. J Neurol 1979;221:39-51.
12. Genain CP, Cannella B, Hauser SL, Raine CS. Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat Med 1999;5:170-5.
13. O'Connor KC, Appel H, Bregoli L, et al. Antibodies from inflamed central nervous system tissue recognize myelin oligodendrocyte glycoprotein. J Immunol 2005;175:1974-82.
14. O'Connor KC, Chitnis T, Griffin DE, et al. Myelin basic protein-reactive autoantibodies in the serum and cerebrospinal fluid of multiple sclerosis patients are characterized by low-affinity interactions. J Neuroimmunol 2003;136:140-8.
15. Kanter JL, Narayana S, Ho PP, et al. Lipid microarrays identify key mediators of autoimmune brain inflammation. Nat Med 2006;12:138-43.
16. Magliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 2007;130:1089-104.
17. Baranzini SE, Jeong MC, Butunoi C, Murray RS, Bernard CC, Oksenberg JR. B cell repertoire diversity and clonal expansion in multiple sclerosis brain lesions. J Immunol 1999;163:5133-44.
18. Colombo M, Dono M, Gazzola P, et al. Accumulation of clonally related B lymphocytes in the cerebrospinal fluid of multiple sclerosis patients. J Immunol 2000;164:2782-9.
19. Corcione A, Casazza S, Ferretti E, et al. Recapitulation of B cell differentiation in the central nervous system of patients with multiple sclerosis. Proc Natl Acad Sci U S A 2004;101:11064-9.
20. Owens GP, Kraus H, Burgoon MP, Smith-Jensen T, Devlin ME, Gilden DH. Restricted use of VH4 germline segments in an acute multiple sclerosis brain. Ann Neurol 1998;43:236-43.
21. Zhang Y, Da RR, Hilgenberg LG, et al. Clonal expansion of IgA-positive plasma cells and axon-reactive antibodies in MS lesions. J Neuroimmunol 2005;167:120-30.
22. Smith-Jensen T, Burgoon MP, Anthony J, Kraus H, Gilden DH, Owens GP. Comparison of immunoglobulin G heavy-chain sequences in MS and SSPE brains reveals an antigen-driven response. Neurology 2000;54:1227-32.
23. Duddy M, Niino M, Adatia F, et al. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. J Immunol 2007;178:6092-9.
24. Reff ME, Carner K, Chambers KS, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 1994;83:435-45.
25. Anderson DR, Grillo-López A, Varns C, Chambers KS, Hanna N. Targeted anticancer therapy using rituximab, a chimaeric anti-CD20 antibody (IDEC-C2B8) in the treatment of non-Hodgkin's B-cell lymphoma. Biochem Soc Trans 1997;25:705-8.
26. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nat Med 2000;6:443-6.
27. Duddy M, Bar-Or A. B-cells in multiple sclerosis. Int MS J 2006;13:84-90.
28. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an Expanded Disability Status Scale (EDSS). Neurology 1983;33:1444-52.
29. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the Diagnosis of Multiple Sclerosis. Ann Neurol 2001;50:121-7.
30. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Online instructions and guidelines. Bethesda, MD: National Cancer Institute, August 9, 2006. (Accessed January 18, 2008, at http://ctep.cancer.gov/forms/CTCAEv3.pdf.)
31. Bar-Or A, Calabresi P, Arnold D, et al. Rituximab in relapsing remitting multiple sclerosis: a 72-week open-label phase 1 trial. Ann Neurol (in press).
32. Meinl E, Krumbholz M, Hohlfeld R. B lineage cells in the inflammatory central nervous system environment: migration, maintenance, local antibody production, and therapeutic modulation. Ann Neurol 2006;59:880-92.
33. Sell S, Max EE. All about B cells. In: Sell S. Immunology, immunopathology, and immunity. 6th ed. Washington, DC: ASM Press, 2001:101.
34. Breedveld F, Agarwal S, Yin M, et al. Rituximab pharmacokinetics in patients with rheumatoid arthritis: B-cell levels do not correlate with clinical response. J Clin Pharmacol 2007;47:1119-28.
35. Winkler U, Jensen M, Manzke O, Schulz H, Diehl V, Engert A. Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8). Blood 1999;94:2217-24.
36. Cohen SB, Emery P, Greenwald MW, et al. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factory therapy: results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis Rheum 2006;54:2793-806.
37. FDA public health advisory: life-threatening brain infection in patients with systemic lupus erythematosus after Rituxan (rituximab) treatment. Rockville, MD: Food and Drug Administration, 2006. (Accessed January 18, 2008, at http://www.fda.gov/cder/drug/advisory/rituximab.htm.)

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