

Randomized, comparative study of interferon β -1a treatment regimens in MS

The EVIDENCE Trial

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Abstract—Background: Interferon β (IFN β) reduces relapses and MRI activity in relapsing-remitting MS (RRMS), with variable effects on disability. The most effective dose regimen remains controversial. **Methods:** This randomized, controlled, multicenter trial compared the efficacy and safety of IFN β -1a (Rebif®) 44 μ g subcutaneously three times weekly (tiw), and IFN β -1a (Avonex®) 30 μ g IM once weekly (qw) in 677 patients with RRMS. Assessors blinded to treatment performed neurologic and MRI evaluations. The primary endpoint was the proportion of patients who were relapse free at 24 weeks; the principal MRI endpoint was the number of active lesions per patient per scan at 24 weeks. **Results:** After 24 weeks, 74.9% (254/339) of patients receiving IFN β -1a 44 μ g tiw remained relapse free compared with 63.3% (214/338) of those given 30 μ g qw. The odds ratio for remaining relapse free was 1.9 (95% CI, 1.3 to 2.6; $p = 0.0005$) at 24 weeks and 1.5 (95% CI, 1.1 to 2.1; $p = 0.009$) at 48 weeks, favoring 44 μ g tiw. Patients receiving 44 μ g tiw had fewer active MRI lesions ($p < 0.001$ at 24 and 48 weeks) compared with those receiving 30 μ g qw. Injection-site reactions were more frequent with 44 μ g tiw (83% vs 28%, $p < 0.001$), as were asymptomatic abnormalities of liver enzymes (18% vs 9%, $p = 0.002$) and altered leukocyte counts (11% vs 5%, $p = 0.003$) compared with the 30 μ g qw dosage. Neutralizing antibodies developed in 25% of 44 μ g tiw patients and in 2% of patients receiving 30 μ g qw. **Conclusions:** IFN β -1a 44 μ g subcutaneously tiw was more effective than IFN β -1a 30 μ g IM qw on all primary and secondary outcomes investigated after 24 and 48 weeks of treatment.

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MS is characterized by multifocal, immune-mediated demyelination and associated axonal injury in the CNS.¹⁻³ Disease activity is monitored by recording the frequency and severity of relapses and by measuring progression of disability. In addition, MRI is an important objective measure of disease activity and severity, and may predict subsequent course and disability.⁴⁻⁷

Clinical and pharmacologic studies support a dose-dependent response to interferon β (IFN β) in the treatment of relapsing-remitting MS (RRMS). Pharmacodynamic measurements of IFN β activity

are greater after a single high dose than after a low dose,^{8,9} and when the same dose of drug is given three times weekly (tiw) compared with once weekly (qw).¹⁰ In clinical trials, IFN β -1b (Betaseron®, Berlex, Richmond, CA/Schering, Berlin, Germany) demonstrated greater benefit when given at a dose of 8 million international units (MIU) compared with 1.8 MIU in terms of both clinical and MRI outcome measures.^{11,12} Similarly, in a previous trial of IFN β -1a (Rebif®, Serono) 44 μ g tiw (132 μ g weekly) and 22 μ g tiw (66 μ g weekly) vs placebo in patients with RRMS, there was a significant difference in

See also pages 1480, 1482, and 1507

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MRI activity between the high- and low-dose arms of the study at 2 years.¹³ When the study period was extended to 4 years, significant dose–response effects were evident for measures of disability, relapse frequency, and MRI.¹⁴ In contrast, a clinical trial of IFN β -1a (Avonex[®], Biogen, Cambridge, MA) 30 μ g or 60 μ g qw failed to demonstrate any difference between doses with respect to disability measures, relapses, or MRI.¹⁵ In order to assess the clinical efficacy and safety of two currently available IFN β -1a dosing regimens, we undertook a large, randomized, assessor-blinded, multicenter trial of 44 μ g subcutaneously (SC) tiw compared with 30 μ g IM qw.

Patients and methods. *Patients.* In this study, 677 IFN-naïve patients with definite RRMS¹⁶ and Expanded Disability Status Scale (EDSS) scores of 0 to 5.5 were enrolled at 56 centers (15 in Europe, 5 in Canada, and 36 in the United States). All patients had experienced at least two exacerbations of MS in the prior 2 years. Principal exclusion criteria were as follows: previous use of IFN, cladribine, or total lymphoid irradiation; use of glatiramer acetate or cytokine therapy in the prior 3 months; use of IV immunoglobulin in the prior 6 months; and use of other immunomodulatory agents in the prior 12 months.

Treatment. Patients were randomized to receive either Rebif[®] 44 μ g SC tiw, or Avonex[®] 30 μ g IM qw for 24 weeks. For the purpose of this report, “44 μ g tiw” denotes the Rebif[®] regimen and “30 μ g qw” denotes the Avonex[®] regimen. Avonex[®] was obtained through commercial suppliers and was administered according to the manufacturer’s recommendations. Rebif[®] was supplied as a liquid formulation in prefilled syringes. Guidelines were provided for the treating physician based on the World Health Organization (WHO) side-effect severity scale for either dose reduction or interruption. Acetaminophen or nonsteroidal anti-inflammatory drugs (NSAIDs) could be used either as prophylaxis or to treat constitutional influenzalike symptoms associated with the study drugs.

Blinding. Separate treating and evaluating physicians were designated prior to randomization. Patients were instructed not to disclose their treatment assignment or symptoms related to their treatment regimen, to the blinded evaluating physician, and to cover injection sites before scheduled and relapse-related neurologic examinations. Evaluating physicians communicated with patients only as needed to complete the neurologic examinations and to calculate the functional systems (FS) and EDSS scores.¹⁷ MRI evaluation was performed centrally (University of British Columbia MS/MRI Research Group, Vancouver, Canada) by blinded examiners who had no knowledge of a patient’s treatment or outcome. A forced choice questionnaire relating to study blinding was circulated to evaluating physicians after week 48.

Study assessments. Screening took place four weeks prior to randomization and initiation of therapy. It included a complete neurologic and medical history, physical and neurologic examination, and MRI scanning with proton-density/T2-weighted and pre- and postgadolinium (Gd) T1-weighted sequences. The neurologic examination and MRI scans were repeated on study day 1. Patients returned to the study center for scheduled follow-up every 4 weeks during the initial 24-week treatment period and

also at 36 and 48 weeks. Between visits, patients were contacted by telephone to inquire if symptoms of a possible relapse had occurred, and if so, they were referred to the clinical center for evaluation. Detailed neurologic assessments by the evaluating physician, including FS and EDSS scoring, were performed at baseline, 12, 24, 36, and 48 weeks, and as needed for relapse assessment. Blood samples were obtained serially for hematologic, biochemical, and thyroid function testing and for determination of neutralizing antibody (NAb) titers. NAb were measured as previously described.¹⁸

A relapse was defined as the appearance of a new symptom or worsening of an old symptom, accompanied by an appropriate objective finding on neurologic examination by the blinded evaluator, lasting at least 24 hours in the absence of fever and preceded by at least 30 days of clinical stability or improvement. An objective finding was defined as an abnormality on examination that was consistent with the reported neurologic symptom. A relapse was recorded only if the blinded evaluator described new findings consistent with the patient’s reported symptoms, and if the treating physician had excluded the possibility of a pseudorelapse.

Relapse severity was based on changes in FS and EDSS scores. A mild relapse was defined as an EDSS increase of one-half point, or a one-point FS change in one to three FS; a moderate relapse as an EDSS increase of one or two points, a two-point FS change in one or two systems, or a one-point change in four or more systems; and a severe relapse as one exceeding the prior criteria. For documented relapses, the unblinded treating physician decided on a course of treatment. Corticosteroids were permitted (methylprednisolone, 1.0 grams IV daily for 3 days). Study-related MRI scans were performed either before beginning methylprednisolone or at least 7 days after the last dose. Therapy for spasticity, depression, pain, bladder control, fatigue, and other MS symptoms was permitted, although the initiation of new medication was discouraged during the study.

MRI scans were performed according to a specific protocol under the direction of the University of British Columbia MS/MRI Research Group. They were done with and without Gd at screening, on study day 1, and every 4 weeks up to week 24. At week 48, a final scan was performed without Gd enhancement. Workshops were conducted to standardize scanning procedures and all sites performed qualifying scans before study initiation. Rejected scans (i.e., those not meeting quality criteria and therefore not acceptable for analysis) were repeated at screening but not at subsequent visits.

Outcome measures. The primary endpoint was the proportion of patients remaining free of relapses during the 24 weeks, with the primary outcome measure being an odds ratio (OR), adjusted for study center. Relapse rate (relapses per patient per time on study), relapse severity, use of steroids for relapses, and time to first relapse were considered secondary and tertiary clinical outcome measures related to the attack rate. Disability was defined as progression by one point on the EDSS scale confirmed at a visit 3 or 6 months later without an intervening EDSS value that would not meet the criteria for progression. Safety evaluations involved the recording of adverse events, withdrawals owing to adverse events, serious ad-

verse events, and monitoring of laboratory abnormalities. These observations were then carried out for the remainder of the study up to week 48.

The principal MRI outcome measure was the number of combined unique (CU) active lesions per patient per scan, a measure of both active T2 and T1 Gd-enhancing lesions.¹⁹ An active T2 lesion was defined as a new or enlarging lesion or a lesion reappearing at a site of previous lesion resolution. A CU lesion was defined as an active lesion on T1 post-Gd or T2 sequences, or both, avoiding double counting. Additional secondary MRI outcome measures included the number of T2 and T1 lesions per patient per scan, the proportion of active scans (T2, T1, and CU) per patient, and the proportion of patients in whom active scans (T2, T1, and CU) either occurred or did not occur during the initial 24 weeks of the trial. At week 48, because Gd was not administered, only T2 lesions were counted and measured.

Statistical considerations. **Sample size.** A sample size of 280 evaluable patients per treatment arm provided 95% power to detect a 30% relative increase in the primary endpoint at 24 weeks in the 44 μg tiw group, compared with the 30 μg qw group. This calculation was performed using a two-sided χ^2 test, assuming a type I error rate of 5%, with 65% of patients exacerbation free at 24 weeks in the 44 μg tiw group and 50% in the 30 μg qw group. These assumptions were derived from data in previous studies of IFN β -1a given SC either qw or tiw.^{13,20} Assuming a 10% withdrawal/nonevaluable rate, 312 patients per group or a total of 624 patients were required. Treatment assignments were determined using a computer-generated randomization list, and were allocated through a centralized telephone randomization system to unblinded site personnel. Patients were allocated equally to the two treatment groups. Randomization was stratified by center, with an initial block size of six followed by block sizes of four in order to reduce the ability of sites to determine subsequent treatment allocation based on prior allocation.

Analysis populations. The primary analysis was conducted on the intent-to-treat cohort. Patients who discontinued therapy were encouraged to continue in the study and undergo protocol-related procedures, including MRI and clinical assessments. The statistical analysis plan was developed and approved prior to locking the database, based on the statistical methodology specified in the study protocol. For patients who withdrew from the study without follow-up, missing data for the primary outcome were imputed using random number allocation based on the overall proportion of patients not experiencing a relapse during the 24- and 48-week treatment periods for both groups combined. Sensitivity analyses were conducted to evaluate the impact of different missing data assumptions on the results for the primary endpoint. All statistical tests were two-sided and were performed at the 5% significance level.

Baseline comparability. Continuous baseline variables were investigated using a two-way analysis of variance model on ranked data, taking into account effects for treatment and center. Nominal scale categorical baseline data were analyzed using the Cochran–Mantel–Haenszel (CMH) general association test, and ordinal scale categorical baseline data were analyzed using the CMH row mean

scores differ test, both adjusted for center. **Efficacy.** The analysis of efficacy was performed when all enrolled patients had either completed 24 weeks of treatment or had stopped treatment before 24 weeks, and again when patients had completed 48 weeks or discontinued treatment prior to week 48. The primary endpoint was analyzed by logistic regression with adjustment for treatment and center. The OR was defined as the ratio of the odds of being relapse free receiving IFN β -1a 44 μg tiw (proportion of patients who were relapse free/proportion of patients experiencing a relapse) divided by the odds of being relapse free on IFN β -1a 30 μg qw. Kaplan–Meier estimates of time to confirmed progression were computed. The hazard ratios, associated CIs, and treatment comparison *p* values were derived from a Cox proportional hazards model with effects for treatment and center. Relapse rate, based on all reported relapses, was analyzed using a Poisson regression model with factors for treatment and center; the log of the time on study was used as the offset variable in the model.

The proportions of patients with no CU active lesions, no T2 active lesions, and no T1 active lesions were also analyzed using a logistic regression model adjusted for treatment and center. All other MRI data were analyzed using a nonparametric analysis of covariance (ANCOVA) model adjusted for treatment and center, with the baseline number of active lesions as the single covariate.²¹ Exacerbation counts were analyzed using a Poisson regression model with factors for treatment and center, with the log of the time on study as the offset variable. The hazard ratio and associated 95% CI for time to first exacerbation was estimated using the Cox proportional hazards model.

Safety. All patients who received at least one injection of drug were included in the safety analyses. Adverse event counts and the number of patients reporting adverse events were summarized for each treatment group. Patients withdrawing prematurely from the study were listed and summarized by the primary reason for withdrawal for each treatment group. Laboratory test results at baseline and changes from baseline were summarized for each treatment group and compared using Fisher's exact test.

Ethics. The study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from the institutional review boards or ethics committees of all participating institutions before study initiation, and written informed consent was obtained from all patients before any study-related procedures were performed.

Results. **Patient disposition and baseline characteristics.** Of 767 patients screened at the start of the study, 339 were randomized to receive IFN β -1a 44 μg SC tiw and 338 to receive IFN β -1a 30 μg IM qw (figure 1). Ninety patients failed screening by not meeting the inclusion/exclusion criteria. The mean time between screening and the first dose of therapy was 29.3 days. One patient randomized to 30 μg qw did not receive treatment. The distribution of patients who completed 24 weeks and 48 weeks of treatment was similar in both groups (95% for 44 μg tiw and 96% for 30 μg qw at 24 weeks; 93% for 44 μg tiw and 94% for 30 μg qw at 48 weeks).

There were no significant differences in any baseline variable between treatment groups (table 1). Approximately 91% of patients were Caucasian and 75% were women. The baseline EDSS scores ranged from 0 to 5.5,

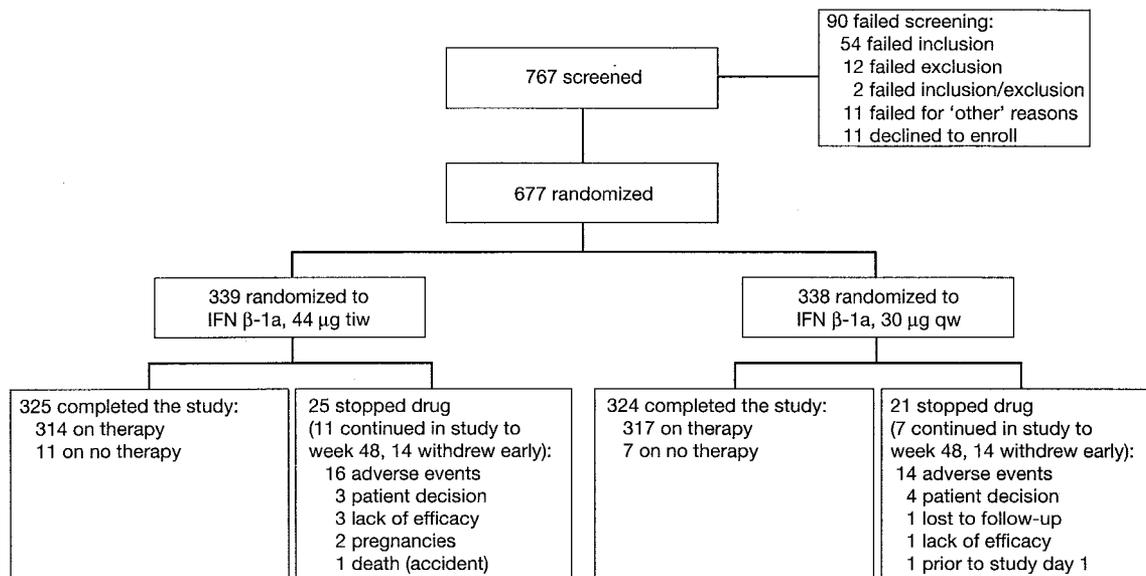


Figure 1. Patient disposition over 48 weeks.

but overall, the level of disability was mild (see table 1). All baseline MRI variables were also similar for the two treatment groups (see table 1). The mean numbers of CU and T1 Gd-enhancing lesions were slightly different because of a few outliers that skewed the mean value; however, median values were identical and the distribution of lesions by group was very similar (see table 1, figure 2). The statistical analysis included baseline MRI activity as a covariate in the regression model so that differences in baseline MRI measures would not bias the results.

Blinding. Evaluating physicians guessed treatment allocation correctly in 52% of patients (47% correct in the 30 μg qw group and 57% in the 44 μg tiw group). Protocol violations wherein treating physicians served as evaluators occurred in 1% of patients in both groups.

Efficacy. Over the initial 24 weeks of treatment, 75% (254/339) of patients in the 44 μg tiw group and 63% (214/338) of those in the 30 μg qw group remained relapse free

(figure 3). The OR, adjusted for center, was 1.9 (95% CI, 1.3 to 2.6; $p = 0.0005$), indicating a relative increase of 90% in the odds of remaining relapse free during the first 24 weeks of therapy for patients receiving 44 μg tiw compared with those receiving 30 μg qw. A similar conclusion (favoring high-dose treatment) was reached when the proportion of relapse-free patients was analyzed using either Fisher's exact test ($p = 0.002$) or the CMH test ($p = 0.001$). Over 48 weeks of treatment, 62% (209/339) of patients in the 44 μg tiw group and 52% (177/338) of those in the 30 μg qw group remained relapse free (see figure 3). The OR, adjusted for center, was 1.5 (95% CI, 1.1 to 2.1; $p = 0.009$), indicating a relative increase of 50% in the odds of remaining relapse free for patients receiving 44 μg tiw compared with those given 30 μg qw.

Relapse status ("yes" or "no") was imputed for patients who dropped out before experiencing a relapse. Of such withdrawals, 2 of the 7 patients treated with 44 μg tiw and

Table 1 Baseline demographic and MRI characteristics by treatment group

Characteristics	IFNβ-1a regimen		p Value
	44 μg tiw, n = 339	30 μg qw, n = 338	
Age, y, mean (range)	38.3 (18–55)	37.4 (18–55)	0.18
Proportion of women, %	74.9	74.6	0.97
Caucasian patients, %	92.3	89.6	0.38
Median (mean) duration of MS, y	4.0 (6.5)	4.1 (6.7)	0.59
Median (mean) time since last relapse, mo	4.4 (5.2)	3.9 (5.0)	0.45
Median (mean) no. relapses in prior 2 years	2.0 (2.6)	2.0 (2.6)	0.44
Median (mean) EDSS at baseline	2.0 (2.3)	2.0 (2.3)	0.99
Median (mean) no. CU-active lesions at baseline	1.0 (2.4)	1.0 (2.9)	0.89
Median (mean) no. T2-active lesions at baseline	0 (1.2)	0 (1.1)	0.73
Median (mean) no. T1-enhancing lesions at baseline	0 (1.9)	0 (2.5)	0.51
Proportion of patients with CU-active lesions at baseline, %	55.1	54.8	0.94

IFN = interferon; EDSS = Expanded Disability Status Scale; CU = combined unique; tiw = three times weekly; qw = once weekly.

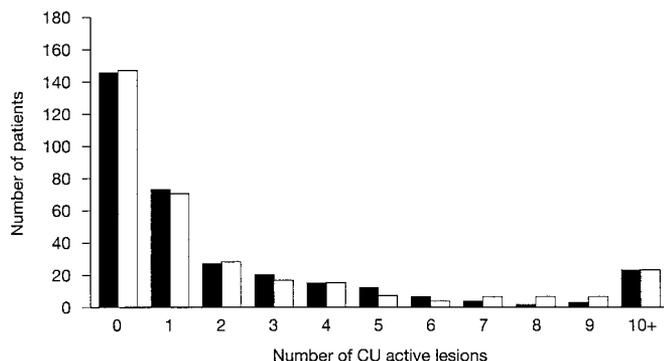


Figure 2. Numbers of patients according to the number of combined unique (CU) active lesions between screening and the first dose of drug (4 weeks). Black bars = interferon (IFN) β -1a 44 μ g three times weekly; white bars = IFN β -1a 30 μ g once weekly.

3 of the 9 patients treated with 30 μ g qw had imputed relapses. Although such imputation of data could alter results, sensitivity analyses showed that even if all patients who withdrew in the 44 μ g tiw group were assumed to have had relapses and all those in the 30 μ g qw group were assumed to have been relapse free over the pivotal 24 weeks, the between-group comparison ($p = 0.006$) still favored the 44 μ g tiw arm. Forty-one percent (40/98) of relapses in the 44 μ g tiw group were detected at unscheduled visits, compared with 36% (48/132) of relapses in the 30 μ g qw group over 24 weeks. This difference was not significant. Over 48 weeks, 45% of relapses in both groups were detected at unscheduled visits.

The time to first relapse was prolonged over the course of the study for patients treated with 44 μ g tiw (hazard ratio = 0.70; 95% CI, 0.55 to 0.88; $p = 0.003$). Outcome data on other relapse-related measures also favored 44 μ g tiw treatment. Relapse rates (mean number of relapses per patient) were 0.29 for the 44 μ g tiw group and 0.40 for the

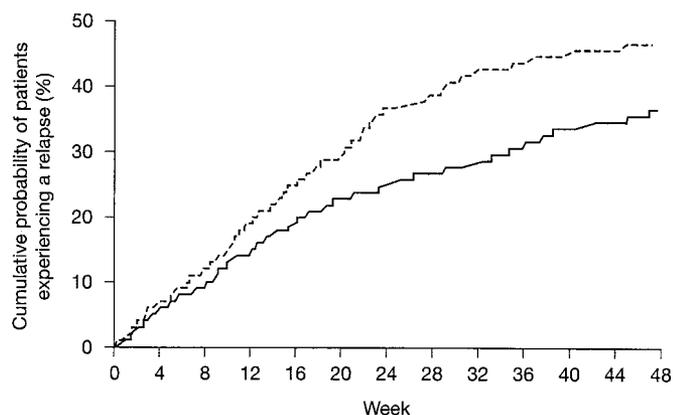


Figure 3. Kaplan-Meier curves illustrating the cumulative probability of patients experiencing a first relapse during the study. There was a 32% relative reduction in the proportion of patients treated with 44 μ g three times weekly (tiw) who experienced a relapse during the initial 24 weeks and a 21% relative reduction after 48 weeks. Hazard ratio = 0.70 ($p = 0.003$). Solid line = interferon (IFN) β -1a 44 μ g tiw; dotted line = IFN β -1a 30 μ g once weekly.

30 μ g qw group at 24 weeks, a 27% relative difference ($p = 0.022$). At 48 weeks, the difference was less pronounced: 0.54 in the 44 μ g tiw group compared with 0.64 in the 30 μ g qw group, a 16% relative reduction ($p = 0.093$). The mean rate of steroid use for relapses was 0.12 courses per relapse in the 44 μ g tiw group and 0.19 in the 30 μ g qw group ($p = 0.017$). The number of relapses was fewer for the 44 μ g tiw group than for the 30 μ g qw group at each severity level, although the proportions of relapses in each group that were mild, moderate, or severe were not different.

Although the study duration was short, disability based on EDSS scores was assessed every 12 weeks for 48 weeks. There were 43 patients in the 44 μ g tiw group and 49 patients in the 30 μ g qw group with confirmed EDSS progression at 2 consecutive visits 3 months apart, indicating a trend toward reduction of progression risk in the 44 μ g tiw group compared with the 30 μ g qw group for this small number of patients (hazard ratio = 0.87; 95% CI, 0.58 to 1.31; $p = 0.51$). Using a more stringent criterion of confirmation after 6 months, 20 patients receiving 44 μ g tiw and 28 patients receiving 30 μ g qw showed disease progression (hazard ratio = 0.70; 95% CI, 0.39 to 1.25; $p = 0.23$).

Patients treated with IFN β -1a 44 μ g tiw had fewer CU, T1, and T2 active lesions per MRI scan compared with those treated with 30 μ g qw at week 24 (nonparametric ANCOVA, $p < 0.0001$ for all activity measures; table 2). To examine the time to onset and persistence of effect, the monthly mean CU active lesion count was assessed. Figure 4 demonstrates a maximal treatment effect within 2 to 3 months of starting therapy, with a persistent reduction in active lesions favoring 44 μ g tiw each month thereafter. The mean number of active scans per patient was also reduced for patients receiving 44 μ g tiw compared with those receiving 30 μ g qw. At baseline, 45% of patients in each group had no CU active lesions on MRI. After 24 weeks of therapy, however, 48% (157/325) of patients treated with 44 μ g tiw had experienced no new MRI activity, compared with 33% (108/325) of the patients treated with 30 μ g qw ($p < 0.0001$). Because Gd was not administered with the 48-week MRI scan, CU data are not available, and only the number of T2-active lesions could be calculated by comparing the baseline and week 48 scans. As shown in table 2, the differences between treatment groups were maintained in favor of the 44 μ g tiw patients ($p < 0.001$ for all comparisons) for mean number of T2 lesions per patient per scan (36% relative reduction), proportion of T2-active scans (38% relative reduction), and proportion of patients with no T2 active lesions over 48 weeks (32% relative increase).

Neutralizing antibodies. Sera obtained at 48 weeks were tested for NAb. Considering any antibody value ≥ 20 neutralizing units (NU)/mL as positive, 84/335 (25%) of 44 μ g tiw patient sera and 7/330 (2%) of 30 μ g qw patient sera had neutralizing activity ($p < 0.001$). Of the 84 patients with NAb receiving 44 μ g tiw, 49 (58%) were positive at week 24, whereas 1 of 7 patients (14%) who became NAb+ on 30 μ g qw did so by week 24. The mean value of NU/mL at week 48 was 537 (median 174) for 44 μ g tiw and 165 (median 34) for 30 μ g qw. Despite the difference in antigenicity of the two treatment regimens, there was no apparent loss of clinical efficacy on relapse activity over the 48-week course of the trial. The proportion of patients re-

Table 2 MRI outcome measures

Measure/IFN β -1a regimen	CU,* wk 24	T1,* wk 24	T2,* wk 24	T2,† wk 48
No. of active lesions per patient per scan‡				
Mean (SEM)§				
44 μ g tiw	0.8 (0.1)	0.6 (0.1)	0.3 (0.1)	0.9 (0.1)
30 μ g qw	1.2 (0.1)	1.0 (0.1)	0.6 (0.1)	1.4 (0.1)
Median				
44 μ g tiw	0.17	0	0	0
30 μ g qw	0.33	0.2	0.2	0.5
Proportion of active scans per patient, %				
Mean (SEM)§				
44 μ g tiw	24 (1.7)	21 (1.6)	15 (1.4)	27 (2.2)
30 μ g qw	37 (1.6)	33 (1.6)	27 (1.4)	43 (2.2)
Median				
44 μ g tiw	17	0	0	0
30 μ g qw	33	20	17	50
Proportion of patients with no active scans, %‡				
44 μ g tiw	48	55	60	63
30 μ g qw	33	38	43	45

* Results based on 7 scans performed every 4 weeks from baseline to week 24.

† Results based on 3 scans at baseline, week 24 and week 48.

‡ p Value < 0.001 for all comparisons between treatment groups on adjusted means, from a nonparametric analysis of covariance A model, with effects for treatment and center with the baseline number of active lesions as covariate.

§ Estimated using a parametric analysis of covariance model on raw data, with effects for treatment and center with the baseline number of active lesions as the covariate.

IFN = interferon; CU = combined unique; tiw = three times weekly; qw = once weekly.

maining relapse free and the probability of first relapse were nearly identical for the patients receiving 44 μ g tiw whether they were NAb+ or NAb- (figure 5). Relapse rates were also identical for NAb+ and NAb- patients in the 44 μ g tiw group. The patients receiving 44 μ g tiw who had NAb titers \geq 20 NU/mL had fewer relapses throughout the study than those in the 30 μ g qw group as a whole or the subgroup of 30 μ g qw patients who remained NAb-.

The data on the effect of NAb on mean numbers of MRI lesions in the 44 μ g tiw group showed that NAb- patients had the fewest T2-active lesions on the week 48 scan (0.6 lesions), whereas those who were NAb+ had more (1.6

lesions, $p = 0.0004$). Differences in lesion count between groups defined by week 48 NAb results were also seen at week 24, but were not different (0.8 for NAb- patients and 1.4 for NAb+ patients, $p = 0.351$) when only 58% of those who became positive at week 48 had developed NAb. No baseline differences were seen between groups. Median

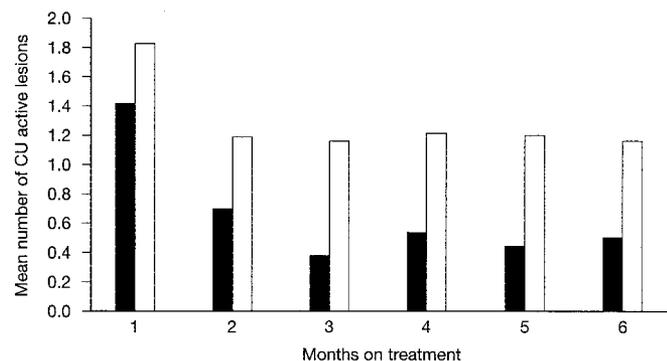


Figure 4. Mean number of combined unique (CU) active lesions per patient per scan from baseline to week 24. Black bars = interferon (IFN) β -1a 44 μ g three times weekly; white bars = IFN β -1a 30 μ g once weekly.

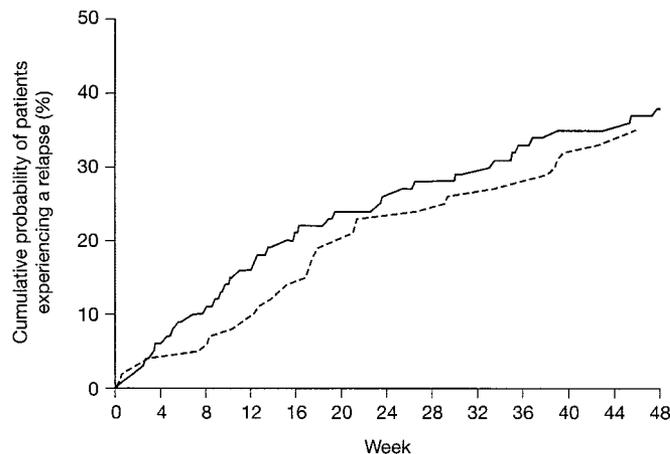


Figure 5. Kaplan-Meier curves illustrating the cumulative probability of patients treated with interferon (IFN) β -1a 44 μ g three times weekly (tiw) experiencing a relapse based on neutralizing antibody status at week 48. Solid line = IFN β -1a 44 μ g tiw NAb⁺; dotted line = IFN β -1a 44 μ g tiw NAb⁻.

Table 3 Safety results at week 48: proportions of patients affected by adverse events

Adverse event	IFN β -1a regimen		p Value
	44 μ g tiw, n = 339	30 μ g qw, n = 337	
Injection-site disorders	83	28	<0.001
Inflammation	44	5	<0.001
Other reaction	35	12	<0.001
Pain	19	10	<0.001
Bruising	8	4	0.020
Influenzalike syndrome	42	49	—
Fever	5	8	—
Rigors	3	7	0.051
Fatigue	18	20	—
Myalgia	12	14	—
Depression	17	18	—
Liver function abnormality	18	9	0.002
Grade 3	1	1	—
Alanine transferase elevation	12	5	0.002
Grade 3	1	1	—
White blood cell abnormality	11	5	0.003
Grade 3	1	<1	—
Lymphopenia	4	<1	0.002
Grade 3	<1	<1	—

Values are expressed as percentages. IFN = interferon; tiw = three times weekly; qw = once weekly.

numbers of T2-active lesions were 0 for NAb subgroups at all time points.

Safety. The mean time on study (46.9 weeks for the 44 μ g tiw group, 47.1 weeks for the 30 μ g qw group) and mean time on treatment (46.0 weeks for the 44 μ g tiw group, 46.3 weeks for the 30 μ g qw group) were similar for both treatment arms. The total amount of drug administered, however, was almost four times higher in the 44 μ g tiw group (mean dose = 5,537 μ g per patient) than in the 30 μ g qw group (mean dose = 1,507 μ g per patient).

Treatment was generally well tolerated in both groups (table 3), although some treatment-emergent adverse events were more common in the 44 μ g tiw group than in the 30 μ g qw group. The most common adverse events were injection-site disorders, which were reported in 83% (282/339) of 44 μ g tiw-treated patients and 28% (93/337) of 30 μ g qw-treated patients. Eighty-four percent of the high-dose events and 92% of the low-dose events were considered mild by physicians. Four patients on 44 μ g tiw and one on 30 μ g qw cited injection-site events as a contributing reason for stopping therapy. There were 160 occurrences of systemic influenzalike symptoms in 143 (42%) patients receiving 44 μ g tiw and 188 similar events in 165 (49%) patients receiving 30 μ g qw ($p = 0.089$). The incidence of subcomponents of the influenzalike syndrome (fever, myalgia, headache) was similar between groups, except for rigors, which were more common in 30 μ g qw-treated patients (7%) than in 44 μ g tiw-treated patients (3%, $p = 0.051$).

Asymptomatic laboratory abnormalities were noted frequently in both treatment arms. Liver function test results reported as adverse events were more common with 44 μ g tiw regimen (60 patients or 18%) than with 30 μ g qw regimen (32 patients or 9%, $p = 0.002$). These events were graded as mild (WHO grade 1, <2.5 times the upper limit of normal [ULN]) in 54% of 44 μ g tiw-treated patients and 48% of 30 μ g qw-treated patients. Severe elevations (WHO grade 3, >5 times but <20 times ULN for transaminases and >1.5 to 3 times ULN for bilirubin) were reported as adverse events in six patients in both groups. Two patients treated with 44 μ g tiw and one patient treated with 30 μ g qw stopped therapy because of raised transaminase levels. Elevated levels of transaminases decreased spontaneously in the majority of patients despite sustained dosing. However, approximately one-fifth of patients with grade 2 elevation progressed to grade 3 elevation if the drug was continued. Dose reductions or interruptions always led to improvement in enzyme levels.

White blood cell abnormalities were reported as adverse events in 38 (11%) patients treated with 44 μ g tiw and in 16 (5%) patients treated with 30 μ g qw ($p = 0.002$), with the events graded as mild in 67% of the patients receiving 44 μ g tiw and in 94% of those receiving 30 μ g qw. Lymphopenia was reported in 13 patients in the 44 μ g tiw group and in 1 patient in the 30 μ g qw group ($p < 0.001$). In 3 patients treated with 44 μ g tiw and 1 patient treated with 30 μ g qw, the lymphopenia was severe.

The number of patients with serious adverse events was 21 (6%) in the 44 μ g tiw group and 18 (5%) in the 30 μ g qw group. Nine of these events were considered by the investigators to be related to the study drug: one case each of lymphopenia, spontaneous abortion, depression, and suicidal ideation for the 44 μ g tiw group; two cases of depression, one MS relapse, one episode of diplopia (not considered a relapse), and one of chest pain for the 30 μ g qw group. Treatment discontinuation because of adverse events occurred in 16 (4.7%) of the 44 μ g tiw patients and 14 (4.2%) of the 30 μ g qw patients. The adverse events contributing to discontinuation (in some cases, more than one per patient) on IFN β -1a 44 μ g tiw were influenzalike syndrome (five patients), injection-site disorders (four patients), white blood cell abnormalities (two patients), liver enzyme abnormalities (three patients), depression (three patients), and unique miscellaneous events (eight patients). Events cited for discontinuation of 30 μ g qw were influenzalike syndrome (six patients), depression (three patients), injection-site reaction (one patient), elevated transaminase level (one patient), and miscellaneous events.¹¹ There was one death (solo pilot airplane crash) of a patient assigned to the 44 μ g tiw group, but no other life-threatening serious adverse events in the study.

Discussion. This randomized, controlled, multicenter trial demonstrated greater efficacy of the IFN β -1a regimen of 44 μ g tiw than for 30 μ g qw on relapse and MRI outcomes assessed at the 24-week primary endpoint and maintained at the 48-week follow-up. In the management of patients with RRMS, these data provide empirical evidence for the clinical importance of 44 μ g SC tiw compared with 30 μ g IM qw, but do not provide specific evidence for each aspect individually—that is, total weekly dose,

frequency of administration, or method of administration. Comparisons within placebo-controlled RRMS studies using more than one dose of IFN consistently showed better results with a higher dose.^{11,14,20} Although a recent dose-comparison study of IFN β -1a did not reveal superiority of 60 μ g qw when compared with 30 μ g qw,¹⁵ this apparent lack of dose effect may be consistent with an earlier pharmacodynamic trial of IFN β -1a, in which the measured biological effect (inhibition of IFN γ and tumor necrosis factor- α secretion by lymphocytes) was greater for 66 μ g when given in divided doses of 22 μ g tiw than when administered as a single weekly injection.¹⁰ These observations argue in favor of the importance of increased frequency of administration. Another potential variable is route of administration, although debate exists as to whether the pharmacokinetic profile with IM administration is superior²² or equivalent²³ to SC administration.

Several design aspects of the current comparative study (use of a single blind, the relatively brief study duration, and the primary use of relapse measures to assess outcome) must be considered. Although the value of the double-blind, placebo-controlled trial is widely recognized, this design is not always appropriate or indicated. Because of the different injection frequency and side-effect profile of IFN β -1a administered IM or SC, it would have been impossible to keep patients blinded in a study of this nature. Similarly, the treating physicians dealing with clinical and laboratory adverse events can easily become unblinded. A recent review of IFN studies in MS concludes that, based on adverse events, so-called double-blind studies should be considered single-blind studies.²⁴ In such a circumstance, the important design element is keeping the assessor blinded, and when this is done (as in the current study), the trial is explicitly defined as providing class I evidence of efficacy by the American Academy of Neurology.²⁵

The double-dummy approach, in which each patient uses the same dosing schedule and injects both placebo and active drug, was considered but rejected by investigators. As discussed above, differences in side-effect profiles would have resulted in essentially the same degree of unblinding as the design we chose. Moreover, such a design would have increased both patient discomfort and adverse events. In view of recently published guidelines for conduct of placebo-controlled trials in MS²⁶ and after careful consideration of these issues, we decided that the use of a placebo-controlled, double-blind design was both impractical and unjustified.

We undertook specific measures to ensure evaluator blinding in this trial. First, patients were consistently reminded not to discuss their symptoms with the evaluating physician. Second, patients were required to cover their injection sites in order to avoid the detection of skin reactions by the evaluating physician. Finally, physicians performed neurologic examinations prior to being informed of any potential

relapse and without access to prior examinations. These blinding measures were used both at scheduled visits and at visits for relapse assessment.

Given the short duration of this trial, it is important to recall that the efficacy of IFN β -1a in the treatment of RRMS had already been established for both drugs in previous studies.^{13,27} The primary purpose of this study was to determine the relative efficacy of two different IFN β -1a products using the standard, clinically proven treatment regimens for each. Previous studies have established the existence of a dose-response effect for IFN β using various pharmacodynamic and immunologic markers.^{8-10,28-36} However, the relevance of such markers to the course of MS was not directly investigated here and remains unknown.³⁷ The importance of the current study, therefore, lies in the fact that it demonstrated a differential effect of IFN β treatment regimens on clinical relapses and MRI activity, disease markers that are clearly related to the underlying biology of MS.

In a short-term clinical trial such as this, only relapse occurrence and MRI-related measures of disease activity can reasonably be expected to change significantly with therapy. The treatment effect difference on relapse rates at 24 weeks was greater than expected (27% relative difference) for a comparison of two active therapies. This decreased to 16% at 48 weeks, possibly reflecting the variability commonly seen in MS attack frequency over short intervals. In terms of proportion of patients remaining relapse free, the absolute difference between groups was slightly less at 48 than at 24 weeks, but remained significant and may better reflect actual treatment differences. The parallel course of the survival curves during the latter portion of the study is typical of the primary endpoint (proportion relapse free), as seen in other studies.³⁸ This does not imply loss of efficacy, as time to first event is delayed for each patient having such an event receiving 44 μ g tiw compared with 30 μ g qw.

Accurate assessment of disability progression would require a study at least 2 years in duration; therefore, assessment of disability at 48 weeks was of limited value but was consistent with effects on relapse and MRI measures. Disability progression in RRMS, particularly at lower EDSS levels, often reflects unresolved relapse-related neurologic dysfunction, distinct from the gradual progression seen in secondary progressive MS (SPMS).

Population-based natural history studies have demonstrated a relationship between early clinical relapse rate and subsequent development of disability.³⁹ It seems reasonable, therefore, to expect that agents capable of preventing relapses in the short term by modulating the early inflammatory phase of MS⁴⁰ would exert a favorable influence on long-term functional outcome by limiting the risk of permanent sequelae of relapses.⁴¹ Indeed, two recent clinical trials in RRMS have provided evidence in support of this hypothesis.^{14,42} Although another study recently suggested that a reduction in relapse rate might not

delay accumulation of disability,⁴³ this conclusion was based on the observation that after reaching a moderate level of disability (EDSS score of 4.0), patients progressed faster when they did not have superimposed relapses; however, clinical course prior to reaching EDSS 4.0 was not explored. This finding also contradicts another study in SPMS,⁴⁴ in which prospectively followed patients with relapses progressed, on placebo or IFN, more quickly than those without relapses.

Lesions detected by MRI in the brains of patients with MS represent a spectrum of pathologic features, but all reflect the disease process. Studies have shown modest but consistent correlations between lesion activity and subsequent clinical relapses, and a recent study demonstrated that T2 lesion number and the total T2 volume of disease at the earliest phase of clinically apparent disease correlated significantly with subsequent disease course and disability.⁴⁵

The development of NAb to IFN β -1a could cause the groups to converge over time, although the relapse data at 48 weeks did not indicate an adverse effect on clinical outcome. This time frame is likely to be too short to draw definitive conclusions about the impact of NAb on efficacy. The MRI data suggest a reduction in efficacy in patients who developed NAb, particularly at the highest titers; however, the differences in mean numbers of T2 MRI lesions were dependent on a small number of outliers, as the median values were identical regardless of antibody status. Reduced efficacy in NAb+ patients is consistent with data from longer-term follow-up studies of patients with MS who develop NAb while receiving IFN therapy.¹⁴

Both dosing regimens of IFN β -1a were well tolerated in this study. Patients in the 44 μ g tiw group had a significantly higher rate of liver function abnormalities—principally transaminase elevations—that were dose related and most apparent at low-grade toxicity levels. All elevations were reversible, either spontaneously or with dose reduction. Cutaneous injection-site reactions were more frequent with 44 μ g tiw, as expected, but necrosis was seen in only one patient. The increase in adverse events had little impact on patient adherence to therapy or retention in the study in contrast to the substantial increases in clinical and MRI benefit.

This comparative trial of two IFN β -1a formulations provides compelling evidence that treatment regimen has an impact on efficacy. Because of the study design, however, the relative contribution of each feature of the regimen (dose, frequency, route) cannot be determined with certainty, nor can the results be used to extrapolate relative benefit beyond the 48-week duration of the study. Nevertheless, the therapeutic benefit of IFN β -1a administered at a higher dose (44 μ g) and more frequently (tiw) compared with IFN β -1a given at 30 μ g qw was robust and was confirmed by each outcome measure investigated in the trial. This is consistent with findings in

another recent comparative study.⁴⁶ These results have important implications for the recommendation and selection of disease-modifying therapies in the management of RRMS.

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Appendix

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