Interferon β-1b–neutralizing antibodies 5 years after clinically isolated syndrome

H.-P. Hartung, M.S. Freedman, C.H. Polman, et al.

Neurology 2011;77;835; Published online before print August 17, 2011;
DOI 10.1212/WNL.0b013e31822c90d7

Interferon β-1b–neutralizing antibodies 5 years after clinically isolated syndrome

H.-P. Hartung, MD
M.S. Freedman, MD
C.H. Polman, MD, PhD
G. Edan, MD
L. Kappos, MD
D.H. Miller, MD
X. Montalbán, MD
F. Barkhof, MD, PhD
J. Petkau, PhD
R. White, MSc
V. Sahajpal, PhD
V. Knappertz, MD
K. Beckmann, MSc
V. Lanius, PhD
R. Sandbrink, MD, PhD*
C. Pohl, MD*
For the BENEFIT Study Group

Address correspondence and reprint requests to Professor Hans-Peter Hartung, Department of Neurology, Heinrich-Heine-Universität, Moorenstraße 5, Düsseldorf 40225, Germany
hans-peter.hartung@uni-duesseldorf.de

ABSTRACT

Objective: To determine the frequency and consequences of neutralizing antibodies (NAbs) in patients with a first event suggestive of multiple sclerosis (MS) treated with interferon β-1b (IFNβ-1b).

Methods: In the Betaseron/Betaferon in Newly Emerging MS For Initial Treatment (BENEFIT) study, patients were randomly assigned to 250 μg IFNβ-1b (Betaferon) or placebo subcutaneously every other day for 2 years or until diagnosis of clinically definite MS (CDMS). Patients were then offered open-label IFNβ-1b for up to 5 years. NAb status was assessed every 6 months by the myxovirus protein A induction assay. A titer >20 NU/mL was considered NAb-positive, with low (≥20–100 NU/mL), medium (≥100–400 NU/mL), and high (≥400 NU/mL) titer categories. Here we examine early-treated patients, who received IFNβ-1b for up to 5 years.

Results: NAbs were measured in 277 of 292 early-treated patients and detected at least once in 88 (31.8%) patients, with 53 (60.2%) reverting to NAb negativity by year 5. Time to CDMS, time to confirmed disability progression, and annualized relapse rate did not differ between NAb-positive and NAb-negative patients or between periods of NAb positivity vs NAb negativity within patients. Increases in newly active lesion number and T2 lesion volume and conversion to McDonald MS were associated with NAb positivity and were more pronounced with higher titers.

Conclusions: Although NAb positivity was associated with increased brain MRI activity, no discernible effects on clinical outcomes were found. This finding may reflect the greater power of MRI compared with clinical outcomes to detect the treatment effects of IFNβ-1b and may also result from temporal changes in NAb titers and biology. *Neurology*® 2011;77:835–843

GLOSSARY

BENEFIT = Betaseron/Betaferon in Newly Emerging MS For Initial Treatment; **CDMS** = clinically definite multiple sclerosis; **CI** = confidence interval; **EDSS** = Expanded Disability Status Scale; **HR** = hazard ratio; **IFNβ** = interferon β; **MS** = multiple sclerosis; **NAb** = neutralizing antibody.

Neutralizing antibodies (NAbs) can develop during interferon β (IFNβ) treatment,[1] as with other injected protein therapies,[2] and reduce or abrogate normal biologic and treatment effects by preventing IFNβ binding to its receptor, thereby blocking receptor activation and expression of IFN-inducible genes.[3–6] NAbs against IFNβ usually develop 3–18 months after treatment initiation and may disappear with continued therapy.[7–18] The effects of NAbs on clinical outcomes in IFNβ-treated patients are inconsistent.[19] Some studies show that clinical outcomes are the same for NAb-negative and NAb-positive patients,[8–11] and others report differences.[7,12,13,16,20–22] Failure to detect consistent NAb effects on clinical outcomes may be a result of most prospective studies being relatively short.

Supplemental data at www.neurology.org

Supplemental Data

*These authors contributed equally to this work.

From the Heinrich-Heine-Universität (H.-P.H., R.S., V.K.), Düsseldorf, Germany; The Ottawa Hospital (M.S.F), Ottawa, Canada; Vrije Universiteit Medical Centre (C.H.P., F.B.), Amsterdam, The Netherlands; Service de Neurologie (G.E.), INSERM, CIC 0203, CHU Rennes, Rennes, France; University Hospital Basel (L.K.), Basel, Switzerland; National Hospital for Neurology and Neurosurgery (D.H.M.), London, UK; Hospital Vall d'Hebron (X.M.), Barcelona, Spain; University of British Columbia (J.P., R.W.), Vancouver, Canada; PAREXEL MMS (V.S.), Worthing, UK, and Bayer HealthCare Pharmaceuticals, Montville, NJ; and Bayer HealthCare Pharmaceuticals (K.B., V.L., R.S., C.P.), Berlin, Germany.

Study funding: Supported in part by the German Ministry of Research and Technology competence network MS and the Walter-und-Ilse Rose Stiftung (H.-P.H). BENEFIT was funded by Bayer HealthCare Pharmaceuticals. Steering committee members and sponsor representatives designed the study protocol. The authors, members of the publication committee, had access to all data and participated in data analysis and interpretation. H.-P.H. made the final decision in the submission of this manuscript.

Disclosure: Author disclosures are provided at the end of the article.

The Betaseron/Betaferon in Newly Emerging MS For Initial Treatment (BENEFIT) study assessed IFNβ-1b (Betaferon, Bayer HealthCare Pharmaceuticals, Wayne, NJ) in patients with a first event suggestive of multiple sclerosis (MS) over 5 years.[22–24] It provided a unique opportunity to prospectively assess the frequency/dynamics of NAb evolution and their long-term consequences.

METHODS **Study design.** Patients were randomly assigned to 250 μg IFNβ-1b subcutaneously every other day or placebo (5:3), within 60 days after onset of the first clinical event, for ≤2 years or until clinically definite MS (CDMS) was diagnosed.[25] Patients were then offered IFNβ-1b for up to 5 years. Neurologic assessments were undertaken at least every 6 months. Up to 10 MRI scans were obtained (screening, annually during years 1–5, and months 3, 6, 9, and 18 in the placebo-controlled phase only). Anti-IFNβ antibodies were evaluated every 6 months using the myxovirus protein A induction assay with IFNβ-1b.[26,27]

Statistical analyses. Analyses were limited to patients initially randomized to IFNβ-1b, to provide a longer, more homogeneous observation period than delayed treatment in patients initially randomized to placebo. *p* Values are for 2-sided tests.

Definitions of NAb status. A titer of 20 NU/mL was the cutoff between NAb negativity (<20 NU/mL) and positivity (≥20 NU/mL). NAb-positive titers were further categorized into low (≥20–100 NU/mL), medium (≥100–400 NU/mL), medium-high (≥100 NU/mL), and high (≥400 NU/mL) titers. Two definitions for eventually NAb-positive patients were evaluated: at least one NAb-positive titer or 2 consecutive (confirmed) positive measurements. Reversion to persistent NAb negativity was assumed if a patient had at least one NAb-negative titer at a subsequent timepoint and no additional NAb-positive titers after reversion. NAbs measured >30 days after the end of study medication were excluded.

Effect of NAbs on outcomes. Differences between "never NAb-positive" and "eventually NAb-positive" subgroups were evaluated cross-sectionally. Because results from unconfirmed and confirmed definitions of NAb positivity were very similar, findings using the confirmed definition are presented in the supplementary material on the *Neurology*® Web site at www.neurology.org.

Because NAb status may have changed over time, further analyses compared NAb-negative with NAb-positive time periods by time-to-event analyses, considering NAb status as a time-dependent covariate, and by longitudinal analyses for repeated outcome measures.[16] All switches between titer levels (dynamic switching) were considered. The effect of NAbs on outcomes was analyzed for NAb positivity ≥20 NU/mL and with respect to different titer categories.

Cross-sectional analyses in eventually NAb-positive patients. In individuals enrolled for >365 days, time to CDMS, McDonald MS, and confirmed progression (Expanded Disability Status Scale [EDSS] score increase ≥1.0 after at least 140 days) were analyzed using Cox proportional hazards regression comparing the NAb-negative vs eventually NAb-positive patients. Relapse rates observed 1) over the entire study period, 2) excluding the first study year, and 3) annually in years 1–5 were compared for eventually NAb-positive and NAb-negative patients by Poisson regression. Five-year MRI outcomes (cumulative number of newly active lesions and T2 volume change) were compared by nonparametric analysis of covariance. The presence or absence of neutralizing activity was compared by the presence of active inflammatory lesions on annual brain MRI scans using Fisher exact test.

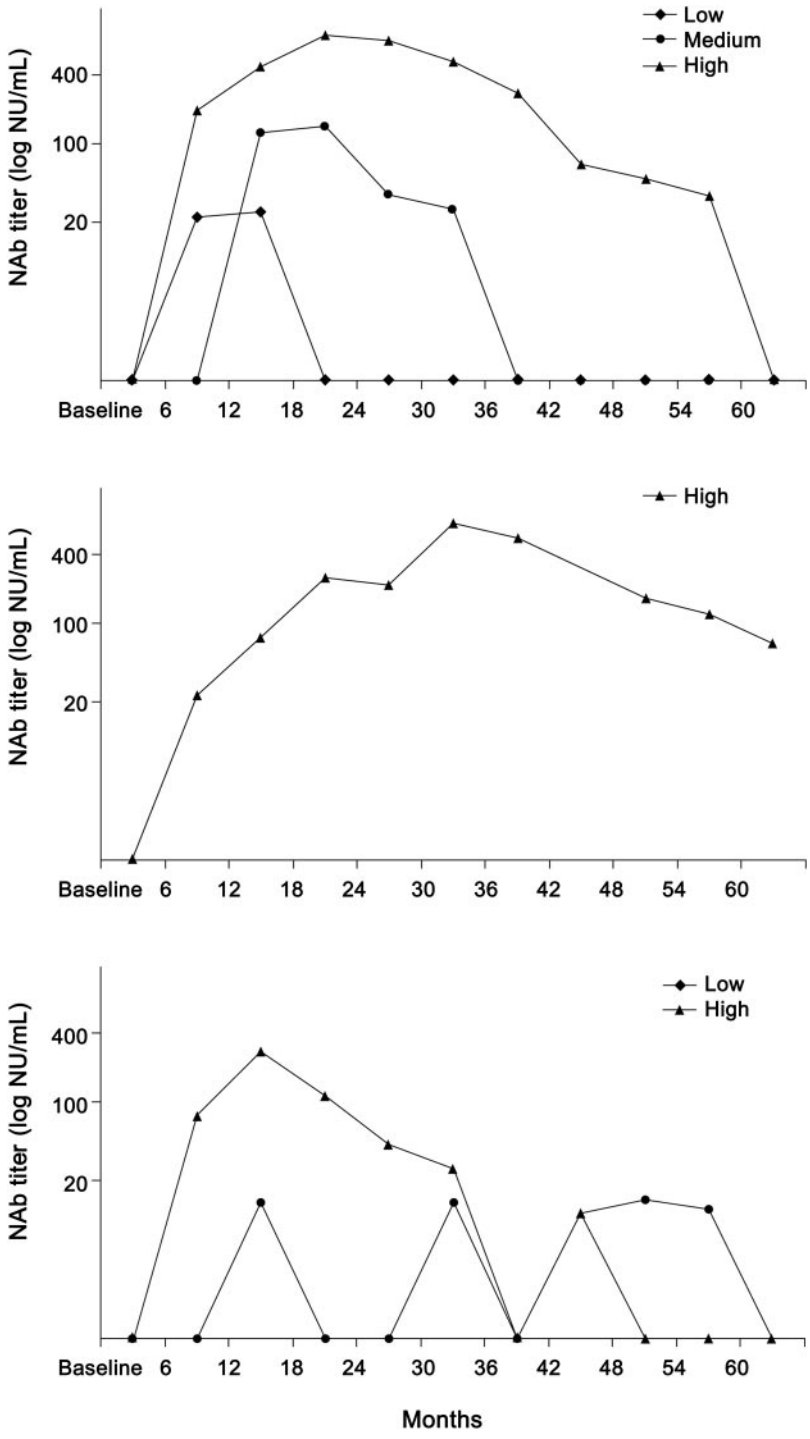
Comparison of outcomes in NAb-negative and NAb-positive periods within patients. The risk during NAb-negative and NAb-positive periods for time to event outcomes was analyzed by baseline-adjusted Cox proportional hazards regression including NAb status as a time-dependent covariate. Hazard ratio (HR) estimates and 95% confidence intervals (CI) indicated whether periods of NAb positivity were associated with increased risk.

Longitudinal analyses for repeated outcome measures was used to further explore the relationships between NAb status and relapse risk, EDSS score change, newly active lesion rate, and T2 volume change. The generalized estimating equation approach, with an exchangeable correlation structure, was used.[16] Specifically, the generalized estimating equation extensions of log-binomial, Poisson, and ordinary regression were used to analyze risk of relapse at any day, rate of development of newly active lesions on successive MRI, and changes in EDSS score or T2 lesion volumes. To account for potential trends over time, a linear relationship was incorporated into all statistical models.

Standard protocol approvals, registrations, and patient consents. Written consent was obtained from all participants for both the original study and the 5-year follow-up phase. The ethics committees and institutional review boards of all participating centers approved the study protocols. The study was registered with ClinicalTrials.gov (identifier NCT00185211).

RESULTS **NAb characteristics.** NAbs were measured in 277 of 292 early-treatment patients. Over 5 years, 31.8% (88 of 277) were NAb-positive at least once; of these, 15.2% had low (42 or 277), 7.2% medium (20 of 277), and 9.4% (26 of 277) high peak titers. By year 5, 60.2% (53 of 88) with ≥1 NAb-positive titer reverted to NAb negativity: −85.7% (36 of 42) with low, 65.0% (13 of 20) with medium, and 15.4% (4 of 26) with high NAb titers. NAbs were measured consecutively at least twice in 26.7% (74 of 277) of patients, with 12.3% (34 of 277), 6.9% (19 of 277), and 7.6% (21 of 277) achieving low, medium, and high titers, respectively. By year 5, 54.1% (40 of 74) of patients with at least 2 consecutive NAb-positive titers reverted to NAb negativity, with 76.5% (26 of 34), 68.4% (13 of 19), and 4.8% (1 of 21) of those with low, medium, and high titers, respectively, reverting. There was a stronger tendency in NAb-negative vs eventually NAb-positive patients to prematurely terminate study medication (34.4% vs 19.3%) or leave the study (14.8% vs 11.4%). NAb-negative compared with NAb-positive patients were more likely to terminate study medication (36.9% vs 11.8%) and the study (21.4% vs 10.0%) because of adverse events.

Figure 1 Percentage of patients with ≥1 gadolinium-enhancing lesion vs neutralizing antibody (NAb) positivity at annual timepoints



Mean NAb titers (NU/mL) in positive patients were lowest in year 1, increased until year 3, and then slightly decreased. Of note, because of reversion to stable NAb status in the majority of patients who eventually became NAb-positive, the number of NAb-positive patients decreased at later timepoints.

Of 88 eventually NAb-positive patients, 48 (54.5%) had a monophasic increase in NAb burden with reversion to NAb negativity by study end; 29 (33.0%) experienced a persisting increase in NAb positivity; and 11 (12.5%) fluctuated between NAb positivity and NAb negativity. In 48 patients with a monophasic increase in NAb positivity, 32 reached low, 13 medium, and 3 high peak titers. Duration of NAb positivity was comparatively short in the low-titer group and increased in a stepwise manner from medium to high titers (mean numbers of consecutively positive NAb measurements in monophasic patients with low, medium, and high peak titers were 2.2, 5.3, and 6.7, respectively). In 29 patients with persisting NAb positivity, 2 achieved low, 6 medium, and 21 high peak titers (5 of them had a <3-year observation period because of premature study termination). Patients with persisting NAb positivity and high peak titers showed declining titers over time.

Effect of NAbs on clinical outcomes. No significantly increased hazard in time to CDMS and time to EDSS score progression occurred in eventually NAb-positive patients for any titer (figure 1). Notably, patients with low peak NAb titers exhibited a significantly reduced hazard in time to CDMS relative to that of never NAb-positive patients (HR 0.536; $p = 0.037$) (figure 1). Similar results for eventually NAb-positive patients with at least 2 consecutively positive NAb measurements are shown in table e-1.

When NAb status was used as a time-dependent covariate, there was no increased risk for CDMS or EDSS score progression during NAb-positive periods (table 1). There was also no measurable increased risk for CDMS or EDSS score progression during low, medium, and high NAb titer periods (table 1). Similar results were obtained when medium to high NAb titer levels were combined in a single category (table e-2).

Annualized relapse rate did not increase in eventually NAb-positive patients, regardless of whether data were analyzed for the entire study period or after year 1 (tables 2 and e-3). A significantly lower annualized relapse rate over the entire study period occurred among eventually NAb-positive individuals, specifically in patients developing low peak NAb titers. There was also no increase in relapse rates in persistently NAb-positive patients (table 2), and there were no systematic differences for NAb-negative vs NAb-positive patients in study years 1–5 with the exception of high NAb titers in year 2 (negative vs high titer 0.18 vs 0.41; $p = 0.03$) and low NAb titers in year 5 (negative vs low titer 0.17 vs 0.46; $p = 0.01$). Notably, patients with medium to high NAb titers in year 4–5 showed low relapse rates (≤0.13; table e-4).

Longitudinal analyses showed that the switch from NAb negativity to positivity at any titer level did not increase relapse risk and EDSS score (table 3). Notably, in NAb-positive periods, estimated ef-

Table 1 Risk of CDMS, confirmed EDSS progression, and McDonald MS by NAb titer[a]

	Risk of event, HR (95% CI), *p* value[b]		
	CDMS	EDSS progression	McDonald MS
Positive (≥20 NU/mL) vs negative	0.82 (0.48–1.42), 0.48	0.97 (0.50–1.89), 0.93	2.84 (1.94–4.14), <0.001
Single model			
Low titer (20–100 NU/mL) vs negative	0.97 (0.51–1.85), 0.93	0.97 (0.41–2.29), 0.95	2.13 (1.36–3.33), <0.001
Medium titer (100–400 NU/mL) vs negative	0.98 (0.35–2.70), 0.96	1.29 (0.39–4.23), 0.67	8.23 (3.94–17.19), <0.001
High titer (≥400 NU/mL) vs negative	0.25 (0.03–1.78), 0.17	0.71 (0.17–2.96), 0.63	6.78 (2.32–19.79), <0.001

Abbreviations: CDMS = clinically definite multiple sclerosis; CI = confidence interval; EDSS = expanded disability status scale; HR = hazard ratio; MS = multiple sclerosis; NAb = neutralizing antibody.
[a] Results are based on the unconfirmed definition of NAb status and the dynamic switching model.
[b] *p* Value by Cox proportional hazards regression with NAb status as a time-dependent covariate adjusted for age, gender, number of T2/gadolinium-enhancing lesions, monofocal or multifocal presentation, and use of steroids at the time of a first clinical event suggestive of MS. HRs > 1.0 indicate increased risk.

fects on EDSS score changes showed disability improvements (table 3).

Because of the relatively low clinical disease activity, the power to detect the effects of NAbs on clinical outcomes was limited. For example, in the cross-sectional analysis comparing eventually NAb-positive (n = 88) vs NAb-negative patients, estimated power (of a 2-sided test at $\alpha = 0.05$) to detect a 50% increase in relapse rate was approximately 62% (this drops to 32% if only the 26 patients with high peak titers are considered; the power for longitudinal analysis is similar).

Effect of NAbs on time to McDonald MS. Cross-sectional analyses showed that eventual NAb positivity in patients with high peak titers was associated with increased risk of McDonald MS (figure 1, table e-1). Progression to McDonald MS was driven primarily by MRI events. NAb-positive periods at each titer level were associated with an increased risk of McDonald MS (tables 1 and e-2), particularly during medium ($p < 0.001$) and high NAb titer periods ($p < 0.001$).

Effect of NAbs on MRI outcomes. Eventually NAb-positive patients had an increased mean number of newly active lesions (12.2 vs 8.6, $p < 0.001$) and change in T2 lesion volume (+82 vs −289 mm^3, $p < 0.001$) over 5 years. Compared with never NAb-positive patients, increases in the mean number of newly active lesions were significant for patients with low (9.3 vs 8.6, $p = 0.048$), medium (15.9 vs 8.6, $p < 0.001$), or high NAb titers (13.6 vs 8.6, $p < 0.001$). For median change in T2 lesion volume, increases relative to those in never NAb-positive patients were significantly greater only for patients with medium (+271 vs −289 mm^3, $p = 0.018$) and high titers (+210 vs −289 mm^3, $p < 0.001$).

Longitudinal analyses showed a significant increase in newly active lesions in NAb-positive vs NAb-negative time periods (table 3). There were no significant increases in T2 lesion volume during NAb-positive time

Table 2 Cross-sectional analysis of annualized relapse rate by NAb titer

		Entire observational period		After year 1	
	No.	Annualized relapse rate (95% CI)	*p* Value[a]	Annualized relapse rate (95% CI)	*p* Value[a]
Negative	189 (182)[b]	0.23 (0.20–0.26)		0.21 (0.18–0.25)	
Positive (≥20 NU/mL)[c]	88	0.17 (0.14–0.22)	0.039	0.17 (0.13–0.23)	0.24
Low (20–100 NU/mL)[c]	42	0.15 (0.10–0.21)	0.021	0.16 (0.10–0.24)	0.20
Medium (100–400 NU/mL)[c]	20	0.18 (0.11–0.29)	0.35	0.18 (0.09–0.30)	0.55
High (≥400 NU/mL)[c]	26	0.21 (0.14–0.31)	0.67	0.20 (0.12–0.30)	0.77
Medium to high (≥100 NU/mL)[c]	46	0.20 (0.14–0.27)	0.38	0.19 (0.13–0.26)	0.57
Persistently positive patients[d]	29	0.19 (0.13–0.28)	0.39	0.18 (0.12–0.28)	0.57

Abbreviations: CI = confidence interval; NAb = neutralizing antibody.
[a] Poisson regression: all *p* values are for comparisons with the never NAb-positive group.
[b] Number in parentheses is the number of never NAb-positive patients who contributed data after year 1.
[c] Results are based on the unconfirmed definition of NAb status.
[d] Persistently positive patients are defined as patients with at least one positive NAb titer (≥20 NU/mL) not reverting to NAb negativity up to the end of the study.

Table 3 Effects of NAb switching on clinical and MRI disease parameters over 5 years[a]

	Estimates (95% CI)	*p* Value
Relapse risk, %		
Positive (≥20 NU/mL) vs negative	15.3 (−20.1 to 66.3)	0.45
Single model		
Low (20–100 NU/mL) vs negative	30.5 (−11.8 to 93.1)	0.18
Medium (100–400 NU/mL) vs negative	17.4 (−45.0 to 150.7)	0.68
High (≥400 NU/mL) vs negative	−31.1 (−66.3 to 41.0)	0.39
EDSS change		
Positive (≥20 NU/mL) vs negative	−0.09 (−0.18 to 0.00)	0.055
Single model		
Low (≥20–100 NU/mL) vs negative	−0.10 (−0.19 to −0.01)	0.032
Medium (≥100–400 NU/mL) vs negative	−0.04 (−0.20 to 0.13)	0.66
High (≥400 NU/mL) vs negative	−0.10 (−0.32 to 0.12)	0.31
Newly active lesion rate, %		
Positive (≥20 NU/mL) vs negative	107.6 (70.5 to 152.8)	<0.001
Single model		
Low (20–100 NU/mL) vs negative	93.6 (54.5 to 142.5)	<0.001
Medium (100–400 NU/mL) vs negative	130.5 (77.6 to 199.3)	<0.001
High (≥400 NU/mL) vs negative	139.8 (76.3 to 226.0)	<0.001
T2 lesion volume change, mm^3		
Positive (≥20 NU/mL) vs negative	80 (−66 to 226)	0.28
Single model		
Low (20–100 NU/mL) vs negative	65 (−82 to 212)	0.39
Medium (100–400 NU/mL) vs negative	73 (−120 to 267)	0.46
High (≥400 NU/mL) vs negative	181 (−140 to 503)	0.27

Abbreviations: CI = confidence interval; EDSS = Expanded Disability Status Scale; NAb = neutralizing antibody.
[a] Results are based on the unconfirmed definition of NAb status and the dynamic switching model.

periods (table 3). This may be due to the resolution of T2 hyperintensity on the screening MRI originating from brain edema induced by the first clinical event leading to an apparent decrease in T2 lesion volume during the first trial year when neutralizing activity was just evolving or below detection.

On annual MRI scans, inflammatory activity indicated by the presence of at least one gadolinium-enhancing lesion was more frequent in NAb-positive patients than in NAb-negative patients (figure 2), with significant between-group differences at years 1 and 3. However, the percentage of NAb-positive patients with MRI activity decreased at years 4 and 5 and was not statistically different from that of NAb-negative patients.

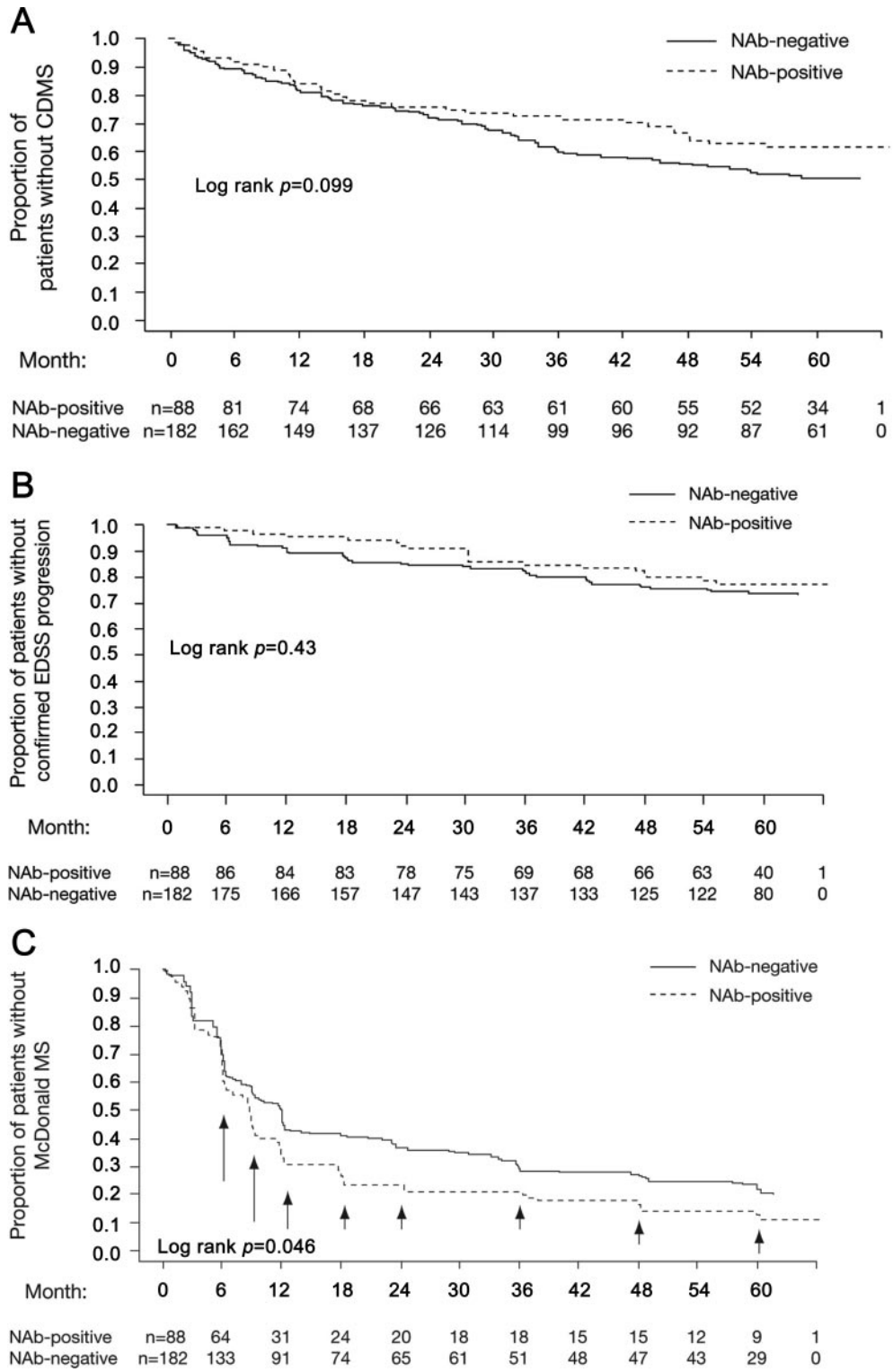
DISCUSSION BENEFIT is the longest prospectively planned follow-up study of patients with a first event suggestive of MS and is thus suited to evaluate the long-term impact of IFNβ-1b–induced NAbs on clinical and MRI outcomes.

We could not show any effect of NAbs on key clinical outcomes (development of CDMS, relapses, and EDSS score progression). No differences in clinical outcomes were found for never NAb-positive and eventually NAb-positive patients or during NAb-positive and NAb-negative time periods within individual patients. In fact, neutralizing activity was associated with numerically better clinical outcomes. In many IFNβ-1b and -1a studies, it has been curiously noted that patients who were eventually NAb-positive experienced a greater reduction in relapses and EDSS score progression before NAb detectability.[10,13,21,22,28] Once measurable, however, NAb positivity was shown to be associated with a higher relapse rate in several IFNβ trials.[7,16,20,21,22,28] The observational Danish patient registry study, combining data from all 3 IFNβ treatments, showed that relapse rates were significantly higher during NAb-positive periods.[28] These results differ from our findings in that over 5 years there was no apparent association with higher relapse rates. An obvious explanation could be that there were fewer overall relapses and little disability progression in BENEFIT, such that the power to detect significant differences between those with and without measurable NAbs was low, especially if the moderate treatment effect of IFNβ products on these clinical outcomes is considered: in the 88 eventually NAb-positive patients, the ability to detect a 50% increase in relapse rate/risk was approximately 60%. The power is further reduced to 32% when only the 26 patients who achieved high peak NAb titers (≥400 NU/mL) are considered.

Conversely, the absent effect of NAbs on clinical outcomes in BENEFIT agrees with several IFNβ-1b and IFNβ-1a prospective studies.[8–11,29] The largest randomized, controlled study of IFNβ in patients with relapsing-remitting MS evaluated NAbs in 1,775 IFNβ-1b–treated patients for up to 3.5 years. This study had more power than BENEFIT to demonstrate the effect of NAbs on relapse rates, because of the higher clinical disease activity of participants and greater number of evaluable NAb-positive patients (n = 659).[29] Increased relapse rates occurred during periods of high NAb titers only in patients receiving an experimental higher dose (500 μg subcutaneously every other day) rather than the approved dose in BENEFIT.[29] Moreover, 2 large observational studies involving 10,000 patients did not demonstrate a relationship between NAb positivity and clinical worsening.[30,31]

The 5-year observation period of our dataset provides insight into the complexity of IFNβ-1b–induced NAbs, because the heterogeneity in NAb evolution and persistence becomes more evident over this time: peak titer levels differed across patients and

Figure 2 Kaplan-Meier curves for time to (A) clinically definite multiple sclerosis (CDMS), (B) confirmed Expanded Disability Status Scale (EDSS) score progression, and (C) McDonald multiple sclerosis (MS)



Time to event outcomes (time to CDMS, time to confirmed progression defined by an EDSS score increase of at least 1.0, and time to McDonald MS) were analyzed by Cox proportional hazards regression comparing the risk in neutralizing antibody (NAb)–negative patients vs that in eventually NAb-positive patients with low, medium, and high titers, while also adjusting for the covariates age, gender, number of T2/gadolinium-enhancing lesions, monofocal or multifocal presentation, and use of steroids at the time of a first clinical event suggestive of MS. Time to McDonald MS is a combined endpoint of clinical and MRI events. Arrows indicate when MRI was performed. Of note, the increase in risk in NAb-positive patients occurred at approximately the time of MRI, indicating that this risk increase was driven primarily by MRI events.

usually did not exceed low to moderate burdens, with a strong tendency for reversion to NAb negativity, particularly with peak low to moderate titers (85% and 65%). High titers occurred in approximately 10% of patients; the reversion rate was only 15% in those with at least 1 high titer and 5% with 2 consecutive high titers. Most IFNβ-1b–induced NAb titers are low and transient; IFNβ-1a is less likely to induce NAbs, but they are more persistent.[15] The reason for more frequent NAb reversion among IFNβ-1b–treated patients is unclear but may involve the earlier induction of immunologic tolerance because of a higher protein load.

As in previous studies, NAb evolution was associated with more inflammatory disease activity on cerebral MRI, which increased with peak titer level.[7,11,13,20,22,29] This was reflected by increased conversion to McDonald MS, a phenomenon driven mainly by the appearance of new lesions over time. The dissociation between clinical and MRI outcomes is partly related to the detection of asymptomatic lesions on brain MRI, which occur more frequently than relapses or EDSS score progression.[32,33] In addition, because the effect of IFNβ is greater on MRI parameters than on clinical outcomes, MRI clearly has the greater power to determine treatment response. Nevertheless, patients reaching high peak NAb titers demonstrated the most pronounced MRI changes, with numerically better relapse rates and decreased relapse risk in periods of high NAb titers. In contrast, many studies have shown a correlation between MRI and relapse-related outcomes.[34–36] In BENEFIT, the effect of NAbs on MRI parameters did not translate into clinical effects over 5 years, indicating limitations to use of MRI activity alone for predicting clinical outcomes.[37,38]

Reasons for the NAb-associated divergence between clinical and MRI findings include the reversion of many NAb-positive patients to seronegativity and a potentially paradoxical benefit of low NAb titers. Furthermore, NAb biology may change in ways that do not necessarily affect the NAb titer, e.g., the binding properties and immunoglobulin G subclass specificity of IFNβ-induced antibodies may change over time and vary across IFNβ preparations.[39] We observed a lower association of NAbs with MRI activity after 4 years of treatment, possibly resulting from such changes in NAb biology or the gradual waning of MRI activity. Moreover, patients destined to develop anti-IFNβ NAbs may be biologically different, with an altered disease course. Finally, divergence between clinical and MRI outcomes might disappear if biomarkers that measure IFNβ bioactivity in vivo are tested. Recent data have shown that measuring IFNβ bioactivity may improve the monitoring of patients and predict therapeutic responses, especially for those with low to moderate NAb titers.[6,40]

Overall, BENEFIT data show that the relationship between NAb status and long-term MS outcomes under IFNβ-1b treatment is complex: despite a significant association of NAb positivity and MRI activity measures, there was still no eventual effect on any clinical parameter over 5 years other than the combined clinical/MRI outcome of time to McDonald MS. Discordance between clinical and MRI outcomes reflects the greater power of MRI to detect reductions in IFNβ-mediated treatment effects in the few patients with persisting NAbs; temporal changes in NAb titers and biology may contribute to this. Given this complexity, treatment decisions regarding IFNβ-1b use should not be based solely on NAb measurements but rather reached within the context of other disease aspects.

AUTHOR CONTRIBUTIONS

Dr. Hartung: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, and study supervision. Dr. Freedman: drafting/revising the manuscript, study concept or design, and analysis or interpretation of data. Dr. Polman: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, and acquisition of data. Dr. Edan: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, and study supervision. Dr. Kappos: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision, and member of steering and writing committee. Dr. Miller: study concept or design and study supervision. Dr. Montalban: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, and study supervision. Dr. Barkhof: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data, and study supervision. Dr. Petkau: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, and statistical analysis. R. White: analysis or interpretation of data and statistical analysis. Dr. Sahajpal: drafting/revising the manuscript. Dr. Knappertz: drafting/revising the manuscript, analysis or interpretation of data, and statistical analysis. K. Beckmann: analysis or interpretation of data and statistical analysis. Dr. Lanius: drafting/revising the manuscript, analysis or interpretation of data, and statistical analysis. Dr. Sandbrink: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, and study supervision. Dr. Pohl: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, and study supervision.

COINVESTIGATORS

Members of the BENEFIT Study Group are the following. Principal investigators: Austria: S. Strasser-Fuchs (Graz), T. Berger (Innsbruck), K. Vass (Vienna); Belgium: C. Sindic (Brussels), B. Dubois (Leuven), D. Dive (Liège), J. Debruyne (Ghent); Canada, L. Metz (Calgary), G. Rice (London), P. Duquette, Y. Lapierre (Montreal), M. Freedman (Ottawa), A. Traboulsee (Vancouver), P. O'Connor (Toronto); Czech Republic: P. Štourač (Brno), R. Taláb (Hradec Kralove); O. Zapletalová (Ostrava), I. Kováøová, E. Medová (Prague), J. Fiedler (Plzen); Denmark: J. Frederiksen (Glostrup); France: B. Brochet (Bordeaux), T. Moreau (Dijon), P. Vermersch (Lille), J. Pelletier (Marseille), G. Edan (Rennes), M. Clanet (Toulouse), P. Clavelou (Clermont Ferrand), C. Lebrun-Frenay (Nice); O. Gout (Paris); Finland: M. Kallela (Helsinki), T. Pirttilä (Kuopio), J. Ruutiainen (Turku), K. Koivisto (Seinäjoki), M. Reunanen (Oulu), I. Elovaara (Tampere); Germany: A. Villringer, H. Altenkirch (Berlin), K. Wessel (Braunschweig), H-P Hartung, W. Steinke (Düsseldorf), H. Kölmel (Erfurt), P. Oschmann (Giessen), R. Diem (Göttingen), A. Dressel (Greifswald), F. Hoffmann (Halle/Saale), K. Baum (Hennigs-

dorf), S. Jung (Homburg/Saar), H. Felicitas Petereit, D. Reske (Cologne), M. Sailer (Magdeburg), J. Köhler (Mainz), N. Sommer (Marburg), R. Hohlfeld (Munich), K.-H. Henn (Offenbach), A. Steinbrecher (Regensburg), H. Tumani (Ulm), R. Gold, P. Rieckmann (Würzburg); Hungary: R. Komoly, G. Gács, G. Jakab (Budapest), L. Csiba(Debrecen), L. Vécsei (Szeged); Israel: A. Miller (Haifa), D. Karussis (Jerusalem), J. Chapman (Tel-Hashomer); Italy: A. Ghezzi (Gallarate), G. Comi (Milan), P. Gallo (Padua), V. Cosi (Pavia), L. Durelli (Turin); the Netherlands: B. Anten (Sittard), L. Visser (Tilburg); Norway: K.-M. Myhr (Bergen); Poland: A. Szczudlik (Kraków), K. Selmaj (Łódź), Z. Stelmasiak (Lublin), R. Podemski (Wrocław), Z. Maciejek (Bydgoszcz); Portugal: L. Cunha (Coimbra); Slovenia: S. Sega-Jazbec (Ljubljana); Spain: X. Montalbán, T. Arbizu, A. Saiz (Barcelona), J. Bárcena (Barakaldo), R. Arroyo (Madrid), O. Fernández (Málaga), G. Izquierdo (Seville), B. Casanova (Valencia); Sweden: J. Lycke (Mölndal); Switzerland; L. Kappos (Basel), H. Mattle (Bern), K. Beer (St. Gallen); United Kingdom: R. Coleman (Aberdeen), J. Chataway (London), J. O'Riordan (Dundee), S. Howell (Sheffield).

ACKNOWLEDGMENT

The authors thank the patients and the BENEFIT investigators for their contributions to the study, Cosima Klein for statistical programming, and Ray Ashton and Tiffany DeSimone, PhD, of PAREXEL, who were funded by Bayer HealthCare Pharmaceuticals, for medical writing assistance.

DISCLOSURE

Dr. Hartung serves on scientific advisory boards for Octapharma AG, Merck Serono, Teva Pharmaceutical Industries Ltd., Biogen Idec, and Eli Lilly and Company and has received speaker honoraria from Biogen Idec, Teva Pharmaceutical Industries Ltd., sanofi-aventis, Merck Serono, Novartis, and Bayer Schering Pharma. Dr. Freedman has received grants and educational support from EMD Serono, Biogen Idec, Genzyme, and Bayer HealthCare; has been on steering committees and acted as an advisory board member for Bayer HealthCare, Merck Serono, sanofi-aventis, and Novartis; and has received honoraria and carried out miscellaneous consultations for Biogen Idec, Bayer HealthCare, sanofi-aventis, Novartis, EMD Serono, and Teva. Dr. Polman serves on scientific advisory boards for and has received funding for travel and speaker honoraria from Actelion Pharmaceuticals Ltd, Biogen Idec, Bayer Schering Pharma, GlaxoSmithKline, Teva Pharmaceutical Industries Ltd., Merck Serono, Novartis, and UCB, Roche; serves on the editorial boards of *Lancet Neurology* and *Multiple Sclerosis*; and receives research support from Biogen Idec, Merck Serono, Novartis, UCB, European Community Brussels, and MS Research Foundation Netherlands. Dr. Edan has served on scientific advisory boards for Bayer Schering Pharma, Biogen Idec, and Teva Pharmaceutical Industries Ltd.; has received speaker honoraria from Merck Serono; and has received research support from Merck Serono, Bayer Schering Pharma, Teva Pharmaceutical Industries Ltd., the French Ministry of Health (Programme Hospitalier de Recherche Clinique), and ARSEP Foundation. Dr. Kappos has received research support through the University Hospital Basel from Acorda Therapeutics Inc., Actelion Pharmaceuticals Ltd, Advancell, Allozyne, Barofold, Bayer HealthCare Pharmaceuticals, Bayer Schering Pharma, Bayhill, Biogen Idec, BioMarin, Boehringer Ingelheim, CSL Behring, Geneuro, Genmab, GlaxoSmithKline, Glenmark, Merck Serono, MediciNova, Novartis, sanofi-aventis, Santhera Pharmaceuticals, Shire Plc, Roche, Teva, UCB, and Wyeth and also from the Swiss MS Society, the Swiss National Research Foundation, European Union, Gianni Rubato, and Roche and Novartis Foundations; and serves on the editorial board of *Multiple Sclerosis Journal, International Journal of Multiple Sclerosis*, and *Swiss Archives of Neurology and Psychiatry*. Dr. Miller serves on scientific advisory boards for Novartis, GlaxoSmithKline, Bayer Schering Pharma, Biogen Idec, and the NIH; has received funding for travel or speaker honoraria from Biogen Idec, Novartis, Bayer Schering Pharma, GlaxoSmithKline, the National MS Society, and Cleveland Clinic; receives publishing royalties for *McAlpine's Multiple Sclerosis, fourth edition* (Churchill Livingstone, 2005); serves as a consultant for GlaxoSmithKline and Biogen Idec; and receives research support through his institution from Biogen Idec, GlaxoSmithKline, Schering-Plough Corp., and Novartis and also from the MS Society of Great Britain and Northern Ireland and the NIH. Dr. Montalban serves on scientific advisory boards for Novartis, Teva Pharmaceutical Industries Ltd., Merck Serono, Biogen Idec, and Bayer Schering Pharma; has received funding for travel and speaker honoraria from Novartis, Teva Pharmaceutical Industries Ltd., Merck Serono, Biogen Idec, sanofi-aventis, and Bayer Schering Pharma; serves on the editorial boards of *Multiple Sclerosis*, *Journal of Neurology*, *The International MS Journal*, *Revista Neurología*, and *Therapeutic Advances in Neurological Disorders*; serves as a consultant to Bayer Schering Pharma, Biogen Idec, Merck Serono, Teva Pharmaceutical Industries Ltd, sanofi-aventis, Novartis, Almirall, and Eli Lilly and Company; has received research support for clinical trials from Genentech, Inc., Genzyme Corporation, Wyeth; and receives research support from the Fundació Esclerosi Múltiple (FEM). Dr. Barkhof serves on scientific advisory boards for Lundbeck Inc., Bayer Schering Pharma, sanofi- aventis, UCB, Novartis, Biogen Idec, BioMS Medical, Merck Serono, and GE Healthcare; serves on the editorial boards of *Brain*, the *Journal of Neurology, Neurosurgery and Psychiatry*, *European Radiology*, the *Journal of Neurology*, and *Neuroradiology*; has received speaker honoraria from Novartis, Merck Serono, and BioClinica; serves as a consultant for sanofi-aventis, UCB, Novartis, Biogen Idec, BioMS Medical, Medicinova, Inc., and GE Healthcare; and receives research support from the Dutch MS Research Foundation. Dr. Petkau has served on scientific advisory boards for Bayer Schering Pharma, Solstice Neurosciences, Merck Serono, Opexa Therapeutics, Eisai Inc., Schering-Plough Corp., and Bayhill Therapeutics; has received funding for travel and speaker honoraria from Bayer Schering Pharma, Solstice Neurosciences, Biogen Idec, and Merck Serono/Pfizer Inc; serves as a consultant for Bayer Schering Pharma, Bayhill Therapeutics, BTG International, Opexa Therapeutics, PRA International, and Solstice Neurosciences; and receives research support from Bayer Schering Pharma, Opexa Therapeutics, Natural Sciences and Engineering Research Council of Canada, Canadian Institutes for Health Research, and Multiple Sclerosis Society of Canada. R. White has served as a consultant for Bayer Schering Pharma, Solstice Neurosciences, and Opexa Therapeutics. Dr. Sahajpal was a salaried employee of PAREXEL when the manuscript was prepared. Dr. Knappertz is a full-time employee of Bayer HealthCare Pharmaceuticals. K. Beckmann is a full-time employee of Bayer HealthCare Pharmaceuticals. Dr. Lanius is a full-time employee of Bayer HealthCare Pharmaceuticals. Dr. Sandbrink is a full-time employee of Bayer HealthCare Pharmaceuticals. Dr. Pohl is a full-time employee of Bayer HealthCare Pharmaceuticals.

Received August 2, 2010. Accepted in final form May 16, 2011.

REFERENCES

1. Schellekens H, Casadevall N. Immunogenicity of recombinant human proteins: causes and consequences. J Neurol 2004;251(suppl 2):4–9.
2. Wolbink GJ, Aarden LA, Dijkmans BA. Dealing with immunogenicity of biologicals: assessment and clinical relevance. Curr Opin Rheumatol 2009;21:211–215.
3. Weinstock-Guttman B, Ransohoff RM, Kinkel P, et al. The interferons: biological effects, mechanisms of action, and use in multiple sclerosis. Ann Neurol 1995;37:7–15.
4. Gilli F, Bertolotto A, Sala A, et al. Neutralizing antibodies against IFN-beta in multiple sclerosis: antagonization of IFN-beta mediated suppression of MMPs. Brain 2004; 127:259–268.
5. Deisenhammer F, Reindl M, Harvey J, et al. Bioavailability of interferon beta 1b in MS patients with and without neutralizing antibodies. Neurology 1999;52:1239–1243.
6. Hesse D, Sellebjerg F, Sorensen PS. Absence of MxA induction by interferon beta in patients with MS reflects complete loss of bioactivity. Neurology 2009;73:372–377.
7. Interferon Beta MS Study Group. Neutralizing antibodies during treatment of multiple sclerosis with interferon beta-1b: experience during the first three years. Neurology 1996;47:889–894.

8. Rudick RA, Simonian NA, Alam JA, et al. Incidence and significance of neutralizing antibodies to interferon beta-1a in multiple sclerosis: Multiple Sclerosis Collaborative Research Group (MSCRG). Neurology 1998;50:1266–1272.
9. PRISMS Study Group. Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. Lancet 1998;352:1498–1504.
10. Durelli L, Verdun E, Barbero P, et al. Every-other-day interferon beta-1b versus once-weekly interferon beta-1a for multiple sclerosis: results of a 2-year prospective randomised multicentre study (INCOMIN). Lancet 2002;359:1453–1460.
11. Panitch H, Goodin DS, Francis G. Randomized, comparative study of interferon-1a treatment regimens in MS: the EVIDENCE Trial. Neurology 2002;59:1496–1506.
12. Malucchi S, Sala A, Gilli F, et al. Neutralizing antibodies reduce the efficacy of beta interferon during treatment of multiple sclerosis. Neurology 2004;62:2031–2037.
13. Kappos L, Clanet M, Sandberg-Wollheim M, et al. Neutralizing antibodies and efficacy of interferon-1a: a 4-year controlled study. Neurology 2005;65:40–47.
14. Sominanda A, Rot U, Suoniemi M, et al. Interferon beta preparations for the treatment of multiple sclerosis patients differ in neutralizing antibody seroprevalence and immunogenicity. Mult Scler 2007;13:208–214.
15. Sørensen PS, Koch-Henriksen N, Ross C, et al. Appearance and disappearance of neutralizing antibodies during interferon-beta therapy. Neurology 2005;65:33–39.
16. Petkau AJ, White RA, Ebers GC, et al. Longitudinal analyses of the effects of neutralizing antibodies on interferon beta-1b in relapsing-remitting multiple sclerosis. Mult Scler 2004;10:126–138.
17. Rice GP, Paszner B, Oger J, Lesaux J, Paty D, Ebers G. The evolution of neutralizing antibodies in multiple sclerosis patients treated with interferon beta-1b. Neurology 1999;52:1277–1279.
18. Bellomi F, Scagnolari C, Tomassini V, et al. Fate of neutralizing and binding antibodies to IFN beta in MS patients treated with IFN beta for 6 years. J Neurol Sci 2003;215:3–8.
19. Goodin DS, Frohman EM, Hurwitz B, et al. Neutralizing antibodies to interferon beta: assessment of their clinical and radiographic impact: an evidence report. Neurology 2007;68:977–984.
20. PRISMS Study Group. PRISMS-4: long-term efficacy of interferon-beta-1a in relapsing MS. Neurology 2001;56:1628–1636.
21. Polman C, Kappos L, White R, et al. Neutralizing antibodies during treatment of secondary progressive MS with interferon-1b. Neurology 2003;60:37–43.
22. Kappos L, Freedman MS, Polman CH, et al. Long-term effect of early treatment with interferon beta-1b after a first clinical event suggestive of multiple sclerosis: 5-year active treatment extension of the phase 3 BENEFIT trial. Lancet Neurol 2009;8:987–997.
23. Kappos L, Polman CH, Freedman MS, et al. Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndromes. Neurology 2006;67:1242–1249.
24. Kappos L, Freedman MS, Polman CH, et al. Effect of early versus delayed interferon beta-1b treatment on disability after a first clinical event suggestive of multiple sclerosis: a 3-year follow-up analysis of the BENEFIT study. Lancet 2007;370:389–397.
25. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227–231.
26. Files JG, Gray JL, Do LT, et al. A novel sensitive and selective bioassay for human type I interferons. J Interferon Cytokine Res 1998;18:1019–1024.
27. Grossberg SE, Kawade Y, Kohase M, Klein JP. The neutralization of interferons by antibody II. Neutralizing antibody unitage and its relationship to bioassay sensitivity: the tenfold reduction unit. J Interferon Cytokine Res 2001;21:743–755.
28. Sørensen PS, Ross C, Clemmesen KM, et al. Clinical importance of neutralising antibodies against interferon beta in patients with relapsing-remitting multiple sclerosis. Lancet 2003;362:1184–1191.
29. Goodin DS, Arnason BGW, Comi G, et al. Neutralizing antibodies during treatment with interferon beta-1b in 1745 patients with relapsing-remitting multiple sclerosis. Neurology 2009;72(suppl 3):A317–A318.
30. Goodin DS, Hurwitz B, Noronha A. Neutralizing antibodies to interferon beta-1b are not associated with disease worsening in multiple sclerosis. J Int Med Res 2007;35:173–187.
31. Rot U, Sominanda A, Fogdell-Hahn A, Hillert J. Impression of clinical worsening fails to predict interferon-beta neutralizing antibody status. J Int Med Res 2008;36:1418–1425.
32. Barkhof F. The clinico-radiological paradox in multiple sclerosis revisited. Curr Opin Neurol 2002;15:239–245.
33. Goodin DS. Magnetic resonance imaging as a surrogate outcome measure of disability in multiple sclerosis: have we been overly harsh in our assessment? Ann Neurol 2006;59:597–605.
34. Sormani MP, Bruzzi P, Comi G, Filippi M. MRI metrics as surrogate markers for clinical relapse rate in relapsing-remitting MS patients. Neurology 2002;58:417–421.
35. Tintoré M, Rovira A, Río J, et al. Baseline MRI predicts future attacks and disability in clinically isolated syndromes. Neurology 2006;67:968–972.
36. Rudick RA, Lee JC, Simon J, Fisher E. Significance of T2 lesions in multiple sclerosis: a 13-year longitudinal study. Ann Neurol 2006;60:236–242.
37. Kappos L, Moeri D, Radue EW, et al. Predictive value of gadolinium-enhanced magnetic resonance imaging for relapse rate and changes in disability or impairment in multiple sclerosis: a meta-analysis Lancet 1999;353:964–969.
38. Daumer M, Neuhaus A, Morrissey S, Hintzen R, Ebers GC. MRI as an outcome in multiple sclerosis clinical trials. Neurology 2009;72:705–711.
39. Gibbs E, Oger J. The IgG subclass-specificities of anti-IFNbeta antibodies change with time and differ between the IFNbeta products in relapsing remitting multiple sclerosis patients. J Neuroimmunol 2007;190:146–150.
40. Pachner AR, Cadavid D, Wolansky L, Skurnick J. Effect of anti-IFNβ antibodies on MRI patients in the BECOME study. Neurology 2009;73:1485–1492.

CORRECTION

Interferon β-1b–neutralizing antibodies 5 years after clinically isolated syndrome

In the article "Interferon β-1b–neutralizing antibodies 5 years after clinically isolated syndrome" by H.-P. Hartung et al. (*Neurology*® 2011;77:835–843), the following errors occurred.

The authors did not plan to publish figure 1 and therefore did not reference it in the text. Moreover, this figure was published with an incorrect title and an incorrect legend. However, the figure presents correct data providing characteristic examples of the development and reversion of NAb positivity over time: (A) monophasic NAb course in 3 patients with peak low, medium, and high titers; (B) sustained NAb increase in a patient with peak high titers; and (C) fluctuating NAb course in 2 patients with peak low and high titers.

Figure 2 in the manuscript is also incorrect as it is an older version of similar analyses but provides less detailed information. See below for the new version of this figure, which should be figure 1 as referenced in the text of the manuscript. The correct version shows Kaplan-Meier curves in NAb-negative patients vs "eventually NAb-positive patients" with low, medium, and high titers for time to (A) CDMS, (B) confirmed EDSS progression, and (C) McDonald MS as presented below.

The figure referenced in the text as figure 2 is missing from the published article. This figure provides the percentage of patients with ≥1 gadolinium-enhancing lesion on brain MRI vs NAb positivity at annual timepoints as presented below.

The version of the paper that was peer-reviewed had the correct figures. Inadvertent substitution of the figures occurred later. The authors regret the errors.

Figure 1 Kaplan-Meier curves for time to (A) CDMS, (B) confirmed EDSS progression, and (C) McDonald MS

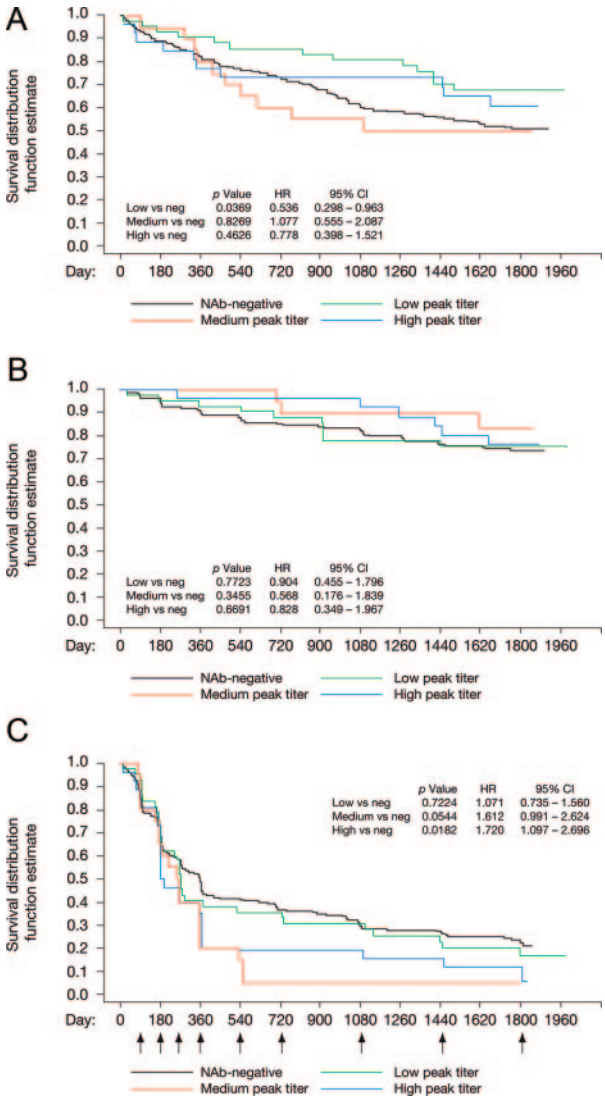


Figure 2 Percentage of patients with ≥1 gadolinium-enhancing lesion vs NAb positivity at annual timepoints

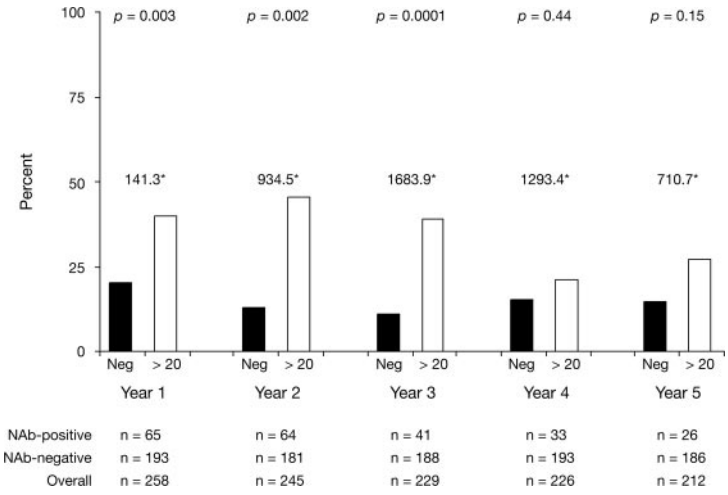


Figure 2 *Mean NAb titers (NU/mL) in positive patients were lowest in year 1, increased until year 3, and then slightly decreased. Of note, due to reversion to stable NAb status in the majority of patients who eventually became NAb-positive, the number of NAb-positive patients decreased at later timepoints.

Figure 1 Time-to-event outcomes (time to CDMS, time to confirmed progression defined by an EDSS increase of at least 1.0, and time to McDonald MS) were analyzed by Cox proportional hazards regression comparing the risk in NAb-negative patients vs "eventually NAb-positive patients" with low, medium, and high titers, while also adjusting for the covariates age, gender, number of T2/gadolinium-enhancing lesions, mono-/multifocal presentation, and use of steroids at the time of a first clinical event suggestive of MS. Time to McDonald MS is a combined endpoint of clinical and MRI events. Arrows indicate when an MRI was performed. Of note, increase in risk in NAb-positive patients occurred around the time of MRI, indicating that this risk increase was driven primarily by MRI events.