

Clinical Effect of Neutralizing Antibodies to Interferon Beta That Persist Long After Cessation of Therapy for Multiple Sclerosis

Laura F. van der Voort, MD; Francesca Gilli, PhD; Antonio Bertolotto, MD; Dirk L. Knol, PhD; Bernard M. J. Uitdehaag, MD, PhD; Chris H. Polman, MD, PhD; Joep Killestein, MD, PhD

Objectives: To confirm that neutralizing antibodies (NAb) to interferon beta can persist after therapy withdrawal and to evaluate whether persisting NAb are associated with a worse clinical disease course in multiple sclerosis (MS).

Design: Retrospective study.

Setting: Tertiary referral center in the Netherlands.

Patients: A total of 71 patients with relapsing-remitting multiple sclerosis treated with interferon beta in the past.

Main Outcome Measures: Persisting NAb after therapy withdrawal were tested using the cytopathic effect assay. Patients with and without persisting NAb were compared on several outcomes: the change in annualized relapse rate from prior to interferon beta treatment initiation to after cessation of treatment, time to sustained disability on the Kurtzke Expanded Disability Status Scale, and the use of disease-modifying treatments after cessation of treatment with interferon beta.

Results: Seventeen of 71 patients (24%) tested NAb positive after a median interval of 25 months (interquartile range, 10-51 months) after interferon beta treatment cessation. Eleven of these 17 patients (15%) were high-titer NAb positive (>150 10-fold reduction units per mL). Persisting NAb were associated with an increase in the annualized relapse rate ($P=.04$) and a reduction in time to reach a sustained Expanded Disability Status Scale score of 6.0, ie, the need for unilateral assistance to walk 100 m ($P=.02$). Moreover, NAb-positive patients were treated with second-line therapy significantly more often, especially mitoxantrone ($P=.006$).

Conclusion: Anti-interferon beta NAb can persist after interferon beta treatment withdrawal and are associated with overt clinical disease activity. This is made apparent by an increase in relapse rate and faster disability progression and is supported by the observed need for more aggressive therapy after interferon beta treatment cessation. Prospective studies are warranted to confirm these results.

Arch Neurol. 2010;67(4):(doi:10.1001/archneurol.2010.21)

Author Affiliations:

Departments of Neurology (Drs van der Voort, Uitdehaag, Polman, and Killestein) and Epidemiology and Biostatistics (Drs Knol and Uitdehaag), VU University Medical Center, Amsterdam, the Netherlands; and Centro di Riferimento Regionale Sclerosi Multipla and Neurobiologia Clinica, ASO San Luigi Gonzaga, Orbassano, Torino, Italy (Drs Gilli and Bertolotto).

MULTIPLE SCLEROSIS (MS) is a chronic, immune-mediated disorder of the central nervous system and one of the leading causes of permanent disability in young adults.¹ Interferon beta has been shown to be a safe and effective treatment for relapsing-remitting MS and is widely used as a first-line treatment.²⁻⁴ Long-term use of recombinant protein-based therapeutics such as interferon beta, insulin, growth hormone, and factor VIII can lead to an immune response directed against the drug⁵ that is mainly based on breaking B-cell tolerance.⁶ Antidrug antibodies may cross-react with their endogenous counterpart,⁷ influence the therapeutic efficacy of the drug,⁸ or be associated with allergic reactions.⁹ The clinical significance of anti-interferon beta neutral-

izing antibodies (NAb) continues to be a controversial issue in the MS community.¹⁰ Increasing evidence suggests that, during treatment, bioactivity of interferon beta is influenced by NAb, and efficacy of the treatment is decreased with persisting NAb.^{6,8,11-13} Some studies have shown that NAb can persist even after cessation of interferon beta treatment. The dynamics and clinical effect of persisting NAb, however, are largely unknown.^{14,15} In this study, our primary aim was to confirm the occurrence of persisting NAb after withdrawal of interferon beta therapy and to evaluate potential predisposing factors. Secondly, as persistent NAb after cessation of interferon beta therapy will probably inhibit endogenous interferon β , we correlated persisting NAb status to measures of clinical disease activity.

Table 1. Patient Characteristics^a

Characteristic	Patients, No. (%)		
	NAb Negative, ≤20 TRU/mL	NAb Positive, >20 TRU/mL	Total
Sample size	54 (76)	17 (24)	71 (100)
Female	40 (74)	11 (65)	51 (72)
Median (IQR) age at disease onset, y	30.8 (25.5-37.5)	37.0 (27.1-40.6)	31.7 (25.9-39.0)
Median (IQR) EDSS score at start of interferon beta therapy	3.0 (2.0-4.0)	3.0 (2.0-4.0)	3.0 (2.0-4.0)
Median (IQR) disease duration at start interferon beta therapy, y	5.6 (1.3-10.2)	3.1 (1.2-5.6)	4.9 (1.3-9.6)
Median (IQR) interferon beta treatment duration, y	2.4 (1.2-4.4)	2.9 (2.2-5.1)	2.6 (1.3-4.4)
Interferon beta-1a dosage, 30 µg IM	18 (33)	2 (12)	20 (28)
Interferon beta-1a dosage, 22 or 44 µg SC	8/2 (19)	5/3 (47)	13/5 (25)
Interferon beta-1b dosage, 250 µg SC	26 (48)	7 (41)	33 (47)

Abbreviations: EDSS, Extended Disability Status Scale; IM, intramuscular; IQR, interquartile range; NAb, neutralizing antibodies; SC, subcutaneous; TRU/mL, 10-fold reduction units per milliliter.

^aA statistical trend was found for an association between NAb positivity and the interferon beta product used ($P=.05$).

METHODS

PROCEDURE

Potential participants were retrospectively identified by a medical record review of all well-monitored patients who had started interferon beta treatment from 1994 to 2006 at the MS center in Amsterdam. Consecutive patients were invited to participate when they were treated with interferon beta for at least 12 months and subsequently ceased treatment for at least 3 months. In general, patients were seen at baseline, 1, 3, 6, and 12 months, and annually thereafter. Patients had additional visits when indicated. We collected data concerning age at disease onset, sex, disease duration at start interferon beta treatment, treatment duration, and interferon beta product used. Furthermore, we evaluated the use of disease-modifying treatment after interferon beta treatment cessation. The number of relapses was assessed in the 2 years before interferon beta treatment initiation and in the period after interferon beta treatment cessation, and both were converted into annualized relapse rates. Disability status was determined at the start of interferon beta treatment for all subjects by using the Kurtzke Expanded Disability Status Scale (EDSS)¹⁶ and repeated at the most recent visit to the outpatient clinic. Neutralizing Ab titer levels were measured by a previously described cytopathic effect assay in the Centro Riferimento Regionale Sclerosi Multipla, Orbassano, Italy. Titers were calculated according to the Kawade formula and expressed in 10-fold reduction units per milliliter (TRU/mL).^{17,18} Measurements were performed with the same type of interferon beta as used in individual patients for therapy. For this study, NAb titers of 20 TRU/mL or less were considered NAb negative; greater than 20 TRU/mL, positive; and greater than 150 TRU/mL, high-titer NAb positive.^{13,19} Clinical researchers were blinded for NAb titer results and laboratory colleagues for the clinical evaluation of patients. This study was carried out with the approval of the Medical Ethical Committee of the VU Medical Center, and written informed consent was obtained from all participants.

STATISTICAL ANALYSIS

Differences in demographic characteristics and disability status (EDSS) at interferon beta treatment initiation between patients with persisting antibodies after cessation of therapy and NAb-negative patients were measured with the Kruskal-Wallis, Mann-Whitney U , χ^2 , and Fisher exact tests, where ap-

propriate. The association between post-interferon beta treatment regimens and NAb status was analyzed with the Fisher exact test. Relapse rates before and after interferon beta therapy cessation were compared using the Wilcoxon signed rank test for related samples. The proportion of patients with a stable or improved relapse rate after interferon beta treatment cessation was compared with patients with a rise in relapse rate in NAb status groups using logistic regression analysis, with treatment regimen after interferon beta as a factor. For the evaluation of disability progression, we used time-to-event analysis in which the event was defined as reaching an EDSS score of 6, sustained for at least 6 months, corresponding with the need for unilateral assistance to walk at least 100 m. We used Cox regression analysis with NAb status as a main independent variable and corrected for the EDSS score at initiation of interferon beta therapy.

RESULTS

PATIENTS

About 525 patients with MS had started interferon beta therapy at the MS center in Amsterdam between 1994 and 2006. We examined the medical records of the 342 patients who had systematic clinical evaluations at least annually. Ninety-seven patients fulfilled the prespecified selection criteria. Seventy-one of these patients, 51 women and 20 men, gave written informed consent (for baseline characteristics see **Table 1**). The reasons for exclusion were either that patients were still receiving interferon beta treatment or that patients had been treated with interferon beta for fewer than 12 months. Twenty-six patients fulfilled the criteria but preferred not to participate because of either work responsibilities ($n=6$) or MS-related conditions ($n=11$). Nine patients declined participation in research without giving a specific reason.

Patients were treated with 30 µg of intramuscular interferon beta-1a (Avonex; Biogen Idec, Cambridge, Massachusetts) once weekly ($n=20$), 22 µg ($n=13$) or 44 µg ($n=5$) of subcutaneous interferon beta-1a (Rebif; Serono, Geneva, Switzerland) 3 times weekly, and 250 µg of subcutaneous interferon beta-1b (Betaferon; Bayer Schering Pharma, Berlin, Germany) every other day ($n=33$).

PERSISTING NAb AFTER CESSATION OF INTERFERON BETA THERAPY

Seventeen patients (24%) were found to be NAb positive (NAb titer median, 320 TRU/mL; range, 22-5120 TRU/mL); 11 of these 17 patients were high-titer positive (range, 152- 5120 TRU/mL). Patients positive for NAb were tested after cessation of interferon beta therapy with a median interval of 25 months (interquartile range, 10-51 months). No differences were found with respect to age at onset, sex, disease duration, duration of interferon beta treatment, EDSS at start of interferon beta treatment, and MS subtype (Table 1). Patients using 22 or 44 µg of subcutaneous interferon beta-1a (Rebif) 3 times weekly were more often persisting NAb titer positive ($P=.05$) and high-titer positive ($P=.003$) than patients using either interferon beta-1b (Betaferon) or intramuscular interferon beta-1a (Avonex) (Table 1).

PERSISTING NAb AND DISEASE-MODIFYING TREATMENT AFTER INTERFERON BETA TREATMENT

Most patients stopped interferon beta treatment because of a disease breakthrough (relapse and/or disability progression) while receiving therapy. Of these 41 patients, 13 (37%) tested positive for NAb after cessation of interferon beta therapy. A smaller group of patients stopped treatment because of adverse effects or because of a desire to become pregnant; 4 of these 26 patients (15%) were positive for persisting NAb. Notably, treatment decisions were made without any knowledge of NAb status during therapy, as NAb measurements were not performed in our clinic during that period.

After interferon beta withdrawal, 13 patients (18%) switched to glatiramer acetate (Copaxone; Teva Pharmaceutical Industries Ltd, Petach Tikva, Israel), 13 (18%) to natalizumab (Tysabri; Elan Pharmaceuticals Inc, Dublin, Ireland) and 10 (14%) patients received courses of intravenous mitoxantrone dihydrochloride (Mitoxantrone; EBEWE Pharma GmbH, Unterach, Austria). Forty-nine percent (35 of 71) of patients remained untreated during follow-up (**Table 2**). Some patients who stopped receiving interferon beta treatment because of perceived treatment failure remained untreated afterwards (15 of 41 [37%]). Most of these patients were considered to not be eligible for other immunomodulatory treatment because they progressed to the secondary progressive phase of MS.

Status of NAb was associated with choice of treatment after abortion of interferon beta treatment (Table 2; $P=.006$). Persisting NAb-positive patients were more often switched to second-line therapy (mitoxantrone or natalizumab) than NAb-negative patients. This difference was mainly driven by the choice for mitoxantrone in these patients.

PERSISTING NAb AND CLINICAL OUTCOME MEASURES

Overall, the mean relapse rate after interferon beta treatment cessation was significantly lower than the relapse rate of the pretreatment phase ($P=.001$). However, within sub-

Table 2. Disease-Modifying Treatment After Cessation of Interferon Beta Therapy and NAb Positivity^a

Treatment After Interferon Beta	Patients, No. (%)		
	Total	NAb Negative, ≤ 20 TRU/mL	NAb Positive, > 20 TRU/mL
Natalizumab	13	8 (62)	5 (38)
Mitoxantrone	10	4 (40)	6 (60)
Glatiramer acetate	13	11 (85)	2 (15)
No treatment	35	31 (89)	4 (11)

Abbreviations: NAb, neutralizing antibodies; TRU/mL, 10-fold reduction units per milliliter.

^aPercentages of NAb positivity (> 20 TRU/mL) in different treatment regimes after cessation of interferon beta therapy. The occurrence of persisting NAb was associated with a treatment switch after interferon beta ($P=.006$).

groups, only NAb-negative patients had a decrease in relapse rate ($P<.001$), whereas the relapse rate in NAb-positive patients remained unchanged ($P=.88$). When comparing the proportion of patients with an increased relapse rate after cessation of interferon beta therapy, correcting for the use of mitoxantrone, a higher proportion was found for NAb-positive patients ($P=.04$; **Table 3**). Compared with patients without persisting NAb, the risk of an increased relapse rate after interferon beta treatment was almost 5 times higher in patients who were positive for NAb (odds ratio [OR], 4.55; 95% confidence interval [CI], 1.10-19.23). This was confirmed when analyzing with cut-off values for high titers (> 150 TRU/mL; OR, 5.99; 95% CI, 1.21-29.41; $P=.03$). Of the 11 patients with an increased relapse rate, 5 of 11 (45%) were NAb positive. Patients positive for NAb who did not show this increase in relapse rate were more frequently treated with mitoxantrone (6 of the remaining 12 NAb-positive patients).

In both NAb status groups, approximately half of the patients reached an EDSS score of 6.0 at the time of study assessment (Table 3). Cox regression analysis, correcting for EDSS at the start of interferon beta treatment, showed that patients positive for persisting NAb after interferon beta treatment withdrawal progress faster than patients who are NAb negative (hazard ratio [HR], 2.94; 95% CI, 1.20-7.14; $P=.02$; **Figure**). For patients who are high-titer NAb positive, a statistical trend was found (HR, 2.36; 95% CI, 0.86-6.49; $P=.10$).

COMMENT

This is the first study reporting that patients with anti-interferon beta NAb that persist after cessation of therapy have a more active disease course. This was indicated by an increase in the annualized relapse rate after interferon beta treatment withdrawal and faster progression to an EDSS score of 6.0. Furthermore, persisting NAb are associated with a different treatment regimen after interferon beta treatment withdrawal; fewer patients remained untreated and more patients switched to second-line treatment, especially mitoxantrone.

Anti-interferon beta NAb develop in a significant proportion of patients with MS who are treated with inter-

Table 3. Clinical Outcome Measures for Patients With and Without Persisting NAb After Cessation of Interferon Beta Treatment

Clinical Outcome Measures Related to NAb Status	NAb Negative, ≤20 TRU/mL (n=54)	NAb Positive, >20 TRU/mL (n=17)	Total (n=71)
Annualized relapse rate before interferon beta therapy, mean (SD)	0.7 (0.6)	1.0 (0.8)	0.8 (0.7)
Annualized relapse rate after interferon beta therapy, mean (SD)	0.3 (0.4) ^a	1.2 (1.6)	0.5 (0.9)
Annual relapse rate before/after interferon beta therapy, proportion (%)			
Decreased or stable	48 (89)	12 (71)	60 (85)
Increased	6 (11)	5 (29) ^b	11 (15)
Patients reaching EDSS score of 6, No. (%)	27 (50)	9 (53)	36 (51)
Time to EDSS score of 6, mean (SD), y	12.5 (1.3)	6.7 (1.0)	12.3 (6.5)

Abbreviations: EDSS, Extended Disability Status Scale; NAb, neutralizing antibodies; TRU/mL, 10-fold reduction units per milliliter.

^aIn NAb-negative patients there was a significant decrease in relapse rate after interferon beta treatment cessation compared with before treatment ($P < .001$).

^bThe proportion of patients with an increased relapse rate after cessation of interferon beta therapy is higher in NAb-positive patients when correcting for the use of mitoxantrone (odds ratio, 4.55; 95% confidence interval, 1.10-19.23).

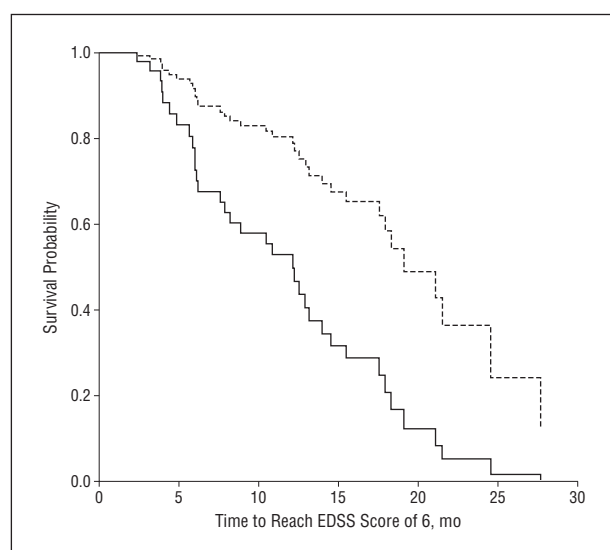


Figure. Survival probabilities for reaching an Expanded Disability Status Scale (EDSS) score of 6 in a Cox proportional hazards model corrected for the EDSS at the start of interferon beta treatment. The black continuous line represents the regression curve of patients positive for persisting neutralizing antibodies (NAb) after cessation of interferon beta treatment. The dashed line represents the regression curve of patients negative for persisting NAb. Patients positive for persisting NAb progress faster to an EDSS of 6.0 than patients who are NAb negative (hazard ratio, 2.94; 95% confidence interval, 1.20-7.14).

feron beta.²⁰⁻²² Median or low titers tend to disappear spontaneously in some patients despite continuous therapy, but high NAb titers persist and are clearly associated with a decrease in efficacy during treatment.⁸

In the absence of antigen exposure, NAb were expected to resolve within a few months of interferon beta treatment withdrawal. Surprisingly, 2 small studies described NAb persisting long after cessation of interferon beta therapy. One described NAb titers persisting in 2 patients up to 54 months after subcutaneous interferon beta-1b therapy was withdrawn.¹⁵ In a Danish retrospective follow-up study of 37 patients with MS, NAb were demonstrated after a mean follow-up of 22 months and up to 59 months.¹⁴ High-titer NAb tended to persist over time, as only 1 of 18 high-titer NAb-positive patients reverted to NAb negativity. Furthermore, the chance of reverting differed between interferon beta products. Simi-

lar to their results, we found a small percentage of patients with persisting NAb after cessation of interferon beta treatment. Most NAb-positive patients were high-titer NAb positive, and persisting high NAb titers were most frequently found with the use of interferon beta-1a subcutaneously.

How NAb are able to persist independently of antigen exposure remains unclear. Repeated antigen presentation (during therapy) can induce long-living plasma cells in the bone marrow that continue to secrete antibodies, outlasting the antigen challenge.²³ Alternatively, a cross-reaction between recombinant and endogenous interferon beta could be the cause of persisting NAb. Endogenous interferon beta produced in response to viral infections could maintain NAb production by activated B cells.²⁴

In this study, both the decision to stop interferon beta treatment and the treatment choice afterwards were made without any knowledge of NAb status, as NAb measurements were not performed in our clinic during that period. The results of this study clearly suggest that persisting NAb are associated with a more aggressive treatment strategy, as mitoxantrone, a treatment reserved for patients with aggressive inflammatory disease in our center, was prescribed significantly more often to patients who turned out to be positive for persisting NAb. However, it must be noted that most patients who discontinued interferon beta treatment because of perceived efficacy failure were not NAb positive. This suggests that treatment failure is often determined by factors other than the persistence of NAb. In our cohort, most NAb-negative patients were progressing during treatment without superimposed exacerbations and were therefore not eligible for alternative immunomodulatory treatment.

Another interesting finding of our study is that in NAb-positive patients who were treated with mitoxantrone, the immunosuppressive properties of this chemotherapeutic agent were not able to abolish persisting anti-interferon beta NAb. One suggested approach to treating NAb-positive patients who are taking interferon beta has been the induction of combination therapies with strong immunosuppressive compounds like mitoxantrone.²⁵ Our data suggest that this approach, at least for the use of mitoxantrone, is not very likely to be successful. This is in line with a recent study showing that treat-

ing NAb after interferon beta therapy withdrawal with monthly pulsed oral methylprednisolone for 6 months has no beneficial effect on NAb status or interferon beta bioactivity.²⁶

The mechanisms through which persisting NAb exert their effect on MS disease activity are unknown. However, anti-interferon beta antibodies probably have an effect on endogenous interferon pathways that may result in a more proinflammatory modification of the immune system and, subsequently, to an increase in MS disease activity. Alternatively, the tendency to develop and sustain anti-interferon beta NAb might be a reflection of a more active immune system. In our study, patients with persisting NAb were treated somewhat earlier and the pretreatment relapse rate was somewhat higher. This could support the hypothesis that these patients have more active disease owing to a previously more active immune system. However, although this alternative explanation cannot be fully excluded, it must be emphasized that there were no significant differences between NAb-positive and -negative patients for any of the clinical features at baseline, including disability status.

Obviously, the retrospective nature and the small sample of our study do not allow for definite conclusions to be drawn, and causality cannot be proven. On the other hand, for the purpose of this analysis, the retrospective nature also provides some strength because it guarantees that all clinical assessments and treatment decisions were not influenced by knowledge of Nab status. Altogether, our findings suggest that NAb that persist after treatment discontinuation may negatively influence the subsequent course of the disease and may require more aggressive treatment. Systematic long-term follow-up of patients exposed to NAb titers that persist after termination of interferon beta therapy is lacking and, given the possible effect on the MS disease course, conclusive studies on persisting anti-interferon beta NAb are warranted.

Accepted for Publication: April 24, 2009.

Published Online: February 8, 2010 (doi:10.1001/archneurol.2010.21).

Correspondence: Laura F. van der Voort, Department of Neurology, VU Medical Center, de Boelelaan 1117, PO Box 7057, 1007 MB Amsterdam (laura.vandervoort@vumc.nl).

Author Contributions: *Study concept and design:* van der Voort, Knol, Polman, and Killestein. *Acquisition of data:* van der Voort, Gilli, and Polman. *Analysis and interpretation of data:* van der Voort, Gilli, Bertolotto, Knol, Uitdehaag, Polman, and Killestein. *Drafting of the manuscript:* van der Voort, Gilli, Knol, and Polman. *Critical revision of the manuscript for important intellectual content:* van der Voort, Gilli, Bertolotto, Knol, Uitdehaag, Polman, and Killestein. *Statistical analysis:* van der Voort, Knol, and Uitdehaag. *Obtained funding:* Bertolotto and Killestein. *Administrative, technical, and material support:* van der Voort and Gilli. *Study supervision:* Gilli, Knol, Polman, and Killestein.

Financial Disclosure: Drs van der Voort, Killestein, and Uitdehaag report being involved in clinical trials of companies that market drugs for multiple sclerosis (Schering AG, Biogen Idec, Serono, Teva) and working with com-

panies that have development programs for future drugs for multiple sclerosis. Dr Polman reports having received consulting fees from Actelion, Biogen Idec, Bayer Schering, Teva, Merck Serono, Novartis, GlaxoSmithKline, Union Chimique Belge, Roche, and Antisense Therapeutics; lecture fees from Biogen Idec, Schering AG, Novartis, and Teva; and grant support from Biogen Idec, Bayer Schering, GlaxoSmithKline, Novartis, UCB, Merck-Serono, and Teva. Dr Gilli reports being reimbursed by Schering, Merck Serono, Sanofi-Aventis, and Biogen Dompè for attending several conferences, and for receiving fees for speaking by Biogen Dompè and Biogen Idec. Dr Bertolotto reports being reimbursed by Schering, Merck Serono, Sanofi-Aventis, and Biogen Dompè for attending several conferences, receiving fees for speaking by Biogen Dompè and Biogen, and receiving funds for research and for members of the staff by Merck Serono, Schering, Sanofi-Aventis, and Biogen Dompè. Dr Knol reports no conflicts of interest.

Funding/Support: This study was supported in part by Neutralizing Antibodies in Multiple Sclerosis, a specific targeted research project on neutralizing antibodies to interferon beta in multiple sclerosis, established by the European Commission under its 6th Framework Programme (contract No. 018926).

Role of the Sponsor: The Neutralizing Antibodies in Multiple Sclerosis group played no role in study design, collection of data, analysis, interpretation, writing of the article or the decision to submit.

Additional Contributions: We would like to thank all participating patients for their cooperation. Furthermore, we thank Marzia Caldano, MPharmD, of 'Centro di Riferimento Regionale Sclerosi Multipla and Neurobiologia Clinica' in Orbassano for performing the neutralizing antibody assays of this study.

REFERENCES

1. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. *N Engl J Med*. 2000;343(13):938-952.
2. The IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis I: clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology*. 1993;43(4):655-661.
3. PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) Study Group. Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. *Lancet*. 1998;352(9139):1498-1504.
4. Jacobs LD, Cookfair DL, Rudick RA, et al; The Multiple Sclerosis Collaborative Research Group (MSCRG). Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. *Ann Neurol*. 1996;39(3):285-294.
5. Ross C, Clemmesen KM, Svenson M, et al; Danish Multiple Sclerosis Study Group. Immunogenicity of interferon-beta in multiple sclerosis patients: influence of preparation, dosage, dose frequency, and route of administration. *Ann Neurol*. 2000;48(5):706-712.
6. Schellekens H. Bioequivalence and the immunogenicity of biopharmaceuticals. *Nat Rev Drug Discov*. 2002;1(6):457-462.
7. Shinohara K, Mitani N, Miyazaki M, et al. Pure red-cell aplasia caused by the antibody to recombinant erythropoietin, epoetin-beta, in a Japanese patient with chronic renal failure. *Am J Hematol*. 2005;78(1):15-20.
8. Bertolotto A, Gilli F, Sala A, et al. Persistent neutralizing antibodies abolish the interferon beta bioavailability in MS patients. *Neurology*. 2003;60(4):634-639.
9. Phillips JT, O'Connor PW, Havrdova E, et al; AFFIRM Investigators. Infusion-related hypersensitivity reactions during natalizumab treatment. *Neurology*. 2006;67(9):1717-1718.
10. Goodin DS, Frohman EM, Hurwitz B, et al. Neutralizing antibodies to interferon beta: assessment of their clinical and radiographic impact: an evidence report: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology*. 2007;68(13):977-984.

11. Kappos L, Clanet M, Sandberg-Wollheim M, et al; European Interferon Beta-1a IM Dose-Comparison Study Investigators. Neutralizing antibodies and efficacy of interferon beta-1a: a 4-year controlled study. *Neurology*. 2005;65(1):40-47.
12. Sorensen PS. Neutralising antibodies to interferon-beta: measurement, clinical relevance, and management. *J Neurol*. 2006;253(suppl 6):vi16-vi22.
13. van der Voort LF, Kok A, Visser A, et al. Interferon-beta bioactivity measurement in multiple sclerosis: feasibility for routine clinical practice. *Mult Scler*. 2009;15(2):212-218.
14. Petersen B, Bendtzen K, Koch-Henriksen N, Ravnborg M, Ross C, Sorensen PS; Danish Multiple Sclerosis Group. Persistence of neutralizing antibodies after discontinuation of IFNbeta therapy in patients with relapsing-remitting multiple sclerosis. *Mult Scler*. 2006;12(3):247-252.
15. Reske D, Walser A, Haupt WF, Peterleit HF. Long-term persisting interferon beta-1b neutralizing antibodies after discontinuation of treatment. *Acta Neurol Scand*. 2004;109(1):66-70.
16. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983;33(11):1444-1452.
17. 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(9):743-755.
18. Kawade Y, Finter N, Grossberg SE. Neutralization of the biological activity of cytokines and other protein effectors by antibody: theoretical formulation of antibody titration curves in relation to antibody affinity. *J Immunol Methods*. 2003;278(1-2):127-144.
19. Sominanda A, Hillert J, Fogdell-Hahn A. In vivo bioactivity of interferon-beta in multiple sclerosis patients with neutralising antibodies is titre-dependent. *J Neurol Neurosurg Psychiatry*. 2008;79(1):57-62.
20. Deisenhammer F, Schellekens H, Bertolotto A. Measurement of neutralizing antibodies to interferon beta in patients with multiple sclerosis. *J Neurol*. 2004;251(suppl 2):II31-II39.
21. Hartung HP, Polman C, Bertolotto A, et al. Neutralising antibodies to interferon beta in multiple sclerosis: expert panel report. *J Neurol*. 2007;254(7):827-837.
22. Sorensen PS, Koch-Henriksen N, Ross C, Clemmesen KM, Bendtzen K; Danish Multiple Sclerosis Study Group. Appearance and disappearance of neutralizing antibodies during interferon-beta therapy. *Neurology*. 2005;65(1):33-39.
23. Manz RA, Arce S, Cassese G, Hauser AE, Hiepe F, Radbruch A. Humoral immunity and long-lived plasma cells. *Curr Opin Immunol*. 2002;14(4):517-521.
24. Maruyama M, Lam KP, Rajewsky K. Memory B-cell persistence is independent of persisting immunizing antigen. *Nature*. 2000;407(6804):636-642.
25. Giovannoni G. Strategies to treat and prevent the development of neutralizing anti-interferon-beta antibodies. *Neurology*. 2003;61(9)(suppl 5):S13-S17.
26. Hesse D, Frederiksen JL, Koch-Henriksen N, et al. Methylprednisolone does not restore biological response in multiple sclerosis patients with neutralizing antibodies against interferon-beta. *Eur J Neurol*. 2009;16(1):43-47.