

# Neutralizing Antibodies to Multiple Sclerosis Treatments

HOWARD S. ROSSMAN, DO, FACN

## ABSTRACT

**OBJECTIVE:** This article reviews the incidence and clinical significance of neutralizing antibodies (NABs) in patients with multiple sclerosis (MS) undergoing treatment with interferon beta (IFN $\beta$ ). Implications for practice are also discussed in light of the currently available data on the clinical consequences of NABs in patients with MS.

**SUMMARY:** As with other recombinant protein drugs used for the treatment of a number of diseases, antibodies commonly develop to IFN $\beta$  products during the treatment of patients with MS. Neutralizing antibodies (NABs) are a subset of antibodies that reduce or diminish the biologic activity of IFN $\beta$ . Three formulations of IFN $\beta$  are currently available for the treatment of relapsing-remitting MS: IFN $\beta$ -1b (Betaseron), intramuscular (IM) IFN $\beta$ -1a (Avonex), and subcutaneous (SC) IFN $\beta$ -1a (Rebif). Individual phase III clinical trials and direct comparison studies have shown that NABs develop more frequently during treatment with IFN $\beta$ -1b than IFN $\beta$ -1a and that between the 2 IFN $\beta$ -1a products, NABs develop more frequently during treatment with SC IFN $\beta$ -1a than IM IFN $\beta$ -1a. Data from clinical trials of IFN $\beta$  products indicate that clinical efficacy of IFN $\beta$  is reduced in NAB-positive patients.

**CONCLUSION:** In light of these data, the immunogenicity of IFN $\beta$  products should be considered prior to initiating treatment with IFN $\beta$ . Also, ongoing laboratory monitoring of patients treated with higher-dose IFN $\beta$  is recommended for early detection of NABs.

**KEYWORDS:** Interferon-beta, Neutralizing antibodies, Multiple sclerosis

*J Manag Care Pharm.* 2004;10(3)(suppl S-b):S12-S18

Therapeutic use of protein products is frequently associated with antibody development. Antibodies develop, albeit at a reduced rate, even with the widespread use of recombinant DNA technology to produce protein drugs that are nearly identical to their endogenous human counterparts. Antibodies to biotherapeutic agents are broadly classified into binding antibodies and neutralizing antibodies (NABs). Binding antibodies include all antibodies that can bind to the drug (and may or may not inhibit the drug), whereas NABs are a subset of binding antibodies that can inhibit or neutralize the biologic activity of the protein drug.<sup>1</sup>

NABs have been shown to develop following treatment with a variety of recombinant human protein drugs, including insulin, erythropoietin, and coagulation factor VIII (Table 1).<sup>1-6</sup> In most cases, the main clinical outcome of NABs is loss of efficacy of the protein drug. For example, the use of interferon alpha (IFN $\alpha$ ) for the treatment of hepatitis or cancer has been associated with NAB development resulting in nonresponsiveness to treatment, disease reactivation, and decreased response duration.<sup>7-10</sup> Similarly, in patients treated for cervical dystonia with botulinum toxin type A, the development of NABs rendered the treatment ineffective.<sup>11</sup>

In addition to reduced efficacy, more severe clinical effects may be observed when NABs form against a protein that has an impor-

**TABLE 1** Clinical Consequences of Antibodies to Various Biotherapeutic Agents

Disease/Condition	Consequence of Antibody	Biotherapeutic Agent	Reference
Diabetes	↓	Insulin	Meager <sup>1</sup> ; Fineberg et al. <sup>2</sup>
Acute myocardial infarction		Streptokinase	Rosenschein et al. <sup>41</sup>
		Staphylokinase	Vanderschueren et al. <sup>42</sup>
Adenosine deaminase deficiency		Adenosine deamidase	Chaffee et al. <sup>43</sup>
Cervical dystonia		Botulinum toxin	Rollnik et al. <sup>11</sup>
Hemophilia A		Factor VIII	Lusher; 2000 <sup>5</sup>
Malignant carcinoid tumors		Interferon alpha-2	Freund et al. <sup>7</sup> ; Quesada et al. <sup>10</sup>
Multiple sclerosis		Interferon beta	IFNB MS Study Group. <sup>34</sup> ; PRISMS Study Group. <sup>20</sup> ; Sorensen et al. <sup>35</sup>
Cancer		Interleukin-2 (IL-2)	Prümmer. <sup>44</sup>
Hypogonadotropic azoospermic men		Gonadotropin-releasing hormone	Blumenfeld et al. <sup>45</sup>
Cutaneous T-cell lymphoma		Denileukin difitox	Olsen et al. <sup>46</sup>
Hypogonadotropic hypogonadism		Human chorionic gonadotropin	Claustrat et al. <sup>47</sup>
Carcinoma		GM-CSF*/IL-3	Ragnhammar and Wadhwa. <sup>48</sup>
Anemia		Neutralization of native protein	Erythropoietin

\* GM-CSF = granulocyte-macrophage colony-stimulating factor.

## Author

HOWARD S. ROSSMAN, DO, FACN, is medical director, Multiple Sclerosis Center, Michigan Institute For Neurological Disorders, Farmington Hills.

**AUTHOR CORRESPONDENCE:** Howard S. Rossman, DO, FACN, Medical Director, Multiple Sclerosis Center, Michigan Institute For Neurological Disorders, 28595 Orchard Lake Rd., Suite 200, Farmington Hills, MI 48334. Tel: (248) 553-0010; Fax: (248) 702-0201; E-mail: hsrossman@aol.com

Copyright© 2004, Academy of Managed Care Pharmacy. All rights reserved.

tant physiologic function. An example of the latter is formation of NAbs to epoetin, a recombinant human erythropoietin that is used for the treatment of anemia in various clinical settings. In a recent collection of case reports, it was reported that, in 13 patients with chronic renal failure treated with epoetin, the formation of NAbs to epoetin was associated with development of red-cell aplasia, a condition that can render a patient transfusion-dependent.<sup>4</sup> Presumably, the NAbs were able to neutralize not only epoetin but also the endogenous erythropoietin in these patients.

NAbs can also develop during treatment with IFN-beta (IFNβ) products, which are first-line therapy for the treatment of patients with multiple sclerosis (MS).<sup>12-14</sup> Three different IFNβ products are currently approved for the treatment of MS in the United States: IFNβ-1b (Betaseron, Berlex Laboratories, Montville, NJ), intramuscular (IM) IFNβ-1a (IM IFNβ-1a [Avonex, Biogen Idec Inc., Cambridge, MA]), and subcutaneous (SC) IFNβ-1a (SC IFNβ-1a [Rebif, Serono, Rockland, MA]). This paper reviews the incidence of NAbs development to IFNβ products during the treatment of MS, some of the factors thought to contribute to the development of NAbs, and implications of NAbs in the care of patients with MS.

### Incidence of Neutralizing Antibodies to Interferon Beta

The incidence of NAbs (titer ≥20) among IFNβ products varies widely, as reported in phase III trials (Table 2). In the pivotal phase III trial of IFNβ-1b, 47% of patients who received IFNβ-1b 1.6 million international units (MIU) and 45% of patients who received 8 MIU SC every other day developed NAbs.<sup>12</sup> In a study conducted by the European Study Group on IFNβ-1b in secondary progressive MS, approximately 28% of patients (100 of 360) who received SC IFNβ-1b 8 MIU every other day tested positive for NAbs at some time during the study, with most patients becoming NAb positive in the first 6 months of treatment.<sup>15</sup>

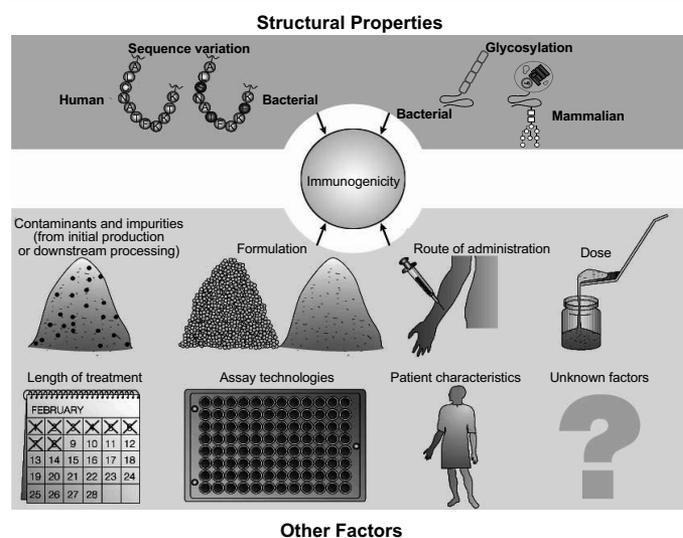
Phase III studies with IM IFNβ-1a have shown consistently lower levels of immunogenicity, with incidences of NAbs ranging from 2% to 5.8%.<sup>16-18</sup> Interestingly, in the earlier pivotal phase III trial of IM IFNβ-1a, NAbs were detected in 14% of patients treated with IM IFNβ-1a 30 mcg once weekly at week 52, 21% at week 78, and 22% at week 104.<sup>13</sup> The reduction in immunogenicity of IM IFNβ-1a after the pivotal phase III trial is thought to be due to improvements in manufacturing, purification, and storage processes of the now commercially available IM IFNβ-1a product.<sup>19</sup> In the pivotal phase III trial of SC IFNβ-1a (Prevention of Relapses and Disability by Interferon β-1a Subcutaneously in Multiple Sclerosis [PRISMS] study), NAbs were observed in 23.8% of patients who received SC IFNβ-1a 22 mcg and 12.5% of patients who received SC IFNβ-1a 44 mcg.<sup>14</sup> In PRISMS-4, which was the extension study of the phase III trial, 23.7% of patients who received SC IFNβ-1a 22 mcg and 14.3% of patients who received SC IFNβ-1a 44 mcg had a positive test result for NAbs.<sup>20</sup> However, in patients who had been on placebo during the first 2 years of the trial and received SC IFNβ-1a 22 mcg or 44 mcg during years 3 and 4, the incidence of NAbs was 27.7%

**TABLE 2** Incidence of Neutralizing Antibodies (NABs; Titer ≥20) in Phase III Clinical Trials of Interferon Beta (IFNβ)

IFNβ Product	Dose and Frequency of Administration	Incidence of NABs (% of Patients)	Reference
IFNβ-1b (Betaseron)	SC* 3.5 times weekly		
	1.6 MIU†	47.0	IFNB MS Study Group <sup>12</sup>
	8 MIU	45.0	
SC IFNβ-1a (Rebif)	8 MIU	27.8	European Study Group <sup>15</sup>
	SC 3 times weekly		
	22 mcg	23.8	PRISMS Study Group <sup>14</sup>
	44 mcg	12.5	
	44 mcg	20.6	SPECTRIMS Study Group <sup>20</sup>
IM‡ IFNβ-1a (Avonex)	44 mcg	14.7	Panitch et al. <sup>24</sup>
	IM once weekly		
	30 mcg	22.0	Jacobs et al. <sup>13</sup>
	30 mcg	2.0	Jacobs et al. <sup>18</sup>
	30 mcg	2.3	Clanet et al. <sup>17</sup>
	60 mcg	5.8	
60 mcg	3.3	Cohen et al. <sup>16</sup>	
30 mcg	2.1	Panitch et al. <sup>24</sup>	

\* SC = subcutaneously. † MIU = million international units. ‡ IM = intramuscularly.

**FIGURE 1** Factors Affecting Immunogenicity of Biotherapeutic Agents



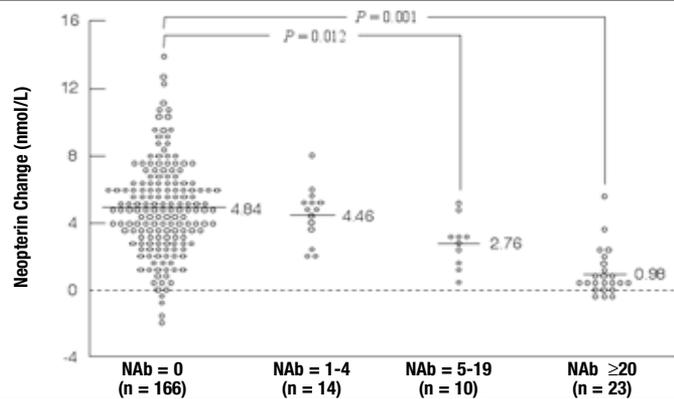
The ability of a biologic protein product to trigger formation of antibodies is influenced by its structural properties as well as other factors, including formulation, presence of contaminants and impurities, route of administration, length of treatment, and dose.

Source: Adapted with permission from Schellekens H. Bioequivalence and the immunogenicity of biopharmaceuticals. *Nat Rev Drug Discov.* 2002;1:457-62.<sup>1</sup>

and 24.4%, respectively.<sup>21</sup>

An evaluation of the relative immunogenicity of the different IFNβ products by comparing incidences among phase III trials is not ideal because of differences in the methods used for detecting and reporting NAbs.<sup>22</sup> However, results from individual phase III

**FIGURE 2** Change in Neopterin Induction After IM Interferon Beta-1a (Avonex) Injections by Neutralizing Antibody (NAb) Titer Status



Source: Reprinted with permission from Rudick RA, Simonian NA, Alam JA, et al. Incidence and significance of neutralizing antibodies to interferon beta-1a in multiple sclerosis. *Neurology*. 1998;50:1266-72.<sup>19</sup>

trials of each IFN $\beta$  product are consistent with data from studies that have directly compared IFN $\beta$  products using the same criteria and methods.<sup>22-25</sup>

For example, Bertolotto et al. conducted a study to directly compare the immunogenicity of the 3 IFN $\beta$  products.<sup>22</sup> Patients with relapsing-remitting MS were treated with SC IFN $\beta$ -1b 8 MIU every other day (n = 29), IM IFN $\beta$ -1a 30 mcg once weekly (n = 44), or SC IFN $\beta$ -1a 22 mcg 3 times weekly (n = 36).<sup>22</sup> Patients were screened for the presence of NAbs at baseline and every 3 months for up to 18 months. The risk of becoming persistent NAb-positive (i.e.,  $\geq 2$  consecutive NAb samples with a titer  $\geq 20$ ) during 18 months of treatment was 31% for patients who received IFN $\beta$ -1b, 15% for patients who received SC IFN $\beta$ -1a, and 2% for patients who received IM IFN $\beta$ -1a (IM IFN $\beta$ -1a versus IFN $\beta$ -1b,  $P = 0.001$ ; IM versus SC IFN $\beta$ -1a,  $P = 0.04$ ); no significant difference in the incidence of NAbs was noted between SC IFN $\beta$ -1a and IFN $\beta$ -1b.<sup>22</sup>

The European North American Comparative Efficacy study, Evidence of Interferon Dose-response (EVIDENCE), compared SC IFN $\beta$ -1a (prefilled syringe) 44 mcg 3 times weekly with IM IFN $\beta$ -1a 30 mcg once weekly in patients with relapsing-remitting MS.<sup>24</sup> NAbs were measured in sera collected after 48 weeks of treatment. Results showed that 25% of patients (84 of 335) who received SC IFN $\beta$ -1a were positive for NAbs (titer  $\geq 20$ ), compared with 2% in the IM IFN $\beta$ -1a group.

In summary, available data show that there are differences in immunogenicity among IFN $\beta$  products. Individual phase III clinical studies and direct comparison studies have shown that 28% to 47% of patients develop NAbs to IFN $\beta$ -1b, 12% to 25% to SC IFN $\beta$ -1a, and 2% to 6% to the commercially available formulation of IM IFN $\beta$ -1a. The incidence of NAbs in the U.S. Food and Drug Administration-approved package insert for each product is 45% for IFN $\beta$ -1b, 24% for SC IFN $\beta$ -1a, and 5% for IM IFN $\beta$ -1a.<sup>26-28</sup>

### Factors Affecting Immunogenicity of Interferon Beta Products

All 3 IFN $\beta$  products are recombinant protein drugs. Some factors affecting the immunogenicity of IFN $\beta$  products are shown in Figure 1.<sup>1</sup> These include the protein sequence and molecular structure of the drug, manufacturing and storage conditions, and route and frequency of administration. Unlike IFN $\beta$ -1a formulations, which are both identical in protein sequence to the natural human IFN $\beta$ , IFN $\beta$ -1b has a serine-to-cysteine substitution at position 17 and a deletion of the N-terminal methionine residue.<sup>29</sup> Also, IFN $\beta$ -1b is produced in *Escherichia coli* bacteria, whereas IM IFN $\beta$ -1a and SC IFN $\beta$ -1a are produced in mammalian cells (Chinese hamster ovarian cells). Because it is produced in bacterial cells, IFN $\beta$ -1b is not glycosylated. The lack of glycosylation is thought to result in an increased tendency of IFN $\beta$ -1b to form aggregates that can trigger the formation of antibodies.<sup>30</sup> The differences in protein sequence and molecular structure between IFN $\beta$ -1b and IFN $\beta$ -1a likely account for the observed greater incidence of NAbs with IFN $\beta$ -1b compared with either of the IFN $\beta$ -1a products.

Given that the 2 IFN $\beta$ -1a formulations have an identical protein sequence, differences in their immunogenicity are likely due to manufacturing, purification, and storage conditions. It is conceivable that differences in these conditions can lead to the production of recombinant proteins with different glycosylation patterns. Also, under differing conditions, oxidation and deamidation of amino acids may vary. Indeed, in the case of IM IFN $\beta$ -1a, improving the manufacturing process resulted in a less immunogenic product. Other factors affecting the immunogenicity of IFN $\beta$  may include dose, route of administration, length of treatment, and frequency of administration. Both IFN $\beta$ -1b and SC IFN $\beta$ -1a are administered 3 to 3.5 times weekly by SC injection. In contrast, IM IFN $\beta$ -1a is administered once weekly by IM injection.

Although Ross et al. showed that SC administration of IFN $\beta$  is more immunogenic than IM administration,<sup>25</sup> Bertolotto et al. showed no differences in immunogenicity between SC and IM administration of IFN $\beta$ .<sup>22</sup> Hence, the route of administration is a potential factor influencing immunogenicity.

### Impact of Neutralizing Antibodies on Biologic Activity and Clinical Efficacy of Interferon Beta Products

#### Biologic Activity

In general, NAbs are detected based on their ability to diminish the biologic activity of IFN $\beta$  in vitro. The ability of NAbs to interfere with the in vivo biologic activity of IFN $\beta$  has also been demonstrated.<sup>18,31-33</sup> Typically, the biologic activity of IFN $\beta$  is determined by measuring levels of biologic markers of IFN $\beta$  activity, such as neopterin, myxovirus resistance protein A (MxA), and  $\beta_2$ -microglobulin.

Measurements of serum neopterin and  $\beta_2$ -microglobulin levels before and 48 hours after IM IFN $\beta$ -1a injection were conducted in

the open-label, safety-extension study of the pivotal phase III trial of IM IFN $\beta$ -1a.<sup>18</sup> The levels of serum NAb titers correlated with measurements of neopterin levels (Figure 2), such that the mean increase in neopterin level after IFN $\beta$ -1a injection was significantly lower in NAb-positive patients (titer 5 to 19 and titer  $\geq 20$ ) compared with NAb-negative patients ( $P = 0.012$  and  $P = 0.001$ , respectively). Similar results were also observed with serum levels of  $\beta_2$ -microglobulin.<sup>18</sup>

Another study used MxA as a marker for the biologic activity of IFN $\beta$  in patients with MS who received IFN $\beta$ -1b and a healthy control group.<sup>33</sup> MxA levels were significantly lower in NAb-positive patients compared with NAb-negative patients ( $P < 0.001$ ). Furthermore, the levels of MxA in NAb-positive patients were similar to those of the untreated healthy control group, suggesting that the biologic activity of IFN $\beta$ -1b in these patients was completely inhibited by NABs. Inhibition of MxA expression by NABs has also been reported at the level of messenger ribonucleic acid (mRNA), with MxA mRNA levels being significantly lower in persistent NAb-positive ( $\geq 2$  consecutive positive samples titer  $\geq 20$ ) patients compared with NAb-negative ( $P < 0.0001$ ) and isolated NAb-positive (1 positive titer  $\geq 20$ ) patients ( $P < 0.005$ ).<sup>31</sup>

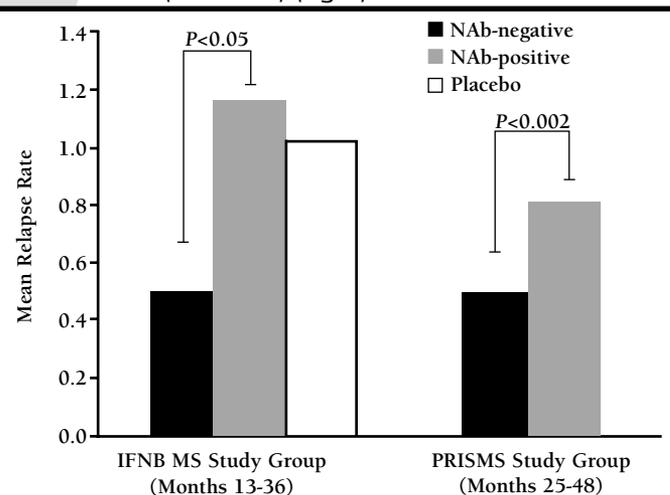
### Clinical Efficacy

NABs have been shown to reduce the clinical efficacy of IFN $\beta$  in MS patients, based on increased relapse rate and lesion activity on magnetic resonance imaging (MRI). In the pivotal phase III trial of IFN $\beta$ -1b, the mean relapse rate during 18 to 36 months of treatment was significantly greater in NAb-positive patients (1.16 per year) compared with NAb-negative patients (0.50 per year) (Figure 3). In fact, the relapse rate in NAb-positive patients was similar to that observed in patients given placebo (1.02 per year,  $P < 0.05$ ).<sup>34</sup> Similarly, in the 2-year extension study of the phase III trial of SC IFN $\beta$ -1a (PRISMS-4), in patients treated with SC IFN $\beta$ -1a 44 mcg, NAb-positive patients experienced a significantly higher mean relapse rate during years 3 and 4 (0.81) compared with NAb-negative patients (0.50,  $P = 0.002$ ).<sup>19</sup>

In addition to data from phase III studies of IFN $\beta$ , a recent study by the Danish Multiple Sclerosis Study Group also provides evidence of diminished clinical efficacy of IFN $\beta$  in NAb-positive patients.<sup>35</sup> The study involved 541 patients randomly selected from all patients in Denmark who started treatment with IFN $\beta$  between 1996 and 1999. Yearly measurements of NABs in these patients revealed that relapse rates were significantly higher during NAb-positive periods (0.64 to 0.70) than they were during NAb-negative periods (0.43 to 0.46,  $P < 0.03$ ). Furthermore, the proportion of relapse-free patients was significantly lower ( $P = 0.0064$ ), and the median time to first relapse was significantly reduced (by 244 days; Kaplan-Meier analysis, log rank test 6.83,  $P = 0.009$ ) in NAb-positive patients compared with NAb-negative patients.<sup>35</sup>

Decreased efficacy of IFN $\beta$  due to NABs has also been noted

**FIGURE 3** Effect of Neutralizing Antibodies (NABs) on Mean Relapse Rates in the Phase III Trial of IFN $\beta$ -1b (left) and the Extension of the Phase III Trial of SC IFN $\beta$ -1a (Rebif) (PRISMS-4) (right)<sup>20,34</sup>

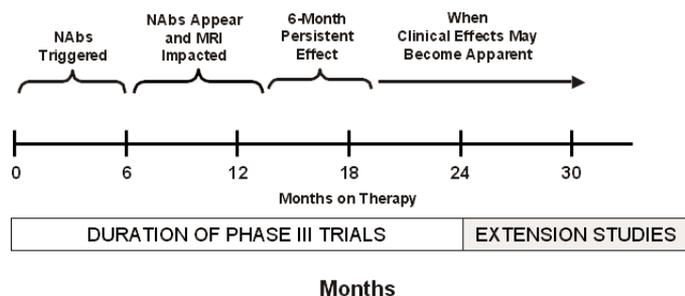


**TABLE 3** Decrease in Efficacy of SC IFN $\beta$ -1a (Rebif) 44 mcg in the Presence of Neutralizing Antibodies (NABs)<sup>20</sup>

	NAB-	NAB+	P Value
Number of T2 active lesions	0.3	1.4	<0.001
Burden of disease	-8.5%	+17.6%	<0.001

using MRI measures.<sup>20,34</sup> Patients who developed NABs to IFN $\beta$ -1b had significantly more enlarging T2 lesions than NAb-negative patients (0.41 versus 0.19 between years 1 and 2 [ $P \leq 0.03$ ], 0.589 versus 0.26 between years 3 and 4 [ $P = 0.01$ ]). Furthermore, NAb-positive patients also showed an increased tendency to form new lesions (mean values of 1.03 in NAb-positive patients versus 0.40 in NAb-negative patients,  $P = 0.067$ ).<sup>34</sup>

Data from the open-label extension study of the pivotal phase III trial of SC IFN $\beta$ -1a (PRISMS-4) provide even stronger evidence of diminished therapeutic effects of IFN $\beta$  on MRI due to NABs (Table 3).<sup>20</sup> After 4 years of treatment with SC IFN $\beta$ -1a, disease burden on MRI was decreased by 8.5% from baseline in NAb-negative patients compared with a 17.6% increase in disease burden in NAb-positive patients ( $P < 0.001$ ). The values in NAb-positive patients approached those of patients treated with placebo during the first 2 years of the study.<sup>20,28</sup> Furthermore, the median number of T2 active lesions was 1.4 in NAb-positive patients compared with 0.3 in NAb-negative patients ( $P < 0.001$ ).<sup>20</sup> Although only a small number of patients develop NABs to IM IFN $\beta$ -1a, results in these patients are similar to those noted with IFN $\beta$ -1b and SC IFN $\beta$ -1a. Patients who developed NABs to IM IFN $\beta$ -1a have

**FIGURE 4** Time Course for Development and Clinical Consequences of Neutralizing Antibodies (NAbs)

*In MS patients undergoing treatment with IFN $\beta$ , NAbs can be detected as early as 3 months. Once formed, the effect of NAbs on MRI measures of disease burden is apparent by 12 months. The effect of NAbs on clinical outcomes is not apparent until after 18 to 24 months of treatment.*

been shown to have an increased number of gadolinium-enhanced lesions on MRI compared with NAb-negative patients (mean values of 1.7 versus 0.6;  $P = 0.062$ ). The number of lesions in NAb-positive patients were similar to that seen in patients who received placebo (mean of 1.6 lesions).<sup>19</sup>

### Temporal Aspects of the Development of Neutralizing Antibodies to Interferon Beta

The time course of the development of NAbs is an important facet of monitoring and assessing the clinical effects of NAbs during treatment with IFN $\beta$ . In general, NAbs become detectable at any time between 3 and 18 months following initiation of treatment with IFN $\beta$  (Figure 4).<sup>19,22,34,36,37</sup> An 18-month study that compared the immunogenicity of the 3 IFN $\beta$  products showed that, although patients continued to develop NAbs to each of the 3 products throughout the study period, 76% developed NAbs during the first 9 months and a further 14% developed NAbs by 12 months. Thus, 90% of the NAb-positive patients in the study developed NAbs during the first year of treatment with IFN $\beta$ .<sup>22</sup> Results from clinical trials indicate that in NAb-positive patients undergoing treatment with IFN $\beta$ , the effects of NAbs on MRI measures of disease burden become apparent at approximately 1 year and effects on clinical outcomes after 18 to 24 months of treatment.<sup>20,34</sup> Thus, short-term studies (<2 years) cannot adequately assess the impact of NAbs on the clinical efficacy of IFN $\beta$ .

An unresolved question with regard to the clinical relevance of NAbs is how long NAbs persist once they are formed. Available data indicate that once formed, NAbs can persist for several years.<sup>34,38</sup> In a recent study by the Danish Multiple Sclerosis Study Group, 45% of patients were NAb-positive to IFN $\beta$ -1b at 1 year, 35% at 3 years, and 28% at 4 years.<sup>35</sup> Thus, approximately 80% of patients who were NAb-positive to IFN $\beta$ -1b remained positive

over 3 years and approximately 70% remained NAb-positive over 4 years. However, these data are difficult to interpret because a large proportion of patients dropped out of the study, and no information was provided regarding the numbers of NAb-positive and NAb-negative patients who discontinued the study.<sup>35</sup> There is evidence that the persistence of NAbs is dependent on both NAb titer (higher-titer NAbs persist longer) and IFN $\beta$  product.<sup>39</sup>

### Implications for Practice

The Consortium of Multiple Sclerosis Centers recently published a list of consensus statements (>70% agreement) regarding the issue of NAbs to IFN $\beta$  in patients with MS; this list was developed based on the opinions of 33 researchers in the area of NAbs.<sup>40</sup> Of note, this group of experts believes that NAbs should be one of the factors that clinicians consider in the ongoing management of MS patients and that future studies should be conducted to determine how best to counteract NAbs.<sup>40</sup> Specific recommendations for NAb testing and the management of NAb-positive patients are provided in the article in this supplement by Sheldon J. Rich et al.

As discussed in the preceding sections, data from a number of clinical studies have shown that NAbs can develop in MS patients undergoing treatment with IFN $\beta$ , but there is no way to predict which patients will develop NAbs. Key evidence from these studies that should be considered when making treatment decisions relating to IFN $\beta$  treatment in MS patients include the following:

- NAbs (titers  $\geq 20$ ) can reduce the bioavailability and clinical efficacy of IFN $\beta$ .
- The incidence of NAbs varies with the 3 IFN $\beta$  preparations. IFN $\beta$ -1b treatment is more immunogenic than IFN $\beta$ -1a treatment, and between the 2 IFN $\beta$ -1a products, SC IFN $\beta$ -1a treatment is more immunogenic than IM IFN $\beta$ -1a treatment. Prior to initiating treatment with an IFN $\beta$  product, these differences in immunogenicity of IFN $\beta$  products should be considered.
- IFN $\beta$ -treated patients who experience worsening in clinical status should be tested for the presence of NAbs. For patients who have a positive test result for NAbs, switching to another IFN $\beta$  product is not recommended because antibodies are cross-reactive among IFN $\beta$ s.<sup>37</sup>
- Given that the clinical effects of NAbs are manifested several months after they develop, ongoing monitoring and early detection of NAbs in patients at higher risk (i.e., those on higher-dosing, more frequently administered, SC IFN $\beta$ s) will likely improve the quality of treatment received by MS patients undergoing treatment with IFN $\beta$ .

### Conclusions

Data from a number of clinical trials indicate that NAbs can reduce the therapeutic benefits of IFN $\beta$  treatment in patients with MS. Loss of clinical efficacy has been observed in these studies in the form of increased relapse rates and disease burden on MRI in NAb-positive patients. Another important issue is the persistence of NAbs once they are formed. Available data indicate that once

formed, NAbs tend to persist for several years. In light of these data, the immunogenicity of IFN $\beta$  products should be considered prior to initiating treatment with IFN $\beta$ . Also, ongoing laboratory monitoring of patients treated with higher-dose IFN $\beta$  is recommended for early detection of NAbs.

### DISCLOSURES

Funding for this paper was provided by Biogen Idec Inc. Author Howard S. Rossman received an honorarium from Biogen. He is a consultant and speaker for Biogen, Teva Neuroscience, and Serono, Inc., and has received compensation for clinical research from these companies.

### REFERENCES

1. Schellekens H. Bioequivalence and the immunogenicity of biopharmaceuticals. *Nat Rev Drug Discov.* 2002;1:457-62.
2. Fineberg SE, Galloway JA, Fineberg NS, Rathbun MJ, Hufferd S. Immunogenicity of recombinant DNA human insulin. *Diabetologia.* 1983;25:465-69.
3. Meager A. Human antibodies to insulin in diabetes. *J Interferon Res.* 1994;14:181-82.
4. Casadevall N, Nataf J, Viron B, et al. Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. *N Engl J Med.* 2002;346:469-75.
5. Lusher JM. Inhibitor antibodies to factor VIII and factor IX: management. *Semin Thromb Hemost.* 2000;26:179-88.
6. Lusher JM, Arkin S, Abildgaard CF, Schwartz RS, and the Kogenate Previously Untreated Patient Study Group. Recombinant factor VIII for the treatment of previously untreated patients with hemophilia A. Safety, efficacy, and development of inhibitors. *N Engl J Med.* 1993;328:453-59.
7. Freund M, von Wussow P, Diedrich H, et al. Recombinant human interferon (IFN) alpha-2b in chronic myelogenous leukemia: dose dependency of response and frequency of neutralizing anti-interferon antibodies. *Br J Haematol.* 1989;72:350-56.
8. Antonelli G, Giannelli G, Currenti M, et al. Antibodies to interferon (IFN) in hepatitis C patients relapsing while continuing recombinant IFN- $\alpha$ 2 therapy. *Clin Exp Immunol.* 1996;104:384-87.
9. Giannelli G, Antonelli G, Fera G, et al. Biological and clinical significance of neutralizing and binding antibodies to interferon-alpha (IFN-a) during therapy for chronic hepatitis C. *Clin Exp Immunol.* 1994;97:4-9.
10. Quesada JR, Rios A, Swanson D, Trown P, Gutterman JU. Antitumor activity of recombinant-derived interferon alpha in metastatic renal cell carcinoma. *J Clin Oncol.* 1985;3:1522-28.
11. Rollnik JD, Wohlfarth K, Dengler R, Bigalke H. Neutralizing botulinum toxin type A antibodies: clinical observations in patients with cervical dystonia. *Neurol Clin Neurophysiol.* 2001;3:2-4.
12. 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;43:655-61.
13. Jacobs LD, Cookfair DL, Rudick RA, et al, and the Multiple Sclerosis Collaborative Research Group (MSCRG). Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. *Ann Neurol.* 1996;39:285-94.
14. 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;352:1498-504.
15. European Study Group on Interferon  $\beta$ -1b in Secondary Progressive MS. Placebo-controlled multicentre randomised trial of interferon  $\beta$ -1b in treatment of secondary progressive multiple sclerosis. *Lancet.* 1998;352:1491-97.
16. Cohen JA, Cutter GR, Fischer JS, et al. for the IMPACT Investigators. Benefit of interferon  $\beta$ -1a on MSFC progression in secondary progressive MS. *Neurology.* 2002;59:679-87.
17. Clanet M, Radue EW, Kappos L, et al., and the European IFN $\beta$ -1a (Avonex) Dose-Comparison Study Investigators. A randomized, double-blind, dose-comparison study of weekly interferon  $\beta$ -1a in relapsing MS. *Neurology.* 2002;59:1507-17.
18. Jacobs LD, Beck RW, Simon JH, et al., and the Controlled high Risk Subjects AVONEX MS Prevention Study (CHAMPS) Study Group. Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. *N Engl J Med.* 2000;343:898-904.
19. Rudick RA, Simonian NA, Alam JA, et al. Incidence and significance of neutralizing antibodies to interferon beta-1a in multiple sclerosis. *Neurology.* 1998;50:1266-72.
20. The PRISMS (Prevention of Relapses and Disability by Interferon- $\beta$ -1a Subcutaneously in Multiple Sclerosis) Study Group and the University of British Columbia MS/MRI Analysis Group. PRISMS-4: Long-term efficacy of interferon- $\beta$ -1a in relapsing MS. *Neurology.* 2001;56:1628-36.
21. Rice GPA, Francis GS, and the PRISMS Study Group. Further evidence of dose effect of IFN beta-1a based on neutralizing antibody status. Poster presented at: The 11th Annual Meeting of the European Neurological Society; April 21-25, 2001; Paris, France.
22. Bertolotto A, Malucchi S, Sala A, et al. Differential effects of three interferon betas on neutralising antibodies in patients with multiple sclerosis: a follow up study in an independent laboratory. *J Neurol Neurosurg Psychiatry.* 2002;73:148-53.
23. Kivisäkk P, Alm GV, Fredrikson S, Link H. Neutralizing and binding anti-interferon- $\beta$  (IFN- $\beta$ ) antibodies. A comparison between IFN  $\beta$ -1a and IFN  $\beta$ -1b treatment in multiple sclerosis. *Eur J Neurol.* 2000;7:27-34.
24. Panitch H, Goodin DS, Francis G, et al. Randomized, comparative study of interferon  $\beta$ -1a treatment regimens in MS. The EVIDENCE Trial. *Neurology.* 2002;59:1496-506.
25. Ross C, Clemmesen KM, Svenson M, et al., and the 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;48:706-12.
26. Betaseron (interferon beta-1b) package insert. Berlex Laboratories, Richmond, CA; 2002.
27. Rebif (interferon beta-1a) package insert. Serono, Inc., Rockland, MA; 2002.
28. Avonex (interferon beta-1a) package insert. Biogen Idec, Inc., Cambridge, MA; 2003.
29. Giovannoni G, Munschauer FE, III, Deisenhammer F. Neutralising antibodies to interferon beta during the treatment of multiple sclerosis. *J Neurol Neurosurg Psychiatry.* 2002;73:465-69.
30. Runkel L, Meier W, Pepinsky RB, et al. Structural and functional differences between glycosylated and non-glycosylated forms of human interferon  $\beta$  (IFN $\beta$ ). *Pharm Res.* 1998;15:641-49.
31. Bertolotto A, Gilli F, Sala A, et al. Persistent neutralizing antibodies abolish the interferon  $\beta$  bioavailability in MS patients. *Neurology.* 2003;60:634-39.
32. Cook SD, Quinless JR, Jotkowitz A, Beaton P, and the Neutralizing Antibody Study Group. Serum IFN neutralizing antibodies and neopterin levels in a cross-section of MS patients. *Neurology.* 2001;57:1080-84.
33. Deisenhammer F, Reindl M, Harvey J, Gasse T, Dilitz E, Berger T. Bioavailability of interferon beta 1b in MS patients with and without neutralizing antibodies. *Neurology.* 1999;52:1239-43.
34. The IFNB Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group. Neutralizing antibodies during treatment of multiple sclerosis with interferon beta-1b: Experience during the first three years. *Neurology.* 1996;47:889-94.

## Neutralizing Antibodies to Multiple Sclerosis Treatments

---

35. Sorensen PS, Ross C, Clemmesen KM, et al. Clinical importance of neutralising antibodies against interferon beta in patients with relapsing-remitting multiple sclerosis. *Lancet*. 2003;362:1184-91.
36. Antonelli G, Bagnato F, Pozzilli C, et al. Development of neutralizing antibodies in patients with relapsing-remitting multiple sclerosis treated with IFN-beta-1a. *J Interferon Cytokine Res*. 1998;18:345-50.
37. Perini P, Facchinetti A, Bulian P, et al. Interferon-beta (IFN $\beta$ ) antibodies in interferon  $\beta$ -1a- and interferon  $\beta$ -1b-treated multiple sclerosis patients. Prevalence, kinetics, cross-reactivity, and factors enhancing interferon- $\beta$  immunogenicity in vivo. *Eur Cytokine Netw*. 2001;12:56-61.
38. Rice GPA, 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;52:1277-79.
39. Goelz SE. The persistence of neutralizing antibodies to interferon beta (IFN $\beta$ ) over 6 years of treatment in MS patients is dependent on titer and IFN $\beta$  product. *Neurology*. 2004;62(suppl 5):A156.
40. Pachner AR. Anti-IFN $\beta$  antibodies in IFN $\beta$ -treated MS patients. Summary. *Neurology*. 2003;61(suppl 5):S1-S5.
41. Rosenschein U, Lenz R, Radnay J, Ben Tovim T, Rozenszajn LA. Streptokinase immunogenicity in thrombolytic therapy for acute myocardial infarction. *Isr J Med Sci*. 1991;27:541-45.
42. Vanderschueren SMF, Stassen JM, Collen D. On the immunogenicity of recombinant staphylokinase in patients and in animal models. *Thromb Haemost*. 1994;72:297-301.
43. Chaffee S, Mary A, Stiehm ER, Girault D, Fischer A, Hershfield MS. IgG antibody response to polyethylene glycol-modified adenosine deaminase in patients with adenosine deaminase deficiency. *J Clin Invest*. 1992;89:1643-51.
44. Prümmer O. Treatment-induced antibodies to interleukin-2. *Biotherapy*. 1997;10:15-24.
45. Blumenfeld Z, Frisch L, Conn PM. Gonadotropin-releasing hormone (GnRH) antibodies formation in hypogonadotropic azoospermic men treated with pulsatile GnRH: diagnosis and possible alternative treatment. *Fertil Steril*. 1988;50:622-29.
46. Olsen E, Duvic M, Frankel A, et al. Pivotal phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma. *J Clin Oncol*. 2001;19:376-88.
47. Claustrat B, David L, Faure A, Francois R. Development of anti-human chorionic gonadotropin antibodies in patients with hypogonadotropic hypogonadism. A study of four patients. *J Clin Endocrinol Metab*. 1983;57:1041-47.
48. Ragnhammar P, Wadhwa M. Neutralising antibodies to granulocyte-macrophage colony stimulating factor (GM-CSF) in carcinoma patients following GM-CSF combination therapy. *Med Oncol*. 1996;13:161-66.
49. Prabhakar SS, Muhlfelder T. Antibodies to recombinant human erythropoietin causing pure red cell aplasia. *Clin Nephrol*. 1997;47:331-35.
50. Secondary Progressive Efficacy Clinical Trial of Recombinant Interferon-beta-1a in MS (SPECTRIMS) Study Group. Randomized controlled trial of interferon-beta-1a in secondary progressive MS. Clinical results. *Neurology*. 2001;56:1496-1504.