

# Identifying responders and nonresponders to interferon therapy in multiple sclerosis

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**Abstract:** Interferon beta is a well established disease-modifying agent used for relapsing-remitting multiple sclerosis. Despite treatment, a relevant proportion of patients continue to experience clinical (ie, relapses, worsening of disability) and magnetic resonance imaging (MRI) activity. Early identification of responders and nonresponders to interferon beta is strongly recommended to select patients who need a prompt switch to another disease-modifying agent and to ultimately avoid accumulation of fixed disability over time. Detecting responders and nonresponders to interferon beta can be challenging, mainly because of the lack of a clear and shared clinical definition of response to treatment. Clinical features at the start of treatment should be considered as prognostic factors, but MRI parameters assessed during treatment, such as contrast-enhancing lesions or new T2-hyperintense lesions, may be sensitive markers of response to interferon beta. Quantitative scoring systems derived from a combination of relapses and MRI activity have recently been proposed as practical tools for use in the everyday clinical setting. Blood biomarkers, such as neutralizing antibodies to interferon beta and Myxovirus resistance protein A, provide further useful information for detecting responders and nonresponders to interferon beta. However, since the presence of neutralizing antibodies can only partially explain the nonresponse to interferon beta, biomarkers of interferon beta activity possibly related to the pathogenesis of the disease could represent a future step toward a tailored, long-lasting effective treatment against multiple sclerosis.

**Keywords:** interferon beta, responders, nonresponders, multiple sclerosis

## Introduction

Multiple sclerosis is a lifelong demyelinating disease typically affecting young adults. It is widely believed to be an autoimmune disorder triggered by environmental factors in genetically predisposed subjects.<sup>1</sup> The clinical onset of multiple sclerosis tends to be between 15 and 50 years of age, with a female predominance at a ratio of 2–3:1 over males.<sup>2</sup>

Symptomatic treatment has been the mainstay of treatment for patients with multiple sclerosis.<sup>3</sup> However, in the last decade, improved diagnostic criteria and availability of effective therapies have led to a paradigm shift toward earlier diagnosis and treatment.<sup>4</sup>

Ten disease-modifying agents are now approved for the treatment of patients with multiple sclerosis: three formulations of interferon beta (IFNB), including subcutaneous IFNB-1b 250 µg every other day (Betaseron®; Bayer, Whippany, NJ, USA, and Schering, Milton Keynes, UK), intramuscular IFNB-1a 30 µg once weekly (Avonex®; Biogen Idec, Cambridge, MA, USA), and subcutaneous IFNB-1a 22 µg or

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44 µg three times per week (Rebif®; Merck Serono, Geneva, Switzerland); subcutaneous glatiramer acetate 20 mg once a day (Copaxone®; Teva, Petach Tikva, Israel); an immunosuppressive agent, mitoxantrone (Novantrone®; Wyeth, Maidenhead, UK); a monoclonal antibody, natalizumab (Tysabri®; Biogen Idec); and three oral drugs, ie, fingolimod 0.5 mg once a day (Gilenya®; Novartis, Basel, Switzerland), teriflunomide 14 mg once a day (Aubagio®; Genzyme, Cambridge, MA, USA), and dimethyl fumarate (Tecfidera®; Biogen Idec).

Despite the increasing availability of new disease-modifying agents, IFNB formulations still remain the standard of care for patients with multiple sclerosis. Phase III randomized controlled trials (RCTs) have consistently shown that IFNB is effective in reducing the relapse rate and new demyelinating lesions as seen on magnetic resonance imaging (MRI). It is also extensively documented that IFNB slows progression of disability when compared with placebo.<sup>5–10</sup> Extension phases of original RCTs and post-marketing studies have confirmed early reports of persistent clinical and MRI efficacy over time.<sup>11–16</sup> Moreover, prolonged survival has recently been observed in patients who initially received IFNB-1b treatment compared with those originally randomized to placebo.<sup>17</sup> Lastly, the available data from RCTs indicate that IFNB is most effective when started early, even in subjects presenting a clinically isolated syndrome suggestive of multiple sclerosis.<sup>18–21</sup> However, response to IFNB is largely heterogeneous, and a significant number of IFNB-treated subjects continue to experience clinical and MRI disease activity despite treatment.<sup>22</sup> Therefore, early identification of patients with a poor response to IFNB and a prompt switch to another disease-modifying agent (if necessary) are required in order to avoid relapses and accumulation of fixed disability over time.<sup>23</sup>

In this review, we summarize the studies that have investigated response and nonresponse to IFNB as a treatment for multiple sclerosis. Studies that merged data of patients treated with IFNB and glatiramer acetate were not included.

## Definition of response or nonresponse to IFNB

A major difficulty in identifying responders and nonresponders to IFNB is that a clear and shared clinical definition of a poor/absent response to IFNB does not exist.<sup>22,24</sup> The definition of response to treatment is commonly based on two fundamental features of multiple sclerosis, ie, relapses and progression of disability (or a combination of both).

Regarding relapses, different definitions of response to IFNB have been used, including frequency and severity of clinical exacerbations, reduction in relapse rate when

compared with pretreatment period, number of patients remaining relapse-free, and degree of recovery after an acute inflammatory event.<sup>25</sup> There are some concerns when clinical criteria based on relapses are adopted to define response and nonresponse to IFNB. First, the relapse rate is influenced by the phenomenon of regression to the mean, which may represent a confounding factor. Second, the clinical severity of a relapse depends on the location of focal demyelinating inflammation in the central nervous system. Third, incomplete recovery after a clinical exacerbation may reflect an individual pathological pattern of disease activity rather than the pattern of response to treatment. Lastly, excluding pseudoexacerbations is not always possible.

Several authors have identified worsening disability as the key indicator of the long-term efficacy of a disease-modifying agent. Criteria for response to IFNB based on assessment of disability have been found more sensitive and more specific in predicting long-term disability.<sup>26</sup> Progression of disability may be defined in different ways, eg, as a sustained increase in Expanded Disability Status Scale (EDSS) score,<sup>27</sup> time to shift to a secondary progressive course, time to reach EDSS milestones of 4.0 or 6.0, or even changes in the multiple sclerosis functional composite score.<sup>28</sup> However, a sustained increase of 1.0 or more in EDSS points that persists for at least two consecutive scheduled visits separated by a 6-month interval is widely accepted as an accurate indicator of lack of response to a disease-modifying agent.<sup>29</sup>

## Baseline clinical features identifying responders and nonresponders to IFNB

Randomized controlled trials have failed to identify any baseline features, other than the arm assignment, associated with response to IFNB. Further, post-marketing studies designed to assess this issue have generally included short-term follow-up (2–4 years), used different criteria for defining response/nonresponse to IFNB, and yielded conflicting results (Table 1).<sup>30–38</sup> In this regard, it has been suggested that the assumption of an “a priori” criterion strongly influences both the proportions of responders and nonresponders and the potential predictors of response and nonresponse to IFNB.<sup>26,34</sup> The regression to the mean phenomenon may occur when relapses are considered as either a response outcome or as potential predictor of response to IFNB. Several studies have found an association between a worse EDSS score and lack of response to IFNB; however, higher early EDSS scores can also be considered a marker of an adverse prognosis, regardless of the disease-modifying agent received by patients.

In conclusion, pre-IFNB clinical characteristics, such as disease duration, EDSS score, and relapse rate prior to starting IFNB should be considered as prognostic factors rather than as markers of response to treatment.<sup>24</sup>

## On-treatment MRI features as a marker of response or nonresponse to IFNB

Conventional MRI is a powerful tool for monitoring the pathological processes involved in multiple sclerosis. New active lesions on MRI are about 5–10 times more frequent than clinical relapses, especially in patients with a relapsing-remitting course of multiple sclerosis.<sup>39</sup>

The concept of monitoring the biological effect of IFNB by MRI is based on the drug's mechanism of action. In fact, although IFNB is a pleiotropic cytokine exerting complex effects, one of its most important actions involves control of the blood–brain barrier.<sup>40</sup> Therefore, persistence

of the inflammatory components of the disease, ie, contrast enhancing lesions or new/enlarged T2-hyperintense lesions, might suggest a lack of biological effect of IFNB, even when the disease is clinically silent.

A post hoc analysis of the original RCT of intramuscular IFNB-1a found worse disability outcomes in patients who had relapses during 2-year follow-up, but no differences were observed between the active group and the placebo group.<sup>41</sup> In contrast, accumulation of more than two new or enlarged T2-hyperintense lesions during the study was associated with poor disability outcome in the active treatment group but not in the placebo group.<sup>41</sup> This latter finding supports the use of MRI as a marker of response to IFNB rather than as a prognostic tool.

Several independent, observational, post-marketing studies confirmed that an MRI scan, performed after 6–12 months of IFNB treatment, is able to predict a subsequent lack of response to IFNB even in the absence of clinical activity

**Table 1** Summary of studies investigating baseline demographic and clinical characteristics associated with response and nonresponse to IFNB in patients with multiple sclerosis

Study	Sample size	Follow-up (years)	Definition of nonresponse	Nonresponders	Baseline features associated with poor response
Roulet et al <sup>30</sup>	116	2	Any relapse	67%	Higher pre-IFNB relapse rate
			No relapse rate reduction with respect to pre-IFNB period	33%	Lower pre-IFNB relapse rate
			Any relapse and one-point EDSS progression	27%	Higher pre-IFNB relapse rate
			No relapse rate reduction with respect to pre-IFNB period and one-point EDSS progression	34%	–
Waubant et al <sup>31</sup>	200	2	No relapse rate reduction with respect to pre-IFNB period	32%	Younger age Shorter disease duration Lower pre-IFNB relapse rate
Trojano et al <sup>32</sup>	378	2	No relapse rate reduction with respect to pre-IFNB period	60%	Longer disease duration Lower pre-IFNB relapse rate
Coppola et al <sup>33</sup>	153	3	Any relapse	60%	≥2 relapses in the pre-IFNB period
Portaccio et al <sup>34</sup>	147	2	One-point EDSS progression	42%	EDSS score of ≥2.0
			No relapse rate reduction with respect to pre-IFNB period	28%	Lower pre-IFNB relapse rate
			Less than 30% relapse rate reduction with respect to pre-IFNB period	27%	Lower pre-IFNB relapse rate
			Any relapse	56%	Higher pre-IFNB relapse rate
O'Rourke et al <sup>35</sup>	175	5	One-point EDSS progression	27%	None
Fromont et al <sup>36</sup>	751	2	One-point EDSS progression	34%	Higher EDSS score
			No relapse rate reduction with respect to pre-IFNB period	28%	Monosymptomatic onset Younger age Lower pre-IFNB relapse rate
Goodin et al <sup>37</sup>	260	16	Shift to SP course or EDSS score ≥6.0	54%	Higher EDSS score Male sex
Mezei et al <sup>38</sup>	81	4.5	No relapse rate reduction with respect to pre-IFNB period	28%	Longer disease duration

**Abbreviations:** IFNB, interferon beta; EDSS, Expanded Disability Status Scale; SP, secondary progressive.

on treatment, and irrespective of the definition of treatment response used (Table 2).<sup>42–48</sup>

Disease activity as seen on MRI has been reported as a valid surrogate marker for clinical activity.<sup>49–51</sup> A post hoc analysis combining data from two RCTs of subcutaneous IFNB-1a showed that treatment effect on new T2-hyperintense lesions after the first year of treatment accounted for a significant proportion of treatment effect on relapses over the subsequent year.<sup>50</sup>

It was recently reported that four-year clinical outcomes in IFNB-treated patients fulfilling the European Medicine Agency criteria for escalating to second-line disease-modifying agents, ie, one or more relapses and either  $\geq 9$  T2-hyperintense lesions on brain MRI or one or more contrast enhancing lesions after 1 year of treatment with IFNB, did not differ from those shown to have isolated MRI activity at the 1-year scan, defined as the presence of at least one contrast enhancing lesion or two or more new T2 lesions.<sup>52</sup> This study suggests that on-treatment MRI monitoring is more sensitive than composite scores for early prediction of nonresponse to IFNB. Lastly, a “dose-effect” with regard to new MRI lesions has also been described, ie, the greater the number of new T2-hyperintense lesions detected after the first year of IFNB treatment, the higher the risk of subsequent accumulation of disability. This finding was replicated even after considering only patients who remained stable (ie, relapse-free) in the first year of IFNB therapy (Figure 1).<sup>45</sup>

## Composite scores to identify response and nonresponse to IFNB

Some investigators still consider that isolated MRI activity is not sufficient to determine IFNB failure, and have proposed

composite scores based on integration of different parameters of disease activity to identify response and nonresponse to IFNB treatment.<sup>24,53–55</sup> This latter suggestion is based on evidence that the effect of treatment on the combination of MRI activity and relapses in the first year accounts for almost 100% of the effect of treatment on progression of disability at 2 years.<sup>56</sup>

The Canadian MS Working Group has updated its recommendations to neurologists for optimal use of disease-modifying agents.<sup>57</sup> While these consensus recommendations were based on a qualitative analogic model that takes into account relapses, disability, and MRI features during treatment, other groups have designed quantitative scoring systems based on combinations of clinical and radiological disease activity.<sup>53,54</sup> The seminal paper suggested that only the concomitant presence of at least two parameters (relapses, progression of disability, activity on MRI) in the first year of treatment with IFNB has significant value for identifying nonresponders in the subsequent 2 years.<sup>53</sup>

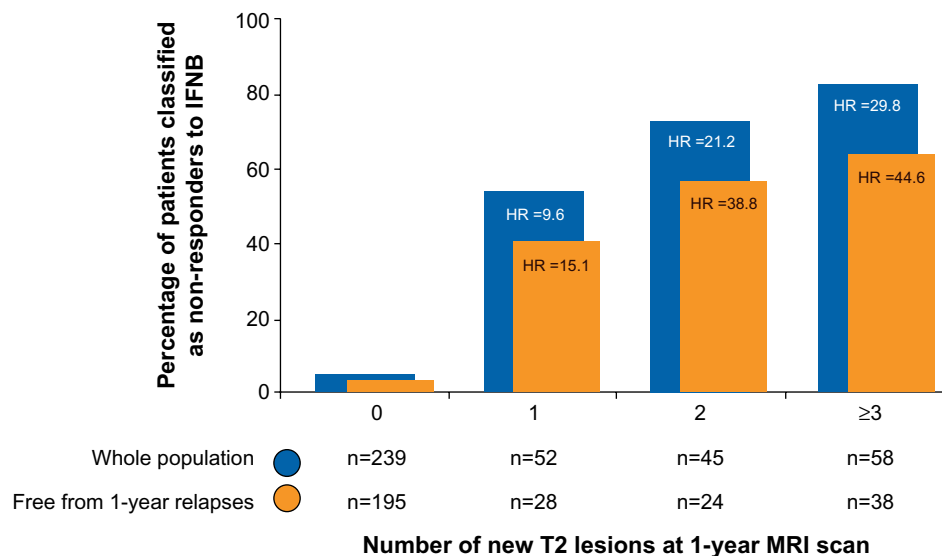
This scoring system was recently refined on the basis of a reanalysis of the active arms of the PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) study,<sup>7</sup> and was then validated in a separate group of patients from the post-marketing Barcelona cohort.<sup>53</sup> This new scoring system, known as the modified Rio score,<sup>54</sup> classified patients into three risk groups for disability progression based on first-year treatment events: low risk, ie,  $\leq 4$  new T2-hyperintense lesions and no relapses; medium risk, ie,  $\leq 4$  new T2-hyperintense lesions and one relapse or  $> 4$  new T2-hyperintense lesions and no relapses; and high risk, ie,  $\leq 4$  new T2-hyperintense lesions and  $\geq 2$  relapses or  $> 4$  new T2-hyperintense lesions and one relapse.<sup>54</sup> However, despite its excellent specificity

**Table 2** Summary of studies investigating on-treatment MRI characteristics associated with response and nonresponse to IFNB in patients with multiple sclerosis

Study	Sample size	Follow-up (years)	Response definition	Nonresponders	On-treatment features associated with poor response
Tomassini et al <sup>42</sup>	68	6	$\geq 2$ relapses One-point EDSS progression	49% 43%	Presence of gadolinium enhancement Development of NABs
Durelli et al <sup>43</sup>	147	2	Any relapse or one-point EDSS progression	43%	Any active MRI lesion
Rio et al <sup>44</sup>	152	2	One-point EDSS progression	16%	$\geq 3$ active MRI lesions
Prosperini et al <sup>45</sup>	394	4.5	One-point EDSS progression	30%	Any new T2 lesion
Freedman et al <sup>46</sup>	268	4	Post-one year relapse rate Post-one year annualized rate of new MRI lesions	NA NA	Relapses Any active MRI lesions
Bermel et al <sup>47</sup>	136	15	EDSS change	32%	Presence of gadolinium enhancement
Horakova et al <sup>48</sup>	217	6	Confirmed disability progression or relapse score $\geq 1$ *	52%	$\geq 3$ new T2 lesions

**Note:** \*Relapse score derived from combining frequency and severity of relapses.

**Abbreviations:** IFNB, interferon beta; EDSS, Expanded Disability Status Scale; NABs, neutralizing antibodies; MRI, magnetic resonance imaging; NA, not available.



**Figure 1** Risk of nonresponse to IFNB over a 4.5-year follow-up according to number of new T2 lesions detected at 1-year MRI scan on treatment.

**Note:** Reprinted with permission from John Wiley and Sons. Prosperini L, Gallo V, Petsas N, et al. One-year MRI scan predicts clinical response to interferon beta in multiple sclerosis. *Eur J Neurol.* 2009;16:1202–1209.<sup>45</sup> Copyright © 2009 Prosperini L, et al. *European Journal of Neurology* © 2009 EFNS.

**Abbreviations:** IFNB, interferon beta; MRI, magnetic resonance imaging; HR, hazard ratio.

(97%), this score demonstrated very low sensitivity (24%), implying a significant risk of misdiagnosing poor responders to IFNB.

The modified Rio score has recently been refined further, with the suggestion that a second MRI scan 6 months on from the 1-year MRI scan enables better classification of the medium-risk group.<sup>24,55</sup> The authors suggest that patients who relapse or accumulate two or more new T2 lesions during months 12–18 of treatment with IFNB can be classified as nonresponders.<sup>24,55</sup>

## Blood-derived biomarkers of response and nonresponse to IFNB

Biological markers of responsiveness to treatment, with IFNB in particular, are landmarks of research in multiple sclerosis. According to the National Institutes of Health Biomarkers Definitions Working Group,<sup>58</sup> a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. Biological activity can be defined as the pharmacological, physiological, and biochemical effects resulting from the interaction of a drug with its target receptor, and is a necessary but not sufficient condition for effectiveness.

Development of neutralizing antibodies (NABs) is one of the main reasons for reduction or loss of biological activity of IFNB.<sup>59–61</sup> NABs develop in 2%–40% of treated patients according to the manufacturers of the product, and the majority of NAB-positive patients are identified within the first 2 years of treatment.<sup>62</sup> The immunogenicity of IFNB

is mainly influenced by the manufacturing process and the formulation, frequency of injection, route of administration (the subcutaneous route being more immunogenic than the intramuscular route) and dose used.<sup>63</sup>

Several studies have shown that the outcome for NAB-positive patients, in terms of clinical and MRI disease activity and accumulation of disability, is worse than for NAB-negative patients.<sup>5,42,43,64–80</sup> Nevertheless, it takes time before the clinical impact of NABs becomes evident, and this could help to explain why some trials did not find significant differences in clinical status between NAB-positive and NAB-negative patients (Table 3).

In this scenario, NAB positivity can represent a negative prognostic factor in terms of therapeutic responsiveness, and should be taken into consideration in a model integrating clinical and MRI parameters to identify poor responders early on.<sup>42,43</sup> According to recent international guidelines, NABs should be tested in all IFNB-treated patients after the first year of treatment, and if positivity is confirmed at high titer in a subsequent test 3–6 months later, patients should be switched to a non-IFNB therapy.<sup>63</sup>

Given that IFNB binds specific receptors on human cells, ie, the interferon alpha/beta receptor, and induces a specific biochemical intracellular pathway that leads to regulation of several genes stimulated by IFNB, monitoring markers of biological activity may be a good method of evaluating the biological response to IFNB.<sup>81</sup>

Molecular markers of the biological activity of IFNB are currently being investigated at the protein



**Table 3** Impact of NABs on clinical and MRI parameters in clinical trials and observational studies

Study	Type of IFNB	Percent NAB-positive	Follow-up (years)	Effect of NABs			
				RR	EDSS	MRI activity	MRI BOD
IFNB MS Study Group <sup>5</sup>	SC IFNB-1b	35%	3	+	NS	NA	NS
Rudick et al <sup>64</sup>	IM IFNB-1a	17%	2	NS	NS	+	NA
Li et al <sup>65</sup>	SC IFNB-1a 22 µg	22%	3	NS	NS	NS	NS
	SC IFNB-1a 44 µg	15%					
Panitch et al <sup>66</sup>	IM IFNB-1a	25%	1	NS	NA	+	NA
	SC IFNB-1a	2%					
Durelli et al <sup>67</sup>	IM IFNB-1a	7%	2	NS	NA	NA	NA
	SC IFNB-1b	30%					
Polman et al <sup>68</sup>	SC IFNB-1b	28%	3	+	NS	NA	+
	SC IFNB-1b 160 µg	32%					
North American Study Group on IFNB-1b in SPMS <sup>69</sup>	SC IFNB-1b 250 µg	23%	3	NS	NS	NS	NS
	SC IFNB-1a 22 µg	24%					
Francis et al <sup>70</sup>	SC IFNB-1a 44 µg	14%	4	+	+	+	+
	SC IFNB-1a 30 µg	2%					
Kappos et al <sup>71</sup>	SC IFNB-1a 60 µg	5%	4	+	+	+	+
	SC IFNB-1b	32%					
Hartung et al <sup>72</sup>	SC IFNB-1b	27%	5	NS	NS	+	+
Sorensen et al <sup>73</sup>	Any IFNB	9%–46%	5	+	NS	NA	NS
Malucchi et al <sup>74</sup>	Any IFNB	17%	3	+	+	NA	NA
Frank et al <sup>75</sup>	SC IFNB-1b	37%	2.5			+	+
Perini et al <sup>76</sup>	Any IFNB	24%	4	+	+	NA	NA
Tomassini et al <sup>42</sup>	SC IFNB-1a	25%	6		+	NA	NA
Boz et al <sup>77</sup>	Any IFNB	13%	3	+	NS	NA	NA
Malucchi et al <sup>78</sup>	Any IFNB	12%	3	+		NA	NA
Durelli et al <sup>43</sup>	SC IFNB-1a	34%	2	+	NS	NA	NA
Sato et al <sup>79</sup>	IM IFNB-1a	35%	4	+	NA	+	NA
	SC IFNB-1b						
Paolicelli et al <sup>80</sup>	Any IFNB	14%	5	+	NS	NA	NA

**Abbreviations:** MS, multiple sclerosis; IFNB, interferon beta; NABs, neutralizing antibodies; RR, relapse rate; MRI, magnetic resonance imaging; EDSS, Expanded Disability Status Scale; BOD, burden of disease; +, outcome significantly worse in NAB-positive group than in NAB-negative group; NS, not significant; NA, not available; IM, intramuscular; SC, subcutaneous.

and messenger (m)RNA levels. A large number of molecules have been proposed as IFNB biomarkers, ie, beta-2-microglobulin and neopterin,<sup>63</sup> Myxovirus resistance protein A (MxA),<sup>82</sup> MxA mRNA,<sup>83</sup> oligoadenylate synthetase,<sup>84</sup> tumor necrosis factor-related apoptosis inducing ligand,<sup>84,85</sup> viperin and interferon alpha-inducible protein 27,<sup>86</sup> CC-chemokine ligand 2, and CXC-chemokine ligand 10.<sup>87</sup>

Of the biomarkers reflecting an in vivo response to IFNB, MxA is the best validated and its use is increasing in clinical practice.<sup>63,88–90</sup> MxA mRNA levels are inhibited in NAB-positive patients,<sup>91</sup> and absence of MxA induction by IFNB reflects complete loss of IFNB bioactivity, as demonstrated by microarray analyses of expression of different IFNB-stimulated genes.<sup>88</sup> The MxA mRNA level after 1 year of treatment with IFNB is predictive of relapse;<sup>78</sup> low levels of MxA mRNA before treatment with IFNB are reported to be associated with occurrence of relapses and contrast enhancing lesions on MRI, whereas high levels of MxA mRNA seem to be associated

with a longer time to relapse and reduction of contrast enhancing lesions.<sup>91</sup>

## Polymorphisms and gene expression signatures associated with response and nonresponse to IFNB

Use of MxA as a biomarker of IFNB activity has been criticized for the lack of evidence of its role in the pathogenesis of multiple sclerosis, so microarray analyses of gene expression to look for biomarkers of IFNB activity (possibly related to the pathogenesis of the disease) represent outstanding research in the field. So far, none of the genes studied fits the definition of a biomarker better than MxA, but some promising molecules are under investigation.

Some molecules, including ubiquitin specific peptidase 18 (USP18) and probably E3 ubiquitin-protein ligase, are significantly induced by IFNB and are underexpressed in patients with multiple sclerosis when compared with controls, suggesting that they have a possible role in the pathogenesis of

multiple sclerosis.<sup>92</sup> In addition, two *USP18* haplotypes have been significantly associated with multiple sclerosis: CG carriers are characterized by lower *USP18* gene expression levels in peripheral blood mononuclear cells and more clinical disease activity, whereas AA homozygosity for the intronic polymorphism *rs2542109* is associated with the responder phenotype in IFNB-treated patients, independent of *USP18* expression levels induced by IFNB.<sup>93</sup>

Two recent interesting studies have analyzed the relationship between different IFNB-stimulated genes discovered by microarray profiles.<sup>94,95</sup> The first combined the results of the main studies of gene profiling in response to treatment with IFNB and identified common differential expression patterns over time in peripheral blood mononuclear cells exposed to IFNB.<sup>94</sup> The majority of the IFNB-responsive gene products were found to be involved in immune modulation or response, and linked with each other in a network, indicating that they also relate to each other at a functional level. Important regulators, such as interleukin-8, signal transducers and activators of transcription 1, Toll-like receptor 7, CC-chemokine ligand 2, and CXC-chemokine ligand 10, appear to be central nodes in the network with mutual connections to each other. Most of these genes are induced in response to treatment with IFNB. The only two genes that are consistently downregulated are *interleukin-8* and *Fc fragment of immunoglobulin (Ig)E, high affinity I, receptor for; alpha polypeptide*.<sup>94</sup>

These changes may not only be directly induced by the drug, but may also result from long-term continuous adaptation of the immune system to treatment over time. Most of the genes respond early to treatment, ie, within the first 24 hours. However, there are a few genes for which modulation has been observed after the first month of treatment. Monitoring expression patterns after 1 or 3 months shows both direct IFNB activity and adaptive immune changes.

It can be postulated that the following scenarios are important for predicting the effectiveness of therapy: the condition of the patient before treatment and adaptive changes during therapy, eg, certain human leukocyte antigen alleles, or single nucleotide polymorphisms in the interferon alpha/beta receptor, large multifunctional peptidase 7, cathepsin-S, or Myxovirus (influenza virus) resistance 1; the expression level of IFNB-responsive genes at baseline; and the induction/reduction of certain genes 24 hours after administration of IFNB.<sup>94</sup>

Another study investigated the IFNB pleiotropic mechanisms of action by combining IFNB-1b-induced gene expression profiles and their biological knowledge bases.<sup>95</sup> Genes involved in immune regulation, metabolism of mitochondrial

fatty acids, and antioxidant activity have been discovered, including *nuclear factor (erythroid-derived 2)-like 2* and *obg-like adenosine triphosphatase 1*, two molecules involved in neuronal protection and antioxidant activity, as well as the antioxidant gene *nicotinamide adenine dinucleotide dehydrogenase 6*, implicated in optic neuropathy and multiple sclerosis-like lesions.<sup>95</sup>

In conclusion, gene profiling studies have demonstrated a wide range of genes regulated by IFNB treatment. These discoveries highlighted the importance of different patterns of gene expression in the short-term and long-term as adaptation to therapy. In particular, overexpression of type I IFN-responsive genes has been associated with a decreased biological and clinical response to IFNB in patients with multiple sclerosis.<sup>88,96</sup>

## Conclusion

Identification of responders and nonresponders to IFNB is still a challenge for neurologists. A standardized definition of response/nonresponse to IFNB is still lacking, thereby making it difficult to identify predictors/markers of a therapeutic response. While clinical features are not considered to be useful, MRI changes during treatment with IFNB are regarded as an accurate marker of treatment response, even if MRI activity occurs in the absence of clinical activity. In addition, use of blood biomarkers such as NABs and MxA could improve the sensitivity and accuracy of early recognition of responders and nonresponders to IFNB. In the near future, microarray analyses of gene expression will expand our knowledge regarding the mechanisms of action of IFNB, and could define a pattern of biological markers to predict the response to IFNB therapy.

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