Journal of Neuroimmunology 292 (2016) 34–39

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Short communication

# A gene expression study denies the ability of 25 candidate biomarkers to predict the interferon-beta treatment response in multiple sclerosis patients

Serena Martire [a,b,*], Nicole D. Navone [c], Francesca Montarolo [a,b], Simona Perga [a,b], Antonio Bertolotto [a,b]

[a] AOU San Luigi Gonzaga, Neurologia 2 – CReSM (Regional Referring Center of Multiple Sclerosis), Neurobiology Unit, Orbassano, TO, Italy
[b] Neuroscience Institute Cavalieri Ottolenghi (NICO), Orbassano, TO, Italy
[c] AOU San Luigi Gonzaga, SCDU Medical Oncology, Orbassano, TO, Italy

ARTICLE INFO

*Article history:*
Received 8 October 2015
Received in revised form 7 January 2016
Accepted 14 January 2016

*Keywords:*
Multiple sclerosis
Interferon-beta
Biomarkers
Treatment response
Gene expression

ABSTRACT

We studied the baseline expression level of 25 interferon-regulated genes (MxA, GPR3, IL17RC, ISG15, TRAIL, OASL, IFIT1, IFIT2, RSAD2, OAS3, IFI44L, TRIM22, IL10, CXCL10, STAT1, OAS1, OAS2, IFNAR1, IFNAR2, IFNβ, ISG20, IFI6, PKR, IRF7, USP18), recurrently proposed in the literature as predictive biomarkers of interferon-beta treatment response, in whole blood of 10 "responders" and 10 "non-responders" multiple sclerosis relapsing–remitting patients, retrospectively selected on the basis of stringent clinical criteria after a five years follow-up. However, we cannot confirm the predictive value of these candidate biomarkers.



## 1. Introduction

Multiple sclerosis (MS) is a chronic, progressive and disabling immune-mediated disorder of the central nervous system. Due to its multifactorial nature, MS is a heterogeneous disease characterized by considerable variation in responsiveness to therapy. Interferon-beta (IFN-beta), a type I IFN, was the first agent to show clinical efficacy in the treatment of relapsing–remitting (RR) MS, and is still the most commonly used therapy. Unfortunately, about 40% of patients respond poorly or not at all to IFN-beta treatment (Rudick et al., 2004; Ann Marrie and Rudick, 2006; Río et al., 2006) Part of the unresponsiveness can be explained by the emergence of neutralizing anti-IFN-beta antibodies (NAbs), (Deisenhammer et al., 1999; Bertolotto et al., 2003; Sorensen et al., 2006), but mostly it is attributed to differences in an individual's genetic response to therapy (Bertolotto and Gilli, 2008). Classifying patients into responders and non-responders to IFN-beta usually happens after one or two years of follow-up, during which patients treated without benefit are exposed to side effects and to the risk of accumulating further disability. Considering also the high socioeconomic costs, there is a strong need to identify biomarkers that predict the success of the therapy. Although several studies suggest that patterns of IFN-stimulated genes in RRMS can predict a clinical response to treatment, most of the suggested biomarkers have not been confirmed in a completely independent analysis. Therefore, currently there is still no definitive biomarker able to discriminate between patients who will optimally respond to treatment and those who are at risk of ongoing disease activity.

In this research we analyzed the baseline expression level of a panel of 25 genes (Table 1) in whole blood of 20 RRMS patients (Polman et al., 2011) (10 responders and 10 non-responders) to verify their potential to predict the clinical response to the drug. The 25 selected genes are recurrently proposed in the literature as optimal biomarkers of IFN-beta responsiveness and patients were retrospectively selected after a five-year follow-up based on their relapse activity, expanded disability status scale (EDSS) and magnetic resonance imaging (MRI). The retrospective selection allowed to clearly discriminate between patients with good and poor response and avoided intermediate phenotypes.

## 2. Materials and methods

### 2.1. Patients and definition of response to IFN-beta therapy

The baseline expression level of a panel of 25 genes (Table 1) was evaluated in 20 RRMS female patients (Polman et al., 2011) immediately before starting treatment with IFN-beta. Basal and longitudinal

* Corresponding author at: AOU San Luigi Gonzaga, Neurologia 2 – CReSM (Regional Referring Center of Multiple Sclerosis), Neurobiology Unit & Neuroscience Institute Cavalieri Ottolenghi (NICO), Regione Gonzole, 10, 10043 Orbassano, Turin, Italy.
*E-mail addresses:* serena.martire@gmail.com (S. Martire), nicolednavone@gmail.com (N.D. Navone), francesca.montarolo@unito.it (F. Montarolo), simona.perga@unito.it (S. Perga), antonio.bertolotto@gmail.com (A. Bertolotto).

http://dx.doi.org/10.1016/j.jneuroim.2016.01.010
0165-5728/

**Table 1**
List of the 25 genes analyzed as candidate biomarkers predictive of the IFN-beta treatment response.

| Gene symbol | Gene name | References |
|---|---|---|
| CXCL10 | Chemokine (C–X–C motif) ligand 10 | Comabella et al. (2009); Cucci et al. (2010) |
| GPR3 | G protein-coupled receptor 3 | Hecker et al. (2011) |
| IFI44L | Interferon-induced protein 44-like | van Baarsen et al. (2008); Hecker et al. (2011) |
| IFI6 | Interferon, alpha-inducible protein 6 | |
| IFIT1 | Interferon-induced protein with tetratricopeptide repeats 1 | van Baarsen et al. (2008); Comabella et al. (2009); Rudick et al. (2011); Hecker et al. (2011) |
| IFIT2 | Interferon-induced protein with tetratricopeptide repeats 2 | van Baarsen et al. (2008); Comabella et al. (2009); Hecker et al. (2011) |
| IFNAR1 | Interferon (alpha, beta and omega) receptor 1 | Comabella et al. (2009); Axtell et al. (2010); Bustamante et al. (2013) |
| IFNAR2 | Interferon (alpha, beta and omega) receptor 2 | |
| IFNb | Interferon beta | Axtell et al. (2010); Bustamante et al. (2011); Bustamante et al. (2013) |
| IL10 | Interleukin 10 | Bartosik-Psujek and Stelmasiak (2006); van Baarsen et al. (2008); Hecker et al. (2011) |
| IL17RC | Interleukin 17 receptor C | Hecker et al. (2011) |
| IRF7 | Interferon regulatory factor 7 | van Baarsen et al. (2008); |
| ISG15 | ISG15 ubiquitin-like modifier | van Baarsen et al. (2008); Comabella et al. (2009); Rudick et al. (2011); Hecker et al. (2011) |
| ISG20 | Interferon stimulated exonuclease gene 20 kDa | |
| MxA | Myxovirus (influenza virus) resistance 1 | van Baarsen et al. (2008); Hecker et al. (2011); Hundeshagen et al. (2012) |
| OAS1 | 2′-5′-oligoadenylate synthetase 1 | van Baarsen et al. (2008); Hecker et al. (2011) |
| OAS2 | 2′-5′-oligoadenylate synthetase 2 | van Baarsen et al. (2008); Hecker et al. (2011) |
| OAS3 | 2′-5′-oligoadenylate synthetase 3 | van Baarsen et al. (2008); Comabella et al. (2009); Hecker et al. (2011) |
| OASL | 2′-5′-oligoadenylate synthetase-like | van Baarsen et al. (2008); Comabella et al. (2009); Rudick et al. (2011); Hecker et al. (2011) |
| PKR | Eukaryotic translation initiation factor 2-alpha kinase 2 | |
| RSAD2 | Radical S-adenosyl methionine domain containing 2 | van Baarsen et al. (2008); Comabella et al. (2009); Hecker et al. (2011) |
| STAT1 | Signal transducer and activator of transcription 1 | Comabella et al. (2009); Hecker et al. (2011) |
| TRAIL | Tumor necrosis factor (ligand) superfamily, member 10 | Wandinger et al. (2003); Hesse et al. (2010); Rudick et al. (2011); Hecker et al. (2011) |
| TRIM22 | Tripartite motif containing 22 | van Baarsen et al. (2008); Hecker et al. (2011) |
| USP18 | Ubiquitin specific peptidase 18 | van Baarsen et al. (2008) |

clinical data were collected at CReSM (Centro Riferimento Regionale Sclerosi Multipla) of the University Hospital San Luigi Gonzaga. The study was performed on blood samples stored in the CReSM Biobank and it was approved by the Bioethic Committee of the University of Turin (prot. June 18, 2014). Patients signed their written informed consent.

Ten responders and ten non-responders were retrospectively selected applying stringent criteria in order to discriminate clearly between the two categories. Patients were classified as responders when they were still treated with IFN-beta after five years and showed at most one new or enlarging T2 lesion on MRI. Moreover, they were free of relapses and showed no increase in the EDSS score in the first three years. Non-responders were defined as patients experiencing two or more relapses while receiving IFN-beta within three years after starting treatment. Attacks occurring in the first three months were not considered.

Patients did not show signs of infection at the time of sampling. They were free of comorbidity and negative for NAbs, whose presence in serum was tested every six months by means of the cytopathic effect (CPE) assay (Bertolotto et al., 2003). Furthermore they were treatment-naïve and none of them suffered exacerbations or received corticosteroids during the month before the start of IFN-beta therapy. Treatment compliance was evaluated by measuring the MxA induction by real-time PCR every three months during the first year and then every six months, eight hours after the last IFN-beta injection. Patients lacking MxA induction were not considered.

### 2.2. Sample collection and real-time PCR analysis

Whole blood samples, collected into Tempus® tubes (Life Technologies, Monza, Italy) immediately before the first IFN-beta injection, were stored at −80 °C until use and then extracted using the ABI Prism 6100 Nucleic Acid Prep Station (Life Technologies, Monza, Italy) following the manufacturer's instructions. Total RNA was reverse-transcribed at final concentration of 10 ng/μl using random hexamer primers. Gene expression analysis was performed by real-time PCR using Applied Biosystems' TaqMan® gene expression products (Life Technologies). Transcriptional expression was normalized using glyceraldehyde-3-phosphate dehydrogenase as reference gene. Expression levels of target genes were calculated by the normalized comparative cycle threshold (Ct) method ($2 - \Delta\Delta Ct$), using the Universal Human Reference RNA(Stratagene, Santa Clara, California) as calibrator.

### 2.3. Statistical analysis

Continuous data are presented as medians and ranges and discrete data are given as counts and percentages. Chi square tests were performed to compare groups of categorical data. Student's t-test or Mann–Whitney U test were used to compare continuous data as appropriate. Correlation between gene expression levels and clinical and demographical data was assessed fitting linear models. Statistical significance was considered at $p < 0.05$. All analyses were carried out using R version 3.1.1.

## 3. Results

Clinical and demographic characteristics of patients included in the study are summarized in Table 2. There were no significant differences between the two groups with respect to age, disease duration, baseline EDSS score, number of relapses the year before the start of therapy, presence of IgG oligoclonal bands in the cerebrospinal fluid and type of IFN-beta formulation administered. Non-responder patients experienced the first attack at 12 months (range 4–21) and the second at 23 months (range 8–33). Five of them (50%) discontinued IFN-beta treatment after 22 months (range 8–46) and then switched to a second line therapy. All non-responder patients showed at least one new/enlarging T2 lesions on MRI during IFN-beta treatment. In particular, six of them (60%) presented two or more new/enlarging T2 lesions. On the other hand, responder patients were relapse-free and neurologically stable for at least three years. Afterwards, four of them (40%) showed an exacerbation, at 45 months (range 38–43), while 2 of them (20%) had a one-point increase in the EDSS score.