Decreased interferon-β induced STAT-4 activation in immune cells and clinical outcome in multiple sclerosis

R. Tanasescu1,3,* | A. Midgley1,* | R. A. Robins2 | C. S. Constantinescu1

1Division of Clinical Neuroscience, Clinical Neurology Research Group, Queen’s Medical Centre, University of Nottingham, Nottingham University Hospitals NHS Trust, Nottingham, UK
2Division of Immunology, Clinical Neurology Research Group, Queen’s Medical Centre, University of Nottingham, Nottingham University Hospitals NHS Trust, Nottingham, UK
3Department of Clinical Neurosciences, University of Medicine and Pharmacy Carol Davila, Department of Neurology, Colentina Hospital, Bucharest, Romania

Correspondence
C. S. Constantinescu, Division of Clinical Neuroscience, Clinical Neurology Research Group, C Floor South Block, Queen’s Medical Centre, Nottingham, UK.
Email: cris.constantinescu@nottingham.ac.uk

Funding information
This study was supported by the MS Society of the UK and Northern Ireland.

Objectives: Interferon-β (IFN-β) is used in the treatment of multiple sclerosis (MS). IFN-β activation of signal transduction and activation of transcription (STAT)-4 is linked to its immunomodulatory effects. Previous studies suggest a type I IFN deficit in immune cells of patients MS, but data on interferon-α/β receptor (IFNAR) expression and the relationship with treatment response are conflicting. Here, we compare IFN-β-mediated STAT4 activation in immune cells of untreated patients with MS and controls.

Materials and methods: Peripheral blood mononuclear cells from 27 untreated patients with relapsing MS, obtained before the initiation of IFN-β treatment, and 12 matched controls were treated in vitro with IFN-β. Total and phosphorylated STAT4 (pSTAT4) and IFNAR were measured by flow cytometry and quantitative PCR. The patients were followed up for 5 years.

Results: pSTAT4 induction by IFN-β was lower in patients with MS than in controls, as was expression of IFNAR. pSTAT4 expression did not correlate with the clinical outcome at 5 years, measured by EDSS change. There was a negative correlation between the baseline IFNAR1 mRNA levels and relapse rate.

Conclusions: The results suggest decreased IFN-β responsiveness in patients with MS, associated with reduced STAT4 activation and reduced IFNAR expression. This reduced responsiveness does not appear to affect the long-term clinical outcome of IFN-β treatment.

KEYWORDS
immunomodulation, interferon beta, multiple sclerosis

1 | INTRODUCTION

The type I interferon, interferon-β (IFN-β) has been used in the treatment of multiple sclerosis (MS) for many years1,2 and remains a mainstay disease-modifying treatment for the relapsing forms of the disease. IFN-β, together with IFN-α, utilizes a shared heterodimeric receptor, IFNAR, composed of two chains, IFNAR1 and IFNAR2, and signals via Janus kinases Tyk2 and Jak1, resulting in the formation of heterodimers of signal transducer and activator of transcription 1 (STAT1) and STAT2, which on translocation to the nucleus associate with IFN regulatory factor 9 (IRF9) forming the heterotrimeric complex IFN-stimulated gene factor 3 (ISGF3). This complex activates IFN-dependent genes. After Janus kinase activation, IFN-β also activates STAT1 homodimers and, primarily in T and NK cells, through docking of STAT2 to STAT4, can induce phosphorylation of STAT4 and activation of gene expression via STAT4. The effect of IFN-β in MS is often partial and variable. Moreover, it is cell type specific, related to differential activation of STATs, sometimes with opposite effects in different cell types.3

We have shown that IFN-β in T cells leads to the induction of anti-inflammatory cytokine IL-10 in a STAT4 activation-dependent manner, explaining in part the immunomodulatory effects of IFN-β.4 This IL-10
induction was required for the suppression by IFN-β of IFN-γ induction by IL-12.5

Production of type I IFN is deficient in patients with MS, and several studies showed a decreased responsiveness of patients with MS to IFN type I,6,7 reflected, for example, in reduced expression of IFN-stimulated genes and decreased phosphorylation of STAT1.7

The aim of this study was to determine the effects of IFN-β on the phosphorylation of STAT4 in patients with MS compared with controls as an indication of response to IFN-β, and to determine its relationship with the expression of interferon type 1 receptor (IFNAR). We also aimed to determine whether the effects of IFN-β on phosphorylation of STAT4 in patients with MS correlate with their 5-year clinical course as measured by the change in the expanded disability scale (EDSS) score and clinical relapses.

2 | MATERIALS AND METHODS

The Nottingham Research Ethics Committee (UK) approved this study.

2.1 | Subjects included in the study

Twenty-seven relapsing-remitting patients with MS (17 women, 10 men; age range 29-60, mean age 43; mean expanded disability status scale, EDSS 3.6, range 1-6) and 12 healthy controls (seven women and five men; mean age 42) were included in the study. The patients with MS had not previously received disease-modifying drugs, were clinically stable at baseline (not in relapse) and had had two relapses in the last 2 years. Participants gave written informed consent prior to participation in the study.

In all MS subjects, treatment with IFN-β was commenced as part of their clinical care. Twenty-four patients were treated (13 with IFN-β 1b and 11 with IFN-β 1a), and all patients were followed up in clinic for 5 years. EDSS at 5 years of follow-up was compared with initial EDSS score.

2.2 | Cytokines and antibodies

The following reagents were obtained: IFN-β (Rebif, a gift from Merck Serono, UK); polyclonal rabbit anti-STAT4 antibody, polyclonal rabbit anti-phospho-STAT4 antibody (Zymed Laboratories, Inc., San Francisco, CA, USA); antihuman IFN-α receptor-1 (IFNAR-1) mAb and mouse IgG isotype control were purchased from R&D Systems (Oxford, UK).

2.3 | Cell preparation

Peripheral blood mononuclear cells (PBMCs) from healthy donors and patients with MS were isolated by standard gradient centrifugation with Histopaque 1077 (Sigma-Aldrich, Dorset, UK). The mononuclear cells were prepared at 1×10⁶ cells/ml in media consisting of RPMI 1640, 2 mM glutamine, 20 mM Hepes, 0.1 mg/ml penicillin and streptomycin, and 10% FCS (Sigma-Aldrich).

2.4 | Cell stimulation

Peripheral blood mononuclear cells were left unstimulated or incubated with 10 ng/ml IFN-β (2.72 U/ml of Rebif®) for 30 minutes at 37°C. Varying IFN-β concentrations were analysed for their effect on STAT4 phosphorylation; concentrations used here were those that produced the peak pSTAT4. Optimal duration of stimulation for pSTAT4 results shown here was 30 minutes.

2.5 | Intracellular staining

Following incubation, 1×10⁶ cells/ml were fixed in 1 ml ice-cold 70% ethanol and incubated on ice for 20 minutes. The cells were then washed by centrifugation once in PBA (PBS, 0.5% bovine serum albumin and 1% sodium azide [Sigma-Aldrich]), once in saponin buffer (PBA + 0.1% saponin [Sigma-Aldrich]) and once in 10% foetal calf serum (FCS) in saponin buffer at 300 g for 5 minutes as in80. 0.5 μg of rabbit polyclonal antiphosphorylated STAT4 pTyr693 or rabbit polyclonal STAT4 (Zymed, San Francisco, CA, USA) [adapted from9] was added and incubated at room temperature for 30 minutes. Following incubation, the cells were washed by centrifugation with saponin buffer. One microgram of PE-conjugated goat anti-rabbit IgG was added for 30 minutes at room temperature. Cells were washed with saponin buffer and resuspended in 0.5% formaldehyde.

2.6 | Surface staining

Following incubation with IFN-β, 1×10⁶ cells/ml were washed by centrifugation in 2% FCS RPMI, and 10 μl IFNAR-1 antibody was added for 30 minutes. After washing, cells were incubated with 5 μl goat anti-mouse FITC or goat anti-rat-PE, for 30 minutes, then washed and resuspended in 0.5% formaldehyde.

2.7 | Quantitative real-time polymerase chain reaction (PCR)

RNA was extracted using RNeasy miniprep kit (Qiagen, Valencia, CA, USA) following manufacturers’ instructions. First-strand cDNA synthesis was initiated from 0.5 μg total RNA, using random hexamers (Promega, Madison, WI, USA), and avian myeloblastosis virus reverse transcriptase (Promega) using conditions as described by the manufacturer, in a final volume of 25 μl as in80. Oligonucleotide primers designed for the GenBank sequences (NM 000629; NM 207585) were as follows: IFNAR1 forward 5’ TGACTGGAAGTCTCCCTGA 3’; reverse 5’ TCTTTTCAACTTCTGGAACA 3’ IFNAR2 forward 5’ CACCAT AGTGACACTG AAATG 3’; reverse 5’ TGGAGCCATGGATATGGT 3’; p2 microglobulin forward 5’ CTCGTTGGCCT AGCTGTG 3’; reverse 5’ ATGTGCTGGTTT CATCCATC 3’. Real-time PCR used SYBRgreen fluorescence method with SYBRgreen master mix (Stratagene, La Jolla, CA, USA) as specified by the manufacturer. PCRs were performed in triplicate on a MX4000® Multiplex System (Stratagene) using standard default thermal cycling conditions. Non-template controls were loaded in triplicate. Quantification of transcripts was carried out using
the relative standard curve method as per Applied Biosystems (Foster City, CA, USA; 1997). An equal aliquot of undiluted cDNA from each sample was pooled together. This pool was serially diluted (neat, 1:2, 1:5, 1:10, 1:20) to produce standards, from which the Ct value was converted to ng total RNA equivalent used for first-strand synthesis. mRNA expression for each gene is normalized to internal standard (β2 microglobulin expression).

2.8 Measurements
Peripheral blood mononuclear cells were evaluated on Epics XL flow cytometer (Beckman Coulter, Fullerton, CA, USA), and the results were analysed using software WINMDI 2.8. Statistical analysis employed the paired t-test. R² was used to assess correlation between IFNAR expression and STAT4 activation. Spearman's rank correlation was used to correlate EDSS change and pretreatment IFNAR expression. P≤.05 was considered significant.

3 RESULTS

3.1 Activation of STAT4 by IFN-β
We first confirmed previous results showing that IFN-β increases pSTAT4. IFN-β stimulation increased pSTAT4 compared with unstimulated cells for MS and controls (Figure 1). No difference in total STAT4 was observed (not shown).

We then compared STAT4 activation in patients and controls. pSTAT4 induction was significantly higher in controls (mean fluorescence intensity, MFI 125±10) than in patients with MS (MFI 98±5) (P=.031) (Figure 1).

3.2 IFNAR expression in patients and controls
We next investigated upstream of STAT4, at the receptor level. IFNAR-positive cells, by flow cytometry with IFNAR1 antibody, were 15%±2% for MS v. 36%±7% for controls. IFNAR expression was lower (P=.034) in patients with MS compared with controls (Figure 2).

We then assessed IFNAR1 and IFNAR2 mRNA expression in PBMCs. Patients with MS and controls had similar IFNAR2 expression; however, IFNAR1 expression was significantly lower in patients with MS when compared to controls (P=.028) (Figure 3).

We also found a positive correlation between IFNAR expression and IFN-β-induced pSTAT4 in patients with MS (P=.03) (Figure 4).

3.3 Clinical outcome at 5 years and IFNAR expression
EDSS scores at baseline and after 5 years of clinical follow-up were compared. Overall mean EDSS change was 1.1 points. 17 patients had an EDSS worsening at 5 years. The change in EDSS did not correlate with total pretreatment IFNAR expression. This lack of correlation was observed in all patient subgroups (with or without change in EDSS; treated with IFN-β1a or IFN-β1b), and both IFNAR subunits. In the 22 patients with reliable documentation of relapses, there was a negative correlation between the baseline IFNAR1 mRNA levels and relapse rate (P=.05).
cumulative induction of myxovirus protein-A (MxA), a marker of interferon-β bioactivity.\textsuperscript{12} Other authors have shown that long-term treatment with IFN-β could increase the levels of IFNAR1 mRNA in subjects in whom MxA has been induced.\textsuperscript{10} Low levels of expression of the IFNAR subunits can be one of the causes of low responsiveness to IFN-β in MS.

STAT4 signalling has been associated with Th1 development, inflammation and autoimmunity; however, its roles are not completely understood. STAT4 Th1 signalling suppresses Foxp3-positive T regulatory cells (Treg) and favours autoimmune disease.\textsuperscript{13} The transition of a subset of Th17 precursors to Th1-like cells is contingent upon STAT4 expression.\textsuperscript{14}

STAT4 represents a core signalling hub in MS.\textsuperscript{6} Mice deficient in STAT4 are resistant to inflammation induction in the animal model of MS.\textsuperscript{15} STAT4 promotes IFN-γ expression in humans. IFN-γ induces inflammation and relapses in MS,\textsuperscript{16} although its role in MS and its experimental models is incompletely elucidated.\textsuperscript{17} IL-12-induced expansion of Th1 cells plays an important role in MS. Responses to IL-12 are mediated mainly through STAT4.\textsuperscript{18} The ability of both IL-12 and IFN-β to activate STAT4 in T cells is relevant to the actions of those cytokines in MS. IL-12 and IFN-β are considered to have biologically opposing effects. IL-12 is increased\textsuperscript{19} and considered detrimental in MS,\textsuperscript{20} but IFN-β treatment does not appear to potentiate IL-12 proinflammatory effects. We have shown that prior exposure to IFN-β enhances STAT4 activation by IL-12, but the net effect is not proinflammatory because of parallel induction of IL-10 by IFN-β.\textsuperscript{4} In progressive stages of MS, IL-10 loses its ability to suppress IFN-γ and IL-12, while IFN-β treatment loses efficacy considerably in this phase of the disease. Therefore, the beneficial immunomodulatory effects of IFN-β may in part be a result of its ability to suppress IL-12.\textsuperscript{5}

The type I IFN pathway of STAT4 activation is more important in human immune responses than in mouse.\textsuperscript{21} Type I IFN-induced STAT4 activation requires interactions of STAT4 with STAT2, and there is genetic dissimilarity between murine and human STAT2.\textsuperscript{22}

Here, we show a decreased IFN-β-STAT4 signalling and IFNAR expression in whole, unseparated PBMCs from patients with MS. There are major differences in the responses of primary human leucocyte subsets to IFN-β.\textsuperscript{23} Although the activation of STAT4 by type I IFN was thought initially to occur only in T and NK cells, STAT4 activation is also shown in endothelial cells, activated monocytes and dendritic cells (DCs).\textsuperscript{24} IFN-β induces distinct gene expression response patterns in human monocytes vs T cells.\textsuperscript{25} Differential activation of a variety of signalling proteins including STATs by IFN-β can explain the significant patient-to-patient variation in gene expression observed after treatment with IFN-β in the PBMCs from patients with MS.\textsuperscript{26} Immune priming can change, positively or negatively, the quantity and quality of signal transduction by type I IFNs.\textsuperscript{24} The decreased STAT4 signalling in response to IFN-β found in the PBMC in the group of untreated patients with MS reported here could therefore reflect the baseline MS immunological milieu in the absence of therapeutic immunomodulation.

Because of the role of STAT4 in mediating inflammation in MS and, at the same time, in mediating IFN-β anti-inflammatory effects, we planned to look at long-term clinical correlates of STAT4 activation in untreated patients with MS just prior to initiating disease-modifying treatment with IFN-β. The patient group was then followed up for 5 years. Disability progression at 5 years is often used as a measure of long-term response to treatments in MS.\textsuperscript{27,28} Of note, the patients in this study were early participants in the UK Multiple Sclerosis Risk Sharing Scheme.\textsuperscript{29} This large observational clinical cohort study recently showed that treatment with IFN-β reduces disability progression measured by EDSS scores over 6 years of treatment.\textsuperscript{27} Early inclusions in the Risk Sharing Scheme (and access to treatment with IFN) consisted predominantly of patients with longer disease duration and
thus more advanced disease, more disability and higher EDSS scores. This explains the relatively high EDSS scores at baseline in our patient group (mean EDSS 3.5). Generally, patients with MS who reach EDSS 3 may already enter secondary progression.30 This may explain why this group showed evidence of disability progression over 5 years; this progression is difficult to quantify and compare to the overall Risk Sharing Scheme results, or what would be expected from the natural history of MS. However, in this cohort, clinical progression was not predicted by the pretreatment in vitro responsiveness to IFN-β. Whether the disease-modifying treatment altered the responsiveness and the expression of STAT4 and IFNAR in the short or medium term is unknown.

The lack of correlation of pSTAT4 expression before treatment with IFN-β to disability status at 5 years suggests the lack of a specific defect in STAT4 signalling that would link with the progression of disability. Of note, it was shown that, although the baseline expression of genes involved in IFN-β-related pathways can differ between different untreated patients with MS, and treatment with IFN-β can differentially induce gene expression (strong induction in those patients with low endogenous IFN-like activity, but only weak induction in patients with high endogenous IFN-like activity before treatment); patients with high and low endogenous IFN-like activity at baseline show similar clinical long-term courses of disease.31

We found a negative, albeit marginal, correlation of relapses with IFNAR1 mRNA expression (r=-.4; P=.05). This may suggest that IFNAR expression can be a predictor of relapses under treatment. Nevertheless, a reliable documentation of relapses was possible only in the 22 of patients followed (81%), and under-reporting of MS relapses cannot be excluded. In a multicentre, retrospective study including 103 patients with MS from 7 UK centres, 46% of patients did not report at least one relapse in the past and 28% of most recent relapses were unreported.32 Several reasons may contribute to this, including rare review schedules.32

The occurrence of relapses remains the principal factor for starting disease-modifying treatments (DMTs) under current MS treatment guidance.33 The risk of severe MS relapses is lower in patients treated with DMTs.34 While cost-effectiveness analyses in MS tend to focus on long-term disability, individual relapses have a major impact on patients’ working and family lives, which is often overlooked.32 This highlights relapse reduction as a worthy treatment goal in MS.32

Regular MRI follow-up data were not available for the group of patients in this study. MRI after the first year of treatment with IFN-β can be a good predictor of the outcome at 4 years,35 and the occurrence of two or more new T2 lesions or new gadolinium-enhancing lesions in patients with variable responses to IFN-β associates a significantly increased risk of future relapses and progression.36

In this study, we intended to determine whether pretreatment activation of pSTAT4 predicts longer term outcome. Therefore, we did not assess the medium- or long-term effects of IFN-β treatment on STAT4 signalling and IFNAR expression. Subnormal responses to type I IFN in MS can be corrected by long-term IFN-β therapy which is able to prime the subnormal IFN response and elevate STAT signalling levels.37 Gene expression profiling studies show that the IFN-β deficiency exhibited by patients with MS can be corrected over time by repeated IFN-β administration38; however, IFN signalling and gene activation pathways are partially resistant to IFN therapy in MS.5 Of note, the decreased IFN-β response in patients with MS can be improved by combination therapy with other treatments with synergistic effects. We have previously shown that prior exposure of PBMC of healthy controls and patients with MS to dexamethasone results in increased sensitivity of the cells to IFN-β, to an increase in pSTAT4 generation and in upregulation of its receptor expression.39 We showed similar enhancement of IFN-β signalling in cells from healthy controls and patients with MS by curcumin.40

In conclusion, here we show for the first time that IFN-β-induced STAT4 signalling is reduced in untreated patients with MS as compared to healthy subjects. The reduced pSTAT4 induction by IFN-β in patients with MS compared with controls indicates a decreased ability to activate IFN-β signalling pathway via STAT4. This, combined with the reduction in IFNAR expression, suggests that untreated patients with MS have decreased IFN-β responsiveness. Therapeutic strategies to improve this responsiveness, for example, by synergistic combinations may be beneficial in the treatment for MS in the future.

ACKNOWLEDGEMENTS

The study was supported in part by the MS Society of Great Britain and Northern Ireland.

CONFLICT OF INTEREST

CS Constantinescu has received research support, travel support for meetings and consultancy fees from Biogen-Idec, Bayer-Schering, Genzyme, Merck Serono, Morphosys, Novartis, Roche, Sanofi-Pasteur MSD and Teva. R Tanasescu has received travel support for meetings from Biogen-Idec, Novartis and Teva. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

REFERENCES

1. Lim SY, Constantinescu CS. Current and future disease-modifying therapies in multiple sclerosis. Int J Clin Pract. 2010;64:637–650.
2. Javed A, Reder AT. Therapeutic role of beta-interferons in multiple sclerosis. Pharmacol Ther. 2006;110:35–56.
3. Hervas-StubbS, Perez-Gracia JL, Rouzaut A, Sanmamed MF, le Bon A, Melero I. Direct effects of type I interferons on cells of the immune system. Clin Cancer Res. 2011;17:2619–2627.
4. Fahey AJ, Robins RA, Constantinescu CS. Reciprocal effects of IFN-beta and IL-12 on STAT4 activation and cytokine induction in T cells. J Leukoc Biol. 2007;81:1562–1567.
5. Tao Y, Zhang X, Chopra M, et al. The role of endogenous IFN-beta in the regulation of Th17 responses in patients with relapsing-remitting multiple sclerosis. J Immunol. 2014;192:5610–5617.
6. Huang YM, Hussien Y, Jin YP, Soderstrom M, Link H. Multiple sclerosis: deficient in vitro responses of blood mononuclear cells to IFN-beta. Acta Neurol Scand. 2001;104:249–256.
7. Feng X, Petraglia AL, Chen M, Byskosh PV, Boos MD, Reder AT. Low expression of interferon-stimulated genes in active multiple sclerosis
is linked to subnormal phosphorylation of STAT1. *J Neuroimmunol.* 2002;129:205–215.

8. Uzel G, Frucht DM, Fleisher TA, Holland SM. Detection of intracellular phosphorylated STAT-4 by flow cytometry. *Clin Immunol.* 2001;100:270–274.

9. A Biosystems. User Bulletin #2: ABI PRISM 7700 Sequence Detection System. Appl Biosyst. 1997. Available at: http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_040980.pdf. Accessed May 4, 2015.

10. Serana F, Sottini A, Ghidini C, et al. Modulation of IFNAR1 mRNA expression in multiple sclerosis patients. *J Neuroimmunol.* 2008;197:54–62.

11. TAO Y, Zhang X, Zivadinov R, et al. Immunologic and MRI markers of the therapeutic effect of IFN-beta-1a in relapsing-remitting MS. *Neural Neuroimmunol Neuroinflamm.* 2015;2:e176.

12. Gilli FVP, Sala A. Correlation between gene expression of interferon alpha/beta receptor and inducibility of four interferon stimulated genes in peripheral blood mononuclear cells from multiple sclerosis patients. *Mult Scler.* 2007;13:565–572.

13. Xu JN, Yang Y, Qiu GX, et al. Stat4 is critical for the balance between Th17 cells and regulatory T cells in colitis. *J Immunol.* 2011;186:6597–6606.

14. Harpston SN, Maynard CL, Zindl CL, Schoeb TR, Weaver CT. TH17 cells give rise to Th1 cells that are required for the pathogenesis of colitis. *Proc Natl Acad Sci USA.* 2015;112:7061–7066.

15. Chitnis T, Najafian N, Benou C, et al. Effect of targeted disruption of STAT4 and STAT6 on the induction of experimental autoimmune encephalomyelitis. *J Clin Invest.* 2001;108:739–747.

16. Panitch HS, Hirsch RL, Schindler J, Johnson KP. Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. *Neurology.* 1987;37:1097–1102.

17. Jagessar SA, Gran B, Heijmans N, et al. Discrepant effects of human interferon-gamma on clinical and immunological disease parameters in a novel marmoset model for multiple sclerosis. *J Neuroimmunol Neuropharmacol.* 2012;7:253–265.

18. Trinchieri G, Pfanz S, Kastelein RA. The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity.* 2003;19:641–644.

19. Musabak U, Demirkaya S, Genc G, Illici RS, Odabasi Z. Serum adiponectin, TNF-alpha, IL-12p70, and IL-13 levels in multiple sclerosis and the effects of different therapy regimens. *Neuroimmunomodulation.* 2011;18:57–66.

20. Sun L, He C, Nair L, Yeung J, Egwuagu CE. Interleukin 12 (IL-12) family cytokines: role in immune pathogenesis and treatment of CNS autoimmune disease. *Cytokine.* 2015;75:249–255.

21. Farrar JD, Murphy KM. Type I interferons and T helper development. *Immunol Today.* 2000;21:484–489.

22. Farrar JD, Smith JD, Murphy TL, Leung S, Stark GR, Murphy KM. Selective loss of type I interferon-induced STAT4 activation caused by a minisatellite insertion in mouse Stat2. *Nat Immunol.* 2000;1:65–69.

23. Van Boxel-Dezaire AH, Zula JA, Xu Y, Ransohooff RM, Jacobberger JW, Stark GR. Major differences in the responses of primary human leukocyte subsets to IFN-beta. *J Immunol.* 2010;185:5888–5899.

24. Frucht DM, Aringer M, Galon J, et al. Stat4 is expressed in activated peripheral blood monocytes, dendritic cells, and macrophages at sites of Th1-mediated inflammation. *J Immunol.* 2000;164:4659–4664.

25. Henig N, Avidan N, Mandel I, et al. Interferon-beta induces distinct gene expression response patterns in human monocytes versus T cells. *PLoS One.* 2013;8:e62366.

26. Reder AT, Velichko S, Yamaguchi KD, et al. IFN-beta1b induces transient and variable gene expression in relapsing-remitting multiple sclerosis patients independent of neutralizing antibodies or changes in IFN receptor RNA expression. *J Interferon Cytokine Res.* 2008;28:317–331.

27. Coles AJ, Fox E, Vladic A, et al. Alemtuzumab more effective than interferon beta-1a at 5-year follow-up of CAMMS223 clinical trial. *Neurology.* 2012;78:1069–1078.

28. Cohen JA, Khatri B, Barkhof F, et al. Long-term (up to 4.5 years) treatment with fingolimod in multiple sclerosis: results from the extension of the randomised TRANSFORMS study. *J Neuro Neurosurg Psychiatry.* 2016;87:468–475.

29. Palace J, Duddy M, Bregenzer T, et al. Effectiveness and cost-effectiveness of interferon beta and glatiramer acetate in the UK Multiple Sclerosis Risk Sharing Scheme at 6 years: a clinical cohort study with natural history comparator. *Lancet Neurol.* 2015;14:497–505.

30. Leray E, Yaoaun, J, le Page E, et al. Evidence for a two-stage disability progression in multiple sclerosis. *Brain.* 2010;133:1900–1913.

31. Hundeshagen A, Hecker M, Paap BK, et al. Elevated type I interferon-like activity in a subset of multiple sclerosis patients: molecular basis and clinical relevance. *J Neuroinflammation.* 2012;9:140.

32. Duddy M, Lee M, Pearson O, et al. The UK patient experience of relapse in multiple sclerosis treated with first disease modifying therapies. *Mult Scler Relat Disord.* 2014;3:450–456.

33. Scolding N, Barnes D, Cadert S, et al. Association of British Neurologists: revised (2015) guidelines for prescribing disease-modifying treatments in multiple sclerosis. *Pract Neurol.* 2015;15:273–279.

34. Vercellino M, Romagnolo A, Mattioda A, et al. Multiple sclerosis relapses: a multivariable analysis of residual disability determinants. *Acta Neurol Scand.* 2009;119:126–130.

35. Prosperini L, Mancinelli CR, de Giglio L, de Angelis F, Barletta V, Pozzilli C. Interferon beta failure predicted by EMA criteria or isolated MRI activity in multiple sclerosis. *Mult Scler.* 2014;20:566–576.

36. Dobson R, Rudick RA, Turner B, Schmierer K, Giovannoni G. Assessing treatment response to interferon-beta: is there a role for MRI? *Neurology.* 2014;82:248–254.

37. Reder AT, Feng X. How type I interferons work in multiple sclerosis and other diseases: some unexpected mechanisms. *J Interferon Cytokine Res.* 2014;34:589–599.

38. Croze E, Yamaguchi KD, Knappertz V, Reder AT, Salamon H. Interferon-beta-1b-induced short- and long-term signatures of treatment activity in multiple sclerosis. *Pharmacogenomics.* 2013;13:443–451.

39. Fahey AJ, Robins RA, Kindle KB, Heery DM, Constantinescu CS. Effects of glucocorticoids on STAT4 activation in human T cells are stimulus-dependent. *J Leukoc Biol.* 2006;80:133–144.

40. Fahey AJ, Robins RA, Constantinescu CS. Curcumin modulation of IFN-beta and IL-12 signalling and cytokine induction in human T cells. *J Cell Mol Med.* 2007;11:1129–1137.

How to cite this article: Tanasescu, R., Midgley, A., Robins, R. A. and Constantinescu, C. S. Decreased interferon-β induced STAT-4 activation in immune cells and clinical outcome in multiple sclerosis. *Acta Neurol Scand.* 2017;136:233–238. https://doi.org/10.1111/ane.12715