

Review of the clinical evidence for interferon β 1a (Rebif[®]) in the treatment of multiple sclerosis

Francesco Manfredonia¹
Livia Pasquali¹
Angela Dardano²
Alfonso Iudice¹
Luigi Murri¹
Fabio Monzani²

¹Department of Neuroscience and
²Department of Internal Medicine,
University of Pisa, Pisa, Italy

Abstract: Interferon (INF) β 1a 22 or 44 μ g (Rebif[®]) administered s.c. 3 times a week (t.i.w) is a well established immunomodulating treatment for relapsing remitting multiple sclerosis (RRMS). This review focuses on its mechanisms of action, evidence of efficacy, safety, and tolerability. Several pharmacodynamic properties explain the immunomodulatory actions of INF β 1a 22 or 44 μ g s.c. t.i.w. Pivotal trials and post-marketing studies proved that the drug is effective in reducing disease activity and likely in slowing disease progression. Head-to-head comparative studies with other marketed INFs β in RRMS suggested a better therapeutic response associated with higher doses and frequency of administration of Rebif[®]. Additional evidence indicated a beneficial effect of INF β 1a in patients with clinically isolated syndromes (CIS) suggestive of MS, as treatment reduced time to conversion to clinically definite (CD) disease. Further, although the drug did not prove to slow time to progression there were benefits on relapse- and MRI-related secondary outcome measures in secondary progressive (SP) MS. Pivotal trials, their cross-over extensions, and post-marketing studies consistently showed that INF β 1a 22 or 44 μ g s.c. t.i.w. is safe and well tolerated, as adverse drug reactions are usually mild and manageable.

Keywords: interferon β 1a, multiple sclerosis, review, clinical trials, post-marketing studies

Introduction

Multiple sclerosis (MS) is an immune mediated disease characterized by a neuroinflammatory process affecting the white matter in the central nervous system (CNS). It results in the occurrence of recurrent acute neurological impairment which remits in the majority of patients with or without sequelae (relapsing remitting [RR] MS) (Compston et al 2002). However, a neurodegenerative process leading to axonal loss and matrix destruction takes place over the course of years, and is implicated in sustained, irreversible neurological disability. Neuronal loss is more prominent when the disease takes a progressive course after years of RR episodes (secondary progressive [SP] MS) (Rovaris et al 2006) or when clinical manifestations are progressive from onset (primary progressive [PP] MS) (Thompson et al 1997).

Over the last 15 years, pivotal randomized, multicenter, double-blind, placebo-controlled studies have led to the market licence of interferons beta (IFNs β) for the treatment of RR MS (The IFNB Multiple Sclerosis Study Group 1993; Jacobs et al 1996; PRISMS Study Group 1998) and to its worldwide use in clinical settings. Additional studies have then assessed efficacy of IFNs β in clinically isolated syndromes (CIS) likely to develop MS (Jacobs et al 2000; Comi et al 2001), and in SP forms of the disease with superimposing relapses (European Study Group on Interferon beta-1b in Secondary Progressive MS 1998; *Secondary Progressive Efficacy Clinical Trial of Recombinant Interferon-beta-1a in MS* (SPECTRIMS) Study Group 2001).

In this review we focused on clinical evidence of efficacy and safety of IFN β 1a 22 and 44 μ g s.c. administered 3 times in a week (t.i.w.), a IFN β preparation that is currently available for treatment of patients with RR MS.

Correspondence: Fabio Monzani
Department of Internal Medicine,
University of Pisa, Via Roma 67, 56126
Pisa, Italy
Tel +39 050 993 490
Fax +39 050 553 235
Email fmonzani@med.unipi.it

Firstly, we examined the mechanisms of action of IFNs β as a class, with reference to *in vivo* and *in vitro* evidence of action of IFN β 1a 22 and 44 μg s.c. Secondly, we discussed the results of trials that led to the marketing approval of IFN β 1a 22 and 44 μg s.c. t.i.w. and their extensions. Thirdly, we focused on selected longitudinal follow-up studies looking at a confirmation of pivotal trial results and trying to infer the long-term benefit of IFN β 1a 22 and 44 μg s.c. t.i.w., by a systematic review of the literature. We then provided an overlook of clinical trials that showed evidence of the benefit of IFN β 1a 22 and 44 μg s.c. t.i.w. beyond RRMS, in both SPMS and CIS. Finally, we considered the safety and tolerability profile of the drug.

Subtypes of interferons

IFNs β can be distinguished into IFN β 1b and 1a on the basis of structural differences that conversely depend on the recombinant technique of production (Runkel et al 1998). IFN β 1b (Betaseron[®], Berlex Laboratories, Montville NJ; Betaferon, Schering AG, Berlin, Germany) is obtained by cloning the molecule in bacterial cells that are unable to glycosylate the recombinant protein, remove the N-terminal methionine during translation and replace one of the three cysteines with serine to maintain structural stability, whereas IFN β 1a is instead produced in mammalian cells, and is identical to the natural form of human IFN β .

Among IFNs β 1a two different treatments are currently available, one requiring a 30 μg dose administered i.m. once weekly (q.w.) (Avonex[®]; Biogen, Cambridge, MA), and the other that is given s.c. t.i.w. (Rebif[®]; Serono International SA, Geneva, Switzerland) at a dose of either 22 or 44 μg .

Because of structural differences, IFN β 1b is less active than IFNs β 1a (Antonetti et al 2002), and higher doses every other day (q.o.d.) are needed to achieve an equivalent effect. These factors may account for the higher degree of immunogenicity and the likelihood of development of binding and neutralizing antibodies (NAbs) over the course of treatment (Bertolotto et al 2004). 28%–47% of patients develop NAbs to IFN β 1b, 12%–28% to IFN β 1a s.c. (Rebif[®]) and 2%–6% to IFN β 1a i.m. (Avonex[®]) (Bertolotto et al 2004), and their production has been associated with deterioration of therapeutic response (Malucchi et al 2004).

Mechanisms of action

IFN β was first tested for treatment of MS due to its antiviral property, as it was thought that the cause of the disease lay in a viral infection. Today, although viral infections are still considered and studied, at least as contributory factors,

IFN β is regarded more as an immunomodulatory and antiproliferative treatment. Laboratory and clinical studies have in fact shown that it inhibits MS activity, acting on a variety of processes and molecular mediators within the immune system. IFN β modifies the cytokine production in favor of the antiinflammatory subset, such as IL-10 and IL-4, inhibiting the release of proinflammatory cytokines such as IFN β and tumor necrosis factor (Rothuizen et al 1999; Yong et al 1998). Other pharmacodynamic properties of IFN β include inhibition of T-cell activation, block of production of oxygen free radicals by mononuclear phagocytes, and reduced expression of major histocompatibility complex class II molecules, which in turn reduces self-antigen presentation in the CNS (Dhib-Jalbut 2002). A recent *ex vivo* and *in vitro* longitudinal study demonstrated that IFN β in its 1a form enhances CD4+ regulatory T cells activity (de Andres et al 2007).

Beneficial effects of IFN β may also be due to a protective role exerted at the level of the blood-brain barrier (BBB), by reducing the activity of metalloproteases that are responsible for BBB disruption, and/or by preventing adhesion and subsequent migration of T-cells into the CNS (Galboiz et al 2001). In particular, it was demonstrated that IFN β 1a regulates the expression of serum and membrane-associated intercellular adhesion molecules (Giorelli et al 2002), and it is associated with up-regulation of vinculin and N-cadherin expression in brain endothelial cells (Harzheim et al 2004) restoring BBB disruption IFN β action-related.

Most of these pharmacodynamic properties depend on the interaction of IFN β with cell surface receptors (Wagstaff and Goa 1998). This interaction induces an intracellular signal cascade leading to the expression of IFN-stimulated genes, whose products such as neopterin, myxovirus resistance protein A, β 2 microglobulin, and 2',5'-oligoadenylate synthetase, besides carrying out the effect of IFN, have also been studied and proposed as a tool to monitor the drug activity, and potentially the biological response to treatment (Bertolotto et al 2001).

However controversial the definition of IFNs β as disease-modifying drugs may be, recent experimental studies have proposed a novel and neuroprotective mechanism of action for IFN β . The survival of retinal ganglion cells in the animal model MS, the experimental autoimmune encephalomyelitis, was enhanced by treatment with IFN β 1a (Sättler et al 2006). In addition, another study proved that IFN β stimulates the secretion of nerve growth factors by endothelial cells (Biernacki et al 2005). This axon protective effect was related to the antiinflammatory properties of the drug.

Pivotal clinical studies of interferon β 1a 22 or 44 μ g s.c. t.i.w. (Rebif®)

The PRISMS trial (*Prevention of Relapses and Disability by Interferon β -1a Subcutaneously in Multiple Sclerosis*) (PRISMS Study Group 1998; Li and Paty 1999) was the pivotal phase III trial that assessed efficacy of IFN β 1a 22 and 44 μ g s.c. t.i.w. in RR MS patients. The study was a randomized, double-blind, placebo-controlled, clinical trial, meeting class I evidence, according to the rating of evidence classification scheme of the AAN.

The EVIDENCE trial (*Evidence of Interferon Dose-response: European North American Comparative Efficacy*) (Panitch et al 2002) was a randomized, controlled, assessor-blinded, parallel-group study, fulfilling as well the AAN criteria for class I data. It reported evidence for the best benefit-to-risk ratio of higher doses and higher frequency of administration of IFN β 1-a 44 μ g s.c. t.i.w. compared to IFN β 1a 30 μ g i.m. q.w., and led to the approval of the drug in the United States.

PRISMS trial

In the PRISMS study (PRISMS Study Group 1998; Li and Paty 1999) 560 RRMS patients with an expanded disability status scale (EDSS) (Kurtzke 1983) score \leq 5.0 were randomized to 1 of 3 treatment arms (IFN β 1a s.c. t.i.w., either at dosages of 22 μ g or 44 μ g, or placebo). Patients with a disease duration \geq 1 year were included in the study, providing they had had at least 2 relapses during the 2 years before enrolment. 22 centers from 9 different countries took part to the study. 189 patients were randomized to the lower dose (22 μ g), 184 to the higher dose (44 μ g) of IFN β 1a s.c. t.i.w., and 187 patients to placebo. Study groups were well balanced for patients' characteristics at baseline. Primary analysis was as intention-to-treat study population.

The primary endpoint in the PRISMS trial was the clinical relapse rate over 2 years. Secondary endpoints consisted of other relapse rate parameters (time to first relapse, percentage of patients remaining relapse free, relapse severity, steroid use, and hospitalization) and MRI attack rate measured by median number of T2 active lesions and volume of white matter disease seen on T2-weighted MRI. Additional secondary outcome measures included time to sustained disability progression (confirmed after three months), findings on an ambulation index and an arm-function index.

Only 10 patients in the placebo group and 17 patients in the treatment group were lost to the follow-up. The study demonstrated a beneficial effect for all major outcomes with either drug dose regimen. The relapse rate in both IFN β 1a

22 μ g and 44 μ g groups were significantly reduced compared to the placebo group (1.82 and 1.73 respectively for treatment groups versus 2.56 in the placebo group, $p < 0.005$). A 27% ($p < 0.05$) and a 32% ($p < 0.005$) reduction in relapse rate was achieved in the 22 μ g group and in the 44 μ g group, respectively, compared with the placebo group. The median time to first relapse was significantly delayed by 3 months in the lower dosage group, and by 5 months in the higher dose treatment group versus placebo (statistical significance not reported). A significant reduction in moderate to severe types of relapses ($p < 0.005$) and in corticosteroids courses ($p < 0.05$ for the 22 μ g group; $p < 0.005$ for the 44 μ g group) was also observed in the active arms compared to placebo. Number of hospitalizations significantly decreased in the 44 μ g group ($p < 0.005$ versus placebo).

Median MRI lesion load significantly decreased in both the active treatment groups (-1.2% in the 22 μ g group and -3.85% in the 44 μ g group; $p \leq 0.0001$), whereas it increased ($+ 10.9\%$) in the placebo group. In addition, T2 active lesion number was reduced in the active treatment groups (-67% for the 22 μ g, -78% for the 44 μ g; $p < 0.0001$) compared to placebo. The decrease was more evident in the higher dosage group compared to the lower dosage group ($p < 0.0003$), suggesting a likely dose-response effect.

Besides clinical or paraclinical measures of disease activity, some measures of disability progression were included in the PRISMS trial. The study showed that time to first progression of disability measured by the EDSS for the 25th percentile of patients was 18.5 months in the IFN β 1a 22 μ g group and 21.3 months in the IFN β 1a 44 μ g group, compared with 11.9 months in the placebo group ($p < 0.05$).

It is worth saying that this kind of fixed disability assessment is not free from misinterpretation, as it does not take into account long term fluctuations or relapses persisting for more than 3 months. In addition, according to natural history of MS, disability take place over the course of many years (Weinshenker et al 1991), while the trial lasted a relatively short time. The EDSS scale, used for detecting disease progression, has also been questioned for its non linearity (Cohen et al 1993), lack of representation of all facets of functional impairment, insensitivity to changes, and other pitfalls such as the poor inter-rater and test-retest reliabilities (Noseworthy et al 1990; Rudick et al 1996), especially at the lower echelons of the scale (range typical of patients included in the trial). In order to address the doubts risen about the protective effect of IFN β 1a 22 and 44 μ g s.c. t.i.w. on disability accrual, further evaluation was carried out on the EDSS scores by a post-hoc analysis of patients

included in the PRISMS trial. The area under disability/time curves was calculated to quantify in-trial changes, which, although potentially advantageous, proved lack of information on direction of changes (Liu and Blumhardt 1999). In another post-hoc analysis, disease trend analysis and categorical classification using serial EDSS scores (Liu and Blumhardt, 2002) confirmed that IFN β 1a 22 and 44 μg s.c. t.i.w. increased the proportion of patients with a stable course and reduced those with prolonged disabling deterioration. Although these calculations were still based on EDSS scores, and inherently incorporated the potential disadvantages of the scale, they reduced the errors associated with conventional confirmed progression definition. They also revealed that baseline disease duration and EDSS levels were predictive of the disability trends, and that the treatment effect was more significant for subjects with entry EDSS of over 3.5 and shorter disease duration. Further evidence from later studies corroborated the results of the PRISMS trial, as they validated the predictive role of short-term outcome variables used in the trial on subsequent long-term disability accumulation.

The relationship between relapses and disease progression is not well defined: Confavreux et al (2000) reported that relapses do not significantly influence the progression of irreversible disability while other authors, analyzing a large database from a combination of previous trials, concluded that MS exacerbations produce a measurable and sustained effect on disability (Lublin et al 2003). Early population-based natural history studies (Weinshenker et al 1989; Weinshenker 1995; Wingerchuk and Weinshenker 1999) have demonstrated a relationship between early clinical relapse rate and subsequent development of disability, thus supporting the hypothesis that a drug that lessens the number of relapses can positively affect MS clinical course over the years.

Among the paraclinical MRI markers of disease activity used in the PRISMS trial, on which IFN β 1a 22 and 44 μg s.c. t.i.w. had proved beneficial effect, disturbance of the blood – brain barrier, as detected by MRI gadolinium enhancement, was shown to be a predictor of the occurrence of relapses, but not a strong predictor of development of cumulative impairment or disability (Kappos et al 1999). On the other hand, some authors have recently demonstrated in a long-term longitudinal study (13 years) that baseline T2 lesions volume in RR MS patients strongly correlated with brain tissue loss and brain integrity, and that changes in T2 lesion volume over the first 2 years correlated with clinical disease severity (Rudick et al 2006). Those results support

the therapeutic benefit of IFN β 1a 22 and 44 μg s.c. t.i.w., as treatment is capable of acting on a MRI surrogate marker of disease linked to long-term progression.

EVIDENCE trial

Some pharmacological and clinical studies have postulated the hypothesis of a dosage-frequency dependent response to IFN β . Whereas pharmacodynamic measurements of IFN β showed a greater activity after a single high dose (Williams and Witt 1998; Stürzebecher et al 1999), studies in healthy volunteers demonstrated a sustained response to IFN β 1a when administered 3 times a week rather than once weekly (Rothuizen et al 1999).

Clinical studies indicated that IFN beta 1b at the dose of 8 million international units (MIU) was superior to 1.8 MIU on both clinical and MRI endpoints (The IFNB Multiple Sclerosis Study Group 1993). Furthermore, the PRISMS trial showed an overall better outcome for IFN β 1a 44 μg vs IFN β 1a 22 μg regimen. In addition, the limited clinical effect of IFN β 1a 22 or 44 μg s.c. q.w. (The Once Weekly Interferon for MS Study Group 1999) contrasted with the positive results obtained in the PRISMS trial, where the same drug was given t.i.w.

This experimental and clinical evidence prompted the EVIDENCE trial (Panitch et al 2002), with the aim to prove the superiority of higher dosages-higher frequency of subcutaneous preparation in a head-to-head comparison of IFN β 1a 44 μg s.c. t.i.w. with IFN β 1a 30 μg i.m. q.w. In the study, 677 patients with RRMS and at least 2 exacerbations of MS in the prior 2 years, and EDSS scores of 0–5.5, were enrolled at 56 centers (15 in Europe, 5 in Canada, and 36 in the United States). Following randomization, 338 patients received IFN β 1a i.m. at the dose of 30 μg q.w. while 339 were given IFN β 1a at the dose of 44 μg s.c. t.i.w. Treatment lasted 48 weeks.

The primary endpoints were the proportion of patients remaining relapse free, and an MRI outcome measure which consisted of the number of combined unique (CU) active lesions per patient per scan, a measure of both active T2 and T1 gadolinium (Gd) enhancing lesions. A CU lesion was defined as an active lesion on T1 post-Gd or T2 sequences, or both, avoiding double counting; an active T2 lesion was defined as a new or enlarging lesion, or a lesion reappearing at a site of previous lesion resolution. Secondary and tertiary clinical outcome measures included relapse rate, relapse severity, use of steroids for relapses, and time to first relapse and disability progression, defined by one point increase on the EDSS scale confirmed at a visit 3 or 6 months

later. Secondary MRI outcome measures included number of T2 and T1 lesions per patient per scan, the proportion of active scans (T2, T1, and CU) per patient, and the proportion of patients in whom active scans (T2, T1, and CU) either occurred or did not occur during the initial 24 weeks of the trial. At week 48 only T2 lesions were counted and measured, as Gd was not administered.

There were no significant differences in baseline characteristics between treatment groups. The results over the initial 24 weeks of treatment showed that 75% of patients in the 44 µg s.c. t.i.w. group and 63% of those in the 30 µg i.m. q.w. group remained relapse free. The odds ratio (OR), adjusted for center, was 1.9 ($p < 0.0005$), indicating a relative increase of 90% in the odds of remaining relapse free during the first 24 weeks of therapy for patients receiving 44 µg s.c. t.i.w. compared with those receiving 30 µg i.m. q.w. The response was maintained over 48 weeks of treatment albeit less marked, as 62% of patients in the 44 µg s.c. t.i.w. group and 52% of those in the 30 µg i.m. q.w. group remained relapse free. The OR, adjusted for center, was 1.5 ($p < 0.009$), indicating a relative increase of 50% in the odds of remaining relapse free for patients receiving 44 µg s.c. t.i.w. compared with those given 30 µg i.m. q.w.

As far as MRI primary outcome is concerned, patients treated with IFN β 1a 44 µg s.c. t.i.w. had fewer CU, T1, and T2 active lesions per MRI scan compared with those treated with 30 µg i.m. q.w. at week 24 ($p < 0.0001$ for all activity measures). Outcome data on other relapse-related measures also favored 44 µg s.c. t.i.w. treatment. The time to first relapse was prolonged over the course of the study for patients treated with 44 µg s.c. t.i.w. (hazard ratio 0.70; $p < 0.003$). Relapse rates were 0.29 in the 44 µg s.c. t.i.w. group and 0.40 in the 30 µg i.m. q.w. group at 24 weeks, with a 27% relative difference ($p < 0.022$), and continued to be lower at 48 weeks, although the difference was less pronounced: 0.54 in the 44 µg s.c. t.i.w. group compared with 0.64 in the 30 µg i.m. q.w. group, with a 16% relative difference ($p < 0.093$). The mean rate of steroid use for relapses was lower for the 44 µg s.c. t.i.w. group compared to the 30 µg i.m. q.w. group ($p < 0.017$). There was a trend toward reduction of risk progression in the 44 µg s.c. t.i.w. group compared with the 30 µg i.m. q.w. group.

Additional MRI outcomes confirmed the superiority of the higher dose-higher frequency preparation. The mean number of active scans per patient was also reduced in patients receiving 44 µg compared with those receiving 30 µg i.m. q.w. Only the number of T2-active lesions after 48 weeks could be calculated by comparing the baseline and

week 48 scans, as Gd was not administered. Differences between treatment groups still favored the 44 µg s.c. t.i.w. patients for mean number of T2 lesions per patient per scan (36% relative reduction), proportion of T2-active scans (38% relative reduction), and proportion of patients with no T2 active lesions over 48 weeks (32% relative increase) ($p < 0.001$ for all comparisons).

In summary, the trial provided evidence of a clinical superior efficacy of IFN β 1a 44 µg s.c. t.i.w. on IFN β 1a 30 µg i.m. q.w. in RRMS patients. However, which aspect of the different IFN β preparations was more relevant remained unsolved: total weekly dose, frequency of administration, or method of administration. Results of EVIDENCE trial were in agreement with another study that compared IFN β 1-b 8MIU s.c. q.o.d. with IFN β 1a 30 µg i.m. q.w., the Independent Comparison of Interferon Study (Durelli et al 2002). Compared to the latter, the EVIDENCE trial has been rated as class I evidence on the AAN rating scale, as assessor blinding was guaranteed.

Theoretically, a head-to-head comparison of disease modifying drugs in MS would require a randomized, double-blind, placebo-controlled trial with a long-term follow-up. However, this study design would not meet ethics' committee approval as it would imply placebo use for long time, while established therapy could be offered to the patients, and would be unpractical for the different route of administration and maintenance of blindness for injection-related side effects. Hence, assessor-blind design represents a valid alternative, as it assures reliability of study results.

PRISMS study extensions

The PRISMS study was further extended to 4 years (PRISMS-4), and patients originally randomized to placebo were re-randomized to either IFN β 1a 22 or 44 µg s.c. t.i.w. (PRISMS Study Group, University of British Columbia MS/MRI Analysis Group 2001). The study provided evidence for a sustained efficacy of IFN β 1a 22 and 44 µg s.c. t.i.w. over the extended time on both clinical (relapse rate and time to disability progression) and MRI outcomes (MRI activity and lesion burden), and a dose-dependent drug response. In addition, outcomes were consistently better for patients who continued the active treatment compared to the cross-over group, suggesting the importance of an early treatment.

Additional evidence comes from the long-term follow-up (LTFU) study that included patients in the original PRISMS trial (Kappos et al 2006). Assessments included clinical and MRI exams of patients who had been enrolled in the PRISMS study, coinciding as close as possible with the seventh or

eighth anniversary of enrollment. To date, this is one of the longest follow-up trial of a pivotal phase III study published. At the LTFU visit, EDSS scores along with documentation of ongoing relapses and clinical data from the final neurological assessment of PRISMS-4 were reviewed. PD/T2 weighted scans were performed for blinded measurement of lesion volume. Brain atrophy was also analysed as brain parenchymal volume derived from the intracranial volume by subtracting the CSF volume. Out of the initial 560 patient cohort, 382 patients were re-evaluated at LTFU, and 72% of attending patients were still receiving IFN β 1a at one of the two dose regimens. A total of 35% of patients receiving IFN β 1a s.c. t.i.w. had converted from one dose to the other, with the majority switching from 22 μ g to 44 μ g; the figure was probably influenced by local treatment guidelines and reimbursement practice in the different countries participating in the trial. One of the most interesting results of the study was that the longest time to reach a confirmed progression was detected in patients originally randomized to 44 μ g, compared to patients who were crossed over to treatment after 2 years of placebo (late treatment group, LTG). Patients were more likely to be relapse free if they started on the higher dose, and both relapse rate and T2 lesion accumulation were similarly lower in the 44 μ g group compared with the LTG. Study results confirmed what had already emerged at 4 years extension, ie, higher drug dose and earlier treatment were the key issues to achieve a better outcome.

Post-marketing clinical studies of interferon β 1a 22 and 44 μ g s.c. t.i.w. (Rebif[®])

Additional evidence on efficacy of IFN β 1a 22 and 44 μ g s.c. t.i.w. in a less strictly selected MS patients populations can be derived from Phase IV studies, as well as from retrospective chart reviews. Overall, these studies adopted wider inclusion/exclusion criteria than pivotal trials, but more adherent to clinical practice settings. Most studies were conducted with the aim of detecting differences between available disease-modifying drugs in MS that were not shown in randomized, controlled clinical trials. Comparison of study results with those from pivotal trials is indeed precluded by the different methodologies they employed. We reviewed 9 open-label, post-marketing, observational, controlled studies that compared the relative long-term efficacy and tolerability of various IFNs β in the treatment of RRMS. We included in our review only published studies that had a follow-up of at least 2 years, a group of patients on IFN β 1a s.c. t.i.w. and included more than 15 patients per treatment group.

Overall, 9 studies were considered (Paolillo et al 2002; Haas et al 2005; Milanese et al 2005; Rio et al 2005; Trojano et al 2005; Bonavita et al 2006; Etemadifar et al 2006; Kock-Henriksen et al 2006; Limmroth et al 2007). Details of these studies are provided in Tables 1 which summarizes trial design, number of patients, main clinical outcomes, and results. A large variability among study characteristics precludes any comparison of the effects of IFN β 1a s.c. t.i.w.. Indeed, the number of patients who reached a certain follow-up and administered dose of IFN β 1a s.c. t.i.w. were sometimes not specified or differed among studies, making impossible to compute a cumulative patient-year treatment number for each IFN β 1a s.c. t.i.w. formulation. Patients selection, sample size, missing data, clinical outcomes, and other issues significantly impair the actual reliability of studies results. Lack of randomization, variation in follow-up duration and changes of drug treatments contribute to limit the assessment of therapeutic response and largely bias the results. An higher number of patients receiving IFN β 1a 30 μ g i.m. q.w. and IFN β 1b 8 MIU s.c q.o.d. than IFN β 1a 22 and 44 μ g s.c. t.i.w. was generally reported, in relation to the earlier availability of these formulations at some study sites.

Most of these studies showed no differences in term of efficacy among the different IFNs β preparations and dose regimens. Others detected a favorable effect of higher frequency and higher doses on clinical outcomes. IFN β 1a 44 μ g s.c. t.i.w. resulted less effective than other IFNs β in one single study (Limmroth et al 2007). In this retrospective, controlled, observational trial, including one of the largest case cohorts, IFN β 1a 44 μ g s.c. t.i.w. was used in a significantly higher number of patients than other treatments, indicating that it was likely substitute for a prior drug in patients with disease progression. Hence, patients in IFN β 1a 44 μ g s.c. t.i.w. could have had a higher disease activity, and thus a poor therapeutic response. As most studies had a follow-up of 2 years it cannot be excluded that comparable efficacy among different IFNs β preparations had not been influenced by development of neutralizing antibodies (NABs), unlikely in this relative short timeframe (Vartanian et al 2004).

Only few of these studies included paraclinical measures of response, and often in a subgroup of patients, further limiting any exhaustive conclusion. However, overall results from these ancillary studies matched the results observed in randomized-controlled trials. Indeed reviews and meta-analysis of disease modifying drugs in MS have confirmed the clinical validity and usefulness of open label trials and their substantial consistency with the results of pivotal trials

Table 1 Post-marketing studies characteristics

Study	FU years	Clinical outcomes	Characteristics of the study	Number of patients and treatment
Bonavita et al 2006	2	<ul style="list-style-type: none"> - Reduction in relapse rate - OR for remaining relapse free - Change of EDSS score - OR for remaining progression free 	Observational, retrospective	58 A, 42 B, 48 R, 22, 67 R, 44
<i>Results</i>				
<ul style="list-style-type: none"> • Patients in R 44 more likely to remain free from relapses OR = 2.23 				
Etemadifar et al 2006	2	<ul style="list-style-type: none"> - Mean relapse rate - Percentage of patients remaining relapse free after 2 years - Change of EDSS score 	Prospective, randomized, assessor-blinded	30 A 30 B 30 R, 44
<i>Results</i>				
<ul style="list-style-type: none"> • Mean relapse rate decreased in all groups (lower in B and R 44 groups compared to A group) • Percentage of patients remaining relapse free after 2 years (A 20%, B 43%, R 44 56%) • EDSS change (A stable, B 0.7 U reduction, R 0.3 U reduction) 				
Haas et al 2005	2	<ul style="list-style-type: none"> - Relapse rate - number of relapse-free patients - mean EDSS change - progression rate (EDSS change \geq 1) 	Observational, retrospective	A, B, R, 22, C Number of patients who reached 2 year FU per each group is not specified
<i>Results</i>				
<ul style="list-style-type: none"> • All groups showed sustained reduction of relapse rate, high percentage of relapse-free patients and reduced progression index • No superiority of one of the INFβ preparations except for highest relapse-rate decline after 24 months in the C group and progression index significantly lower in R 22 and C groups compared with B group 				
Kock-Henriksen et al 2006	2	<ul style="list-style-type: none"> - annualized relapse rate - time to first relapse - time to sustained progression 	Open-label, prospective, randomized	188 B 110 R, 22
<i>Results</i>				
<ul style="list-style-type: none"> • No superiority of one of the INFβ preparations in any of clinical outcomes 				
Limmroth V et al 2007	2	<ul style="list-style-type: none"> - Change from baseline EDSS score - percentage of progression-free patients (< 1.0 EDSS score) - annualized relapse rate - percentage of relapse-free patients 	Observational, retrospective	As initial therapy A 1469, B 1484, R22 784, R44 254 As FU therapy A 224, B 182, R, 22 126 R, 44 130 (after another DMT they had received for less than 2 years)

(Continued)

Table 1 (Continued)

Study	FU years	Clinical outcomes	Characteristics of the study	Number of patients and treatment
<i>Results</i>				
		<ul style="list-style-type: none"> No significant differences among IFNs β when used as initial or FU therapy on almost all outcome variables (percentage of progression-free patients lower in R 44 group, but R 44 was used more often as FU treatment, in patients with a likely higher disease activity) 		
Milanesi et al 2005	1–3	<ul style="list-style-type: none"> annual relapse frequency compared to pre-treatment values EDSS change 	Observational, retrospective	115 A, 67 B, 45 R22, 18 R44, 49 C
<i>Results</i>				
		<ul style="list-style-type: none"> Annual relapse frequency compared to pre-treatment values decreased by 63%, 67%, 40%, 58%, and 32% in A, B, R22, R44 and C after 1 year and continued to decrease in the subsequent years (81%, 86%, 79%, 79%, and 76% respectively after 5 years) EDSS does not consistently change in any treatment group 		
Paolillo et al 2002	6	<ul style="list-style-type: none"> Mean annual relapse rate compared with the 2-year period before treatment sustained progression defined as an increase of at least 1 point in EDSS scores 6 months apart 	Observational, retrospective 68 patients	All received R for the first 2 years at different doses – 16 continued with R 33 μg t.i.w – 52 continued with B – 11 drop-outs
<i>Results</i>				
		<ul style="list-style-type: none"> Mean annual relapse rate compared with the 2 year period before treatment reduced (68.6%) Sustained progression: 60% of patients remain stable or improved 		
Rio et al 2005	8	<ul style="list-style-type: none"> proportions of relapse-free patients relapse rate compared to 2 years before treatment probability of remaining progression free 	Observational, retrospective	At year 5 A 37 B 114 R 17 (dose not specified)
<i>Results</i>				
		<ul style="list-style-type: none"> No differences between the different products were observed 		
Trojano et al 2005	3–6	<ul style="list-style-type: none"> Relapse rate EDSS change from baseline 	Observational, retrospective	395 A, 294 B, 397 R 22, 87 R 44
<i>Results</i>				
		<ul style="list-style-type: none"> All groups showed significant reduction in relapse rate (differences between groups not significant) All groups showed increased EDSS change from baseline after 2 years (increase higher in B group) 		
Abbreviations: A, Avonex; B, Betaferon; Betaseron; EDSS, expanded disability status scale; R 22, Rebif 22 μg ; R 44 Rebif 44 μg ; C, Copaxone; DMT, disease-modifying treatment; FU, follow-up; OR, odds ratio; RRRMS, relapsing-remitting multiple sclerosis.				

(Benson and Hartz 2000; Concato et al 2000; Juni et al 2001). The extent of relapse rate reduction in these post-marketing trials was generally greater than that reported in pivotal trials. This effect was likely related to the selective exclusion from the analysis of non-responder patients, and hence an overestimation of benefit. Interestingly, most of these studies confirmed beside a relapse rate reduction, a favorable effect of IFN β 1a 22 or 44 μg s.c. t.i.w. on disability measures, foreseeing a protective role on disease progression, in accordance with the results of long-term analysis conducted by Kappos in the 7–8 years long-term follow-up study of the PRISMS cohort.

Besides improving our knowledge and validating the results of pivotal trials in clinical practice it is important to mention that some post-marketing studies have also explored the possible extension of IFNs β indication in early-onset MS for which no treatment is currently approved. Few studies have assessed safety and tolerability of IFN β 1a 22 or 44 μg s.c. t.i.w. in this particular subtype of MS patients (Pohl et al 2005; Tenembaum et al 2006) and provided efficacy evidences (Ghezzi et al 2005; Ghezzi et al 2007), although limited by the absence of a randomized double blind control design, and the treatment is now being offered off-label in many MS centers while waiting for more methodologically robust trials.

ETOMS trial

Recent randomized controlled trials have provided evidence on efficacy of IFN β 1a 22 and 44 μg s.c. t.i.w. in other MS subtypes. The drug was tested in a cohort of patients with CIS likely to develop MS (Comi et al 2001), and in SPMS patients (SPECTRIMS Study Group 2001). The rationale for treating patients earlier in the course of the disease is based upon several evidences. Previous pathological (Trapp et al 1999) and MRI studies (De Stefano et al 1999) have shown that axonal damage secondary to inflammation occurs early in the course of MS. Further, longitudinal observational (Weinshenker et al 1995) and MRI studies (O’Riordan et al 1998) have indicated that early pathological events are predictive of the future course of the disease. Imaging studies (Miller et al 1988; Paty et al 1988; Baum et al 1990) have contributed to the evidence that inflammatory activity underlying clinical relapses reflects a chronic process starting before the first episode of acute neurological impairment. It continues between relapses, becoming increasingly entrenched and difficult to control due to the early tissue injury that leads to the exposure of a progressively wider variety of autoantigens, in accordance to a phenomenon known as epitope spreading (Tuohy et al 1998).

The ETOMS trial (*Early Treatment Of Multiple sclerosis Study group*) (Comi et al 2001) studied a cohort of 309 patients who had presented with a single neurological episode suggestive of MS, and whose MRI scan was compatible with spatial disseminated demyelination. The inclusion criteria identified patients who were at high risk of developing clinically definite multiple sclerosis (CDMS) according to Poser’s diagnostic criteria (Poser et al 1983). Patients were randomized in two balanced groups receiving either IFN β 1a 22 μg q.w. or placebo. The study lasted 2 years, and 278 (90%) patients completed the follow-up according to the study protocol. The primary outcome measure was the conversion to CDMS, as defined by the occurrence of a second relapse. Secondary study outcomes included change in the Scripps Neurologic Rating Scale (SNRS) score (Kozioł et al 1999), and MRI measures such as the number of T2 active lesions – defined as new or enlarging T2 lesions, the number of enhancing T1 lesions, the number of patients without an active MRI scan, and the yearly changes of hyperintense T2 lesion volume. IFN β 1a 22 μg showed advantages over placebo on both primary and secondary outcomes. The percentage of patients converting to CDMS was significantly lower in the IFN β 1a 22 μg group compared to placebo (34% versus 45%, $p = 0.047$). Other clinical endpoints, although not stated as primary or secondary outcomes, confirmed the therapeutic effect of the medication. Time to conversion to CDMS was more than doubled in the IFN β 1a 22 μg group (569 days for the treated group versus 252 days for the placebo group in the 30th centile; $p = 0.034$), and annual relapse rate was 0.33 in the IFN β 1a 22 μg group and 0.43 in the placebo group ($p = 0.045$). Neuroimaging results were available for a large subgroup of patients in each of the two arms, and supported the benefit of active treatment versus placebo, as both T2 active lesions and lesion volume were significantly reduced by IFN β 1a. Furthermore, a post-hoc imaging analysis of brain volume by the ETOMS study group (Filippi et al 2004) demonstrated that early treatment reduced the progressive loss of brain tissue, a marker of axonal injury and matrix destruction associated with irreversible disability.

The results of the trial were in agreement with those of the Controlled High-risk subjects Avonex Multiple sclerosis Prevention Study (CHAMPS) (Jacobs et al 2000), which assessed the potential of IFN β 1a 30 μg i.m. q.w. in reducing the risk of developing CDMS in patients with CIS. Compared to the CHAMPS trial, the results from the ETOMS study were more remarkable for several reasons. The patient cohort at baseline was representative of a more severe and active disease, as it included patients with multifocal onset and a

higher MRI lesion numbers, the study follow-up was longer, the patient retention higher, and the beneficial effect of treatment associated with a frequency of treatment administration that had proved of limited efficacy in RRMS, suggesting that the approved standard frequency of administration for RRMS might have achieved even better outcome. Consistent results came from similar studies with the INF β 1-b 8 MIU s.c q.o.d. preparation which not only showed how early treatment in patients with CIS delayed conversion to clinically definite MS (Kappos et al 2006) but recently underscored its potential to prevent the development of fixed disability (Kappos et al 2007).

The suitability of the drug therapy after a first disease episode has gained more relevance following the introduction of the McDonald's diagnostic criteria (McDonald et al 2001) and its recent revision (Polman et al 2005). These criteria, accepting the Barkhof/Tintore criteria for providing MRI evidence of dissemination in space, allow an early diagnosis of multiple sclerosis in patients with CIS, that otherwise would have to await a second clinical episode for MS diagnosis. According to longitudinal MRI and clinical studies of CIS cohorts (O'Riordan et al 1998) it is now possible to estimate the risk of conversion to CDMS. Fullfillment of MRI criteria of dissemination in space and in time after a first episode suggestive of MS could lead to a screening of patients in terms of risk of conversion (Kortweg et al 2006) and to an adequate and reasonable efficacy-risk weighted early initiation of treatment.

SPECTRIMS trial

Evidence of efficacy of INFs β in SPMS has been more controversial. An European randomized, placebo-controlled, double-blind trial (European Study Group on Interferon beta-1b in Secondary Progressive MS 1998) demonstrated that INF β 1b reduced the confirmed 1-point EDSS progression rate (-22%; $p = 0.0008$), the study primary endpoint, as well as the clinical attack rate (-31%; $p = 0.0002$), the MRI attack rate (-78%; $p = 0.0008$), the white matter lesion burden (-13%; $p = 0.0001$), and more relevant the likelihood of becoming wheelchair bound during the study (-33%; $p = 0.01$). At variance with these results, a North American trial (Goodkin et al 2000) failed to replicate the favorable impact of INF β 1b on the 1-point confirmed EDSS progression rate (trial primary endpoint), although positive findings were reported in secondary outcomes clinical attack rate, MRI attack rate and white matter lesion burden. Discrepancy between results of the two trials was attributed to the fewer attack rate in the North American cohort, and was taken as a proof of efficacy of INF β 1b on SPMS with superimposed relapses.

On the basis of these previous trials, the SPECTRIMS study group (SPECTRIMS Study Group 2001) conducted a multicenter, randomized, parallel-group, placebo-controlled study, assessing INF β 1a 22 and 44 μg s.c. t.i.w. in SPMS patients. The trial failed to show a benefit on the primary endpoint time to confirmed progression, defined as an increase from baseline by at least 1 point or 0.5 if baseline EDSS was ≥ 5.5 (the hazard ratio 0.8 for 44 μg s.c. t.i.w. did not significantly differ from that in the placebo group; $p = 0.146$). However, a favorable effect was reported on clinical attack rate (0.50 per year with the active treatment versus 0.71 per year with placebo; $p < 0.001$ for both doses), on other secondary exacerbation-related outcomes, and on a composite score resulting from the combination of five major clinical and MRI measures. MRI findings (Li et al 2001) were detailedly reported apart, and demonstrated a favorable effect of the treatment (reduced median numbers of active lesions per patient per scan and white matter lesion burden). When the results were reconsidered in a post-hoc analysis by separating patients into those with and without superimposed relapses, a significant benefit on the confirmed EDSS progression rate was detected in patients who continued to experience relapse, and positive MRI results were also of greater magnitude. However caution should be used to interpret these positive findings as this analysis was not planned when the study was designed and both treatment doses were combined in the calculation.

Surprisingly, the study showed also a significant benefit on disability progression in women, and treatment-by-sex interactions were also reported for MRI measures. The authors could not provide a definite explanation for this finding, as neither the effect of chance nor a better responsiveness of women to INF could be excluded.

On the basis of these results, the treatment was not acknowledged as effective in SPMS notwithstanding the potential benefit in patients with superimposed relapses which suggested that treatment in the early phase of the disease, when relapses are a dominant feature, may be paramount to achieve a better therapeutic response. Discrepancy of these SPECTRIMS trial findings with the European INF β 1b trial could be explained by the shorter follow-up of the latter, due to early termination, higher rate of treatment discontinuation, and higher proportion of patients with pre-study relapses. Percentage of patients with pre-study relapse was more comparable to the North American Trial (European INF β 1b 70%, North American INF β 1b study 45%, SPECTRIMS 48%). The positive effect on relapses and MRI activity not accompanied by an impact on disability was interpreted as evidence

of a neurodegeneration dissociated from inflammatory events or of the existence of a time lag between inflammation and degeneration, which again strongly supported the usefulness of an early treatment. Table 2 shows a summary of clinical trials that have assessed IFN β 1a 22 or 44 μg s.c. t.i.w. in different clinical forms of MS.

The influence of NAbS on IFN β 1a efficacy

The detrimental effect of NAbS was observed after 18 months of treatment, and thereby short term clinical trials could lead to misinterpret the real impact of their development on treatment response (Vartanian et al 2004). Indeed, in the PRISMS trial the development of NAbS did not affect the mean relapse count for the 22 μg nor for 44 μg groups, and did not influence the overall results. However, at the 4 years follow-up the effect of NAbS became more relevant, as NAbS-positive patients experienced slightly more relapses than NAbS-negative in both dose groups. NAbS were less frequently associated to the 44 μg dose regimen, but their impact on relapse rate was greater, and exclusion of NAbS-positive patients strengthened the dose effect on relapse. NAbS impact was appreciated also on MRI measures, as NAbS-positive patients had a higher number of T2 active lesions, the lesion burden being increased in NAbS-positive patients and decreased in NAbS-negative ones within the 44 μg group.

Whereas the appearance of NAbS is not an early phenomenon, and its significance can be appreciated over longer follow-up, it is noteworthy that longer follow-up also documented a higher proportion of patients who developed NAbS seroreverted to NAbS-negative status. Kappos et al (2006) reported in their 7- to 8-year follow-up study of the PRISMS cohort that fewer patients in the LTFU cohort were NAbS-positive at their LTFU assessment than at earlier assessments. They observed also that at this time point an analysis of the impact of NAbS on the efficacy of the drug was hindered by many factors: few patients from a large cohort were interested, differential withdrawal of patients by NAbS status, the post hoc nature of the NAbS efficacy analysis, NAbS development not always preceding EDSS progression, and variability in titer measures. In the EVIDENCE trial, NAbS developed more frequently in the IFN β 1a 44 μg s.c. t.i.w group than in the lower dose regimen. By using the anytime positive definition of NAbS-positivity, ie, considering only patients who remained NAbS positive until the last assessment, it could be appreciated that their clinical impact was minimal during the study and only some effects were evident on MRI outcomes. In the SPECTRIMS trial, patients were

tested for NAbS at 6-month intervals. The study shared the same limits of the PRISMS trial, a short duration of follow-up and a small number of patients involved, for a reliable analysis of the impact of NAbS development. However, the results confirmed that the rate of NAbS development was higher for the 22 μg group, the seroconversion occurred generally after the first 18 months, and the positive status tended to affect drug efficacy on relapse rate in the 44 μg group, without any impact on the primary outcome.

Review of the selected phase IV studies do not provide additional information. According to a recent consensus (Hartung et al 2005) the immunogenicity of IFN β 1a 22 and 44 μg s.c. t.i.w. seems to be intermediate between that of IFN β 1b 8 MIU s.c. q.o.d., the most immunogenic drug, and IFN β 1a 30 μg i.m. q.w., the least immunogenic drug. Reasons behind immunogenicity differences are manifold and not yet completely understood. It is likely that difference of dose, frequency and route of administration, structural drug characteristics, level of homology to the natural human IFN, differences in manufacturing, purification, and formulation processes, all contribute to the variable immunogenicity of compounds.

Little information exists regarding how long NAbS last over continued treatment, as in some cases NAbS disappear over time (Rice et al 1999). It is likely that during the period of NAbS positive status, clinical benefits of the drug are abolished or diminished. Evidence that NAbS may affect drug efficacy encouraged the research of different IFN β 1a 22 or 44 μg formulations, hopefully less immunogenic and alternative to the current preparation. An ongoing phase III trial is assessing the antigenicity of a new fetal bovine serum (FBS)-free/human serum albumin (HSA)-free formulation of IFN β 1a. The producer has already submitted a supplemental Biologics Licence Application (sBLA) to the US Food and Drug Administration (FDA) and a variation to the current Marketing Authorization to the European Medicines Agency (EMA) for the new IFN β 1a formulation. An interim analysis of data from a Phase III clinical trial in patients with RR MS presented at the last ECTRIMS meeting (Giovannoni et al 2006) showed a substantial improvement in overall tolerability of the new formulation, as measured by injection site reactions, and a remarkable lower incidence of NAbS.

Safety and tolerability

Data on safety and tolerability of IFN β 1a 22 or 44 μg s.c. t.i.w. in MS patients were derived by PRISMS study and its cross-over extensions, EVIDENCE, ETOMS, and SPECTRIMS trials, and selected post-marketing studies.

Table 2 Summary of trials with IFN β 1a 22 μ g and 44 μ g in the different indications

CIS	ETOMS
Primary outcome	Proportion of patient developing CDMS reduced in IFN β 1a group compared to placebo group (34% vs 45%); p = 0.047
Secondary clinical outcomes	Time at which 30% of patients had converted to CDMS longer for IFN β 1a group compared to placebo group (569 vs 252 days) p = 0.034 Annual relapse rate reduced in IFN β 1a group compared to placebo group (0.33 vs 0.43) p = 0.045
Secondary MRI outcomes	Median number of active T2 lesion per patient per scan reduced in IFN β 1a group compared to placebo group (2.0 vs 3.0) p < 0.001 Median absolute change in T2 lesion volume (mm ³) reduced in IFN β 1a group compared to placebo group (-487 vs -299) p = 0.002
RRMS	PRISMS
Primary outcome	Relapse rate at year 2 lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (1.82, 1.73 vs 2.56) p < 0.005
Secondary clinical outcomes	Percentage relapse free patients over 1 year higher in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (37%, 45% vs 22%) p < 0.005 Percentage relapse free patients over 2 years higher in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (27%, 32% vs 16% placebo) p \leq 0.05 and p < 0.005 Mean moderate or severe relapses in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (0.71, 0.62 vs 0.99) p < 0.005 Mean steroid courses lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (0.97, 0.75 vs 1.39) p \leq 0.05 and p < 0.005 Mean hospital admissions lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (0.38, 0.25 vs 0.48) p < 0.005 only for IFN β 1a 44 μ g group compared to placebo Mean changes in EDSS lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (0.23, 0.24 vs 0.48) p \leq 0.05
Secondary MRI outcomes	Median lesion volume percentage change over baseline lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (-1.2%, -3.8% vs +10.9%) p < 0.0001 Median number of active lesions lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (-67%, -78%) p < 0.0001
SPMS	SPECTRIMS
Primary outcome	Time to confirmed progression in disability not significantly affected by treatment with IFN β 1a 44 μ g compared to placebo (HR 0.83; 95 CI 0.65-1.07) p = 0.146
Secondary clinical outcomes	Mean relapse rate lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (0.50, 0.50 vs 0.71) p < 0.001 Median time to first exacerbation longer in the IFN β 1a 44 μ g group compared to placebo (494 vs 281 days, HR 0.77) p = 0.034 Median time between first and second exacerbation lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (572, 511 vs 279 days) p \leq 0.001 Mean moderate and severe exacerbations per person-year lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (0.26, 0.27 vs 0.39) p = 0.002, p = 0.003 Mean steroid courses lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (0.31, 0.34 vs 0.52) p = 0.001 and p = 0.006 Mean hospital admission lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (0.14, 0.15 vs 0.22) p = 0.006 and p = 0.005
Secondary MRI outcomes	Lesion burden change over three years in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (-32, -4 vs +263) p < 0.001 T2 active lesions/patient/scan lower in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (0.17, 0.20 vs 0.60) p < 0.001 Median CU active lesions/patient/ in IFN β 1a 22 μ g and 44 μ g groups compared to placebo (0.11, 0.22 vs 1.0) p < 0.001 and p < 0.01

Abbreviations: CIS, clinically isolated syndrome; CI, confidence interval 95%; CDMS, clinically definite multiple sclerosis; CU, combined unique; ETOMS, Early Treatment Of Multiple sclerosis Study group; HR, hazard ratio; PRISMS, Prevention of Relapses and disability by Interferon β -1a Subcutaneously in Multiple Sclerosis; RRMS, Relapsing Remitting Multiple Sclerosis; SPMS, Secondary Progressive Multiple Sclerosis; SPECTRIMS, Secondary Progressive Efficacy Clinical Trial of Recombinant Interferon-beta-1a in MS.

In the PRISMS trial and its extensions, treatment with both doses of IFN β 1a s.c. t.i.w. was generally well tolerated. Adverse events were common but mild and manageable. These events included headache, flu-like symptoms, injection-site reactions, fatigue, myalgia, and fever. Laboratory abnormalities without clinical manifestations were also reported, as lymphopenia, increased alanine aminotransferase, leucopenia, increased aspartate aminotransferase, granulocytopenia. Similar adverse reactions were observed in the placebo arm, except injections-site reactions and laboratory abnormalities significantly more frequent in active treated patients. No difference was reported between the two dose regimens with regard to injection-site reactions, but laboratory abnormalities were more frequently associated with the higher dose. Hematologic abnormalities and elevation of liver enzymes were reduced during the second year of treatment.

The 4-year follow-up safety assessment confirmed that treatment was generally well tolerated, and flu-like symptoms, injection-site reactions, and laboratory abnormalities occurred to a lower extent over time. As expected, adverse events were more frequent in the cross-over group compared to early placebo treatment. Drug discontinuation due to adverse events was slightly higher at the higher dose, but more patients at the lower dose withdrew for disease progression. The most common adverse events leading to patient discontinuation were depression and injection-site reactions. Very rarely a skin necrosis was reported. Injection-site reactions decreased in frequency at 3 and 4 years of treatment, and were more often associated with the higher dose regimen.

Depression was not significantly associated with active drug, and over 3 and 4 years of treatment rate was comparable with that observed among patients receiving placebo at the initial phase of the study. Depressive symptoms were strongly associated with depression at baseline (Patten and Metz 2001). Association between treatment and depression was ruled out also in a study conducted on the SPMS cohort of the SPECTRIMS trial (Patten and Metz 2002). Depression ratings were obtained from 365 subjects treated either with IFN β 1a or with placebo: no significant differences between groups emerged over 36-month follow-up.

Data of a 7- to 8-year follow-up study confirmed a generally manageable drug tolerability profile and no new safety concerns were identified. Injection-site reactions were the most frequently reported adverse events, while others events including laboratory abnormalities occurred less frequently.

The EVIDENCE trial showed that IFN β 1a 22 and 44 μ g s.c. t.i.w. were more frequently associated with injection-site reactions and asymptomatic hepatic and white blood cell abnormalities, compared to IFN β 1a 30 μ g i.m. q.w. These events tended to reduce over time, and patients with NAb had fewer IFN-related adverse events. Analysis of ETOMS and SPECTRIMS trials did not add any additional information on safety and tolerability of the drug.

Thyroid, hepatic, and hematological abnormalities

Pre-existing and incident thyroid disease in multiple sclerosis patients receiving IFN β 1b has been reported (Monzani et al 2004). Similarly, clinically significant abnormal thyroid laboratory values were described during the PRISMS study but none caused drug discontinuation or dose reduction. A recent study confirmed that both incidental thyroid autoimmunity and dysfunction occurred in a large sample of RR MS patients treated with both IFN β 1a and 1b, namely within the first year of treatment. However, thyroid dysfunction was generally subclinical and transient, and never associated with treatment discontinuation (Caraccio et al 2005).

Two reviews have addressed hepatic dysfunction and hematologic events associated with IFN β 1a, combining data from randomized clinical trials and post-marketing surveillance (Francis et al 2003; Rieckmann et al 2004). Raised hepatic aminotransferase levels were mostly asymptomatic, occurred more commonly in the first 12 months of treatment, were frequency-dose related, and resolved mostly spontaneously or with dose adjustment. Post-marketing studies confirmed these findings, but generally reported a greater elevation of liver enzymes (Tremlett and Oger, 2004a; Tremlett and Oger, 2004b; Tremlett et al 2004c). Post-marketing surveillance of IFN β 1a included 130,000 patient-years, and reported 30 cases of serious symptomatic hepatic dysfunction, 2 cases requiring liver transplantation.

A review of pooled data on hematological events (Rieckmann et al 2004) showed only mild, asymptomatic, reversible, dose-related abnormalities, with little impact on adherence to treatment. They tend to occur during the first 6 months of treatment and consisted of decreases in white blood cells, neutrophils, lymphocytes, and platelet counts. Post-marketing surveillance data of IFN β 1a showed that hematological accounted for 8.6% of all reported adverse events, and only 12.8 of them were severe (Murdoch and Lyseng-Williamson 2004).

In our selected ancillary studies, IFN β 1a was well tolerated and adverse events were comparable with those reported

in pivotal trials. Injection-site reactions were more frequent with for IFN β 1a s.c. preparations. Rio et al (2005) reported 3 cases of urticaria and angioedema that were not observed in pivotal trials: reactions subsided with drug discontinuation and appropriate treatment. Limmoth et al (2007) considered number of patients who switched therapy as a partial indirect measure of tolerability, and showed fewer switches due to injection-site reaction in IFN β 1a i.m group compared to s.c. preparations. However, the authors acknowledged that drug discontinuation and switch to alternative therapy was often related to poor efficacy, and that IFN β 1a 44 μ g s.c. t.i.w. was often used as second treatment in these cases. Milanese et al (2005) claimed that shift to lower dose and lower frequency occurred more often when discontinuation of previous treatment was due to toxicity and lack of compliance, whereas shift to higher doses and frequencies was driven mainly by clinician decision toward a perceived poor response.

Conclusions

Pivotal trials and post-marketing studies have established the efficacy of INF β 1a 22 and 44 μ g s.c. t.i.w. on reduction of disease activity measured by clinical outcomes and MRI surrogate measures. Theoretically less immunogenicity compared to INF β 1b, and treatment at higher doses and higher frequency favor INF β 1a 22 and 44 μ g s.c. t.i.w. compared to other INF β formulations, but further head-to-head comparison studies are warranted to confirm this potential. Additional studies show that early drug treatment is beneficial in patients with early clinical manifestations and extends time to conversion to CDMS. Current evidences of benefit in patients with SPMS are not strong enough to support its use in this clinical MS subtype. Results of clinical trials and post-marketing surveillance of INF β 1a 22 and 44 μ g s.c. consistently show that the treatment is generally well tolerated, even on long-term basis. Adverse reactions generally occur at treatment initiation and are mild and manageable.

Disclosures

The authors have no conflicts of interest to disclose.

References

- Antonetti F, Finocchiaro O, Mascia M, et al. 2002. A comparison of the biologic activity of two recombinant IFN-beta preparations used in the treatment of relapsing-remitting multiple sclerosis. *J Interferon Cytokine Res*, 22:1181-4.
- Baum K, Nehrig C, Schörner W, et al. 1990. Long-term follow-up of MS: disease activity detected clinically and by MRI. *Acta Neurol Scand*, 82:191-6.
- Benson K, Hartz AJ. 2000. A comparison of observational studies and randomized, controlled trials *N Engl J Med*, 342:1878-86.
- Bertolotto A, Gilli F, Sala A, et al. 2001. Evaluation of bioavailability of three types of IFN- β in multiple sclerosis patients by a new quantitative-competitive-PCR method for MxA quantification. *J Immunol Methods*, 256:141-52.
- Bertolotto A, Deisenhammer F, Gallo P, et al. 2004. Immunogenicity of interferon beta:differences among products. *J Neurol*, 251(Suppl 2):15-24.
- Biernacki K, Antel JP, Blain M, et al. 2005. Interferon beta promotes nerve growth factor secretion early in the course of multiple sclerosis. *Arch Neurol*, 62:119-24.
- Bonavita S, Dinacci D, Lavorgna L, et al. 2006. Treatment of multiple sclerosis with interferon beta in clinical practice:2-year follow-up data from the South Italy Mobile MRI Project. *Neurol Sci*, 27:365-8.
- Caraccio N, Dardano A, Manfredonia F, et al. 2005. Long-term follow-up of 106 multiple sclerosis patients undergoing interferon-beta 1a or 1b therapy:predictive factors of thyroid disease development and duration. *J Clin Endocrinol Metab*, 90:4133-47.
- Cohen RA, Kessler HR, Fisher M. 1993. The expanded disability status scale (EDSS) as a predictor of impairments of functional activities of daily living in multiple sclerosis. *J Neurol Sci*, 115:132-5.
- Comi G, Filippi M, Barkhof F, et al. 2001. Effect of early treatment on conversion to definite multiple sclerosis:a randomized study. *Lancet*, 357:1576-82.
- Compston A, Coles A. 2002. Multiple sclerosis. *Lancet*, 359:1221-31.
- Concato J, Shah N, Horwitz RI. 2000. Randomized, controlled trials, observational studies, and the hierarchy of research design. *N Engl J Med*, 342:1887-92.
- Confavreux C, Vukusic S, Moreau T, et al. 2000. Relapses and progression of disability in multiple sclerosis. *N Engl J Med*, 343:1430-8.
- de Andres C, Aristimuno C, de Las Heras V, et al. 2007. Interferon beta-1a therapy enhances CD4+ regulatory T-cell function:an ex vivo and in vitro longitudinal study in relapsing-remitting multiple sclerosis. *J Neuroimmunol*, 182:204-11.
- De Stefano N, Narayanan S, Pelleties D, et al. 1999. Evidence of early axonal damage in patients with multiple sclerosis [abstract]. *Neurology*, 52(Suppl 2):378.
- Dhib-Jalbut S. 2002. Mechanisms of action of interferons and glatiramer acetate in multiple sclerosis. *Neurology*, 23(8 Suppl 4):S3-9.
- Durelli L, Verdun E, Barbero P, et al. 2002. Every-other-day interferon beta-1b versus once-weekly interferon beta-1a for multiple sclerosis: results of a 2-year prospective randomised multicentre study (INCOMIN). *Lancet*, 359:1453-60.
- Filippi M, Rovaris M, Inglesse M, et al. 2004. Interferon beta-1a for brain tissue loss in patients at presentation with syndromes suggestive of multiple sclerosis:a randomized, double-blind, placebo-controlled trial. *Lancet*, 364:1489-96.
- Francis GS, Grumser Y, Altieri E, et al. 2003. Hepatic reactions during treatment with interferon- β -1a:incidence and clinical significance. *Drug Saf*, 26:815-27.
- Etemadifar M, Janghorbani M, Shaygannejad V. 2006. Comparison of Betaferon, Avonex, and Rebif in treatment of relapsing-remitting multiple sclerosis. *Acta Neurol Scand*, 113:283-7.
- European Study Group on Interferon beta-1b in Secondary Progressive MS. 1998. Placebo-controlled multicentre randomized trial of interferon beta-1b in treatment of secondary progressive multiple sclerosis. *Lancet*, 352:1491-7.
- Galboiz Y, Shapiro S, Lahat N, et al. 2001. Matrix metalloproteinases and their tissue inhibitors as markers of disease subtype and response to interferon- β therapy in relapsing and secondary-progressive multiple sclerosis patients. *Ann Neurol*, 50:443-51.
- Ghezzi A, Amato MP, Capobianco M, et al. 2005. Immunomodulatory Treatment of Early onset MS Group. Disease-modifying drugs in childhood-juvenile multiple sclerosis:results of an Italian co-operative study. *Mult Scler*, 11:420-4.
- Ghezzi A, Amato MP, Capobianco M, et al. 2007. Immunomodulatory Treatment of Early-onset MS (ITEMS) Group. Treatment of early-onset multiple sclerosis with intramuscular interferonbeta-1a:long-term results. *Neurol Sci*, 28:127-32.

- Giorelli M, De Blasi A, Defazio G, et al. 2002. Differential regulation of membrane bound and soluble ICAM 1 in human endothelium and blood mononuclear cells: effects of interferon beta-1a. *Cell Commun Adhes*, 9:259–72.
- Giovannoni G, Barbarash OL, Jaber A, et al. 2006. Reduced immunogenicity with a new formulation of interferon-beta-1a (Rebif): 24 week results of a phase IIIb trial study [abstract]. *Mult Scler*, 12(Suppl 1):192.
- Goodkin DE, North American Study Group. 2000. Interferon beta-1b in secondary progressive MS: clinical and MRI results of a 3-year randomized controlled trial [abstract]. *Neurology*, 54:2352.
- Haas J, Firzlaiff M. 2005. Twenty-four-month comparison of immunomodulatory treatments – a retrospective open label study in 308 RRMS patients treated with beta interferons or glatiramer acetate (Copaxone). *Eur J Neurol*, 12:425–31.
- Hartung HP, Munschauer III F, Schellekens H. 2005. Significance of neutralizing antibodies to interferon beta during treatment of multiple sclerosis: expert opinions based on Proceedings of an International Consensus Conference. *Eur J Neurol*, 12:588–601.
- Harzheim M, Stepien-Mering M, Schroder R, et al. 2004. The expression of microfilament-associated cell-cell contacts in brain endothelial cells is modified by IFN-beta1a (Rebif). *J Interferon Cytokine Res*, 24:711–16.
- Jacobs LD, Cookfair DL, Rudick RA, et al. 1996. Intramuscular interferon beta-1a for disease progression in exacerbating-relapsing multiple sclerosis. *Ann Neurol*, 39:285–94.
- Jacobs LD, Beck RW, Simon JH, et al. 2000. Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. *N Eng J Med*, 343:898–904.
- Juni P, Altman DG, Egger M. 2001. Systematic reviews in health care: assessing the quality of controlled trials. *Br Med J*, 323:42–6.
- Kappos L, Moeri D, Radue EW, et al. 1999. Predictive value of gadolinium-enhanced magnetic resonance imaging for relapse rate and changes in disability or impairment in multiple sclerosis: a meta-analysis. Gadolinium MRI Meta-analysis Group. *Lancet*, 353:964–9.
- Kappos L, Polman CH, Freedman MS, et al. 2006. Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndromes. *Neurology*, 67:1242–9.
- Kappos L, Traboulsee A, Costantinescu C, et al. 2006. Long-term subcutaneous interferon beta-1a therapy in patients with relapsing-relapsing MS. *Neurology*, 67:944–53.
- Kappos L, Freedman MS, Polman CH et al. BENEFIT Study Group. 2007. Effect of early versus delayed interferon beta-1b treatment on disability after a first clinical event suggestive of multiple sclerosis: a 3-year follow-up analysis of the BENEFIT study. *Lancet*, 370:389–97.
- Koch-Henriksen N, Sorensen PS, Christensen T, et al. 2006. A randomized study of two interferon-beta treatments in relapsing-relapsing multiple sclerosis. *Neurology*, 66:1056–60.
- Korteweg T, Tintore M, Uitdehaag B, et al. 2006. MRI criteria for dissemination in space in patients with clinically isolated syndromes: a multicentre follow-up study. *Lancet Neurol*, 5:221–7.
- Koziol JA, Lucero A, Sipe JC, et al. 1999. Responsiveness of the Scripps neurologic rating scale during a multiple sclerosis clinical trial. *Can J Neurol Sci*, 26:283–9.
- Kurtzke JF. 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*, 33:1444–52.
- Li DKB, Paty DW. 1999. Magnetic resonance imaging results of the PRISMS trial: a randomized, double-blind, placebo-controlled study of interferon-beta1a in relapsing-relapsing multiple sclerosis. *Ann Neurol*, 46:197–206.
- Li DKB, Zhao GJ, Paty DW et al. 2001. Randomized controlled trial of interferon-beta-1a in secondary progressive MS: MRI results [abstract]. *Neurology*, 56(Suppl 3):148–9.
- Limmroth V, Malessa R, Zettl U, et al. 2007. Quality Assessment in Multiple Sclerosis Therapy (QUASIMS). A comparison of interferon beta therapies for relapsing-relapsing multiple sclerosis. *J Neurol*, 254:67–77.
- Liu C, Blumhardt LD. 1999. Randomized, double-blind, placebo-controlled study of interferon beta-1a in relapsing-relapsing multiple sclerosis analyzed by area under disability/time curves. *J Neurol Neurosurg Psychiatry*, 67:451–6.
- Liu C, Blumhardt LD. 2002. Randomized, double-blind, placebo-controlled study of subcutaneous interferon beta 1a in relapsing-relapsing multiple sclerosis: a categorical disability trend analysis. *Mult Scler*, 8:10–14.
- Lublin FD, Baier M, Cutter G. 2003. Effect of relapses on development of residual deficit in multiple sclerosis. *Neurology*, 61:1528–32.
- Malucchi S, Sala A, Gilli F, et al. 2004. Neutralizing antibodies reduce the efficacy of beta1FN during treatment of multiple sclerosis. *Neurology*, 62:2031–7.
- McDonald WI, Compston A, Edan G, et al. 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol*, 50:121–7.
- Milanese C, La Mantia L, Palumbo R, et al. 2003. A post-marketing study on interferon b 1b and 1a treatment in relapsing-relapsing multiple sclerosis: different response in drop-outs and treated patients. *J Neurol Neurosurg Psychiatry*, 74:1689–92.
- Miller DH, Rudge P, Johnson G, et al. 1988. Serial gadolinium enhanced magnetic resonance imaging in multiple sclerosis. *Brain*, 111:927–39.
- Monzani F, Caraccio N, Dardano A, et al. 2004. Thyroid autoimmunity and dysfunction associated with type I interferon therapy. Review. *Clin Exp Med*, 3:199–210.
- Murdoch D, Lyseng-Williamson KA. 2005. Subcutaneous recombinant interferon-beta-1a (Rebif): a review of its use in relapsing-relapsing multiple sclerosis. *Drugs*, 65:1295–312.
- Noseworthy JH, Vandervort MK, Wong CJ, et al. 1990. Interrater variability with the expanded disability status scale (EDSS) and functional systems (FS) in a multiple sclerosis clinical trial. *Neurology*, 40:971–5.
- O’Riordan JI, Thompson AJ, Kingsley DP, et al. 1998. The prognostic value of brain MRI in clinically isolated syndromes of the CNS. A 10-year follow-up. *Brain*, 121:495–503.
- Paolillo A, Pozzilli C, Giugni E, et al. 2002. A 6-year clinical and MRI follow-up study of patients with relapsing-relapsing multiple sclerosis treated with Interferon-beta. *Eur J Neurol*, 9:645–55.
- Panitch H, Goodin DS, Francis G, et al. for the EVIDENCE Study Group and the University of British Columbia MS/MRI Research Group. 2002. Randomized, comparative study of interferon beta-1a treatment regimens in MS. The EVIDENCE trial. *Neurology*, 59:1496–506.
- Patten SB, Metz LM. 2001. Interferon beta-1a and depression in relapsing-relapsing multiple sclerosis: an analysis of depression data from the PRISMS clinical trial. *Mult Scler*, 7:243–8.
- Patten SB, Metz LM. 2002. Interferon beta-1a and depression in secondary progressive MS: Data from the SPECTRIMS Trial. *Neurology*, 59: 744–6.
- Paty DW. 1988. Magnetic resonance imaging in the assessment of disease activity in multiple sclerosis. *Can J Neurol Sci*, 15:266–72.
- Pohl D, Rostasy K, Gärtner J, Hanefeld F. 2005. Treatment of early onset multiple sclerosis with subcutaneous interferon beta-1a. *Neurology*, 65:888–90.
- Polman CH, Reingold SC, Edan G, et al. 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the “McDonald Criteria”. *Ann Neurol*, 58:840–6.
- Poser CM, Paty DW, Scheinberg L, et al. 1983. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol*, 13:227–31.
- PRISMS study group. 1998. Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. PRISMS (Prevention of Relapses and Disability by interferon beta-1a in Subcutaneously in Multiple Sclerosis) Study Group. *Lancet*, 352:1498–504.
- PRISMS Study Group, University of British Columbia MS/MRI Analysis Group. 2001. PRISMS-4: long term tolerability of interferon beta-1a in relapsing MS. *Neurology*, 56:1628–36.
- Rice GPA, Paszner B, Oger J, et al. 1999. The evolution of neutralizing antibodies in multiple sclerosis patients treated with interferon beta-1b. *Neurology*, 52:1277–9.

- Rieckmann P, O'Connor P, Francis GS, et al. 2004. Haematological effects of interferon- β -1a (Rebif) therapy in multiple sclerosis. *Drug Saf*, 27:745–56.
- Rio J, Tintore M, Nos C, T  lez N, et al. 2005. Interferon beta in relapsing-remitting multiple sclerosis – an eight years experience in a specialist multiple sclerosis center. *J Neurol*, 252:795–800.
- Rothuizen LE, Buclin T, Spertini F, et al. 1999. Influence of interferon β -1a dose frequency on PBMC cytokine secretion and biological effect markers. *J Neuroimmunol*, 99:131–41.
- Rovaris M, Confavreux C, Furlan R, et al. 2006. Secondary progressive multiple sclerosis:current knowledge and future challenges. *Lancet Neurol*, 5:343–54.
- Rudick RA, Antel J, Confavreux C, et al. 1996. Clinical outcomes assessment in multiple sclerosis. *Ann Neurol*, 40:469–79.
- Rudick RA, Lee JC, Simon J, et al. 2006. Significance of T2 lesions in multiple sclerosis:a 13-year longitudinal study. *Ann Neurol*, 60:236–42.
- Runkel L, Meier W, Pepinsky, et al. 1998. Structural and functional differences between glycosylated and non-glycosylated forms of human interferon-beta (IFN-beta). *Pharm Res*, 15:641–649.
- Simon JH, Jacobs LD, Campion M et al. and the Multiple Sclerosis Collaborative Research Group (MSCRG). 1998. Magnetic resonance studies of intramuscular interferon b-1a for relapsing multiple sclerosis. *Ann Neurol*, 43:79–87.
- SPECTRIMS Study Group (Secondary Progressive Efficacy Clinical Trial of Recombinant Interferon-beta-1a in MS). 2001. Randomized controlled trial of interferon-beta-1a in secondary progressive MS. *Neurology*, 56:1505–13.
- S  ttler MB, Demer I, Williams SK, et al. 2006. Effects of interferon-beta-1a on neuronal survival under autoimmune inflammatory conditions. *Exp Neurol*, 201:172–81.
- St  rzebecher S, Maibauer R, Heuner A et al. 1999. Pharmacodynamic comparison of single doses of IFN- β 1a and IFN- β 1b in healthy volunteers. *J Interferon Cytokine Res*, 19:1257–64.
- Tenembaum SN, Segura MJ. 2006. Interferon beta-1a treatment in childhood and juvenile-onset multiple sclerosis. *Neurology*, 67:511–13.
- The IFN β Multiple Sclerosis Study Group. 1993. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis:I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. The IFN β Multiple Sclerosis Study Group. *Neurology*, 43:655–61.
- The Once Weekly Interferon for MS Study Group. 1999. Evidence of interferon beta-1a dose response in relapsing-remitting MS:the OWIMS Study. The Once Weekly Interferon for MS Study Group. *Neurology*, 53:679–86.
- Thompson AJ, Polman CH, Miller DH, et al. 1997. Primary progressive multiple sclerosis. *Brain*, 120:1085–96.
- Trapp BD, Ranschoff RM, Fisher et al. 1999. Neurodegeneration in multiple sclerosis:relationship to neurological disability. *Neuroscientist*, 5:48–57.
- Tremlett HL, Oger J. 2004. Elevated aminotransferases during treatment with interferon-beta for multiple sclerosis:actions and outcomes. *Mult Scler*, 10:298–301.
- Tremlett HL, Oger J. 2004. Hepatic injury, liver monitoring and the beta-interferons for multiple sclerosis. *J Neurol*, 251:1297–303.
- Tremlett HL, Yoshida EM, Oger J. 2004. Liver injury associated with the β -interferons for MS: a comparison between the three products. *Neurology*, 62:628–31.
- Trojano M, Paolicelli D, Zimatore GB, et al. 2005. The IFNbeta treatment of multiple sclerosis (MS) in clinical practice. The experience at the MS center of Bari, Italy. *Neurol Sci*, 26(Suppl 4):179–82.
- Tuohy VK, Yu M, Yin L. 1998. The epitope spreading cascade during progression of experimental autoimmune encephalomyelitis and multiple sclerosis. *Immunol Rev*, 164:93–100.
- Vartanian T, Solberg Sorensen P, Rice G. 2004. Impact of neutralising antibodies on the clinical efficacy of interferon beta in multiple sclerosis. *J Neurol*, 251 (Suppl 2):25–30.
- Wagstaff AJ, Goa KL. 1998. Recombinant interferon- β -1a:a review of its therapeutic efficacy in relapsing-remitting multiple sclerosis. *Biodrugs*, 10:471–94.
- Weinshenker BG. 1995. The natural history of multiple sclerosis. *Neurol Clin*, 13:119–46.
- Weinshenker BG, Bass B, Rice GPA, et al. 1989. The natural history of multiple sclerosis:a geographically based study: I. Clinical course and disability. *Brain*, 112:133–46.
- Weinshenker BG, Rice GPA, Noseworthy JH, et al. 1991 The natural history of multiple sclerosis:a geographically based study. 4. Applications to planning and interpretation of clinical therapeutic trials. *Brain*, 114:1057–67.
- Williams GJ, Witt PL. 1998. Comparative study of the pharmacodynamic and pharmacologic effects of Betaseron and Avonex. *J Interferon Cytokine Res*, 18:967–75.
- Wingerchuk DM, Weinshenker BG. 1999. The natural history of multiple sclerosis. Implications for trial design. *Curr Opin Neurol*, 12:345–9.
- Yong VW, Chabot S, Stuve O, et al. 1998. Interferon beta in the treatment of multiple sclerosis:mechanisms of action. *Neurology*, 51:682–9.