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RESEARCH PAPER

Subcutaneous interferon β-1a in the treatment of clinically isolated syndromes: 3-year and 5-year results of the phase III dosing frequency-blind multicentre REFLEXION study

Giancarlo Comi,[1] Nicola De Stefano,[2] Mark S Freedman,[3] Frederik Barkhof,[4,5] Bernard M J Uitdehaag,[6] Marlieke de Vos,[7] Kurt Marhardt,[8] Liang Chen,[9] Delphine Issard,[10] Ludwig Kappos[11]

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ABSTRACT

Objective Early treatment following a first clinical demyelinating event (FCDE) delays further disease activity in patients with multiple sclerosis (MS). This study determined the effects of early versus delayed treatment (DT) with subcutaneous interferon (sc IFN) β-1a 44 µg in patients with an FCDE up to 60 months postrandomisation.

Methods Patients who completed the 24-month double-blind REFLEX (REbif FLEXible dosing in early MS) study entered an extension (REFLEXION, REbif FLEXible dosing in early MS extensION): patients initially randomised to sc IFN β-1a and not reaching clinically definite MS (clinically definite MS, CDMS (second attack or sustained Expanded Disability Status Scale (EDSS) score increase)) continued original treatment (three times weekly (tiw) or once weekly (qw)); placebo patients switched to tiw (DT); patients with CDMS switched to tiw. Clinical, MRI and adverse event data up to month 60 are reported.

Results 402/517 (77.8%) REFLEX patients entered REFLEXION (DT, n=133; tiw, n=127; qw, n=142). At month 60, cumulative probability of CDMS was: DT 44.6%; qw 40.7% (nominal p=0.084 vs DT); tiw 39.2% (nominal p=0.032 vs DT). Cumulative probability of McDonald MS conversion (CDMS or new MRI activity) at month 60 was also reduced for tiw versus DT (nominal p<0.001). At month 60, mean cumulative numbers of new T2, gadolinium-enhancing and T1 hypointense lesions were lower with sc IFN β-1a qw (nominal p<0.05) and tiw versus DT (nominal p<0.001); T2 and T1 hypointense lesion volume change was lower for sc IFN β-1a tiw versus DT (nominal p<0.01). Treatment was well tolerated; fewer patients receiving tiw versus qw were positive for neutralising or binding antibodies.

Conclusions Over 5 years in patients presenting with an FCDE, early sc IFN β-1a tiw administration versus DT prolonged time to CDMS and McDonald MS, and reduced overall MRI activity.

Trial registration number NCT00813709; Results.

INTRODUCTION

The initial manifestation of multiple sclerosis (MS) is often a first clinical demyelinating event (FCDE), also known as a clinically isolated syndrome (CIS), which frequently affects the optic nerve, brainstem or spinal cord.[1] Clinical trials performed in CIS have shown that between 38% and 45% of untreated patients convert to clinically definite MS (CDMS (a new attack or sustained increase in Expanded Disability Status Scale (EDSS) score)) within 2 years.[2–4]

The diagnosis of CDMS is made after the exclusion of other differential diagnoses and the occurrence of either a second clinical attack at least 1 month after the first, based on evidence of involvement of more than one area in the central nervous system, or evidence of disease progression, as noted by a sustained increase in EDSS score.[5] The McDonald criteria, first introduced in 2001,[6] and revised in 2005[7] and 2010,[8] take into account lesions on MRIs, allowing earlier diagnosis even before a second clinical attack occurs.[8]

Treatment recommendations advise that patients should be treated as early as possible after an FCDE,[9] and previous studies have shown that early treatment (ET) with interferon (IFN) β, glatiramer acetate, oral teriflunomide or oral cladribine has beneficial effects in patients with an FCDE for reducing the risk of developing MS.[3 4 10–14] The REFLEX (REbif FLEXible dosing in early MS) phase III study[2] demonstrated that ET with IFN β-1a 44 µg, initiated after the FCDE and administered subcutaneously (sc) three times weekly (tiw) or once weekly (qw) over 24 months, delayed conversion to McDonald MS (2005 criteria)[7] and CDMS compared with placebo.[2] More frequent treatment (tiw vs qw) prolonged time to McDonald MS conversion. REFLEXION (REbif FLEXible dosing in early MS extensION), a preplanned extension of the REFLEX study, was designed to evaluate the benefits of more frequent and early dosing versus delayed treatment (DT) on disease course over a prolonged period. It compared the efficacy, in terms of progression to CDMS and McDonald MS, proportion of patients remaining relapse-free, EDSS progression and MRI outcomes, safety and immunogenicity of ET with sc IFN β-1a 44 µg (tiw or qw) versus DT over 60 months since randomisation in REFLEX. The results of an integrated analysis from REFLEX and REFLEXION are presented here.

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METHODS

REFLEXION (NCT00813709) was a multicentre, double-blind, controlled extension of the phase III REFLEX study (NCT00404352),[2] conducted at 70 centres in 24 countries (Argentina, Austria, Belgium, Bulgaria, Canada, Croatia, the Czech Republic, Estonia, Finland, France, Germany, Greece, Israel, Italy, Latvia, Lebanon, Morocco, Poland, Portugal, Romania, Russia, Serbia, Slovakia and Spain) in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice, applicable local regulations and the Declaration of Helsinki. All participants provided written informed consent to continue follow-up in REFLEXION. REFLEX was initiated on 16 November 2006 (first patient first visit), and REFLEXION on 22 December 2008, with the last patient completing the month 60 visit on 30 August 2013.

Patients and procedures

Inclusion/exclusion criteria and the randomisation procedure for REFLEX have been described.[2] Briefly, REFLEX recruited patients aged 18–50 years who had an EDSS score of ≤5.0, a single clinical event suggestive of MS within 60 days of study entry, and ≥2 clinically silent lesions ≥3 mm on T2-weighted brain MRI (at least one of which was ovoid, periventricular or infratentorial). Patients were randomised (1:1:1) to the serum-free formulation of sc IFN β-1a 44 µg tiw or qw (plus placebo twice weekly for blinding), or placebo, for 24 months or until CDMS (defined by a second attack or a sustained increase in EDSS score of ≥1.5 points). On conversion to CDMS, patients from any treatment arm were switched to open-label sc IFN β-1a 44 µg tiw without unblinding of the initial randomisation. Exclusion criteria included: any disease other than MS that could better explain the patient's signs and symptoms; a primary progressive course of MS; a medical objection to continuing in the study (laboratory abnormalities and clinically significant liver, renal or bone marrow dysfunction).

All patients who completed the 24-month period of REFLEX were eligible for the REFLEXION extension. Patients initially randomised to sc IFN β-1a 44 µg tiw or qw who had not converted to CDMS continued their original dosing regimen (figure 1). Patients originally receiving placebo who had not converted to CDMS were switched to sc IFN β-1a 44 µg tiw, forming the REFLEXION DT group; patients who converted to CDMS during REFLEX or REFLEXION received open-label sc IFN β-1a 44 µg tiw from then on. Patients who completed REFLEX but were not on treatment (ie, withdrew from treatment but not from the study) could restart treatment at entry to REFLEXION, and received double-blind IFN β-1a (qw or tiw) if they had not converted to CDMS (those who had converted to CDMS restarted with open-label IFN β-1a tiw). Blinding to the initial treatment assignment in REFLEX was maintained in all cases. To maintain initial blinding, all patients who had not converted to CDMS entered a 4-week retitration phase, whereby they received 20% of the full dose (8.8 µg qw or tiw) for the first 2 weeks and 50% (22 µg qw or tiw) for the following 2 weeks. Other procedures were as previously described.[2]
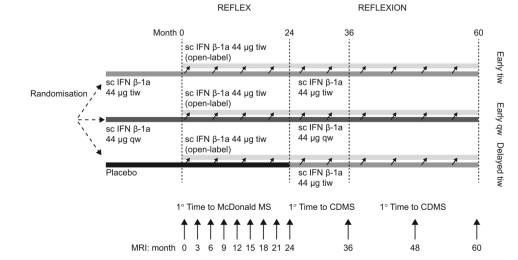
Study assessments

In REFLEX, efficacy and safety data were collected every 3 months up to conversion and then every 6 months. In REFLEXION, EDSS scores and CDMS assessments were recorded at extension baseline (month 24) and every 6 months thereafter. MRIs were performed every 3 months during REFLEX, at extension baseline and then yearly at months 36, 48 and 60 (or at the end-of-treatment visit in patients who discontinued). MRIs were analysed centrally at the VU University Medical Center, Amsterdam, the Netherlands, to ensure homogeneity, as described previously.[15] Adverse events (AEs) were monitored at months 25 and 27 and then every 3 months to the study end.

Study end points

The primary end point was time to CDMS conversion (defined in REFLEX)[2] from first randomisation to month 36; time to CDMS to month 60 was a secondary end point. Additional secondary end points included (months 36 and 60): time to conversion to McDonald MS (2005 criteria, defined previously);[2 7] proportion of patients remaining relapse-free; time to confirmed EDSS progression (increase of ≥1.0 point, confirmed during a visit 6 months later) and EDSS change from baseline; mean total T2 lesions and mean number of new T2, T1 hypointense and gadolinium-enhancing (Gd+) lesions/patient/scan; change from baseline in volume of T2, T1 hypointense and Gd+ lesion volume; percentage brain volume change at months 36 and 60 vs baseline; development of neutralising and binding antibodies (nAbs; bAbs, respectively) to IFN β-1a over time (samples for immunogenicity assessments were taken at 6-month intervals until conversion to CDMS); and incidence of AEs, serious AEs (SAEs) and AEs leading to treatment discontinuation.

Figure 1 REFLEX and REFLEXION study design. Short arrows indicate conversion to CDMS and the consequent switch to open-label treatment, which could happen at any time throughout the study. CDMS, clinically definite multiple sclerosis; IFN, interferon; MS, multiple sclerosis; qw, once weekly; REFLEX, REbif FLEXible dosing in early MS; REFLEXION, REbif FLEXible dosing in early MS extension; sc, subcutaneous; tiw, three times weekly.

Statistical analyses

Study populations included the integrated intent-to-treat (ITT) population consisting of all patients randomised in REFLEX, including those who did not enrol in REFLEXION; the integrated double-blind safety population, which included all patients who had received at least one dose of double-blind treatment in REFLEX; the REFLEXION ITT population, which included all patients enrolled in REFLEXION; the REFLEXION double-blind safety population, which included all patients who had received at least one dose of double-blind treatment in REFLEXION.

For the primary end point, statistical testing was performed comparing the three treatment groups pairwise in a hierarchical order to control for family-wise type I error rate (tiw ET and DT; then qw ET and DT; then tiw ET and qw ET). Probabilities of CDMS conversion, McDonald MS conversion and EDSS progression over time were determined for each treatment group in the form of cumulative incidence curves estimated using the non-parametric Kaplan-Meier method; pairwise comparison of the treatment groups and estimation of treatment effect was performed as described previously.[2]

For MRI lesion number end points, pairwise treatment differences were analysed using a negative binomial regression model, with treatment and randomisation stratification factors as covariates. The number of scans was used as an offset variable. An approximate χ^2 test based on Wald statistics was used for the pairwise comparison of treatment groups. For MRI volume end points, change from baseline to month 36 was analysed using a non-parametric analysis of covariance (ANCOVA) adjusted for treatment, randomisation stratification factors and baseline value as covariates. Estimates of size in treatment differences were obtained using a parametric ANCOVA. Month 60 statistical analyses were exploratory; therefore, all p values were nominal, with no formal hypothesis testing for month 60 analysis and no multiplicity adjustment for the p value (p values <0.05 are reported).

RESULTS

In total, 402/517 (77.8%) patients randomised in REFLEX continued in REFLEXION: 127/171 originally randomised to sc IFN β-1a 44 µg tiw; 142/175 originally randomised to sc IFN β-1a 44 µg qw; and 133/171 in the DT arm (originally randomised to placebo); 371/402 (92.3%) completed the month 60 visit. Patient disposition for the integrated ITT population in the two studies is presented in figure 2. The baseline demographics and clinical characteristics of patients (at the time of randomisation in REFLEX) were comparable across treatment groups (table 1). Those patients who converted to CDMS during REFLEX and received open-label treatment were similar with respect to age, sex, race and history of FCDE (see online supplementary table S1).

The baseline characteristics of those who did not continue in REFLEXION were similar to those of the overall population at the start of REFLEX, although a higher proportion of women and higher MRI activity was apparent in those who did not continue in REFLEXION (see online supplementary table S1). There was no indication that clinical outcomes in REFLEX (conversion to CDMS or McDonald MS) were associated with patients not continuing to REFLEXION, indicating no bias in the REFLEXION population, as shown by disease characteristics in those patients who, at the end of REFLEX, did or did not continue to REFLEXION (see online supplementary table S2).

In the REFLEXION double-blind safety population comprising 300 patients (tiw, n=99; qw, n=117; DT, n=84),

Figure 2 Patient disposition in REFLEX and REFLEXION (integrated intent-to-treat population). IFN, interferon; MS, multiple sclerosis; qw, once weekly; REFLEX, REbif FLEXible dosing in early MS; REFLEXION, REbif FLEXible dosing in early MS extension; sc, subcutaneous; tiw, three times weekly.

REFLEX
517 patients randomised
    sc IFN β-1a 44 µg, tiw, n=171
    sc IFN β-1a 44 µg, qw, n=175
    Placebo, n=171

448 completed Month 24 visit
    sc IFN β-1a 44 µg, tiw, n=146
    sc IFN β-1a 44 µg, qw, n=156
    Placebo, n=146

402 entered **REFLEXION**
    sc IFN β-1a 44 µg, tiw, n=127
    sc IFN β-1a 44 µg, qw, n=142
    Delayed treatment, n=133

8 withdrew prematurely prior to Month 36
    sc IFN β-1a 44 µg, tiw, n=4
    sc IFN β-1a 44 µg, qw, n=2
    Delayed treatment, n=2

394 completed Month 36 visit
    sc IFN β-1a 44 µg, tiw, n=123
    sc IFN β-1a 44 µg, qw, n=140
    Delayed treatment, n=131

23 withdrew prematurely prior to Month 60
    sc IFN β-1a 44 µg, tiw, n=4
    sc IFN β-1a 44 µg, qw, n=9
    Delayed treatment, n=10

371 completed Month 60 visit
    sc IFN β-1a 44 µg, tiw, n=119
    sc IFN β-1a 44 µg, qw, n=131
    Delayed treatment, n=121

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Table 1 Baseline demographics and disease characteristics of the REFLEXION intent-to-treat population (at the time of randomisation to REFLEX)

Characteristic	sc IFN β-1a tiw n=127 (74.3% of REFLEX)	sc IFN β-1a qw n=142 (81.1% of REFLEX)	DT n=133 (77.8% of REFLEX)	Overall n=402 (77.8% of REFLEX)
Age, years	31.8 (8.6)	31.4 (8.2)	31.0 (8.2)	31.4 (8.3)
Women, n (%)	78 (61.4)	88 (62.0)	82 (61.7)	248 (61.7)
EDSS score	1.5 (0–4.0)*	1.5 (0–3.5)	1.5 (0–3.5)	1.5 (0–4.0)*
Time since FCDE, days	57.6 (3.83)	57.7 (3.40)	57.8 (4.04)	57.7 (3.75)
Classification of FCDE as monofocal,† n (%)	66 (52.0)	76 (53.5)	72 (54.1)	214 (53.2)
Steroid use at FCDE, n (%)	90 (70.9)	99 (69.7)	95 (71.4)	284 (70.6)
Presence of ≥1 T1 Gd+ lesion, n (%)	48 (37.8)	62 (43.7)	52 (39.1)	162 (40.3)
T1 Gd+ lesion number	1.2 (2.5)	1.5 (3.4)	1.0 (1.8)	1.2 (2.7)
T1 Gd+ lesion volume, mm³	147.94 (430.64)	169.20 (384.88)	142.93 (453.35)	153.79 (422.07)
T1 hypointense lesions	6.4 (7.3)	6.1 (7.6)	5.6 (8.1)	6.0 (7.7)
T1 hypointense lesion volume, mm³	787.05 (1163.61)	806.48 (1344.18)	705.40 (1115.41)	766.90 (1213.36)
T2 lesion number	22.6 (17.4)	24.3 (21.8)	20.7 (19.7)	22.6 (19.8)
≥9 T2 lesions, n (%)	100 (78.7)	104 (73.2)	95 (71.4)	299 (74.4)
T2 lesion volume, mm³	3312.00 (3370.44)	3798.88 (4490.66)	3296.99 (3937.93)	3479.02 (3977.55)
Normalised brain volume, cm³	1533.12 (76.83)	1532.72 (65.95)	1541.25 (64.95)	1535.65 (69.21)

Data are presented as mean (SD) or median (range), unless indicated otherwise.
*Data missing for one patient.
†As classified by the adjudication committee.
DT, delayed treatment; EDSS, Expanded Disability Status Scale; FCDE, first clinical demyelinating event; Gd+, gadolinium-enhancing; IFN, interferon; MS, multiple sclerosis; qw, once weekly; REFLEX, REbif FLEXible dosing in early MS; REFLEXION, REbif FLEXible dosing in early MS extensION; sc, subcutaneously; tiw, three times weekly.
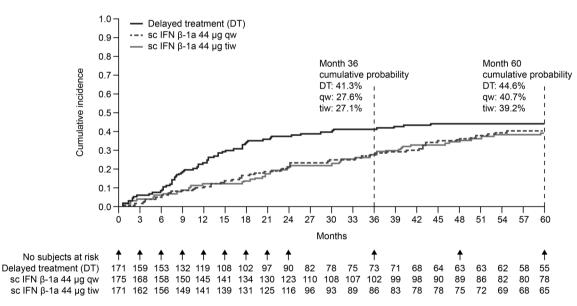

Figure 3 Time to CDMS conversion during the double-blind period: Kaplan-Meier cumulative incidence curves (integrated intent-to-treat population, N=517). Arrows indicate MRI assessments. CDMS, clinically definite multiple sclerosis; IFN, interferon; qw, once weekly; sc, subcutaneously; tiw, three times weekly.

197/295 (66.8%) completed double-blind treatment (and 5 patients did not receive double-blind treatment during REFLEXION (stopped treatment during REFLEX but were enrolled in REFLEXION)). Of those who discontinued prematurely (45/295 (15.3%)), more patients discontinued from DT (21 (25.3%)) than from tiw (12 (12.2%)) or qw (12 (10.5%)). Reasons for discontinuation were treatment-emergent AEs (TEAEs; 9 patients (20.0%)), lost to follow-up (7 (15.6%)) and other reasons (29 (64.4%)). Median compliance (proportion of scheduled doses administered) in the safety population (months 24–60) was >99% for all three treatment groups.

Time to CDMS conversion from baseline to study end in the integrated ITT population is shown in figure 3. At month 36 (primary end point), there were lower proportions of patients who had converted to CDMS by month 36 with tiw and qw than with DT (25.1%, 25.7% and 38.6%, respectively), and lower cumulative probability of CDMS conversion (27.1%, 27.6% and 41.3%, respectively). The risk of CDMS conversion was significantly reduced for patients receiving ET (tiw or qw) compared with DT (p≤0.006 for both tiw and qw vs DT), with no significant difference between the ET groups (p=0.941; table 2). Results for the month 60 analysis also showed longer times to CDMS conversion in both ET groups compared with

DT. The proportion of patients who converted to CDMS had increased at month 60, although it was still lower in those receiving tiw and qw ET than with DT (32.2%, 36.0% and 40.4%). Similarly, cumulative probability of conversion was lower with tiw and qw ET than with DT (39.2%, 40.7% and 44.6%), with a relative risk reduction of 31.7% for tiw versus DT (nominal p=0.032; figure 3; table 2). A preplanned exploratory analysis of time to CDMS evaluated possible treatment effects after adjusting for the randomisation stratification factors. This analysis showed that the presence of Gd+ lesions at screening was a factor that may contribute to the effect of treatment (HR point estimate (95% CI) 1.457 (1.017 to 2.086); nominal p=0.040).

Time to McDonald MS conversion from baseline to study end in the integrated ITT population is shown in figure 4. A higher percentage of DT patients had converted to McDonald MS compared with the ET groups at most of the assessments. At month 36, the cumulative probability of conversion to McDonald MS was significantly lower with both sc IFN β-1a dosing regimens than DT (tiw 66.8% (nominal p<0.001); qw 79.1% (nominal p=0.009); DT 86.5%) and remained lower with tiw at month 60 (79.2%) versus DT (86.5%), but not for qw (89.5%). sc IFN β-1a tiw was associated with a relative risk reduction of time to conversion to McDonald MS of 30.8% versus qw at month 60 (nominal p=0.005; table 2).

Minimal differences were seen for numbers with confirmed EDSS progression between groups (see online supplementary table S3). Mean EDSS scores from baseline are shown in online supplementary table S3; the mean change in EDSS score from baseline to month 60 was −0.05, −0.03 and −0.16 for tiw, qw and DT, respectively.

Table 2 Effects of treatment on conversion to CDMS and McDonald MS (integrated analysis, intent-to-treat population, double-blind period)

	sc IFN β-1a tiw n=171	sc IFN β-1a qw n=175	Delayed treatment n=171
Conversion to CDMS			
Month 36			
Patients converting (%)	25.1	25.7	38.6
Cumulative probability of CDMS (%)	27.1	27.6	41.3
HR (95% CI) vs DT	0.555 (0.38; 0.82)	0.573 (0.39; 0.84)	–
Relative risk reduction vs DT (%)	44.5	42.7	–
p Value	0.002	0.006	–
HR (95% CI) vs qw	0.993 (0.65; 1.51)	–	–
Relative risk reduction vs qw (%)	0.7	–	–
p Value	0.941	–	–
Month 60			
Patients converting (%)	32.2	36.0	40.4
Cumulative probability of CDMS (%)	39.2	40.7	44.6
HR (95% CI) vs DT	0.683 (0.48; 0.98)	0.752 (0.53; 1.06)	–
Relative risk reduction vs DT (%)	31.7	24.8	–
Nominal p value	0.032	0.084	–
HR (95% CI) vs qw	0.940 (0.65; 1.35)	–	–
Relative risk reduction vs qw (%)	16.0	–	–
Nominal p value	0.852	–	–
Conversion to McDonald MS			
Month 36			
Patients converting (%)	66.7	76.0	84.2
Cumulative probability of McDonald MS (%)	66.8	79.1	86.5
HR (95% CI) vs DT	0.508 (0.40; 0.65)	0.698 (0.55; 0.89)	–
Relative risk reduction vs DT (%)	49.2	30.2	–
Nominal p value	<0.001	0.009	–
HR (95% CI) vs qw	0.722 (0.56; 0.93)	–	–
Relative risk reduction vs qw (%)	27.8	–	–
Nominal p value	0.024	–	–
Month 60			
Patients converting (%)	72.5	82.9	84.2
Cumulative probability of McDonald MS (%)	79.2	89.5	86.5
HR (95% CI) vs DT	0.546 (0.43; 0.70)	0.759 (0.60; 0.96)	–
Relative risk reduction vs DT (%)	45.4	24.1	–
Nominal p value	<0.001	0.040	–
HR (95% CI) vs qw	0.692 (0.54; 0.88)	–	–
Relative risk reduction vs qw (%)	30.8	–	–
Nominal p value	0.005	–	–

Cumulative probability: Kaplan-Meier estimate of the cumulative probability of CDMS or McDonald MS; HR: multivariate Cox proportional hazards model with treatment and randomisation stratification factors as covariates; p value: two-sided log-rank test, stratified for randomisation stratification factors. Month 60 statistical analyses were exploratory; therefore, all p values for this time point were nominal (p values <0.05 are reported).
CDMS, clinically definite multiple sclerosis; DT, delayed treatment; IFN, interferon; MS, multiple sclerosis; qw, once weekly; sc, subcutaneously; tiw, three times weekly.
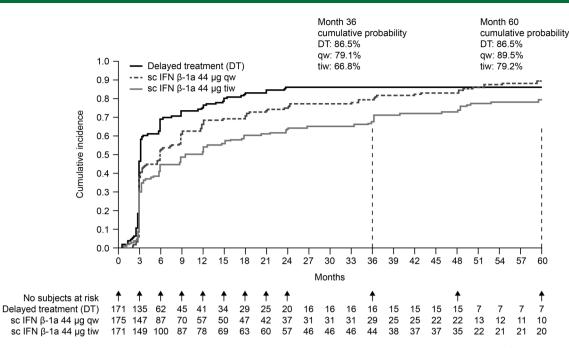
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Figure 4 Time to conversion to McDonald MS during the double-blind period: Kaplan-Meier cumulative incidence curves (integrated intent-to-treat population, N=517). Arrows indicate MRI assessments. IFN, interferon; MS, multiple sclerosis; qw, once weekly; sc, subcutaneously; tiw, three times weekly.

Table 3 summarises the effect of treatment on MRI end points. Treatment with tiw was associated with a lower adjusted mean total number of T2 lesions than DT at month 36 (nominal p=0.031) and month 60 (nominal p=0.029). At both time points, both tiw and qw regimens were associated with lower numbers of new T2 lesions (months 36 and 60, nominal p<0.001), new Gd+ lesions (months 36 and 60, nominal p<0.001) and new T1 hypointense lesions (Months 36 and 60, nominal p<0.05). The mean number of lesions per patient per scan by year is shown in online supplementary table S4.

In all treatment groups, there was a reduction in mean T2 lesion volume and an increase in T1 hypointense lesion volume from baseline to the last observed value (LOV) during the study (figure 5). Patients in the tiw ET group had greater mean reductions in T2 lesion volume than patients in the qw ET group (month 36, nominal p=0.009; month 60, nominal p=0.013) and patients in the DT group (months 36 and 60, nominal p<0.001); there were no differences between the qw and DT groups. In contrast, T1 hypointense lesion volume increased in all groups, but the increase was consistently less in only the tiw group compared with the DT group (months 36 and 60, nominal p=0.007; figure 5).

There were no notable differences between groups for the mean change in Gd+ lesion volume from study baseline (month 0) to month 36 LOV and month 60 LOV. There were no differences between ET and DT in mean percentage change from baseline in brain volume. However, qw treatment was associated with less brain volume reduction than tiw treatment (months 0–36, −0.889% vs −1.114%, nominal p=0.026; months 0–60, −1.409% vs −1.777%, nominal p=0.032). Brain volume reduction during the REFLEXION study period (using month 24 as baseline) was more pronounced at each time point in the DT group than in either ET group (see online supplementary table S5).

In the integrated double-blind safety population, the proportion of patients who were nAb-positive (≥20 neutralising units/

mL) at any time during the 60-month study period was 16.0% (27/169) for tiw, 21.4% (36/168) for qw and 14.8% (25/169) for DT. During the first 18 months of treatment (months 24–42 for DT), the proportion of nAb-positive patients increased across all groups, and then remained relatively constant and similar (see online supplementary figure S1). The proportion of patients bAb-positive was highest in the tiw group after 6 months of treatment; with DT, the proportion was highest at month 30 (reflecting the first 6 months after patients switched to tiw), before declining over time to month 60. The proportion of patients bAb-positive remained relatively constant for the qw group (see online supplementary figure S2).

Table 4 presents TEAEs reported between months 24 and 60 (REFLEXION double-blind safety population). Approximately 83% of patients within each treatment group experienced at least one TEAE, with more DT patients reporting drug-related TEAEs versus the ET groups. The percentage of patients with any AE of severe intensity, SAEs or an AE that led to discontinuation was comparable between the three treatment groups. Only six TEAEs occurred in ≥10% of patients in any group: influenza-like illness, headache, upper respiratory tract infection, nasopharyngitis, injection-site erythema and leucopenia. A higher percentage of patients in the DT group had at least one prespecified TEAE (MeDRA preferred term), with more patients experiencing influenza-like syndrome, injection-site reactions and hypersensitivity reactions than in the ET groups. Prespecified AEs were transient and resolved over time.

DISCUSSION

REFLEXION, a preplanned extension of the phase III REFLEX study,[2] was designed to evaluate how two different dosing frequencies of sc IFN β-1a 44 μg initiated at the time of an FCDE influence later disease course compared with DT. The results from REFLEXION are consistent with the findings from REFLEX and further suggest that, over 5 years, initiating ET with sc IFN β-1a within 60 days of an FCDE significantly

Table 3 Effects of treatment on MRI end points (integrated analysis, intent-to-treat population, double-blind period)

	sc IFN β-1a tiw n=171	sc IFN β-1a qw n=175	Delayed treatment n=171
T2 lesions, mean (SE)			
Month 36	21.16 (1.44)	24.61 (1.62)	25.79 (1.73)
Rate ratio (95% CI) vs DT	0.82 (0.69; 0.98)	0.95 (0.80; 1.14)	–
	Nominal p=0.031	Nominal p=0.606	
Month 60	21.46 (1.45)	24.95 (1.62)	26.19 (1.74)
Rate ratio (95% CI) vs DT	0.82 (0.69; 0.98)	0.95 (0.80; 1.14)	–
	Nominal p=0.029	Nominal p=0.591	
New T2 lesions, mean (SE)			
Month 36	0.47 (0.06)	0.61 (0.07)	1.14 (0.13)
Rate ratio (95% CI) vs DT	0.41 (0.30; 0.56)	0.53 (0.39; 0.73)	–
	Nominal p<0.001	Nominal p<0.001	
Month 60	0.55 (0.07)	0.66 (0.08)	1.11 (0.13)
Rate ratio (95% CI) vs DT	0.49 (0.36; 0.68)	0.59 (0.43; 0.81)	–
	Nominal p<0.001	Nominal p<0.001	
New Gd+ lesions, mean (SE)			
Month 36	0.14 (0.03)	0.29 (0.05)	0.74 (0.13)
Rate ratio (95% CI) vs DT	0.18 (0.11; 0.30)	0.39 (0.24; 0.63)	–
	Nominal p<0.001	Nominal p<0.001	
Month 60	0.16 (0.03)	0.28 (0.05)	0.69 (0.13)
Rate ratio (95% CI) vs DT	0.23 (0.14; 0.38)	0.41 (0.25; 0.67)	–
	Nominal p<0.001	Nominal p<0.001	
New T1 hypointense lesions, mean (SE)			
Month 36	0.33 (0.05)	0.47 (0.06)	0.73 (0.09)
Rate ratio (95% CI) vs DT	0.45 (0.32; 0.65)	0.64 (0.45; 0.91)	–
	Nominal p<0.001	Nominal p=0.012	
Month 60	0.35 (0.05)	0.49 (0.06)	0.70 (0.09)
Rate ratio (95% CI) vs DT	0.50 (0.35; 0.70)	0.70 (0.50; 0.97)	–
	Nominal p<0.001	Nominal p=0.035	

Data are per patient per scan unless stated otherwise. The negative binomial regression model is corrected for overdispersion with treatment and randomisation stratification factors as covariates and number of scans as an offset variable. Month 60 statistical analyses were exploratory; therefore, all p values for this time point were nominal (p values <0.05 are reported).

DT, delayed treatment; Gd+, gadolinium-enhancing; IFN, interferon; qw, once weekly; sc, subcutaneous; tiw, three times weekly.

prolonged time to conversion to MS compared with a treatment delay of up to 2 years.

Furthermore, both in REFLEX and REFLEXION, there was a difference between the two dose frequencies of sc IFN β-1a in the time to McDonald MS, in favour of tiw dosing. This difference was accounted for by a difference in MRI outcome between the two groups. Indeed, by systematically incorporating MRI lesions as surrogates of clinical events, and as lesions occur more frequently than relapses,[16] [17] the McDonald criteria provide a sensitive tool to measure disease activity and anti-inflammatory drug effects.[6–8] No difference between the two groups was observed for the time to CDMS.

Analysis of MRI outcomes in this study also showed benefits with ET, as previously suggested from the findings of the MRI analysis of REFLEX.[15] ET was associated with marked reductions in new lesions compared with DT over 5 years. Although the mean number of lesions per patient per scan by year shows fewer lesions in the DT group than the tiw and qw groups, patients in the DT group may have less active disease (patients converting to CDMS would already have been switched to tiw and remaining patients received tiw at start of REFLEXION). Furthermore, tiw was associated with greater reductions in T2 lesion volume compared with qw, confirming the short-term dose effect previously observed in REFLEX. It is possible that the reductions in T2 lesion volume observed were related to patients with more active disease being

lost to follow-up, skewing the patient population included in these analyses. Further analysis of the MRI data is warranted, as previous studies have suggested a role for lesions at the time of an FCDE in predicting future disease activity.[18] Analyses of the BENEFIT study[19] and the ETOMS study[20] showed that the presence of Gd+ lesions or ≥9 T2 lesions at baseline led to higher percentages of patients converting to CDMS. IFN β has been shown to have a robust treatment effect in patients with CIS, including those with greater MRI disease burden or activity.[21–23] The dose effect observed in REFLEX and confirmed in REFLEXION is consistent with the finding that higher dose sc IFN β-1a (44 µg tiw) is associated with better MRI outcomes than lower dose (22 µg tiw)[24] or less frequent intramuscular IFN β-1a.[25]

Changes in brain volume did not differ between groups but should be interpreted with caution; brain volume changes during the first year of treatment, especially with high frequency DT sc IFN β-1a, may have a stronger anti-inflammatory effect, causing accelerated, non-tissue-related brain volume decline (pseudoatrophy).[26] [27] This phenomenon was observed in patients switching in other extension studies.[11] [28]

These results from REFLEXION add to a small but growing number of trials, which suggest that ET of an FCDE with IFN β is associated with improved long-term clinical, paraclinical and MRI outcomes to at least 5 years.[4] [11] [28–31] In the 5-year active treatment extension of the phase 3 BENEFIT trial, ET with IFN
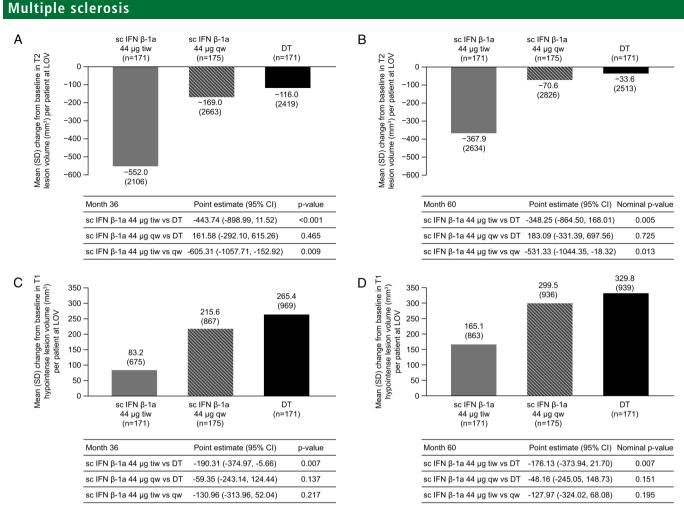
Figure 5 Change from baseline to LOV in mean T2 lesion volume at (A) month 36 and (B) month 60, and mean T1 hypointense lesion volume at (C) month 36 and (D) month 60 by original treatment group (integrated intent-to-treat population, N=517). Data are unadjusted raw means. Point estimate (95% CI) estimated using a parametric ANCOVA model with treatment, randomisation stratification factors and baseline value as covariates. p Values obtained using a non-parametric ANCOVA (ANCOVA on ranked data) with treatment, randomisation stratification factors and baseline value as covariates. ANCOVA, analysis of covariance; DT, delayed treatment; IFN, interferon; LOV, last observed value; qw, once weekly; sc, subcutaneous; tiw, three times weekly.

β-1b every other day reduced the risk of CDMS by 37%, although DT by up to 2 years did not affect long-term confirmed disability progression, as measured by the EDSS.[28] In a 10-year follow-up of the open-label CHAMPIONS study (an extension of the CHAMPS trial), ET with intramuscular IFN β-1a reduced the rate of CDMS; however, there was no differential effect on disability, MRI T2-weighted lesions or the proportion of patients developing progressive disease.[30][31] Further analysis identified patients with low T2 lesion counts as having a significantly less severe disease course.[32] It is important to consider that CHAMPS enrolled primarily patients with monofocal disease presentation (some were later redefined as multifocal), whereas patients in BENEFIT and REFLEX could have had monofocal or multifocal disease onset at the study start.[33]

The absence of a continuation placebo group, as patients initially randomised to placebo who had not converted to CDMS were switched to tiw treatment for REFLEXION, is a limitation of this study. Patients randomised to qw treatment were also switched to tiw on CDMS, limiting the possibility to observe differences between the two active arms, and resulting in a bias towards patients in the tiw arm with potentially more active disease. Furthermore, patients in the DT group were biased towards less active disease as they had not yet converted to CDMS at the start of REFLEXION. Nevertheless, a treatment

effect is still evident on MRI, but it could not be determined that tiw dosing was superior to qw dosing for delaying conversion to CDMS. However, tiw dosing consistently reduced MRI activity to a greater extent than was seen with qw dosing. Additionally, tiw dosing led to fewer TEAEs, particularly influenza-like symptoms and reduced immunogenicity. While REFLEX and REFLEXION used the 2005 McDonald criteria,[7] the most recent criteria are McDonald 2010,[8] meaning that some patients would now be diagnosed with MS and excluded from the study. However, a subgroup analysis of REFLEX suggests that had the McDonald 2010 criteria been available at the time of the study design, the main findings from REFLEX would not have been affected.[34]

Immunogenicity and safety data were consistent with those reported in REFLEX,[2] and in agreement with the well-established safety profile of sc IFN β-1a, involving 20 years of follow-up data.[24][35–37] The higher proportion of patients with TEAEs in the DT group most likely reflects the typical time course of TEAEs shortly after starting IFN β treatment to which the majority of patients can adjust, and to which those in the REFLEX tiw and qw groups had already adjusted.

The results of REFLEXION extend the clinical and MRI findings of the REFLEX study and show that, over 5 years in patients with an FCDE, early initiation of treatment with sc IFN

Table 4 Patients (n (%)) reporting AEs during months 24–60 of the double-blind treatment period (REFLEXION safety population)

	sc IFN β-1a tiw n=99	sc IFN β-1a qw n=117	Delayed treatment n=84
Any TEAE	84 (84.8)	96 (82.1)	70 (83.3)
Any drug-related TEAE*	43 (43.4)	63 (53.8)	56 (66.7)
AEs of severe intensity	6 (6.1)	6 (5.1)	6 (7.1)
SAEs	9 (9.1)	7 (6.0)	7 (8.3)
AEs leading to death	0	0	0
AEs leading to treatment discontinuation	2 (2.0)	0	3 (3.6)
Most common TEAEs (≥10% of patients)			
Headache	16 (16.2)	19 (16.2)	11 (13.1)
Influenza-like illness	17 (17.2)	39 (33.3)	45 (53.6)
Injection-site erythema	8 (8.1)	6 (5.1)	17 (20.2)
Upper respiratory tract infection	10 (10.1)	11 (9.4)	10 (11.9)
Nasopharyngitis	9 (9.1)	14 (12.0)	12 (14.3)
Leucopenia	2 (2.0)	2 (1.7)	9 (10.7)
Prespecified AEs (prespecified group)†			
Any prespecified AE	44 (44.4)	55 (47.0)	53 (63.1)
Influenza-like syndrome	17 (17.2)	39 (33.3)	45 (53.6)
Cytopenia	12 (12.1)	6 (5.1)	11 (13.1)
Injection-site reactions	12 (12.1)	8 (6.8)	20 (23.8)
Hypersensitivity reactions	8 (8.1)	9 (7.7)	12 (14.3)
Skin rashes	7 (7.1)	4 (3.4)	5 (6.0)
Depression and suicidal ideation	5 (5.1)	8 (6.8)	1 (1.2)
Hepatic disorders	4 (4.0)	4 (3.4)	4 (4.8)
Thyroid disorders	4 (4.0)	4 (3.4)	1 (1.2)

*Probable or possible relationship with double-blind treatment according to the investigator.
†Medical Dictionary for Drug Regulatory Activities, V.16.0.
AE, adverse event; IFN, interferon; MS, multiple sclerosis; qw, once weekly; REFLEXION, REbif FLEXIble dosing in early MS extensION; SAE, serious adverse event; sc, subcutaneous; TEAE, treatment-emergent adverse event; tiw, three times weekly.

β-1a prolonged time to CDMS and McDonald MS, and reduced MRI activity compared with DT. This further supports the early initiation of treatment with sc IFN β-1a at the time of the FCDE, with patients receiving ET benefiting from improved outcomes.

Author affiliations
[1]Department of Neurology, Scientific Institute H.S. Raffaele, Milan, Italy
[2]Department of Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy
[3]Department of Medicine (Neurology), University of Ottawa, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada
[4]Department of Diagnostic Radiology, VU University Medical Center, Amsterdam, The Netherlands
[5]The Institutes of Neurology and Healthcare Engineering, UCL, London, UK
[6]Department of Neurology, VU University Medical Center, Amsterdam, The Netherlands
[7]Department of Radiology and Nuclear Medicine, VU University Medical Center, Amsterdam, The Netherlands
[8]Global Medical Affairs, Merck GmbH, Vienna, Austria
[9]Global Biostatistics, EMD Serono Inc., Billerica, Massachusetts, USA
[10]Department of Biostatistics, Cytel Inc., Geneva, Switzerland
[11]Departments of Medicine, Clinical Research, Biomedicine, and Biomedical Engineering, University Hospital Basel, Basel, Switzerland

interpretation. All authors actively contributed to the writing and review of the manuscript, and have seen and approved the final version.

REFERENCES

1 Noseworthy JH, Lucchinetti C, Rodriguez M, et al. Multiple sclerosis. N Engl J Med 2000;343:938–52.
2 Comi G, De Stefano N, Freedman MS, et al. Comparison of two dosing frequencies of subcutaneous interferon beta-1a in patients with a first clinical demyelinating event suggestive of multiple sclerosis (REFLEX): a phase 3 randomised controlled trial. Lancet Neurol 2012;11:33–41.
3 Comi G, Filippi M, Barkhof F, et al. Effect of early interferon treatment on conversion to definite multiple sclerosis: a randomised study. Lancet 2001;357:1576–82.
4 Kappos L, Polman CH, Freedman MS, et al. Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndromes. Neurology 2006;67:1242–9.
5 Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227–31.
6 McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001;50:121–7.
7 Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 2005;58:840–6.
8 Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69:292–302.
9 Goodin DS, Frohman EM, Garmany GP Jr, et al. Disease modifying therapies in multiple sclerosis: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology and the MS Council for Clinical Practice Guidelines. Neurology 2002;58:169–78.
10 Jacobs LD, Beck RW, Simon JH, et al. Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. CHAMPS Study Group. N Engl J Med 2000;343:898–904.

Multiple sclerosis

11 Kappos L, Freedman MS, Polman CH, *et al*. 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* 2007;370:389–97.

12 Comi G, Martinelli V, Rodegher M, *et al*. Effect of glatiramer acetate on conversion to clinically definite multiple sclerosis in patients with clinically isolated syndrome (PreCISe study): a randomised, double-blind, placebo-controlled trial. *Lancet* 2009;374:1503–11.

13 Miller AE, Wolinsky JS, Kappos L, *et al*. Oral teriflunomide for patients with a first clinical episode suggestive of multiple sclerosis (TOPIC): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Neurol* 2014;13:977–86.

14 Leist TP, Comi G, Cree BA, *et al*. Effect of oral cladribine on time to conversion to clinically definite multiple sclerosis in patients with a first demyelinating event (ORACLE MS): a phase 3 randomised trial. *Lancet Neurol* 2014;13:257–67.

15 De Stefano N, Comi G, Kappos L, *et al*. Efficacy of subcutaneous interferon β-1a on MRI outcomes in a randomised controlled trial of patients with clinically isolated syndromes. *J Neurol Neurosurg Psychiatry* 2014;85:647–53.

16 Sormani MP, Bonzano L, Roccatagliata L, *et al*. Magnetic resonance imaging as a potential surrogate for relapses in multiple sclerosis: a meta-analytic approach. *Ann Neurol* 2009;65:268–75.

17 Smith ME, Stone LA, Albert PS, *et al*. Clinical worsening in multiple sclerosis is associated with increased frequency and area of gadopentetate dimeglumine-enhancing magnetic resonance imaging lesions. *Ann Neurol* 1993;33:480–9.

18 Freedman MS, Comi G, De Stefano N, *et al*. Moving toward earlier treatment of multiple sclerosis: Findings from a decade of clinical trials and implications for clinical practice. *Mult Scler Relat Disord* 2014;3:147–55.

19 Moraal B, Pohl C, Uitdehaag BM, *et al*. Magnetic resonance imaging predictors of conversion to multiple sclerosis in the BENEFIT study. *Arch Neurol* 2009;66:1345–52.

20 Barkhof F, Rocca M, Francis G, *et al*. Validation of diagnostic magnetic resonance imaging criteria for multiple sclerosis and response to interferon beta1a. *Ann Neurol* 2003;53:718–24.

21 Polman C, Kappos L, Freedman MS, *et al*. Subgroups of the BENEFIT study: risk of developing MS and treatment effect of interferon beta-1b. *J Neurol* 2008;255:480–7.

22 Beck RW, Chandler DL, Cole SR, *et al*. Interferon beta-1a for early multiple sclerosis: CHAMPS trial subgroup analyses. *Ann Neurol* 2002;51:481–90.

23 O'Connor P. The effects of intramuscular interferon beta-Ia in patients at high risk for development of multiple sclerosis: a post hoc analysis of data from CHAMPS. *Clin Ther* 2003;25:2865–74.

24 PRISMS Study Group and the University of British Columbia MS/MRI Analysis Group. PRISMS-4: Long-term efficacy of interferon-beta-1a in relapsing MS. *Neurology* 2001;56:1628–36.

25 Schwid SR, Panitch HS. Full results of the Evidence of Interferon Dose-Response-European North American Comparative Efficacy (EVIDENCE) study: a multicenter, randomized, assessor-blinded comparison of low-dose weekly versus high-dose, high-frequency interferon beta-1a for relapsing multiple sclerosis. *Clin Ther* 2007;29:2031–48.

26 Zivadinov R, Reder AT, Filippi M, *et al*. Mechanisms of action of disease-modifying agents and brain volume changes in multiple sclerosis. *Neurology* 2008;71:136–44.

27 De Stefano N, Arnold DL. Towards a better understanding of pseudoatrophy in the brain of multiple sclerosis patients. *Mult Scler* 2015;21:675–6.

28 Kappos L, Freedman MS, Polman CH, *et al*. Long-term effect of early treatment with interferon beta-1b after a first clinical event suggestive of multiple sclerosis: 5-year active treatment extension of the phase 3 BENEFIT trial. *Lancet Neurol* 2009;8:987–97.

29 Barkhof F, Polman CH, Radue EW, *et al*. Magnetic resonance imaging effects of interferon beta-1b in the BENEFIT study: integrated 2-year results. *Arch Neurol* 2007;64:1292–8.

30 Kinkel RP, Kollman C, O'Connor P, *et al*. IM interferon beta-1a delays definite multiple sclerosis 5 years after a first demyelinating event. *Neurology* 2006;66:678–84.

31 Kinkel RP, Dontchev M, Kollman C, *et al*. Association between immediate initiation of intramuscular interferon beta-1a at the time of a clinically isolated syndrome and long-term outcomes: a 10-year follow-up of the Controlled High-Risk Avonex Multiple Sclerosis Prevention Study in Ongoing Neurological Surveillance. *Arch Neurol* 2012;69:183–90.

32 Simon JH, Kinkel RP, Kollman C, *et al*., CHAMPIONS Investigators Group. Ten-year follow-up of the 'minimal MRI lesion' subgroup from the original CHAMPS Multiple Sclerosis Prevention Trial. *Mult Scler* 2015;21:415–22.

33 Freedman MS. Evidence for the efficacy of interferon beta-1b in delaying the onset of clinically definite multiple sclerosis in individuals with clinically isolated syndrome. *Ther Adv Neurol Disord* 2014;7:279–88.

34 Freedman MS, De Stefano N, Barkhof F, *et al*. Patient subgroup analyses of the treatment effect of subcutaneous interferon β-1a on development of multiple sclerosis in the randomized controlled REFLEX study. *J Neurol* 2014;261:490–9.

35 PRISMS Study Group. 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 Subcutaneously in Multiple Sclerosis) Study Group. *Lancet* 1998;352:1498–504.

36 Kappos L, Traboulsee A, Constantinescu C, *et al*. Long-term subcutaneous interferon beta-1a therapy in patients with relapsing-remitting MS. *Neurology* 2006;67:944–53.

37 Kappos L, Kuhle J, Multanen J, *et al*. Factors influencing long-term outcomes in relapsing–remitting multiple sclerosis: PRISMS-15. *J Neurol Neurosurg Psychiatry* 2015;86:1202–7.

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Subcutaneous interferon β-1a in the treatment of clinically isolated syndromes: 3-year and 5-year results of the phase III dosing frequency-blind multicentre REFLEXION study

Giancarlo Comi, Nicola De Stefano, Mark S Freedman, Frederik Barkhof, Bernard M J Uitdehaag, Marlieke de Vos, Kurt Marhardt, Liang Chen, Delphine Issard and Ludwig Kappos

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