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Original article

No evidence of disease activity status in patients treated with early vs. delayed subcutaneous interferon β-1a

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ABSTRACT

Background: Clinically isolated syndrome (CIS) is defined as a monophasic clinical episode highly suggestive of multiple sclerosis (MS). Regardless, studies have shown that treatment at this early stage of MS can delay a second event and prolong the transition to clinically diagnosed MS. The objective of this *post-hoc* analysis was to determine the effect of early CIS treatment with once weekly (qw) or three times weekly (tiw) subcutaneous interferon (scIFN) β-1a vs. delayed treatment (DT) on the composite endpoint of no evidence of disease activity (NEDA)-3.

Methods: In REFLEX, patients with CIS were randomized to double-blind scIFN β-1a 44 μg tiw, qw, or placebo for 24 months. Upon clinically-definite MS, patients switched to open-label scIFN β-1a tiw. Patients who completed REFLEX entered an extension (REFLEXION). Patients initially randomized to placebo switched to tiw (DT); scIFN β-1a patients continued their initial qw/tiw regimen for up to 60-months post-randomization. This *post-hoc* analysis was conducted in the integrated intent-to-treat REFLEX plus REFLEXION population (tiw, $n = 171$; qw, $n = 175$; DT, $n = 171$). All p values are nominal. CIS was defined using the McDonald 2010 criteria.

Results: Patients receiving early treatment (ET) with scIFN β-1a tiw and qw were more likely to achieve NEDA-3 than DT at year 2 (tiw vs. DT: OR 4.26, 95% CI 2.02–8.98, $p = 0.0001$; qw vs. DT: OR 2.99, 95% CI 1.39–6.43, $p = 0.005$). Compared with DT, ET with scIFN β-1a tiw was more likely to achieve NEDA-3 at year 3 (OR 3.73, 95% CI 1.63–8.55, $p = 0.002$) and year 5 (OR 12.96, 95% CI 1.66–101.04, $p = 0.015$). Between ET regimens, the odds of achieving NEDA-3 were not significantly improved by scIFN β-1a 44 μg tiw at year 2 (OR 1.42, 95% CI 0.81–2.50, $p = 0.22$) but were at year 3 (OR 2.26, 95% CI 1.11–4.60, $p = 0.024$) and year 5 (OR 3.22, 95% CI 1.01–10.22, $p = 0.048$), indicating that the beneficial effects of more frequent scIFN β-1a dosing become more apparent over time in patients with CIS. In the subgroup of patients with Gd+ lesions at baseline the odds for achieving NEDA-3 were higher for ET up to year 2 compared with DT (tiw: OR 10.21, 95% CI 1.23–84.82, $p = 0.03$; qw: OR 8.97, 95% CI 1.08–74.28, $p = 0.04$). In patients without Gd+ lesions at baseline, those receiving ET were more likely to achieve NEDA-3 at year 2 (OR 3.56, 95% CI 1.56–8.10, $p = 0.003$), year 3 (OR 2.54, 95% CI 1.05–6.18, $p = 0.04$) and year 5 (OR 9.63, 95% CI 1.19–77.79, $p = 0.034$) than patients who received DT.

Conclusions: ET with scIFN β-1a tiw was associated with a higher likelihood of achieving NEDA-3 not only at 2 but also at 3 and 5 years.

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1. Introduction

Clinically isolated syndrome (CIS) is the first clinical manifestation of relapsing remitting multiple sclerosis (RRMS) or the first clinical demyelinating event (FCDE) and is consistent with the presence of one or more subclinical white matter lesions in the central nervous system (CNS) (Miller et al., 2005). Although CIS is highly suggestive of multiple sclerosis (MS) it does not necessarily fulfill the accepted diagnostic criteria for MS, primarily due to a lack of evidence of multiplicity over time.

Traditionally, the diagnosis of MS required clinical evidence of lesions disseminated in space and time (Poser et al., 1983). However, the acceptance of magnetic resonance imaging (MRI) lesions being indicative of new disease activity, and advances in MRI techniques and cerebrospinal fluid analysis have led to the development of more sensitive diagnoses (Mahajan and Ontaneda, 2017, Rammohan, 2009). The McDonald RRMS criteria, introduced in 2001 and revised in 2005, 2010, and 2017 (McDonald et al., 2001, Polman et al., 2005, Polman et al., 2011, Thompson et al., 2018) indicate that a diagnosis of MS can be made despite the FCDE if the MRI shows evidence of old and new lesions (thus satisfying the "dissemination in time" or DIT criteria), or since 2017 if there is dissemination in space and the presence of CNS-specific oligoclonal banding. This allows for a diagnosis to be made before a second clinical attack occurs. Regardless of the criteria, the overall goal for early treatment (ET) is to initiate effective therapy before patients experience further attacks. The ET of CIS with disease modifying treatment (DMTs) is recommended in the current guidelines from the European Committee for Treatment and Research in MS (ECTRIMS) / European Academy of Neurology (EAN) (Montalban et al., 2018). The American Academy of Neurology (AAN) also recommends prescribing DMTs to patients with CIS following discussions about the benefits and risks (Rae-Grant et al., 2018).

"No evidence of disease activity" (NEDA) − 3 as a composite endpoint of clinical (no relapses and confirmed worsening of disability) and MRI outcomes (no gadolinium-enhancing [Gd +] lesions and no new/enlarging T2-hyperintense lesions) was first derived from the *post-hoc* analyses of a phase 3 clinical trial (Giovannoni et al., 2015). In the meantime, NEDA-3 has been evaluated in patients treated with subcutaneous interferon (scIFN) β-1a (Coyle et al., 2017), natalizumab (Havrdova et al., 2009, Prosperini et al., 2017), fingolimod (Prosperini et al., 2017, Nixon et al., 2014, Fox et al., 2017), dimethyl fumarate (Nixon et al., 2014, Fox et al., 2017, Havrdova et al., 2017), teriflunomide (Nixon et al., 2014), intramuscular IFN β-1a (Uher et al., 2017), peginterferon β-1a (Arnold et al., 2017), ocrelizumab (Havrdova et al., 2018), cladribine (Giovanonni et al., 2019) and alemtuzumab (Havrdova et al., 2017). However, the long-term impact of early vs. delayed treatment (DT) with scIFN β-1a on overall NEDA-3 status, and its radiological and clinical subcomponents, in patients treated following their FCDE has not been fully determined. In addition, the impact of the presence or absence of Gd + lesion activity at baseline on rates of NEDA-3 warrants investigation.

In this *post-hoc* analysis of the phase 3 REFLEX (REbif FLEXible dosing in early MS) (NCT00404352) (Comi et al., 2012) clinical trial and the preplanned extension study REFLEXION (REbif FLEXible dosing in early MS extension; NCT00813709) (Comi et al., 2017), we compared the effects of two dosing frequencies of scIFN β-1a (three-times weekly [tiw] or once weekly [qw]) and placebo (DT) on achieving NEDA-3 and no evidence of radiological activity.

2. Methods

2.1. Patients and study design

The methodology of REFLEX and REFLEXION has been described elsewhere (Comi et al., 2012, Comi et al., 2017). Briefly, the phase 3, multicenter, double-blind, randomized, placebo-controlled REFLEX trial recruited patients from 80 centers in 28 countries aged 18–50 years and had an EDSS score of 0–5.0, 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 scans (Comi et al., 2012).

After diagnosis of CIS, eligible patients were randomized (1:1:1) to early treatment (ET) with scIFN β-1a 44 μg tiw or qw (plus placebo twice weekly for blinding), or placebo, for 24 months or until clinically definite MS (CDMS, defined as a distinct second attack or confirmed EDSS score worsening). At CDMS, patients switched to open-label scIFN β-1a 44 μg tiw without unblinding of the initial randomization. Patients' demographics and medical history were obtained at the initial screening and efficacy and safety data were collected every 3 months up to conversion and then every 6 months thereafter.

Patients completing REFLEX at 24 months were eligible to participate in the multicenter, dose-blinded, controlled extension trial, REFLEXION, conducted at 70 centers in 24 countries (Comi et al., 2017). REFLEX was initiated at the first patient visit on 16 November 2006, and REFLEXION commenced on 22 December 2008, with the last patient completing the Month-60 visit on 30 August 2013. Patients from REFLEX who received placebo and those who reached CDMS switched to open-label scIFN β-1a 44 μg tiw (DT), otherwise patients who received scIFN β-1a 44 μg tiw or qw continued with their initial regimen; blinding to initial treatment in REFLEX was maintained in all cases. EDSS scores and CDMS assessments were recorded at the extension baseline (Month 24) and then every 6 months thereafter. MRI scans were performed at the extension baseline and then yearly at months 36, 48 and 60 (or at end of treatment for discontinuing patients).

The present analysis was a 60-month, *post-hoc* analysis of the integrated intent-to-treat (ITT) population from REFLEX (Comi et al., 2012) and REFLEXION (Comi et al., 2017), i.e., those randomized in REFLEX: tiw n = =171; qw n = =175; DT n = =171.

2.2. Study endpoints

The primary endpoint was the occurrence of NEDA-3 status; defined as no confirmed relapses, no disability worsening, and no MRI activity (no new/enlarging T2-hyperintense lesions or Gd + lesions). Clinical activity was defined as confirmed relapses or disability worsening, and radiological activity was defined as MRI activity. Time to first disease activity was defined as time to first occurrence of confirmed relapse, disability worsening, or new/enlarging T2-hyperintense lesions or Gd + lesions. The primary objective of this analysis was to examine the relationship of early vs. delayed scIFN β-1a 44 μg on NEDA-3 at years 2, 3, and 5 after randomization.

Secondary objectives were to examine this relationship by presence or absence of Gd + lesions at baseline and to examine NEDA-3 by baseline disease course. Disease course included both patients classified as RRMS or CIS which was retrospectively determined according to whether patients did or did not, respectively, fulfil the McDonald 2010 RRMS criteria (hereby known as the 2010 criteria) (Polman et al., 2011) at baseline. CIS was defined using the 2010 criteria as this was the criteria in effect at the time that these analyses were performed.

2.3. Statistical analyses

The statistical analysis is based on the ITT population from the REFLEX study. Patient visits were used to derive the year time point (i.e. 2, 3, or 5 years post-randomization). All statistical tests, including non-overlapping 95% confidence intervals (CIs), were exploratory and two-sided for comparison of treatment arms; p values are nominal. Patient characteristics are presented as number (%), mean (± standard deviation [SD]) and median (range).

NEDA-3 and no evidence of radiological activity at years 1–5 were calculated for each treatment group. Data are presented descriptively as the proportion (95% CI) of patients; the proportions of NEDA and no evidence of radiological activity are cumulative by definition. Best-fit

logistic regression models, adjusted for treatment, age, monofocal vs. multifocal classification of first clinical demyelinating event according to the investigator, steroid use at first event, and presence of at least one Gd+ lesion at baseline, assessed the odds of achieving NEDA-3 and no evidence of radiological activity, at years 2, 3 and 5 respectively. The interactions between treatment and presence of baseline Gd+ lesions were considered in model selection and ultimately excluded from the models due to overfitting. Presence of baseline Gd+ lesions was included in models instead of retrospective CIS vs. RRMS diagnosis according to McDonald 2010 criteria since they were highly correlated. Data are presented as adjusted odds ratios (ORs) and 95% CI, with corresponding p values. To examine the relationship of early NEDA and long-term NEDA, the proportion of patients who were NEDA-3 at 2 years and remained NEDA-3 at 3 and 5 years was reported by treatment arm.

The impact of Gd+ lesions at baseline on NEDA-3 status was assessed by calculating the proportions of patients with cumulative NEDA-3 at years 2, 3 and 5, according to the presence or absence of Gd + lesions at baseline. Adjusted ORs (95% CIs) are presented. Time to first disease activity was displayed for all patients and separately by treatment arm using Kaplan Meier methods. Median times to first disease activity and 95% CIs were calculated using Cox's proportional hazards model. Since early MRI may not reflect treatment's onset of action during the first 3–4 months, disease activity occurring in the initial period after starting treatment but before therapy is effective can be omitted from analysis and considered as disease activity prior to treatment's onset of action (Giovannoni et al., 2015). To examine the data without the influence of the 3-month MRI, which could reflect disease activity prior to treatment's onset of action, time to first disease activity event after 105 days post-randomization was calculated as a sensitivity test.

To examine the impact of baseline disease course, defined as CIS or the McDonald 2010 RRMS 2010 (Polman et al., 2011), the proportion and 95% CI of patients with NEDA-3 and no evidence of radiological activity at years 1–5 were reported by baseline disease course. The present analysis stratified patients according to the 2010 criteria which resulted in the inclusion of some patients who had been excluded from REFLEX based on the 2005 McDonald definition of RRMS.

All p-values reported are considered nominal. Statistical analyses were performed using SAS 9.3 (Cary, NC, USA).

3. Results

3.1. Patients

517 patients were randomized in REFLEX. Patient demographics and clinical characteristics were comparable between treatment groups (Table 1).

3.2. NEDA-3

Patients that received ET with scIFN β-1a tiw and qw were more likely to achieve NEDA-3 than those receiving DT up to 2 years (tiw vs. DT: OR 4.26, 95% CI 2.02–8.98, $p = =0.0001$; qw vs. DT: OR 2.99, 95% CI 1.39–6.43, $p = =0.005$; Fig. 1). Compared with DT, patients treated with ET with scIFN β-1a tiw were also more likely to achieve NEDA-3 at year 3 (OR 3.73, 95% CI 1.63–8.55, $p = =0.002$) and year 5 (OR 12.96, 95% CI 1.66–101.04, $p = =0.015$; Fig. 1). At all time-points, a dose-dependent, numerically greater proportion of patients that received ET (tiw or qw) had NEDA-3 than those that received DT (Supplementary Figure 1).

Between ET regimens, the odds of achieving NEDA-3 with scIFN β-1a 44 μg tiw vs. qw were higher at year 3 (OR 2.26, 95% CI 1.11–4.60, $p = =0.024$) and year 5 (OR 3.22, 95% CI 1.01–10.22, $p = =0.048$), than at year 2 (OR 1.42, 95% CI 0.81–2.50, $p = =0.22$; Fig. 1).

3.3. The proportion of patients with NEDA-3 at 2 years that remained NEDA-3 at 3 and 5 years

In patients with NEDA-3 at 2 years, the proportion of patients who still had NEDA-3 at 5 years was dose-dependent and numerically greater in those who received ET compared with those who received DT (tiw: 34.3%, 95% CI 0.19–0.52; qw: 14.8%, 95% CI 0.04–0.34; DT: 10.0%, 95% CI 0.00–0.45; Table 2).

3.4. Impact of the presence or absence of Gd+ lesions at baseline

At baseline there were similar proportions of patients free of Gd+ lesions across all treatment groups (tiw 60%, qw 59%, DT 57%; Table 1). Overall the median time to first MS defining event was shorter in those with baseline Gd+ lesions ($n = =213$) than without ($n = =301$): 0.5 years (95% CI 0.5–0.7) vs 1.0 year (95% CI 0.8–1.4), respectively (Fig. 2A).

With scIFN β-1a tiw the median time to first MS defining event was shorter in the higher risk group (patients with Gd+ lesions at baseline; $n = =68$; 0.7 years [95% CI 0.5–1.2]) than in those without baseline Gd+ lesions ($n = =102$; 1.7 years [95% CI 1.5–2.0]; Fig. 2B). This was also the case with scIFN β-1a qw where the median time to first MS defining event was 0.7 years (95% CI 0.5–0.8) for patients with baseline Gd+ lesions ($n = =72$) vs 1.0 year (95% CI 1.0–1.5) for patients without ($n = =102$; Fig. 2C). However, for DT, there was no difference observed for median time to first MS defining event between patients with ($n = =73$) and without ($n = =97$) baseline Gd+ lesions: 0.5 years (95% CI 0.5–0.5) vs 0.7 years (95% CI 0.5–0.7; Fig. 2D).

In patients with Gd+ lesions at baseline, there was a benefit of ET compared with DT on the odds of achieving NEDA-3 up to 2 years with tiw (OR 10.21, 95% CI 1.23–84.82, $p = =0.03$) and qw (OR 8.97, 95% CI 1.08–74.28, $p = =0.04$; Fig. 3A). The odds of achieving NEDA at years 2, 3 and 5 were similar between ET regimens (tiw vs. qw; Fig. 3A).

In patients without Gd+ lesions at baseline, those treated early with scIFN β-1a tiw were more likely to achieve NEDA-3 by year 2 (OR 3.56, 95% CI 1.56–8.10, $p = =0.003$), year 3 (OR 2.54, 95% CI 1.05–6.18, $p = =0.04$) and year 5 (OR 9.63, 95% CI 1.19–77.79, $p = =0.34$) than patients who received DT (Fig. 3B). Furthermore, there was a trend towards higher odds of achieving NEDA-3 at year 2 for scIFN β-1a qw vs. DT (OR 2.24, 95% CI 0.95–5.24, $p = =0.06$). However, at years 3 and 5, there were no differences between qw and DT for odds of achieving NEDA-3 (Fig. 3B). Compared with qw dosing, treatment with scIFN β-1a tiw increased the odds of achieving NEDA-3 at year 5 for patients without Gd+ lesions at baseline (OR 4.9, 95% CI 1.03–23.31, $p = =0.05$; Fig. 3B).

3.5. Impact of disease course at baseline (CIS or RRMS)

Regardless of baseline disease course, there was a dose frequency-dependent, numerically higher proportion of patients who achieved NEDA-3 with early scIFN β-1a 44 μg compared with DT (**Supplementary Figure 2A, B**), and a numerically higher proportion of patients who achieved no evidence of radiological activity (**Supplementary Figure 2C, D**). For both analyses, the proportions of patients with NEDA-3 and no evidence of radiological activity were higher at all time-points in patients with CIS compared with RRMS at baseline.

4. Discussion

Overall, the results support previous studies showing that ET with IFN β-1a improves overall outcomes (Comi et al., 2012, Comi et al., 2017, Trojano et al., 2009, Comi et al., 2001). This supports clinical practice guidelines which recommend that patients are treated as early as possible after the FCDE (Goodin et al., 2002).

Compared with DT, ET with scIFN β-1a was associated with higher

Table 1
Baseline clinical characteristics and demographics of the ITT population of REFLEX/REFLEXION.

	sc IFN β-1a tiw (*n* = =171)	sc IFN β-1a qw (*n* = =175)	Delayed treatment (*n* = =171)	Overall (*n* = =517)
Age, years	30.6 (8.5)	30.7 (8.1)	30.9 (7.9)	30.7 (8.2)
Women, n (%)	114 (67)	106 (61)	112 (65)	332 (64)
EDSS score	1.50 (0–4.0)	1.50 (0–3.5)	1.50 (0–3.5)	1.50 (0–4.0)
Time since FCDE, days	57.6 (3.7)	57.7 (3.4)	57.6 (4.2)	57.6 (3.8)
Classification of FCDE as monofocal (according to the investigator), n (%)	99 (58)	104 (59)	97 (57)	300 (58)
Classification of FCDE as monofocal (according to the adjudication committee), n (%)	96 (56)	90 (51)	91 (53)	277 (54)
Steroid use at FCDE, n (%)	121 (71)	123 (70)	121 (71)	365 (71)
Gd+ lesions				
Patients with at least one T1 Gd+ lesion, n (%)	68 (40)	72 (41)	73 (43)	213 (41)
Number of Gd+ lesions	1.3 (2.5)	1.5 (3.5)	1.2 (2.7)	1.3 (2.9)
Gd + lesion volume, mm^3	156.54 (427.33)	194.15 (593.66)	193.68 (588.50)	181.56 (541.68)
T2 hyperintense lesions				
Number of T2 lesions, n (%)	22.0 (18.8)	23.6 (21.0)	21.3 (20.2)	22.3 (20.0)
Patients with at least nine T2 lesions, n (%)	129 (75)	126 (72)	122 (71)	377 (73)
T2 lesion volume, mm^3	3110.53 (3410.74)	3853.12 (4716.71)	3334.92 (3990.41)	3436.11 (4083.90)

Data are presented as mean (standard deviation) or median (range), unless indicated otherwise.
EDSS, Expanded Disability Status Scale; FCDE, first clinical demyelinating event; Gd+, gadolinium-enhancing; IFN, interferon; ITT, intention-to-treat; qw, once weekly; sc, subcutaneous; tiw, three times weekly.

odds of achieving NEDA-3 in the short-term (up to 2 years), which was also found in the long-term (up to 5 years). This benefit of ET with scIFN β-1a on freedom from evidence of disease activity was primarily driven by the effect on the MRI-detected component of NEDA-3. Differences in clinical disease activity with ET vs. DT were not maintained for all time-points during the follow-up of 5 years. This finding is consistent with previous studies that showed that NEDA-3 is primarily driven by the more frequently occurring MRI events rather than the less frequent clinical end points (Uher et al., 2017, Bevan and Cree, 2014, Rotstein et al., 2015).

The odds of achieving NEDA-3 significantly favored more frequent dosing with scIFN β-1a tiw compared with scIFN β-1a qw. Interestingly, the odds of achieving NEDA-3 were not significantly improved by scIFN β-1a 44 μg tiw at year 2 but were at year 3 and 5. This either suggests that the beneficial effects of scIFN β-1a (including the more frequent dosing effect) become more apparent over time in patients with CIS or that the development of more lesions over time increased the power to see the differences. The reason for the difference in NEDA-3 between arms not being strong at year 2 may also be related to the small sample size and the fact that a longer follow-up period allows better assessment with this sample size and endpoint.

Sub-analysis results suggest that the presence of baseline Gd+ lesions was associated with a lower chance of remaining NEDA-3 after the 3-month MRI compared with those without baseline Gd+ lesions, irrespective of treatment assignment. Regardless of baseline Gd+ lesion status, early scIFN β-1a treatment was associated with a higher chance of NEDA-3 beyond the 3-month MRI than DT. These data complement those from a recent *post-hoc* analysis of the EVIDENCE study, in which baseline Gd+ lesions predicted NEDA status (Coyle et al., 2017).

The results from the sub-analysis of baseline disease characteristics showed that the proportions of patients with NEDA-3 and no evidence of radiological activity were higher at all time-points in patients with CIS compared with RRMS at baseline. This supports the value of the MS diagnostic criteria revisions for diagnosing patients as MS who have a high risk of further disease activity. In a study that investigated the impact of intramuscular IFN β-1a on NEDA-3, fewer patients with RRMS than CIS achieved NEDA-3 at 1 year (20.4% vs 40.1%, respectively) and 4 years (3.3% vs 10.1%, respectively) (Uher et al., 2017).

Regarding the predictive utility of NEDA-3, in patients with NEDA-3 at 2 years, ET with sc IFN β-1a 44 μg tiw was associated with a higher

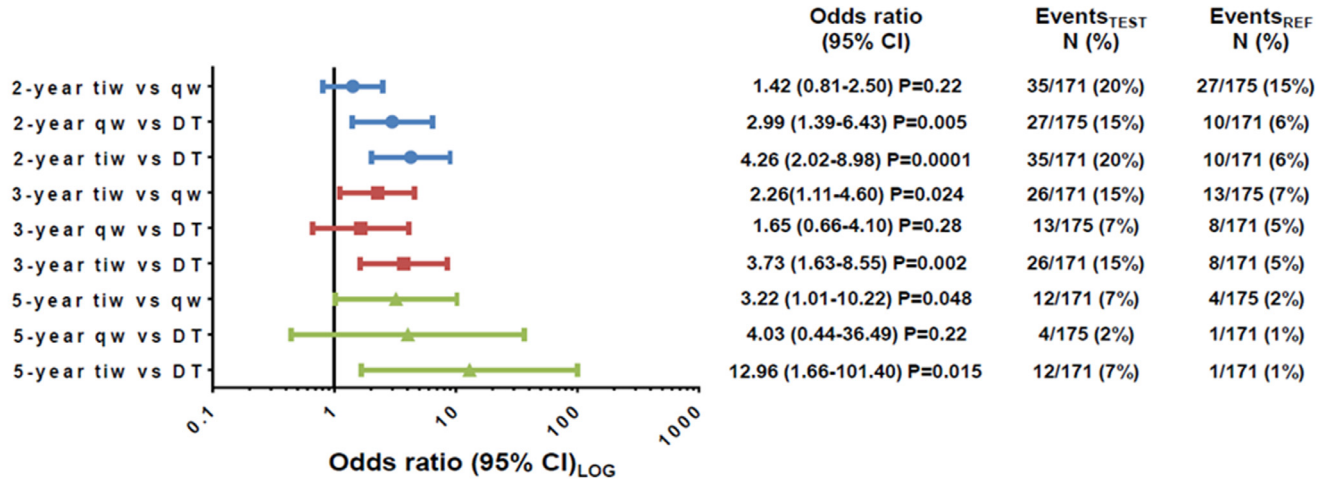


Fig. 1. Effect of early vs. delayed scIFN β-1a on achieving NEDA-3.

Table 2
Proportion of patients remaining NEDA-3 at 3 and 5 years among those with NEDA-3 at 2 years.

	sc IFN β-1a tiw		sc IFN β-1a qw		Delayed treatment	
	Yes, n	Proportion of patients at Year 2,% (95% CI)	Yes, n	Proportion of patients at Year 2,% (95% CI)	Yes, *n*	Proportion of patients at Year 2,%(95% CI)
NEDA at 2 years	35	100 (0.90–1.00)	27	100 (0.87–1.00)	10	100 (0.69–1.00)
Up to year 3	26	74.29 (0.57–0.88)	13	48.15 (0.29–0.68)	8	80.00 (0.44–0.97)
Up to year 5	12	34.29 (0.19–0.52)	4	14.81 (0.04–0.34)	1	10.00 (0.00–0.45)

CI, confidence interval; IFN, interferon; NEDA, no evidence of disease activity; qw, once weekly; sc, subcutaneous; tiw, three times weekly.

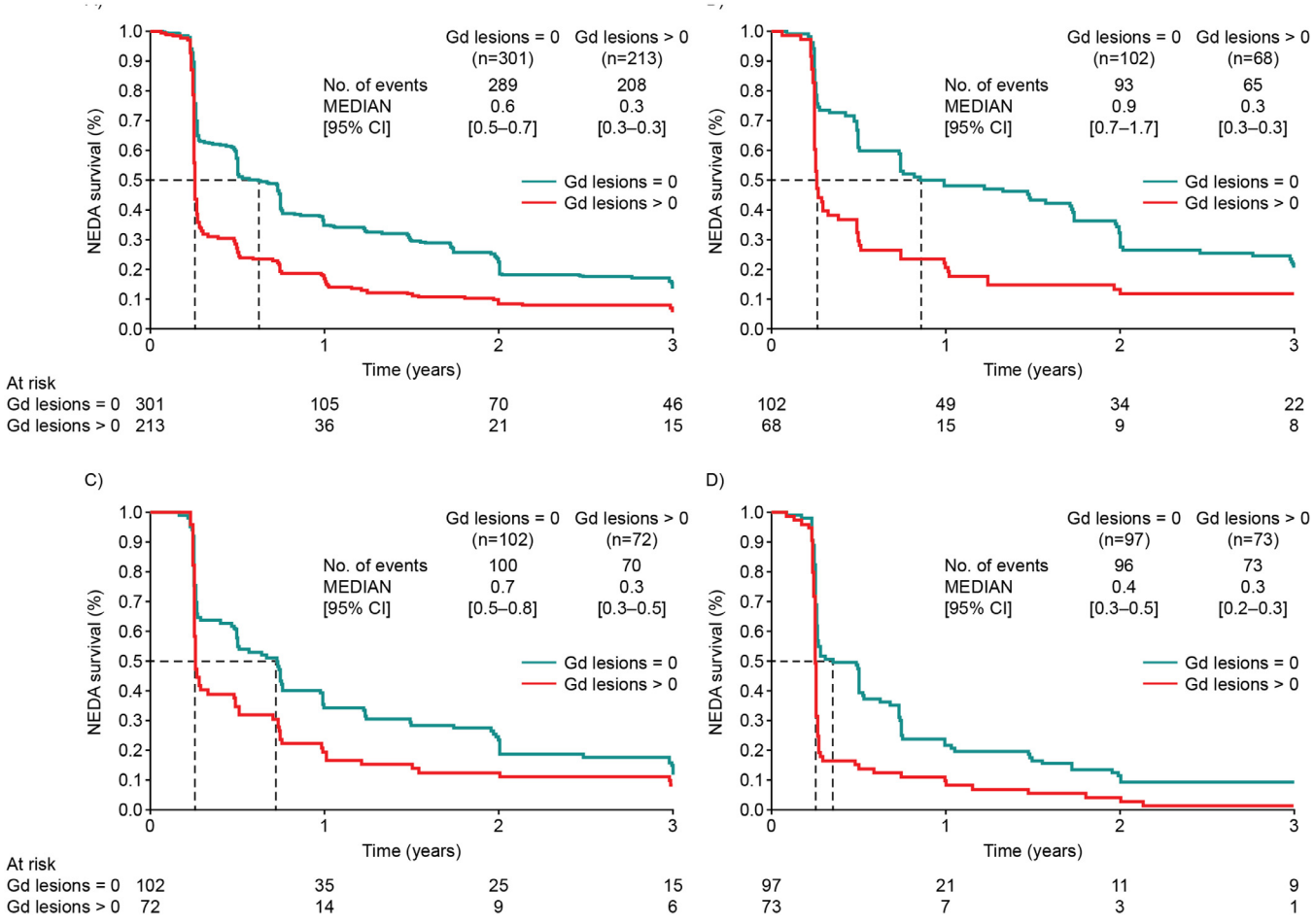
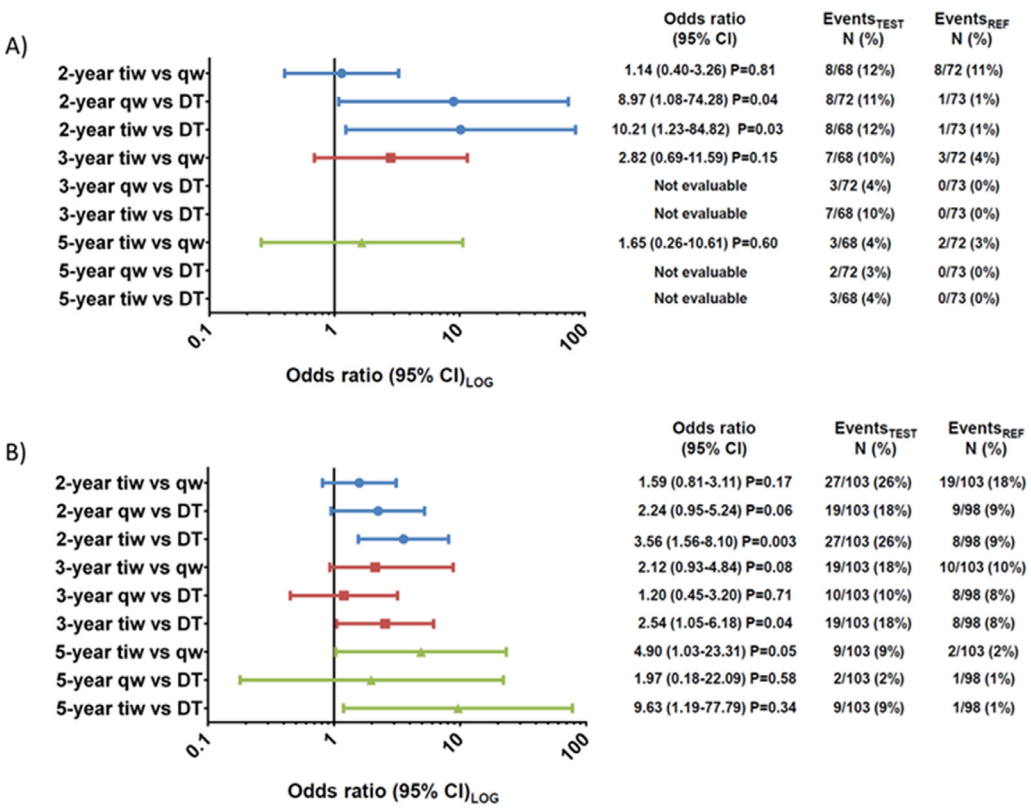


Fig. 2. Kaplan-Meier curves for median time to first MS defining event in patients with or without Gd+ lesions at baseline for A) all patients (ITT population), B) scIFN β-1a 44 μg tiw, C) scIFN β-1a 44 μg qw, and D) delayed treatment.

proportion of patients who remained NEDA-3 at 5 years compared with DT. However, the proportion of patients receiving scIFN β-1a tiw and qw who were NEDA-3 at year 2 and remained NEDA-3 at 5 years was relatively low (34% and 15%, respectively). Findings by Uher et al. showed that the majority of patients who received im IFN β-1a lost NEDA-3 status at an early stage with approximately 40% of patients with CIS and 20% of patients with RRMS losing their NEDA-3 status after 1 year (Uher et al., 2017). In addition, the CLIMB study showed that only 7.9% of patients maintained NEDA status after 7 years (Rotstein et al., 2015). Overall, these findings suggest that maintaining NEDA-3 over long-term follow-up is a challenging treatment goal.

At all time-points, there were a dose-dependent, numerically greater proportion of patients who received ET (tiw or qw) that had no evidence of radiological activity than those who received DT (Supplementary Figure 2C). Furthermore, ET with scIFN β-1a 44 μg tiw increased the odds of achieving no evidence of radiological activity up to year 2, 3 and 5 compared with DT (Supplementary Figure 3). However, no significant differences were observed between scIFN β-1a 44 μg qw and DT at year 3 and 5 (Supplementary Figure 3).

It is important to note that our analysis has limitations. The analysis is *post-hoc* and retrospective in nature. Patients in the DT group were heterogeneous as patients who developed clinical activity during REFLEX and REFLEXION had the option to switch to active tiw treatment (Comi et al., 2012, Comi et al., 2017); therefore, any treatment differences are potentially underestimated. Results from the subgroup analysis assessing the impact of the Gd+ lesions at baseline on NEDA should be interpreted with caution owing to small patient numbers, particularly in the long term. It is important to note that the definitions of CIS have evolved and consecutive revisions of diagnostic criteria have resulted in a reduction in the number of patients diagnosed as CIS. Therefore, as this analysis includes both CIS patients and patients satisfying the 2010 criteria, it was decided to examine if ET with scIFN β-1a 44 μg has an impact on a composite outcome applicable to CIS and MS alike. A significant conceptual limitation of an analysis using NEDA-



CI, confidence interval; DT, delayed treatment; NEDA-3, no evidence of disease activity; qw, once weekly; sc, subcutaneous; tiw, three times weekly.

Fig. 3. Effect of early vs. delayed scIFN β-1a on achieving NEDA-3 in patients A) with and B) without Gd+ lesions at baseline.

3 is that relapses, disability worsening, Gd+ lesions, and new/enlarging T2-hyperintense lesions are all weighted equally in terms of their impact on NEDA-3 status. As the loss of NEDA-3 status was primarily driven by the detection of a new lesion on MRI, it may have been better to weight the detection of lesions on MRI differently and to focus only on relapses or disease progression to more accurately reflect the treatment responses. It is also important to consider that REFLEX and REFLEXION were not designed to study NEDA-3; therefore, these results should be interpreted with caution.

In conclusion, this *post-hoc* analysis suggests that ET with scIFN β-1a was associated with higher odds of achieving NEDA-3 compared with DT, and treatment differences were sustained in the long-term (up to 5 years). The presence of Gd+ lesions at baseline was associated with a lower chance of remaining NEDA-3 compared with those without baseline Gd+ lesions. This observation adds to the evidence supporting their inclusion in the diagnostic criteria of MS with later iterations of the McDonald criteria (2010 and 2017).

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CRediT authorship contribution statement

Mark S. Freedman: Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Giancarlo Comi:** Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Patricia K. Coyle:** Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Julie Aldridge:** Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Liang Chen:** Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Kurt Marhardt:** Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Ludwig Kappos:** Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interests

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2019.101891.

References

Miller, D., Barkhof, F., Montalban, X., Thompson, A., Filippi, M., 2005. Clinically isolated syndromes suggestive of multiple sclerosis, part I: natural history, pathogenesis, diagnosis, and prognosis. Lancet Neurol. 4 (5), 281–288.

Poser, C.M., Paty, D.W., Scheinberg, L., McDonald, W.I., Davis, F.A., Ebers, G.C., et al., 1983. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann. Neurol. 13 (3), 227–231.

Mahajan, K.R., Ontaneda, D., 2017. The role of advanced magnetic resonance imaging techniques in multiple sclerosis clinical trials. Neurotherapeutics 14 (4), 905–923.

Rammohan, K.W., 2009. Cerebrospinal fluid in multiple sclerosis. Ann. Indian Acad. Neurol. 12 (4), 246–253.

McDonald, W.I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., et al., 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. Ann. Neurol. 50 (1), 121–127.

Polman, C.H., Reingold, S.C., Edan, G., Filippi, M., Hartung, H.P., Kappos, L., et al., 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald criteria" Ann. Neurol. 58 (6), 840–846.

Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J.A., Filippi, M., et al., 2011. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann. Neurol. 69 (2), 292–302.

Thompson, A.J., Banwell, B.L., Barkhof, F., Carroll, W.M., Coetzee, T., Comi, G., et al., 2018. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 17 (2), 162–173.

Montalban, X., Gold, R., Thompson, A.J., Otero-Romero, S., Amato, M.P., Chandraratna, D., et al., 2018. ECTRIMS/EAN guideline on the pharmacological treatment of people with multiple sclerosis. Mult. Scler. J. Exp. Transl. Clin. 24 (2), 96–120.

Rae-Grant, A., Day, G.S., Marrie, R.A., Rabinstein, A., Cree, B.A.C., Gronseth, G.S., et al., 2018. Practice guideline recommendations summary: disease-modifying therapies for adults with multiple sclerosis: report of the guideline development, dissemination, and implementation subcommittee of the American academy of neurology. Neurology 90 (17), 777–788.

Giovannoni, G., Turner, B., Gnanapavan, S., Offiah, C., Schmierer, K., Marta, M., 2015. Is it time to target no evident disease activity (NEDA) in multiple sclerosis? Mult. Scler. Relat. Disord. 4 (4), 329–333.

Coyle, P.K., Reder, A.T., Freedman, M.S., Fang, J., Dangond, F., 2017. Early MRI results and odds of attaining 'no evidence of disease activity' status in MS patients treated with interferon β-1a in the evidence study. J. Neurol. Sci. 379, 151–156.

Havrdova, E., Galetta, S., Hutchinson, M., Stefoski, D., Bates, D., Polman, C.H., et al., 2009. Effect of natalizumab on clinical and radiological disease activity in multiple sclerosis: a retrospective analysis of the natalizumab safety and efficacy in relapsing-remitting multiple sclerosis (AFFIRM) study. Lancet Neurol. 8 (3), 254–260.

Prosperini, L., Sacca, F., Cordioli, C., Cortese, A., Buttari, F., Pontecorvo, S., et al., 2017. Real-world effectiveness of natalizumab and fingolimod compared with self-injectable drugs in non-responders and in treatment-naive patients with multiple sclerosis. J. Neurol. 264 (2), 284–294.

Nixon, R., Bergvall, N., Tomic, D., Sfikas, N., Cutter, G., Giovannoni, G., 2014. No evidence of disease activity: indirect comparisons of oral therapies for the treatment of relapsing-remitting multiple sclerosis. Adv. Ther. 31 (11), 1134–1154.

Fox, R.J., Chan, A., Zhang, A., Xiao, J., Levison, D., Lewin, J.B., et al., 2017. Comparative effectiveness using a matching-adjusted indirect comparison between delayed-release dimethyl fumarate and fingolimod for the treatment of multiple sclerosis. Curr. Med. Res. Opin. 33 (2), 175–183.

Havrdova, E., Giovannoni, G., Gold, R., Fox, R.J., Kappos, L., Phillips, J.T., et al., 2017. Effect of delayed-release dimethyl fumarate on no evidence of disease activity in relapsing-remitting multiple sclerosis: integrated analysis of the phase III define and confirm studies. Eur. J. Neurol. 24 (5), 726–733.

Uher, T., Havrdova, E., Sobisek, L., Krasensky, J., Vaneckova, M., Seidl, Z., et al., 2017. Is no evidence of disease activity an achievable goal in ms patients on intramuscular interferon beta-1a treatment over long-term follow-up? Mult. Scler. 23 (2), 242–252.

Arnold, D.L., Calabresi, P.A., Kieseier, B.C., Liu, S., You, X., Fiore, D., et al., 2017. Peginterferon beta-1a improves MRI measures and increases the proportion of patients with no evidence of disease activity in relapsing-remitting multiple sclerosis: 2-year results from the advance randomized controlled trial. BMC Neurol. 17 (1), 29.

Havrdova, E., Arnold, D.L., Bar-Or, A., Comi, G., Hartung, H.P., Kappos, L., et al., 2018. No evidence of disease activity (NEDA) analysis by epochs in patients with relapsing multiple sclerosis treated with ocrelizumab vs interferon beta-1a. Mult. Scler. J. Exp. Transl. Clin. 4 (1), 2055217318760642.

Durability of NEDA-3 status in patients with relapsing multiple sclerosis receiving cladribine tablets: clarity extension. In: Giovanonni, G., Keller, B., Jack, D (Eds.), ACTRIMS Forum. Dallas, TX.

Havrdova, E., Arnold, D.L., Cohen, J.A., Hartung, H.P., Fox, E.J., Giovannoni, G., et al., 2017. Alemtuzumab care-MS I 5-year follow-up: durable efficacy in the absence of continuous MS therapy. Neurology 89 (11), 1107–1116.

Comi, G., De Stefano, N., Freedman, M.S., Barkhof, F., Polman, C.H., Uitdehaag, B.M., et al., 2012. 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. 11 (1), 33–41.

Comi, G., De Stefano, N., Freedman, M.S., Barkhof, F., Uitdehaag, B.M., de Vos, M., et al., 2017. Subcutaneous interferon β-1a in the treatment of clinically isolated syndromes: 3- and 5-year results of the phase III, dosing frequency-blind, multicentre reflexion study. J. Neurol. Neurosurg. Psychiatry 88 (4), 285–294.

Trojano, M., Pellegrini, F., Paolicelli, D., Fuiani, A., Zimatore, G.B., Tortorella, C., et al., 2009. Real-life impact of early interferon beta therapy in relapsing multiple sclerosis. Ann. Neurol. 66 (4), 513–520.

Comi, G., Filippi, M., Barkhof, F., Durelli, L., Edan, G., Fernandez, O., et al., 2001. Effect of early interferon treatment on conversion to definite multiple sclerosis: a randomised study. Lancet 357 (9268), 1576–1582.

Goodin, D.S., Frohman, E.M., Garmany Jr., G.P., Halper, J., Likosky, W.H., Lublin, F.D., et al., 2002. 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 58 (2), 169–178.

Bevan, C.J., Cree, B.A., 2014. Disease activity free status: a new end point for a new era in multiple sclerosis clinical research? JAMA Neurol. 71 (3), 269–270.

Rotstein, D.L., Healy, B.C., Malik, M.T., Chitnis, T., Weiner, H.L., 2015. Evaluation of no evidence of disease activity in a 7-Year longitudinal multiple sclerosis cohort. JAMA Neurol. 72 (2), 152–158.