ORIGINAL RESEARCH

Clinical effectiveness of different natalizumab interval dosing schedules in a large Italian population of patients with multiple sclerosis

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ABSTRACT

Introduction Natalizumab (NTZ) is one of the most effective treatment options for multiple sclerosis (MS) treatment. Our study aimed to evaluate the effectiveness of NTZ when administered according to the extended dosing strategy compared with standard 4-weekly administration in a large Italian MS population.

Materials and methods This retrospective multicentre study included patients with relapsing-remitting MS (RR-MS) who received NTZ administrations between the 1 June 2012 and the 15 May 2018 and were followed by the 'Italian MS Register'. All patients with MS were stratified into two groups based on NTZ administration schedule: standard interval dosing (SID) patients who received infusions on average from 28 to 32 days (median 30) and extended interval dosing (EID) including patients who have been infused with interval between 33 and 49 days (median 43). Clinical data were assessed at baseline (before starting NTZ), after 12 (T1) and 24 months (T2) of treatment.

Results Out of 5231 patients with RR-MS screened, 2092 (mean age 43.2±12.0, 60.6% women) were enrolled. A total of 1254 (59.9%) received NTZ according to SID, and 838 (40.1%) according to EID. At 12 and 24 months, no differences in terms of annualised relapse rate and disability status were found between the two groups. Progression index and confirmed disability worsening were similar between the two groups.

Discussion The use of NTZ with an extended interval schedule showed similar effectiveness compared with SID. Unchanged clinical efficacy of EID schedule may raise the question of a possible advantage in terms of tolerability and safety.

INTRODUCTION

Natalizumab (NTZ; Tysabri) is a humanised anti-α4 integrin monoclonal antibody that blocks lymphocyte adhesion to endothelial cells, thereby preventing their migration to the central nervous system (CNS) and reducing inflammation.[1–4] The NTZ safety and efficacy in relapsing-remitting multiple sclerosis study (Safety and Efficacy of Natalizumab in the Treatment of Multiple Sclerosis - AFFIRM study) showed that NTZ, compared with placebo, was able to reduce the annualised relapse rate (ARR) by 68% relative to placebo, the accumulation of new or enlarging hyperintense lesions by 83%, 12-week sustained disability progression by 42% and 24-week sustained disability progression by 54% over 2 years.[5]

The beneficial effects of NTZ on relapse rate have been further confirmed in several studies.[4–9] In a meta-analysis of three randomised clinical trials and five observational studies, NTZ was compared with fingolimod, showing a greater reduction in the ARR and a lower probability of disability progression at 2 years.[9] In another study of a US cohort, patients treated with glatiramer acetate or interferon-β were 34% more likely to have a relapse than those on NTZ.[10] The high efficacy of NTZ was confirmed also in a postmarketing study, which demonstrated in over 793 patients of 18 French MS centres on NTZ for almost 2 years, a reduction of ARR of 78.6% in the first year.[8]

Since its approval in 2006/2007, NTZ has demonstrated higher efficacy in reducing the progression of MS compared with second-line drugs, although safety issues have imposed a strict clinical surveillance. The potential occurrence of progressive multifocal leucoencephalopathy (PML) in NTZ-treated patients has prompted an intense search for the best strategy to reduce such a serious complication and to prevent the clinical and radiological relapses associated to NTZ discontinuation, in particular the risk of a clinical rebound.[11] In this regard, an early study has proved that a progressive return of subclinical MRI activity may occur after approximately 7 weeks from the last NTZ infusion in patients with MS,[12] suggesting that the therapeutic window of NTZ could be larger than that approved based on clinical trials. Thus, a reasonable delay of time between infusions could provide advantages in terms of safety (ie, reduced risk of PML due to subliminal entrance of protective immune cells within the central nervous system), likely without exposing patients to a risk of MS relapse. Therefore, in real-world clinical practice, the neurologists of Italian MS centres across the country have begun to treat patients with MS using various extended interval dosing (EID) schedules.[11 13]

This multicentre retrospective observational study aims to provide additional information on NTZ effectiveness in the real-world clinical practice and to evaluate the therapeutic durability of different extended dosing strategies in a large Italian population of patients with MS.

MATERIALS AND METHODS

Study population

This retrospective observational multicentre study included patients with MS from 11 Italian MS centres contributing to the Italian MS Register on NTZ treatment from 1 June 2012 to 15 May 2018. The iMed software was used by all contributing centres in order to collect standardised information about all patients with MS followed during the observation period.[14] All data were prospectively collected by well-trained neurologists.

A further stratification according to the NTZ treatment schedule was carried out, dividing the cohort in:

- Standard interval dosing (SID) patients who received infusions on average from 28 to 32 days (median 30 days).
- EID including patients infused on a schedule between 33 days and 49 days (median 43 days).

Our cohort included all patients with a diagnosis of RR-MS according to McDonald's 2010 criteria,[15] treated with NTZ according to SID or EID. All enrolled patients in EID had undergone standard dose schedule of NTZ infusions for at least 6 months prior to the start of EID. Moreover, at least three consecutive extended dose infusions were counted for stratification into one of the extended dose groups. The analysis excluded patients with a diagnosis of secondary progressive MS or primary progressive MS, alternative shift between SID and EID schedules or patients' Expanded Disability Status Scale (EDSS) >6.5.

A minimum of two visits per patient spanning at least 12 months, with full EDSS evaluation, was required to define a minimum 12-month confirmed disability-worsening event. EDSS evaluation was assessed by certified and qualified neurologists at each centre.

Study design

Clinical data at baseline (before starting NTZ), after 12±3 months and 24±3 months of treatment were retrospectively collected from the Italian MS register (iMed software). The iMed database allows to store all clinical and demographical data and to prospectively collect new information about treatment, EDSS status, relapse, discontinuation therapy and so on. In particular, this study focused on demographic data, age at onset, age at diagnosis, MS phenotype, date of disease onset, disease duration, EDSS scores at onset, at 12 and 24 months after the first infusion of NTZ and at each follow-up visits, total number of relapses, number of relapses in the year before NTZ and during NTZ after 12 and 24 months of treatment, immunosuppressive exposure prior to NTZ (yes/no), and NTZ treatment data (start date NTZ, number of doses administered and NTZ administration dates). Data about the John Cunningham virus (JCV) antibody status and index value at NTZ treatment onset were collected in an ad-hoc dataset.

ARR and percentage of patients showing no evidence of disease activity (NEDA-2) were also calculated. NEDA-2 was defined as lack of clinical relapses and of disease progression measured by EDSS.[16]

Considering the whole population with baseline EDSS, the cumulative probability of confirmed EDSS worsening (CEW), defined as either a ≥1-point or ≥2-points increase in EDSS score from baseline that was confirmed at 12 and 24 months of treatment, was evaluated.[17] In patients with baseline EDSS scores of ≤2.0, the cumulative risk of confirmed transition to an EDSS score ≥3.0 was evaluated; the cumulative risk of confirmed transition to an EDSS score of ≥4.0 was considered in patients with a baseline EDSS score of 2.5–3.0; finally, in patients with an EDSS score ≥4.0, the cumulative risk of confirmed transition to an EDSS score of ≥6.0 was calculated. The cumulative probabilities of CEW were also evaluated in each of the administration protocol subgroups (SID vs EID).

In addition, a progression index (PI) was calculated as EDSS score divided by duration of the disease at the time of the last follow-up.[18]

Confirmed disability improvement (CDI), defined as a 1.0-point decrease from baseline confirmed over 6 months, was also assessed only in patients with baseline EDSS scores ≥2.0.

Differences in terms of clinical effectiveness were also assessed stratifying the EID patients in two different subgroups: early EID (EEID) infused on a schedule of 33–41 days and late EID (LEID) infused on a schedule ≥42 days.

Statistical analysis

Statistical analysis was performed using Stata V.16.0 software.

In descriptive analyses, continuous variables were summarised as mean and SD or median and IQR, while categorical variables were expressed as percentages. All clinical and demographical characteristics were compared with Pearson's χ^2 test and Mann-Whitney U test for categorical and continuous variables, respectively.

The mean adjusted ARR was calculated using descriptive statistics. With regard to mean adjusted ARRs, a negative binomial regression was used to account for overdispersion of the relapse count data. The multivariate Poisson regression model was carried out to assess incidence of relapses in the two groups during NTZ treatment, whereas multivariate Cox proportional hazards regression was used to model the time to reach the first relapse and the 1-point and 2-point CEW at 12 and 24 months. All the models were adjusted for the following baseline covariates: sex, age, disease duration, EDSS, immunomodulant exposure prior to NTZ (yes/no), immunosuppressive exposure prior to NTZ (yes/no), number of relapses in the year before, during NTZ, number of NTZ infusions and NTZ administration schedules (SID and EID).

The Kaplan-Meier method was used to estimate the cumulative probability of first relapse occurrence and EDSS worsening of 1-point and 2-points at 24 months.

Risks were reported in terms of incidence rate ratios or HRs along with their 95% CIs. A two-sided p value of <0.05 was considered as statistically significant.

RESULTS

At the extraction date, 5231 patients with RR-MS who had received NTZ from 1 June 2012 to 15 May 2018 in 30 Italian MS centres were recruited. A total of 2092 patients (mean age of 43.2±12.0 years) met the inclusion criteria and were finally enrolled. A percentage of 60.6 were women. The remaining 3139 patients were excluded because of missing data.

We found that 1254 (59.9%) patients received NTZ according to SID and 838 (40.1%) according to EID.

EID patients had a longer disease duration and a higher EDSS before starting NTZ compared with SID. Moreover, the percentages of patients drug-naïve and of patients treated with immunosuppressant drugs before starting NTZ treatment were higher in the EID compared with the SID group (table 1). At baseline, the

Table 1 Demographical and baseline clinical characteristics of the study population

	Total=**2092**	**SID 1254 (59.9)**	**EID 838 (40.1)**	**P value**
Women (%)	1268 (60.6)	753 (60.1)	515 (61.5)	0.8
Age (years); mean±SD	43.2±12.0	41.6±11.5	42.3±13.4	0.2
Disease duration (months); mean±SD	191.3±101.2	200.5±105.8	294.3±97.6	0.0001
Age at onset (years); mean±SD	33.5±13.7	32.5±19.4	33.9±16.7	0.09
Age at diagnosis (years); mean±SD	38.0±13.3	36.9±14.7	37.2±12.3	0.6
Patients JC virus positive (%)	562 (26.9)	245 (19.5)	317 (37.8)	0.0001
JC virus antibody index; mean±SD	1.6±1.1	1.1±1.4	2.0±0.9	0.0001
JC virus antibody index <0.9 (%)	225 (10.8)	132 (10.5)	93 (11.1)	0.7
JC virus antibody index 0.9–1.5 (%)	139 (6.6)	75 (6)	64 (7.6)	0.2
JC virus antibody index >1.5 (%)	198 (9.5)	38 (3)	160 (19.1)	0.0001
EDSS pre-NTZ; mean±SD	3.3±2.1	3.8±1.8	3.0±2.0	0.0001
Patients with EDSS ≤2.0 at baseline (%)*	689 (41.7)	432 (41.5)	257 (41.6)	0.2
Patients with EDSS 2.0–4.0 at baseline (%)*	643 (38.9)	402 (38.7)	241 (39.4)	0.2
Patients with EDSS ≥4.0 at baseline (%)*	319 (19.3)	206 (19.8)	113 (18.5)	0.1
N relapses at baseline; mean±SD	2.3±1.9	2.3±1.3	2.2±1.5	0.1
Drug-näive patients (%)	625 (29.9)	451 (36)	174 (20.8)	0.0001
Prior use of immunosuppressive drugs (%)	622 (29.7)	326 (26)	296 (35.3)	0.001

*The percentage is referred to 1651 patients for which EDSS values were available at baseline.
EDSS, Expanded Disability Status Scale; EID, extended interval dosing; NTZ, natalizumab; SID, standard interval dosing.

EID patients were more frequently JC virus positive with higher JC virus antibody index compared with SID patients.

At 12 and 24 months after start of NTZ, no differences in terms of ARR and of EDSS were found between the two groups. No statistically significant differences in terms of percentage of patients reaching NEDA-2, PI and CDI were found between the two groups. Moreover, at 24 months, EID patients showed a statistically significant higher JCV index compared with SID (table 2).

Overall, at 24 months, the percentage of patients JCV positive slightly increased from 26.9% to 29.5% (p=0.14), with a significant higher JCV index compared with baseline (p<0.001). Stratifying according to the two different administrations schedules, after 24 months, both SID and EID groups showed a significant increase of JCV index values compared with baseline (1.1±1.4 vs 1.4±1.1, p<0.001 and 2.0±0.9 vs 2.2±1.5, p<0.001, respectively).

At 24 months, in the patients with baseline EDSS scores available (n=1651, 78.9% of 2092), the cumulative probabilities of 12-month and 24-month CEW were 14.3% and 11.6%, respectively, with worsening defined as an increase in EDSS score of ≥1.0 point. When worsening was defined as an increase of ≥2.0 points, the cumulative probabilities were 8.1% and 6.1%, respectively. No differences in terms of cumulative risk of CEW were found between SID and EID (table 3).

In the overall population, 689 (41.7% of 1651) patients had a baseline EDSS score 0.0–2.0, 432 (41.5% of 1040) in SID and 257 (41.6% of 611) in EID. Among these patients, the

Table 2 Clinical outcomes of the study population stratified according to the different administration protocols

	Total=**2092**	**SID 1254 (59.9)**	**EID 838 (40.1)**	**P value**
NTZ interval dosing (days); mean±SD	34.3±3.2	30.8±1.9	39.8±4.3	**0.001**
Number of NTZ doses*; mean±SD	38.6±19.9	31.9±23.8	51.3±22.9	**0.001**
Patients JC virus positive at 24 months (%)	618 (29.5)	279 (22.2)	339 (40.5)	**0.0001**
JC virus antibody index at 24 months; mean±SD	1.9±1.3	1.4±1.1	2.2±1.5	**0.0001**
JC virus antibody index at 24 months <0.9 (%)	219 (10.5)	132 (10.5)	87 (10.4)	0.9
JC virus antibody index 0.9–1.5 at 24 months (%)	169 (8.1)	85 (6.8)	84 (10)	**0.01**
JC virus antibody index >1.5 at 24 months (%)	230 (11)	62 (4.9)	168 (20)	**0.0001**
Patients with clinical relapses at 12 months (%)	83 (4.0)	54 (4.3)	29 (3.5)	0.4
Patients with clinical relapses at 24 months (%)	73 (5.1)	47 (3.7)	26 (3.1)	0.5
N relapses at follow-up; mean±SD	0.7±0.8	0.7±1.3	0.8±1.4	0.09
EDSS at 12 months; mean±SD	3.4±2.0	3.1±2.9	3.2±2.5	0.4
EDSS at 24 months; mean±SD	3.1±2.6	3.3±2.8	3.4±2.1	0.3
EDSS at last follow-up; mean±SD	2.9±2.5	2.9±2.5	3.0±2.6	0.4
ARR at 12 months; mean±SD	0.12±0.08	0.10±0.09	0.11±0.08	0.8
ARR at 24 months; mean±SD	0.09±0.05	0.09±0.05	0.10±0.05	0.7
Patients reached NEDA-2 at 24 months (%)	1658 (79.3)	995 (79.3)	663 (79.1)	1.0
Progression index at 24 months; mean±SD	0.39±0.31	0.38±0.28	0.40±0.31	0.1
Confirmed disability improvement at 24 months (%)	341 (16.3)	221 (17.6)	125 (14.9)	0.2

ARR, annualised relapse rate; EDSS, Expanded Disability Status Scale; EID, extended interval dosing; NEDA-2, no evidence of disease activity; NTZ, natalizumab; SID, standard interval dosing.

Table 3 Cumulative probability of 12-month and 24-month confirmed EDSS worsening in SID and EID patients

	Overall (n=1651)		SID (n=1040)		EID (n=611)			
CP%	N	%	N	%	N	%	HR (95% CI)	P value
12-month confirmed worsening								
EDSS score by ≥1.0 point	249	15.1	160	15.4	89	14.6	1.01 (0.85 to 1.86)	p=0.42
EDSS score by ≥2.0 point	139	8.4	91	8.8	48	7.9	0.99 (0.75 to 2.22)	p=0.39
24-month confirmed worsening								
EDSS score by ≥1.0 point	192	11.6	125	12.0	67	11.0	0.96 (0.71 to 2.81)	p=0.52
EDSS score by ≥2.0 point	101	6.1	68	6.5	33	5.4	1.12 (0.89 to 2.75)	p=0.29

CP, cumulative probability; EDSS, Expanded Disability Status Scale; EID, extended interval dosing; SID, standard interval dosing.

cumulative risk of confirmed transition to an EDSS score ≥3.0 was 7.3% at 12 months and 8.1% at 24 months. Among the 643 (38.9% of 1651) patients with a baseline EDSS score of 2.5–3.0, 402 (38.7% of 1,040) in SID and 241 in EID (39.4% of 611), the cumulative risk of confirmed transition to an EDSS score of ≥4.0 was 12.4% at 12 months and 13.2% at 24 months with a trend towards a higher probability to proceed from 2.0 to 3.0 to >4.0 EDSS score in the EID (16.9% at 12 months and 16.9% at 24 months) versus the SID (12.4% at 12 months and 13.6% at 24 months) group. For the 319 (19.3% of 1,651) patients with a baseline EDSS score of ≥4.0, 206 (19.8% of 1040) in SID and 113 (18.5% of 611) patients in EID, the cumulative risk of confirmed transition to an EDSS score of ≥6.0 was 18.4% at 12 months and 23.6% at 24 months with no differences between SID and EID patients. Similar risks were found when stratifying for different administration schedules, with the exception of a statistical trend between SID and EID for the cumulative risk of confirmed transition to an EDSS score of ≥4.0 in patients with a baseline EDSS score of 2.0–3.0 (table 4).

Moreover, Kaplan-Meier estimates for the first relapse occurrence, CEW of 1 point and CEW of 2 points showed no statistically significant differences between the two groups (figures 1–3).

No differences in terms of clinical effectiveness were found between the EEID and the LEID subgroups. These results were provided as online supplementary materials.

DISCUSSION

In our retrospective multicentre study, EID does not appear to statistically diminish the effectiveness of NTZ, and comparable results were registered across all measured outcomes, including ARR, CEW risks and NEDA-2 outcome between the SID and EID groups. In particular, PI and CDI were similar between the two different administration schedules. This is, at least in the short period of the 2 years considered, a reassuring finding due to the widespread use of extending the interval between NTZ infusions in the majority of MS centres of the world.

The soundness of this conclusion obviously relies on how well the balance between the two populations studied is achieved. In our cohort, actually, the EID group included patients with longer disease duration, higher risk for PML at baseline as showed by higher mean JCV antibody index, higher proportion of patients positive to JCV, higher proportion of patients with disease duration ≥2 years and prior use of immunosuppressants. These discrepancies derive from the fact that MS treating neurologists tend to prefer to assign patients with a high PML risk (JCV positive and with a higher number of NTZ administrations) to the EID schedule programme hoping to allow an even minimal immune reconstitution/surveillance within the CNS. This hypothetical PML preventive strategy has been supported by several retrospective studies showing that the EID schedule actually reduces the risk of PML while maintaining NTZ efficacy.[19 20] A recent analysis of a large dataset from the Tysabri Outreach: Unified Commitment to Health programme has confirmed with a class III of evidence the lower PML risk with NTZ EID schedule.[21] Noteworthy, in our cohort, after 24 months of treatment, a significant increase in JCV index was observed in both SID and EID groups. This is in line with other studies showing that JCV seroprevalence rises with treatment duration.[22–24] However, our study did not actually aim to evaluate the impact of different administration schedules on the safety profile (ie, PML incidence) but primarily focused on the NTZ efficacy profile of EID and SID administration schedules. Although the finding of a similar clinical effectiveness between SID and EID could have been influenced by the abovementioned differences, this was not, since no statistical differences between the two groups were detected.

The use of EID schedules is further justified by previous pharmacokinetics studies, demonstrating that the maximal receptor

Table 4 Cumulative probabilities of 12-month and 24-month confirmed worsening to specific EDSS milestones at 24 months

	Overall (n=1651)		SID (n=1040)		EID (n=611)			
	N	CP, %	N	CP, %	N	CP, %	HR (95% CI)	P value
12-month confirmed worsening								
From EDSS score of 0.0–2.0 to ≥3.0	50	7.3	31	7.2	19	7.5	1.05 (0.61 to 2.13)	0.79
From EDSS score of 2.0–3.0 to ≥4.0	105	12.6	53	12.4	52	16.9	1.39 (0.99 to 2.39)	0.13
From EDSS score of 4.0–5.0 to ≥6.0	105	18.4	71	18.5	34	18.3	1.12 (0.79 to 6.32)	0.71
24-month confirmed worsening								
From EDSS score of 0.0–2.0 to ≥3.0	56	8.1	34	7.8	22	8.7	1.12 (0.76 to 3.68)	0.63
From EDSS score of 2.0–3.0 to ≥4.0	110	13.2	58	13.6	52	16.9	1.32 (1.00 to 3.19)	0.08
From EDSS score of 4.0–5.0 to ≥6.0	135	23.6	91	23.8	44	23.7	1.09 (0.85 to 5.69)	0.78

CP, cumulative probability; EDSS, Expanded Disability Status Scale; EID, extended interval dosing; SID, standard interval dosing.

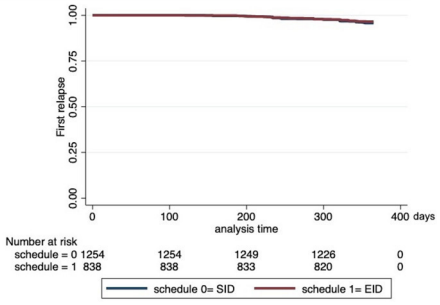

Figure 1 Kaplan-Meier analysis of first relapse occurrence in SID and EID. EID, extended interval dosing; SID, standard interval dosing.

saturation is reached during the first 3–4 weeks after NTZ dose when more than 80% integrins are bound by the drug. Between the following 4–8 weeks, the integrin saturation progressively declines, although maintaining levels between 50% and 80%.[25] The functional integrins' desaturation is reached when less than 50% are bound by the drug and occurs when NTZ blood levels fall below 1 µg/mL, usually around 8 weeks after the infusion.[25–27] Thus, the rising concentration of NTZ, the individual metabolism and body mass index (BMI) positively influence blood levels over time. The correlation between PML risk and the level of integrins saturation[21] has raised the question whether the use of different EID schedules may actually mitigate the overall burden of AEs, especially PML, in NTZ-treated MS patients.[28–30] A prospective observational cohort study has measured the NTZ trough serum concentrations in 80 patients, showing high and stable intraindividual NTZ levels (≥10 µg/mL) in 94% of patients and no correlation between spread in concentrations and disease activity.[31] More recently, a cross-sectional evaluation of serum NTZ concentrations and α4-integrin receptor saturations in patients with MS receiving SID or EID has shown that preservation of adequate trough NTZ saturation and concentration levels were guaranteed by at least nine NTZ infusions/year. Interestingly, a higher BMI was a predictor of suboptimal trough saturation on EID,[32] a finding that at least for another drug used to treat MS has led to a dosage adapted to the weight.[33]

Other studies have already reported the clinical and neuroradiological outcomes, such as adjusted ARR, MRI activity and NEDA-2, of reducing NTZ frequency of infusion up to 8 weeks and found no difference between MS patients with standard administration schedule and several alternative schedules.[20] In

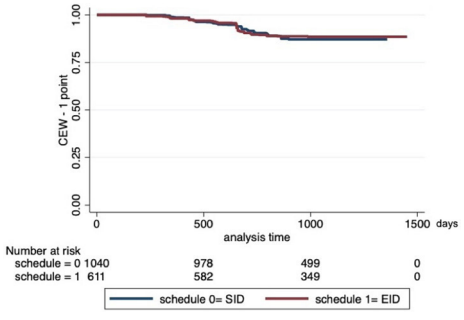

Figure 2 Kaplan-Meier analysis of CEW 1 point in SID and EID. CEW 1 point, cumulative Expanded Disability Status Scale (EDSS) worsening defined as a ≥1-point increase in EDSS score from baseline that was confirmed at 24 months of treatment; EID, extended interval dosing; SID, standard interval dosing.

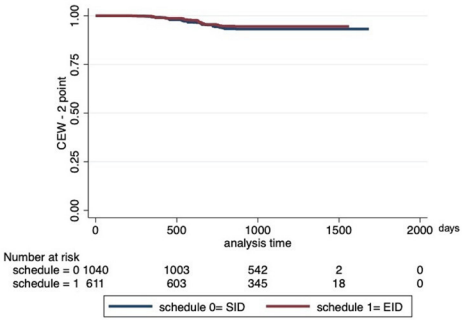

Figure 3 Kaplan-Meier analysis of CEW 2 points in SID and EID. CEW 2 points, cumulative Expanded Disability Status Scale (EDSS) worsening defined as ≥2-point increase in EDSS score from baseline that was confirmed at 24 months of treatment. EID, extended interval dosing; SID, standard interval dosing.

this study, EDSS was not used as outcome measure as it could be affected by inconsistency between evaluators of the participating centres. In our larger study, the participation of the most highly specialised national centres in diagnosis and treatment of MS should, at least partially, warrant a sufficient standard of assessment allowing the detection of significant findings within patients' clinical picture.[34]

Our conclusion that the ARR during 2 years of observation was not different in patients on EID or SID, the former almost 50% longer than the latter (a median difference of 13 days), is in line with other studies.[19 20 35] In a retrospective study of 361 MS patients treated with NTZ at two MS centres, the relapse rate was not different in patients on EID or on monthly dosing.[19] In a study evaluating 85 patients with MS treated with NTZ with EID for at least 6 months, all patients were shifted after an initial SID period to the EID ranging from 5 to 8 weeks. Despite the different study design, these authors also found that the ARR and MRI inflammatory activity was also unchanged between SID and EID.[35]

However, even if we did not find statistically significant differences between SID and EID regarding EDSS and ARR values, we cannot exclude that patients with less active disease, showing an overall lower ARR but a higher PML risk based on the JCV index and more likely to be switched to the EID protocol, may present a somewhat delayed clinical activity over the 2 years of our observation.[19] Clearly, studies with an extended follow-up are needed to confirm our conclusions.

In our study, the CEW showed similar results between the two different administration schedules. Results of a recent analysis of Tysabry Observational Program (TOP) patients also indicated that 48-week confirmed worsening is a robust outcome measure that may reliably capture irreversible disability worsening.[17] Thus, our results may add value to the current literature showing the similar effectiveness of different NTZ administration schedules, in particular because of the similar CEW risks. This is also supported by the finding of similar PI values between the two groups, which to the best of our knowledge is reported for the first time.

Also unreported is our observation that roughly 1/5 of patients showed an improvement in the disability status, as previously reported,[36] with no difference between the two treatment schedules. Moreover, a statistical trend between SID and EID for the cumulative risk of confirmed transition to an EDSS score of ≥4.0 in patients with a baseline EDSS score of 2.0–3.0 was also found. This could be explained by the fact that this group of patients may include those who have a transitional phase of MS clinical

course, that is the anteroom of a secondary progressive form. It could be speculated that, with regard to slowing disability progression, patients with a baseline EDSS score of 2.0–3.0 may benefit from SID rather than EID, possibly because they have a higher underlying inflammatory activity better contained with a more aggressive confinement strategy. Longitudinal randomised studies are needed in order to confirm or reject this hypothesis.

This study has several limitations. First, the retrospective non-randomised design did not allow to control for baseline confounders and, thus, may have skewed our results. Indeed, patients with less aggressive MS could be more likely to move on to the EID schedule, as suggested by the longer disease duration and the higher number of NTZ administrations in EID than in SID observed in our cohort. In addition, the clinicians participating in the study may have chosen to use EID protocol in those patients more likely to stay on NTZ treatment anyway, considering their clinical stability and a higher risk of PML, as demonstrated by the higher percentage of patients with a JCV index >1.5 in EID compared with SID. Second, the use of apparently early EID (≥33 days) in our study might have prevented from properly estimating the effect of wider interval dosing schedules on NTZ effectiveness. Although, further analyses were carried out stratifying the EID patients in two subgroups (EEID and LEID), confirming no differences in terms of clinical effectiveness among the different intervalling-dosing groups (see online supplementary materials). It should be also noted that, as a result of the retrospective design of the study, all patients with MS in EID group have been treated with at least 6 NTZ doses, and this may have impacted the clinical evaluations at 12 months. Nonetheless, SID and EID patients exhibited similar results in terms of clinical effectiveness even at 24 months. Moreover, our study did not aim to investigate the difference of PML risk among different administration schedules. However, the demonstration of maintenance of clinical effectiveness of EID encourages a continued evaluation for PML risk reduction. Finally, our study did not include the MRI data, as the count of T2 lesions and new/enlarging T2 lesions may be susceptible of high variability in terms of evaluators and MRI machines (ie, different magnetic field strength, slices thickness and so on).

In conclusion, this multicentre retrospective study assessed the largest population of MS patients treated with NTZ in MS centres distributed across the Italian territory and the clinical effect that different dosing schedules may have on the progression of MS within a 2-year time frame. Physicians treating patients with MS are consistently attempting to balance the risks and benefits of this highly effective medication.[19] The high number of patients on EID in our cohort already reflects the propensity of Italian clinicians to diverge from the monthly SID and to delay NTZ administration according to different patient conditions.

Randomised controlled trials comparing the efficacy of both regimens, such as the NOVA study (NCT03689972), that will prospectively evaluate the efficacy of EID NTZ administration in subjects who have previously been treated with NTZ according to the SID for at least 12 months, compared with continued SID treatment, will definitively contribute to clarify this issue.[37]

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