Prospective trial of natalizumab personalized extended interval dosing by therapeutic drug monitoring in relapsing-remitting multiple sclerosis (NEXT-MS)

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ABSTRACT

Background: Extended interval dosing (EID) of natalizumab is a promising strategy to optimize treatment in multiple sclerosis (MS). Personalized EID by therapeutic drug monitoring can enable further extension of treatment intervals.

Methods: The NEXT-MS trial is an investigator-initiated prospective phase IV nonrandomized study. Adults with a diagnosis of relapsing-remitting MS who received ≥6 natalizumab infusions were included in three groups: personalized EID with a target drug trough concentration of 10 µg/mL (EID10), an exploratory group of personalized EID with a target of 5 µg/mL (EID5), and standard interval dosing (SID) of 4 weeks. The primary outcome is radiological disease activity (new/newly enlarged T2 lesions) comparing the EID10 group to a historical cohort of SID (HSID).

Results: Results of the first phase of the NEXT-MS trial are reported here (n=376) as the study will continue with an amended protocol. In the EID10 group (n=251), incidence rate of radiological activity was 10.0 per 1000 person-years, which was non-inferior to the HSID cohort (24.7 per 1000 person-years (n=87), incidence rate difference 14.7, 90% CI -4.5 to 34.0). Incidence rate of radiological activity was 10.0 per 1000 person-years in the EID5 group (n=65), and 47.0 per 1000 person-years in the SID group (n=60). Serum neurofilament light levels did not increase over time within the EID groups. There were no cases of PML.

Conclusions: MS disease activity is adequately controlled with personalized natalizumab EID. Interval extension to a drug trough concentration of 5 µg/mL is likely a safe target to extend natalizumab treatment intervals >6 weeks.

Keywords: Multiple Sclerosis, Natalizumab, Extended Interval Dosing, Therapeutic Drug Monitoring, Neurofilament Light.

- **What is already known on this topic**: Comparable efficacy has been shown between natalizumab every 4 versus 6 weeks. However, a proportion of patients still have high natalizumab drug concentrations after 6 weeks intervals.
- **What this study adds:** Personalized EID based on drug concentrations can enable further extension of treatment intervals with adequate control of MS disease activity. With a lower target (5 µg/mL), one-third of participants were able to extend beyond a 6 week interval.
- **How this study might affect research, practice or policy:** Therapeutic drug monitoring can be used by clinicians to (further) extend natalizumab treatment intervals, thereby lowering treatment burden for patients and healthcare costs, and potentially further reducing treatment risks.

Trial Registration Information: ClinicalTrials.gov identifier NCT04225312.

INTRODUCTION

Natalizumab is an effective therapy for relapsing-remitting multiple sclerosis (RRMS).(1) By binding to the α4-integrin receptor, natalizumab prohibits lymphocytes from entering the central nervous system (CNS). Receptor saturation falls between 80-100% after a 4 week infusion interval,(2, 3) and disease activity potentially returns when saturation drops below 20-40% (natalizumab serum concentration <1-2 µg/mL).(4-7) As most patients on standard interval dosing (SID) have high natalizumab trough concentrations prior to re-dosing (mean 26-44 µg/mL),(2, 8, 9) extended interval dosing (EID) could be feasible without losing efficacy.(10) Importantly, EID is associated with a decreased risk of progressive multifocal leukoencephalopathy (PML) compared to SID (relative risk reduction 88-94%).(11)

There exists a large inter-individual variation in drug trough concentrations (range 0.1-110 µg/mL).(2, 8) Natalizumab drug concentrations are usually stable intra-individually within a set treatment interval, indicating that natalizumab drug metabolism is highly variable between patients but is usually stable within one patient.(12) Therefore, a fixed infusion interval for all patients is likely suboptimal. In a previous study, we applied personalized EID by therapeutic drug monitoring (target 10 µg/mL) and showed preserved maximal efficacy (interval 5-7 weeks).(13)

The objectives of this study (NEXT-MS trial) were to evaluate the efficacy of personalized EID by therapeutic drug monitoring in a larger than previously studied cohort.(13) A lower target trough concentration (5 µg/mL) was investigated in a subgroup. Results of the first phase of the NEXT-MS trial are presented here, as all participants will be asked to participate in the second phase of the study with an amended study protocol.

METHODS

Study design

The NEXT-MS trial is an investigator-initiated prospective phase IV nonrandomized study containing three study groups: personalized EID with a target drug trough concentration of 10 µg/mL (EID10), an exploratory subgroup with a target drug trough concentration of 5 µg/mL (EID5), and SID of 4 weeks. Planned follow-up (FU) was 104 weeks for the EID10 and SID group with an extension phase of 104 weeks, and 52 weeks in the EID5 group with an extension phase of 52 weeks. A nonrandomized study design was chosen as we wanted to offer as many patients as possible EID by therapeutic drug monitoring. We hypothesized EID10 to be non-inferior to standard dosing but safer in terms of PML risk. Furthermore, the efficacy of natalizumab is widely known as it has been extensively studied in RCTs and real world cohorts.(1) In addition, we compared the EID10 group to a historical SID cohort (HSID) containing prospective longitudinal data.(14) An independent data safety monitoring board was appointed for a six-monthly evaluation. Changes to the study protocol are summarized in table S1 (supplemental material).

Participants

Participants were included in 21 hospitals specialized in MS in the Netherlands (table S2, supplemental material). Inclusion criteria were as follows: adults with a diagnosis of RRMS according to the 2017 McDonald criteria who had thus far received six or more consecutive natalizumab infusions.(15) Additional inclusion criteria for the EID5 group were no radiological

and/or clinical disease activity in the past 52 weeks before inclusion, as an extra precaution in this subgroup with a lower target trough concentration.(13) Patients were excluded in case of high (>100 AU/mL) natalizumab antidrug antibodies (ADAs) or any contraindication for magnetic resonance imaging (MRI).(16)

Study procedures

Natalizumab concentration was measured at baseline prior to two consecutive natalizumab infusions. Treatment intervals were based on baseline drug trough concentrations (figure 1). During the study, treatment intervals were adjusted based on FU trough drug concentrations. Natalizumab (300 mg) was administered following standard local protocols.

Participants received a brain MRI scan and clinical assessment every 26 weeks (EID5 group) or 52 weeks (EID10 group and SID group), including Expanded Disability Status Scale (EDSS) scores (evaluated by a neurologist or subinvestigator, physical or by telephone). The MRI protocol consisted of 3D fluid-attenuated inversion recovery and axial PD/T2-weighted sequences without gadolinium following international guidelines.(17) Local radiologists of the participating centers evaluated the scans during the study. Relapses were defined as new neurological symptoms lasting more than 24 hours that were evaluated by a neurologist and were not attributable to other factors than MS.(15)

Blood samples were analyzed for serum natalizumab drug concentrations and ADAs at Sanquin Diagnostic Services, Biologics Laboratory in Amsterdam. A cross-linking assay using polyclonal rabbit anti-natalizumab fragments and mouse anti-IgG4 monoclonal antibodies for detection were used.(6, 16) Serum neurofilament light (sNfL) levels were measured in available blood samples at baseline, year 1, and last available follow-up in one batch after the first phase of the study as a biomarker for neuro-axonal damage and MS disease activity (Simoa® Neurology 4-Plex E Advantage Kit).(18)

Study outcomes

The primary outcome was radiological activity (new or newly enlarging T2 lesions) comparing the EID10 group to the HSID cohort. Secondary outcomes included radiological disease activity in the EID5 and SID group, incidence rate of relapses, EDSS progression, John-Cunningham (JCV) conversion rate, course of JCV index, incidence of PML, and sNfL levels and treatment intervals in the extended groups. Data of participants switching to subcutaneous natalizumab were reported elsewhere.(19)

Sample size calculation and statistical analyses

A power calculation for the EID10 group was performed using a one-side one proportion exact test assuming that 5% of the HSID cohort would develop radiological disease activity during two years of natalizumab treatment.(14) With a non-inferiority margin of 5%, 184 participants needed to be included (power 80%). To account for 10% missing data, a minimum of 205 participants in the personalized EID group of 10 µg/mL was warranted. Inclusion of additional participants was approved by the medical ethics committee. The EID5 group was included as an explorative group to study a lower target trough concentration.

For the primary outcome, radiological disease activity in the personalized EID10 group versus the HSID cohort are expressed as incidence rates of new or newly enlarged T2 lesions on MRI to account for differences in FU duration. Confidence intervals (CI) for the incidence rate and

their difference between the EID10 group and HSID cohort (90% CI) are calculated. The non-inferiority margin of 5% in the sample size calculation was transformed to 27.2 per 1000 person-years for the incidence rate difference. For the secondary outcomes, incidence rates of radiological disease activity in the EID5 group and SID group are calculated, as well as incidence rates of relapses in all study groups. Mean change (95% CI) in EDSS scores between baseline and FU within study groups are calculated. When the 95% CI of the mean change for each study group is between -0.5 and 0.5, the change in EDSS score within a group is evaluated as stable over time. Mean change in EDSS score between study groups are compared with an independent samples T-test. Incidence rates of JCV conversion in JCV-negative participants and incidence rate differences (90% CI) between study groups are calculated to study superiority of EID on JCV conversion rate. Mean change in JCV index in JCV-positive participants between study groups are compared with an independent samples T-test. Changes in JCV index over time within study groups are tested with a paired-samples T-test. The course of sNfL levels (Ln-transformed) over time in the EID groups are analyzed with linear mixed effect models with fixed effect for time and random effect for subjects. Sex, age, body mass index (BMI), and treatment duration at baseline are explored as effect modifiers by including each as fixed effect as well as the interaction with time. Data are stratified in case of a significant association. Sex, age, BMI, and treatment duration at baseline are explored as (random) confounders as well. For all secondary outcomes except JCV conversion rate, a *p*-value <0.05 based on two-tailed statistical tests is considered statistically significant. Statistical analyses were conducted with SPSS statistic software version 28.0 (IBM, Armonk, NY). Figures were designed in GraphPad Prism version 9.3.1 for Windows (GraphPad software, San Diego, California USA).

Ethics approval, registrations, and patient consent

The study protocol was approved by the medical ethics committee (VUMC Ethics committee number 2019.552). Oral and written informed consent were obtained from all participants. The NEXT-MS trial was registered at ClinicalTrials.gov (identifier NCT04225312).

Data availability statement

Anonymized data will be shared upon reasonable request from any qualified investigator.

RESULTS

Participants

A total of 381 participants were included, and 376 participants started with the study (figure 2). Participants in the EID5 group had a longer pre-study treatment duration with natalizumab, longer duration of radiological and clinical stability, and were positive for JCV more often than participants in the EID10 and SID group (table 1). The most frequent reason for discontinuation of the study was JCV seroconversion (8.5%). Six participants of the EID groups (1.9%) dropped out due to wearing-off symptoms.(20) Median FU of all study groups at last FU was 86.9 weeks (IQR 51.9 to 105.0 weeks).

Table 1. Baseline characteristics

	EID10 (n=251)	**EID5** (n=65)	**SID** (n=60)	**Total** (n=376)	**Historical SID cohort**[e] (n=87)
Age, y	40.0 ± 10.7	41.6 ± 11.6	42.3 ± 10.3	40.7 ± 10.8	35.6 ± 9.0
Sex, female	204 (81.3)	48 (73.8)	46 (76.7)	298 (79.3)	58 (66.7)
Body weight, kg	75.9 ± 15.7	76.3 ± 16.8	74.9 ± 14.2	75.8 ± 15.6	NA
BMI, m^2/kg	25.3 ± 5.1	25.3 ± 5.2	25.4 ± 4.4	25.3 ± 5.0	NA
Time since diagnosis, y	8.5 (4.6-14.8)	11.4 (7.3-18.1)	11.4 (6.5-15.5)	9.5 (5.4 to 15.5)	6.2 (3.3-9.7)
Duration of previous NTZ treatment, y	3.2 (1.1-6.8)	6.7 (2.2-9.8)	5.1 (1.8-9.6)	4.0 (1.5-8.1)	1.0 (0.93-1.1)
Duration of radiological stability, y[a]	2.7 (0.9-5.8)	5.3 (2.3-9.0)	4.3 (1.8-7.5)	3.4 (1.3-7.3)	0.99 (0.92-1.1)
Duration of clinical stability, y[b]	3.5 (1.7-6.5)	6.9 (3.1-9.8)	5.4 (2.0-8.6)	3.9 (2.0-8.0)	0.99 (0.92-1.1)
EID before start study[c], n	14 (5.6)	26 (40.0)	0 (0)	40 (10.6)	0 (0)
Natalizumab drug trough concentration, µg/mL	23.5 (16.0-34.5)	15.5 (12.0-26.3)	21.5 (15.3-32.8)	22.5 (14.0-33.0)	NA
Serum neurofilament light level, pg/mL	9.8 (7.2-12.7)	9.2 (6.7-15.3)	10.4 (7.8-13.5)	9.8 (7.2-13.1)	NA
EDSS score[d]	2.5 (2.0-4.0)	3.5 (2.0-4.8)	3.3 (2.0-5.9)	3.0 (2.0-4.0)	3.5 (2.5-4.8)
JCV positive status, n	28 (11.2)	19 (29.2)	10 (16.7)	57 (15.2)	NA
JCV index in JCV positive participants	0.43 (0.32-0.76)	1.3 (0.6-3.2)	0.46 (0.3-1.4)	0.56 (0.36-1.4)	NA
NTZ ADAs, detectable, n	6 (2.4)	0 (0)	1 (1.7)	7 (1.9)	NA
Median level detectable NTZ ADAs in positive patients, AU/mL	30.5 (20.0-50.5)	-	23.0	28.0 (20.0-50.0)	NA

[a]Time in years between baseline and last brain MRI scan with new or newly enlarging T2 lesions and/or gadolinium-enhancing lesions.

[b]Time in years between baseline and last relapse.

[c]At baseline, 5.6% (EID10) and 40.0% (EID5) of participants had a treatment interval >4 weeks before start of this study.

[d]EDSS scores were evaluated by the treating neurologist or delegated sub-investigator.

[e]Baseline was one year after start natalizumab; duration of radiological/clinical stability were calculated between start of natalizumab and one year FU.

Values are presented as mean ± SD, median (Interquartile range) or frequency (%). y=years; n=count; NTZ = natalizumab; EID = extended interval dosing; EID10 = target trough concentration of 10 µg/mL; EID5 = target trough concentration of 5 µg/mL SID = standard interval dosing; EDSS = Expanded Disability Status Scale; ADAs = antidrug antibodies; JCV = John-Cunningham virus; NA = not available.

Personalized treatment intervals and natalizumab trough concentrations

Of the 381 participants that were included, 84% (n=321) chose personalized EID. In the EID10 group (n=251), the median treatment interval at last FU was 5 weeks (IQR 5 to 6 weeks, figure 3A). Treatment intervals ranged from 4 to 8 weeks to reach the target trough concentration of 10 µg/mL (figure 3B). In the EID5 group, (n=65), the median treatment interval was 6 weeks (IQR 5 to 7 weeks, figure 3A). Treatment intervals ranged from 4 to 9 weeks to reach the target trough concentration of 5 µg/mL (figure 3B). In the SID group (n=60), baseline median natalizumab trough drug concentration was 21.0 µg/mL (IQR 15.3 to 32.8 µg/mL). Natalizumab drug concentrations decreased in most participants after switching to subcutaneous administration (n=15) as was reported elsewhere.(19)

Radiological and clinical disease activity

Participants with at least one available FU scan were evaluated for radiological disease activity (n=283, figure 4). Of the 235 participants in the EID groups (median radiological FU 61.6 weeks, IQR 43.0 to 95.3 weeks), radiological disease activity was observed in three participants (1.3%). Radiological disease activity in the EID10 group (n=171) was non-inferior to the HSID cohort (n=87, incidence rate difference 14.7 per 1000 person-years, 90% CI -4.5 to 34.0). In the EID10 group (median radiological FU 102.0 weeks, IQR 52.0 to 105.8 weeks), radiological disease activity was observed in two participants (incidence rate 10.0 per 1000 person-years, 95% CI 2.5 to 39.9). In the original data of the HSID natalizumab cohort (median radiological FU 157.3 weeks, IQR 108.3 to 264.1 weeks), radiological disease activity on MRI was observed in seven participants (incidence rate 24.7 per 1000 person-years, 95% CI 11.8 to 59.9).

Radiological disease activity in the EID5 group (n=64) was non-inferior to the HSID cohort (incidence rate difference 14.7 per 1000 person-years, 90% CI -7.9 to 37.3). In the EID5 group (median radiological FU 64.9 weeks, IQR 45.0 to 100.7 weeks), radiological disease activity was observed in one participant (incidence rate 10.0 per 1000 person-years, 95% CI 1.4 to 71.2). In the SID group (n=48, median radiological FU 64.9 weeks, IQR 45.0 to 100.7 weeks), radiological disease activity was observed in three participants (incidence rate 47.0 per 1000 person-years, 95% CI 15.1 to 145.6). There was no evidence for differences in radiological disease activity between all study groups (figure 4).

Of the 316 participants in the EID groups (median clinical FU 83.9 weeks, IQR 51.6 to 104.1 weeks), clinical disease activity was observed in one participant (0.3%). Incidence rate of relapses in the EID10 group (n=251) was lower compared to the HSID cohort (n=87, incidence rate difference 26.0 per 1000 person-years, 90% CI 9.6 to 42.3). In the EID10 group, one relapse occurred in one participant (5-week interval) after 27.3 weeks without corresponding radiological activity (incidence rate 2.7 per 1000 person-years, 95% CI 0.4 to 19.2). In the HSID cohort, nine participants experienced a relapse during FU (incidence rate 28.7 per 1000 person-years, 95% CI 14.9-55.1).

Incidence rate of relapses in the EID5 group (n=65) was lower compared to the HSID cohort (incidence rate difference 28.7 per 1000 person-years, 90% CI 13.0 to 44.4). No relapses occurred in the EID5 and SID group.

EDSS progression

Changes in EDSS scores between baseline and last available FU within the study groups and HSID cohort were evaluated as stable over time, as the mean change and 95% CI of each group was between -0.5 and 0.5 (figure 5). Mean change in EDSS scores in the EID10 group

was 0.03 (95% CI -0.06 to 0.11). In this group, fifteen participants (9.0%) had EDSS progression and eleven participants (6.6%) EDSS improvement.
Mean change in the EID5 group was 0.05 (95% CI -0.07 to 0.16). In this group, five participants had EDSS progression (7.9%) and five participants EDSS improvement (7.9%).
Mean change in the SID group was -0.02 (95% CI -0.14 to 0.10). In this group, two participants had EDSS progression (4.2%) and four participants had EDSS improvement (8.3%).
Mean change in the HSID cohort in EDSS score between year 2 and year 1 after start of natalizumab was 0.12 (95% CI -0.04 to 0.28). In this group, fifteen participants (19.5%) had EDSS progression and six participants (7.8%) EDSS improvement.
Mean difference in EDSS scores between the EID10 group and HSID cohort (mean difference -0.09, 95% CI -0.27 to 0.09, *p*=0.32), and the EID5 group and HSID cohort (mean difference -0.07, 95% CI -0.27 to 0.13, *p*=0.49) were comparable.

Serum neurofilament light levels over time
sNfL levels in the EID10 group were stable over time (figure 6). The interaction term with treatment duration was significant (*p*=0.012), and further analyses with a median split for treatment duration at baseline showed lower sNfL levels at year 1 compared to baseline in participants with a shorter treatment duration (year 1 Exp(estimate) 0.91 (95% CI 0.86 to 0.96), *p*=0.001; last follow-up Exp(estimate) 0.95 (95% CI 0.88 to 0.103), *p*=0.18). There were no other relevant effect modifiers or confounders. sNfL levels in the EID5 group significantly decreased over time compared to baseline, although changes were marginal (year 1 Exp(estimate) 0.94 (95% CI 0.89 to 0.996), *p*=0.035; last follow-up Exp(estimate) 0.93 (95% CI 0.88 to 0.99), *p*=0.032). There were no relevant effect modifiers or confounders. Changes in sNfL levels over time were comparable between the EID10 and EID5 group (figure 6).

JC virus antibodies and PML
JCV conversion rate within the first year was 7.3% in the EID10 group, 7.0% in the EID5 group and 12.1% in the SID group. However, we found no evidence in our study for a statistical difference in JCV conversion (figure 7). The incidence rate of JCV conversion was 78.6 per 1000 person-years (95% CI 53.1 to 116.3) in the EID10 group (n=218), 38.9 per 1000 person-years (95% CI 12.5 to 120.5) in the EID5 group (n=46), and 95.6 per 1000 person-years (95% CI 47.8 to 191.2) in the SID group (n=50). There was no evidence for superiority of JCV conversion rate in the EID10 group compared to the SID group (incidence rate difference 17.1 per 1000 person-years, 90% CI -44.3 to 78.4), the EID5 group compared to the SID group (incidence rate difference 56.7 per 1000 person-years, 90% CI -10.0 to 123.5), and the EID5 group compared to the EID10 group (incidence rate difference 39.7 per 1000 person-years, 90% CI -5.4 to 84.7, figure 7).
There was no evidence for changes between baseline and last available FU JCV index in JCV-positive participants (n=50) within groups (JCV index mean change EID10 (n=23) 0.017, 95% CI -0.07 to 0.10, *p*=0.68; EID5 (n=19) 0.06, 95% CI -0.18 to 0.31, *p*=0.60; SID (n=8) 0.32, 95% CI -0.07 to 0.71, *p*=0.097). There was a trend towards a smaller change in JCV index during the study in the EID5 compared to the SID group (mean difference -0.38, 95% CI -0.81 to 0.05, *p*=0.080). We found no evidence for a difference in JCV index between the EID10 and SID group (mean difference -0.30, 95% CI -0.69 to -0.09, *p*=0.12), and EID10 and EID5 group (mean difference 0.08, 95% CI -0.17 to 0.33, *p*=0.52). There were no cases of PML.

DISCUSSION

The first results of our investigator-initiated nonrandomized study showed that a large majority of participants (82%) were able to extend natalizumab treatment intervals with personalized EID by therapeutic drug monitoring. We found that radiological disease activity during personalized EID was non-inferior to a historical cohort of SID. Radiological disease activity was low in all study groups. sNfL levels did not increase over time, supporting effective control of MS disease activity with personalized EID.(21, 22) Furthermore, clinical disease activity was lower in the EID groups, and we found no evidence for differences in mean changes in EDSS scores over time between the study groups.

EID of natalizumab with various treatment intervals (range 4-8 weeks) was mostly studied in retrospective studies and did not cause increased MS disease activity compared to SID.(23-30) A recent randomized controlled study (NOVA trial) reported that natalizumab administered every 6 weeks provides a high level of efficacy.(10) Another study randomized patients treated with natalizumab between 4 and 12 weeks, and clearly showed that EID until 12 weeks induces recurrence of disease activity in at least some patients.(9) Thus, the optimal treatment interval probably lies somewhere between 6-12 weeks, although a small percentage might still have adequate MS disease control >12 weeks. Natalizumab trough concentrations on SID range from 0.1-110 µg/mL, and decrease with EID (1-11 µg/mL on a 6 weeks interval).(2, 8, 31) In our study, median natalizumab trough concentrations roughly fell to 5 µg/mL when extending intervals to 6 weeks, although higher concentrations were also prevalent in 6 weeks intervals.(6, 7) As PML can occur in JCV positive patients during extended treatment with 6 week intervals,(10, 32) personalized EID based on individual natalizumab metabolism is likely superior to fixed EID as it warrants natalizumab concentrations within the therapeutic range, but also induces some receptor desaturation to increase immune surveillance in the CNS, potentially lowering PML risk.(4, 11) Treatment intervals were extended >6 weeks in 7% (EID10) and 32% (EID5) of participants, thereby further reducing hospital visits and healthcare costs (Dutch medication costs of one natalizumab 300 mg dose lie around €1400,- and measurement of natalizumab concentration is less than €100,-).(33) Based on these data and following the recommendation of the NEXT-MS steering committee, participants of the NEXT-MS trial will continue with an amended study protocol of personalized EID starting from 6 weeks, with further interval extension to a target trough concentration of 5 µg/mL. EID of subcutaneous natalizumab will also be studied in the NEXT-MS trial and extension phase of the NOVA-trial (NCT03689972).

In our study, we found no evidence for a difference in JCV conversion in the personalized EID groups compared to the SID group. However, JCV conversion rate within the first year was 7% in the EID groups, and 12% in the SID group. Furthermore, there was a trend towards a smaller change in JCV index in the EID5 group compared to the SID group. JCV seroconversion is higher in natalizumab treated patients than in the general MS population, which could be related to a possible decrease in lymphocyte trafficking in the gut.(34, 35) Possibly, JCV seroconversion could be decreased by extending natalizumab intervals by increasing immune surveillance in the gut. Additional data on JCV seroconversion and indices during EID would be of great interest to strengthen the benefits of EID. In our study, there were no cases of PML. The number of included patients limits interpreting these results due to the low overall incidence of PML.

Limitations of this study include the nonrandomized design, introducing a selection bias in our study groups. However, we wanted to offer personalized EID to as many patients as possible. With therapeutic drug monitoring, we ensured adequate natalizumab concentrations. We also compared the EID10 group to a historical SID cohort, containing prospectively collected longitudinal data of patients treated with SID.(14) Furthermore, due to more strict inclusion criteria, disease characteristics of the exploratory EID5 group were different from the EID10 and SID group with respect to treatment duration and disease activity. Patients who recently started therapy with natalizumab or with recent MS disease activity might benefit from close monitoring after initiation of EID. Median radiological FU in the EID10 group was 57 weeks. Although this is shorter than the initially planned FU of the study, disease activity measured with MRI was low in all study groups with a comparable FU to previous trials.(10, 13) In addition, based on previous studies on MS disease activity after discontinuation of natalizumab or interval extension to 12 weeks, MS disease activity would be expected in the first months if treatment with EID of natalizumab was not sufficient.(9, 36) Timing of the annual scan was based on the most recent scan at baseline and was therefore not always performed 52 weeks after start of EID. We therefore evaluated sNfL levels during EID to detect additional evidence of neuroinflammation and found that sNfL levels did not increase over time, supporting effective control of MS disease activity with personalized EID. Strengths include the prospective study design and relatively large study group on personalized EID. Most participants were able to extend treatment intervals without return of significant disease activity, even with treatment intervals up to 7-9 weeks. Furthermore, this study was an investigator-initiated trial performed independently from the pharmaceutical industry. The large number of included participants (n=381) in 21 hospitals demonstrates implementation of personalized EID in clinical practice is feasible.

In conclusion, personalized EID of natalizumab by therapeutic drug monitoring is a promising approach to optimize treatment intervals, thereby lowering treatment burden and healthcare costs, and potentially further reducing treatment risks. A 6 week interval with personalized interval extension >6 weeks (target 5 µg/mL) will be further studied with an amended study protocol in the NEXT-MS trial.

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Data sharing statement
Anonymized data will be shared upon reasonable request from any qualified investigator.

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Figure 1. Personalized EID treatment interval algorithm.

In the EID10 group, drug trough concentrations were measured at baseline and after 12 weeks (range 12-16 weeks), 24 weeks (range 24-28 weeks), and every 24 weeks thereafter (range 24-28 weeks depending on the infusion interval). Treatment intervals during blood sample FU were adjusted when the trough concentration exceeded 20 µg/mL (plus one week), or was below 2 µg/mL (minus one week). In this group, clinical FU was every 52 weeks. In the EID5 group, trough concentrations were measured at baseline and before every natalizumab treatment in the first 52 weeks after start of personalized EID, and approximately every 12 weeks thereafter (range 9-16 weeks depending on the infusion interval). Treatment intervals during blood sample FU were adjusted when the trough concentration exceeded 10 µg/mL (plus one week), or was below 2 µg/mL (minus one week). In this group, clinical FU was every 26 weeks. For participants in the SID group, blood was collected once to measure natalizumab trough concentration and ADAs. In this group, clinical FU was every 52 weeks. Participants were allowed to choose one of the study groups when they were eligible for inclusion. EID = extended interval dosing; SID = standard interval dosing; NTZc = natalizumab concentration (serum trough concentration in µg/mL); ADA = antidrug antibodies; NA = not applicable.

Figure 2. Flowchart of the inclusion process.

Inclusion of participants started in February 2020. Median FU was calculated until dropout, end of study, or last FU (data extraction on 13 December 2022). Median FU in the historical SID cohort was 187.9 weeks (IQR 129.0 to 290.4 weeks). SID = standard interval dosing; EID = extended interval dosing; NTZ = natalizumab; FU = follow-up; JCV = John-Cunningham Virus.

Figure 3. Natalizumab treatment intervals and concentrations in the personalized EID groups.

A. Percentage of participants in each study group (EID10 depicted in red; EID5 depicted in blue) are displayed on the x-axis. The y-axis indicates natalizumab treatment intervals at last available FU. Of the 316 participants who started EID, 82.3% of participants were able to extend natalizumab treatment intervals (EID10 78.5%, EID5 96.9%).
In the EID10 group, treatment intervals were ≥6 weeks in 33.5% of participants, and >6 weeks in 7.2%. Treatment intervals were adjusted in 19 participants (7.6%) after the start of EID during the study (per protocol: extended n=13, shortened n=1; patient preference: extended n=1; shortened n=4). In the EID5 group, treatment intervals were ≥6 weeks in 72.3% of participants, and >6 weeks in 32.3%. Treatment intervals were adjusted in 33 participants (50.8%) after the start of EID during the study (per protocol: extended n=24, shortened n=7; patient preference: extended n=1, 1.5%; shortened n=1, 1.5%).
B. Number of infusions after start of EID are displayed on the x-axis. The y-axis indicates natalizumab drug trough concentrations in µg/mL. Values are presented as median with ranges (min-max).
In the EID10 group, median natalizumab trough concentration was 11.0 µg/mL (IQR 8.9 to 14.0 µg/mL) after 52 weeks FU (7-13 infusions) and 11.0 µg/mL (IQR 9.2 to 14.0 µg/mL) after 104 weeks FU (13-26 infusions).
In the EID5 group, median natalizumab trough concentration after 10 infusions (40-90 weeks) was 4.9 µg/mL (IQR 3.5 to 6.6 µg/mL). NTZ = natalizumab; EID = extended interval dosing.

Figure 4. Time to new MRI disease activity per study group.

Proportions of participants with an available FU scan with no new disease activity using the Kaplan-Meier method. Study groups are displayed separately. Participants were indicated as censored when the last available scan during FU was performed or when radiological disease activity occurred (n=13). Twelve participants (3.2% of n=376) included during the COVID-19 pandemic had no available re-baseline scan after start of natalizumab and were excluded in analyses for radiological disease activity (EID10 n=10; SID n=2). Eighty-one participants (22.3%) did not receive a follow-up scan (n=26 (32.1%) discontinued the study before the FU scan was performed; n=55 (67.9%) did not receive a FU scan yet). In the EID10 group, two small new T2 lesions were observed in one participant on a 5 weeks interval after 38.6 weeks, with a stable scan after 95.6 weeks, and one expanded periventricular T2 lesion was observed in one participant on a 6 weeks interval after 77.1 weeks. In the EID5 group, one new subcortical T2 lesion was observed in one participant in the left cerebellar lobe after 54 weeks, with a stable scan after 64.9 weeks (8 weeks interval). In the SID group, two small new frontal T2 lesions were observed in one participant after 31.9 weeks, with a stable scan after 82.7 weeks, one new T2 lesion in the medulla oblongata was observed in one participant after 26.9 weeks, with a stable scan after 101 weeks, and one new T2 lesion in the pons was observed in one participant after 40 weeks.
There was no evidence for differences in radiological disease activity between the EID10 and EID5 group (incidence rate difference 0.043 per 1000 person-years, 90% CI -20.1 to 20.2), and EID10 and SID group (incidence rate difference 37.0 per 1000 person-years, 90% CI -9.1 to 83.1). There was no evidence for differences in radiological disease activity between the EID5 and SID group (incidence rate difference 36.9 per 1000 person-years, 90% CI -10.6 to 84.5). There was no evidence for differences in radiological disease activity between the SID group and the HSID cohort (incidence rate difference 22.2 per 1000 person-years, 90% CI -25.0 to 69.4). EID = extended interval dosing; SID = standard interval dosing; HSID = historical SID cohort.

Figure 5. EDSS scores in the study groups and HSID cohort over time.

Boxplot of baseline (blue) and FU (orange) EDSS scores depicted as median with interquartile ranges. Participants with available baseline and follow-up EDSS scores were included (n=356). Study groups (SID n=48, EID10 n=168, EID5 n=63, HSID n=77) are displayed separately. Disability progression was defined as a ≥1.5-point increase if baseline EDSS was 0, a ≥1-point increase if baseline EDSS was 1.0-5.0, and a ≥0.5-point increase if baseline score was ≥5.5.(37)
Mean difference in EDSS scores between the EID10 and EID5 group (mean difference -0.02, 95% CI -0.17 to 0.13, *p*=0.79), and EID10 and SID group (mean difference 0.05, 95% CI -0.12 to 0.22, *p*=0.58) were comparable. Mean difference in EDSS scores between the EID5 and SID group was comparable (mean difference 0.07, 95% CI -0.10 to 0.23, *p*=0.42). Mean difference in EDSS scores between the SID group and HSID cohort was comparable (mean change -0.14, 95% CI -0.33 to 0.06, *p*=0.17).
FU = follow-up; EDSS = Expanded Disability Status Scale; SID = standard interval dosing; EID = extended interval dosing; HSID = historical cohort of SID.

Figure 6. Serum neurofilament light levels in the EID10 and EID5 group over time.

Boxplot of sNfL levels in the EID10 (red) and EID5 (blue) group over time depicted as median with interquartile ranges.
EID10 group: baseline (n=232) median sNfL level 9.8 pg/mL (IQR 7.2 to 12.7 pg/mL), year 1 (n=229) median interval since baseline 48.1 weeks (IQR 29.6 to 54.0 weeks) and median sNfL level 9.2 pg/mL (6.9 to 12.5 pg/mL), last follow-up (n=116) median interval since baseline 80.9 weeks (74.5 to 103.3 weeks) and median sNfL level 9.7 pg/mL (7.4 to 12.2 pg/mL).
EID5 group: baseline (n=65) median sNfL level 9.2 pg/mL (IQR 6.7 to 15.3 pg/mL), year 1 (n=64) median interval since baseline 51.3 weeks (IQR 49.1-53.6 weeks) and median sNfL level 9.7 (6.6 to 14.9 pg/mL), last follow-up (n=49) median interval since baseline 102.3 weeks (IQR 91.6 to 105.4 weeks) and median sNfL level 9.3 pg/mL (IQR 6.4 to 14.3).
Linear mixed effect models analyses showed changes in sNfL levels were comparable between the EID10 and EID5 group (Exp(estimate) 1.05 (95% CI 0.92 to 1.20), p=0.45), and stable over time as the interaction term between group and time was not significant (p=0.76). sNfL = serum neurofilament light; EID = extended interval dosing; FU = follow-up.

Figure 7. Time to JCV conversion per study group.

Proportions of JCV-negative participants with no JCV conversion during the study until conversion or last available FU using the Kaplan-Meier method. Study groups are displayed separately. Participants with at least one available FU JCV status are displayed. Participants were indicated as censored when JCV conversion occurred or at last available FU. Thirty-six participants (11.5%) had persistent JCV conversion from negative to positive during the study (EID10 11.5% (n=25), EID5 6.5% (n=3), SID 16% (n=8)). There was no evidence in our study for a statistical difference in JCV conversion between the study groups (log-rank test p=0.38).
FU = follow-up; SID = standard interval dosing; EID = extended interval dosing; JCV = John-Cunningham Virus.