Published Ahead of Print on July 20, 2020 as 10.1212/WNL.0000000000009995

Neurology®

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The Official Journal of the American Academy of Neurology

AMERICAN ACADEMY OF NEUROLOGY®

Neurology Publish Ahead of Print
DOI: 10.1212/WNL.0000000000009995

Personalized extended interval dosing of natalizumab in MS - a prospective multicenter trial

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Titel character count: 92
Number of references: 28
Number of tables: 2
Number of figures: 4

Word count abstract: 250
Word count paper: 3724

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Statistical analysis conducted by: Z. van Kempen, J. Killestein and B. Lissenberg-Witte at the Amsterdam UMC, The Netherlands.

Search terms: multiple sclerosis, natalizumab, extended interval dosing

Acknowledgements

We want to thank all patients included in this study for their participation.
Rogier Hintzen from the Erasmus MC helped design the study and reviewed the study protocol, he sadly passed away before the end of the study. We want to thank Henry Weinstein, Brechtje Jelles and Geert Lycklama a Nijeholt for the evaluation of the study as part of the DSMB. We gratefully acknowledge the help of Djoeke Doesburg, Iris Dekker, Danco Coric, Ka-Hoo Lam, Cyra Leurs, Martijn Wijburg and Marloes Hagens in the acquisition of the data. We want to thank Gianina Eissens, Michelle Simons and Sanne Gouderjaan for their support in this study as clinical research nurses. We express our gratitude to Asma Kalei, Kim van Houten and Iris Claessen in their continuous support surrounding the blood assays performed at Sanquin Laboratory.

At last, we want to thank the Brain Foundation Netherlands for the complete funding of this study.

Study Funding

This study was fully funded by the Brain Foundation Netherlands (HA2015.01.05). This sponsor had no role in the study design, data collection, analyses or interpretation of data, writing the report or decision to submit the paper for publication. The corresponding author has full access to all the data in the study and has the final responsibility on the decision to submit for publication.

Disclosures

Z. van Kempen and E. Hoogervost report no disclosures relevant to the manuscript.

M. Wattjes has received consultancy or speaking fees from Biogen, Novartis, Roche, Celgene, IXICO, Sanofi Genzyme, Bayer Healthcare, Biologix, Genilac and Merck Serono.

N. Kalkers received consulting fees from Sanofi-Genzyme, Roche, Teva, Novartis and Biogen and participated in medication trials from: Biogen, Sanofi-Genzyme, Merck.

J. Mostert, B. Lissenberg-Witte, A. de Vries, A. ten Brinke and B. van Oosten report no disclosures relevant to the manuscript.

F. Barkhof received support by the NIHR UCLH BRC; consultancy fees from Bayer, Roche, Merck, Sanofi-Genzyme, Teva, IXICO, Biogen, GeNeuro, Apitope, and Novartis, either as central reader or by serving on steering committees, advisory boards, and data safety monitoring committees. F. Barkhof is supported by the NIHR biomedical research centre at UCLH

C. Teunissen has functioned in advisory boards of Roche, received non-financial support in the form of research consumables from ADxNeurosciences and Euroimmun, performed contract research or received grants from Probiodrug, Biogen, Esai, Toyama, Janssen prevention center, Boehringer, AxonNeurosciences, EIP farma, PeopleBio, Roche.

B. Uitdehaag has received consultancy fees and/or research support from Biogen Idec, Sanofi Genzyme, Merck Serono, Novartis, Roche and Teva.

T. Rispens has received speaking fees from Pfizer and AbbVie.

J. Killestein has received speaker and consulting fees from Merck-Serono, Biogen Idec, TEVA, Genzyme, Roche and Novartis.

Abstract

Objective: We aimed to determine whether natalizumab efficacy is maintained when switching to personalized extended interval dosing based on individual natalizumab trough concentrations in relapsing remitting MS patients.

Methods: This was a prospective multicenter single-arm trial with one year follow-up and a one year extension phase. Participants were adult persons with MS treated with natalizumab without disease activity in the year prior to enrollment. The natalizumab treatment interval was based on longitudinal natalizumab trough concentrations. Patients received three monthly MRI scans, relapse assessments and disability scoring during follow-up. The primary endpoint was the occurrence of gadolinium enhancing lesions on MRI. Secondary endpoints were new/enlarging T2 lesions on MRI, relapses and progression on the Expanded Disability Status Scale (EDSS) during follow-up and extension phase.

Results: Sixty-one patients were included. Eighty-four percent extended the interval from a 4-week interval to a 5-7-week interval. No patient developed gadolinium enhancing lesions (95% CI 0 – 7.4%) during follow-up. No new/enlarging T2 lesions (95% CI 0 – 7.4%) or relapses (95% CI 0 – 7.4%) were reported during follow-up and in the extension phase. Median EDSS was comparable at baseline (3.0, IQR 2.0-5.0) and after follow-up (3.0, IQR 2.0-5.0).

Conclusion: Personalized extended interval dosing did not induce recurrence of MS disease activity. Natalizumab efficacy was maintained in stable MS patients receiving personalized extended interval dosing based on individual natalizumab concentrations.

Classification of evidence: This study provides class IV evidence that personalized extended interval dosing of natalizumab does not result in recurrence of disease activity in stable RRMS patients.

Introduction

Natalizumab is effective in treating relapsing remitting multiple sclerosis (RRMS),[1] but is associated with an increased risk of progressive multifocal leukoencephalopathy (PML).[2] The approved treatment regimen of natalizumab consists of a 300mg 4-weekly dose (standard interval dosing). However, in recent years with the aim of reducing the risk of PML, extended interval dosing is gaining ground.[3] Recent retrospectively collected data show a reduction of PML in extended interval dosing in comparison to standard interval dosing.[4] Retrospective studies do not show a decrease of natalizumab efficacy in extended interval dosing,[3, 5, 6] but prospective data are lacking. In light of these findings, the regulatory authorities have requested further data on efficacy and safety of extended interval dosing.[7]

Natalizumab binds to the α4-subunit (CD49d) of the α4ß1-integrin on leukocytes, hereby inhibiting binding of α4ß1-integrin to vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells which mediates migration of leukocytes into the central nervous system (CNS). Receptor desaturation is associated with disease activity[8] and occurs when the natalizumab concentration falls below 1-2.5µg/ml.[9, 10] The median trough concentration after a 4 week interval (>25µg/ml) lies far above the therapeutic threshold, which implies that most patients receive natalizumab while still having high drug concentrations.[11, 12] We hypothesized that extending infusion intervals based on natalizumab concentrations leads to decreased infusions without losing drug efficacy.

The objective of this study was to investigate the efficacy of natalizumab in clinically and radiologically stable RRMS patients after switching to personalized extended interval dosing guided by longitudinal natalizumab trough concentrations.

Methods

Study design

This is a prospective multicenter single-arm trial involving four hospitals in the Netherlands. The study was designed without a control group as all included patients were free of disease activity under

natalizumab at least one year prior to enrollment and minimal disease activity was expected if patients would have continued standard interval dosing. We prospectively collected patient data during one year of follow-up, after which patients entered an extension phase of a second year. Study data was entered in an online database (Castor Electronic Data Capturing) by the local investigator or research nurse. The database was closed when all patients reached the primary endpoint after one year follow-up. Data of patients completing the extension phase up to that time was included for analyses.

Standard Protocol Approvals, Registrations and Patient Consents

This study was conducted in accordance with the Declaration of Helsinki and the International Good Clinical Practice guideline. The study was approved by the ethics committee of the Amsterdam University Medical Centers location VUmc (reference 2016-161) and approval of the study protocol was granted by the ethics committee of each participating center. Written informed consent was obtained from all participants. The study protocol was made public online (ClinicalTrials.gov Identifier: NCT03516526, EudraCT Identifier: 2016-000345-31). For this study an independent data safety monitoring board (DSMB) was installed to ensure patient safety. This board reviewed collected data of the study every six months. An interim analysis was performed after the 15th patient received the 6 month scan. In case of disease activity (clinical and/or radiological) ≥20% of patients, the study would be prematurely terminated.

Participants

The eligibility criteria were age of 18 years or older; a current diagnosis of relapsing remitting multiple sclerosis[13] and a minimum natalizumab treatment duration of 12 months at the time of enrollment.

Exclusion criteria included gadolinium enhancing (Gd+) lesions or active (new/enlarging) T2 lesions on brain MRI in the year prior to enrollment; clinical relapses in the year prior to enrollment; an EDSS score higher than 6,5; extended interval dosing (interval ≥5 weeks) for ≥3 consecutive infusions in the year prior to enrollment; the use of immunomodulatory medication other than natalizumab;

contraindications to undergo frequent MRI and unwillingness or inability to comply with the study protocol.

Procedures

Blood was collected through the intravenous drip prior to each natalizumab infusion during follow-up. All sera were sent to Sanquin Laboratory in Amsterdam, for measurement of the natalizumab concentration using a cross-linking assay using polyclonal rabbit anti-natalizumab F(ab)2 fragments for capture and a mouse anti-IgG4 monoclonal antibody for detection.[14] The interval was prolonged with one week if the natalizumab trough concentration ≥15µg/ml. The aim was a natalizumab trough concentration of approximately 10µg/ml. This aim was based on a pharmacokinetic study conducted by our study group showing a large range of natalizumab trough concentrations in standard interval dosing without increase of disease activity in the patients in the lower range (2-10µg/ml), compared to the higher range (>10µg/ml).[12] If baseline trough concentration was below 15µg/ml, patients were categorized as screen failure and did not receive further follow-up. After the first year, patients remained on their personalized interval with six monthly measurements of natalizumab trough concentration to evaluate stability of trough concentrations during the extension phase.

Clinical information from the period prior to enrollment (date of diagnosis, date of start natalizumab and JCV status) was retrieved from the medical records. At baseline, body weight was assessed. Patients received 12 weekly assessments (including baseline) with a brain MRI scan, a relapse assessment, scoring on the Expanded Disability status scale (EDSS) and adverse events assessment during follow-up. The MRI protocol consisted of a 3D fluid-attenuated inversion recovery (FLAIR), axial PD/T2-weighted sequences and Gd+ enhanced T1-weighted sequences. All MRI acquisition parameters (e.g., pulse sequences, spatial resolution etc.) were according to the MAGNIMS guidelines for MS treatment monitoring.[15] All images were initially read by the local neuroradiologist. Clinical decisions and DSMB assessments during the study were based on these imaging evaluations. At the end of the study one neuroradiologist specialized in neuroinflammatory disorders (MPW) who was masked for the different treatment intervals reread all scans (including the baseline scan) for Gd+

enhancing lesions, active T2 lesions, black holes and PML characteristics. A relapse was defined as a period of new neurological deficit, existing longer than 24 hours and not attributable to another cause than MS. EDSS assessments were scored by certified physicians. Significant EDSS progression was defined as an increase of 1.5 points for a baseline EDSS of 0, an increase of 1 point for baseline EDSS of 1-5.5 and an increase of 0.5 for baseline EDSS >5.5. At baseline and at 12 months, patients were scored on the Multiple Sclerosis Functional Composite (MSFC); consisting of the timed 25-foot walk (T25FW), the 9 hole peg test (9HPT) and the paced auditory serial addition test (PASAT). For the T25FW assessments and 9HPT, each assessment had two trials and the average score was used for statistical analyses. At baseline and 12 months patients completed two questionnaires; the Multiple Sclerosis Impact Scale (MSIS-29) and Short Form Health Survey (SF-36). The scores (ranging 29-145) of the MSIS-29 increase with greater disability. The SF-36 consists of eight subscales (physical function, role limitations due to physical or emotional health problems, social function, bodily pain, vitality, mental health and general health perceptions), items are transformed onto a scale of 0–100, with scores decreasing with greater disability. In the extension phase, an annual brain MRI scan, relapse assessment and EDSS were performed. In all patients, JCV status was measured every six months by the STRATIFY-2 test (Unilabs, Copenhagen, Denmark), as is current protocol. JCV results were communicated by the treating neurologist and in case of JCV positivity a treatment switch was considered.

In all patients included at the Amsterdam UMC, additional blood was drawn for assessment of natalizumab trough α4ß1-integrin receptor saturation and CD49d expression using freshly isolated CD8 T cells. CD49d expression was assessed by flow cytometry and α4ß1-integrin receptor saturation was determined by measuring cell-bound natalizumab on the CD8 effector/effector memory population in isolated PBMC samples, of which part was post-saturated with saturating amounts of natalizumab to create a fully saturated reference sample.[16] Natalizumab binding was expressed as a percentage relative to saturated conditions for each sample. To evaluate the course of serum neurofilament light (NfL) with different treatment schedules, NfL was measured at baseline of the study andat the end of one year of personalized extended interval dosing. NfL concentrations were quantified in serum by the neurochemistry laboratory of the Amsterdam UMC, using an in-house

developed homebrew fourth-generation SiMoA technology as described in detail elsewhere,[17] validated according to standardized international protocols.[18] The assay had a lower limit of quantification of 3.1 pg/mL.

Outcomes

The primary outcome measure was the occurrence of Gd+ lesions on brain MRI during follow-up. Gd+ lesions were chosen as a primary outcome for detection of acute inflammation.[15] Secondary outcome measures included active T2 lesions on brain MRI, clinical relapses, disability progression (EDSS and MSFC) and quality of life (MSIS-29 and SF-36) during follow-up and extension phase. The course of serum NfL, natalizumab α4ß1-integrin saturation and CD49d expression were measured to establish a possible influence of personalized extended interval dosing on these biomarkers.

Sample size calculation and statistical analyses

The sample size calculation was based on the principle that extended intervals should not induce any Gd+ lesions during one year follow-up. In particular, if we were to find that none of subjects included in this study developed Gd+ lesions during follow-up, we would like to be confident that this is due to a very low probability of developing Gd+ lesions rather than the number of subjects included being too low. Sample size was therefore chosen such that the probability of Gd+ lesions being detected in at least one study subject is at least 90% in case the true probability for a subject developing Gd+ lesions during one year follow-up is 5%. Based on a binomial distribution the required number of subjects was calculated to be 45. Mathematically, with n = sample size, x = number of patients with ≥ 1 lesion, π = probability of 'developing' at least one lesion, we want $P(x = 0 \mid \pi \geq 0.05) \leq (1 - 0.05)^n \leq 0.1$. Hence, the sample size satisfies $n \geq \log [0.1] / \log [1 - 0.05]$, i.e. $n \geq 45$. With a sample size of size 45, $P(x = 0 \mid \pi = 0.01) = 0.64$, $P(x = 0 \mid \pi = 0.005) = 0.80$ and $P(x = 0 \mid \pi = 0.001) = 0.96$. As we expected 15% of patients with a low baseline trough natalizumab concentration[12] not able of extending the interval and 10% missing data or dropouts we included 15 more patients than needed based on calculations. Therefore, the sample size was set at 60 patients. Analyses were per-protocol. Occurrence of Gd+ lesions, active T2 lesions and relapses is reported with a 95% Clopper-Pearson confidence

interval (CI). The mean differences in EDSS, T25FW, 9HPT and PASAT between baseline and follow-up are reported with 95% CIs. The association of baseline natalizumab concentration with final treatment interval and body weight with final treatment interval was analyzed using a Spearman correlation coefficient. The association of the baseline natalizumab concentration with body weight was analyzed using a Pearson correlation coefficient. The association of natalizumab receptor saturation and CD49d expression with the treatment interval was investigated with an independent samples T test. The mean difference of NfL is reported with a 95% CI. Mean differences between baseline and 12 months follow-up in the questionnaires are reported with 95% CIs. Categorical data is described by frequencies and percentage, continuous data by mean and standard deviation (SD) if normally distributed or median and interquartile range (IQR) if not normally distributed. SPSS statistics software version 22.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analyses. All reported *p*-values were based on two-tailed statistic tests, with a significance level set at $p<0.05$.

Classification of evidence

Our primary research question was to determine whether personalized extended interval dosing of natalizumab leads to recurrence of radiological disease activity (Gd+ lesions) in stable RRMS patients. This study provides class IV evidence that personalized extended interval dosing based on individual natalizumab trough concentrations with an aim of 10µg/ml does not lead to recurrence of radiological (or clinical) disease activity.

Data Availability

No de-identified patient data will be shared. No related study documents will be shared. Anonymized data will be shared by request from any qualified investigator.

Results

Sixty-one patients were included in this study between November 2015 and June 2018. See figure one for an overview of included patients and follow-up. See table one for baseline patient characteristics.

Radiological and clinical endpoints

No patient in this study developed disease activity during follow-up (95% CI 0 – 7.4, n=48), see table 2. In two patients, both on the first follow-up MRI 12 weeks after inclusion, a new lesion was identified (by MPW) that was not confirmed on an additional sequence. The first lesion was a small right cerebellar lesion seen on T2 sequence, not visible on the PD or FLAIR. The second lesion was a brainstem lesion visible on the FLAIR but not on PD- and T2-weighted sequences. Our neuroradiologists (MPW and FB) did not consider these inconclusive lesions as active T2 lesions. Disability was comparable between baseline and follow-up (table 2). Three patients experienced significant EDSS progression during follow-up.

Of the patients completing the one year extension phase (n=26) none developed Gd+ enhancing lesions, active T2 lesions or experienced a relapse (95% CI: 0 – 13.2). None of the patients who did not complete the follow-up or the extension phase experienced clinical or radiological disease activity while on extended interval dosing. Mean difference of EDSS between baseline and 24 months was -0.1 (95% CI 0.4 – 0.2) points. No patient experienced significant EDSS progression during the extension phase compared to baseline. None of the patients developed clinical or radiological signs suspect of PML during follow-up and extension phase.

Biomarkers

Baseline mean±SD natalizumab concentration of all patients was 25.4±11.5µg/ml. Ten patients (16%) could not extend the treatment interval because of a baseline natalizumab trough concentration <15µg/ml. Median time to final interval was five weeks (IQR 5-11). Baseline natalizumab trough concentration was associated with the final treatment interval (*rho* 0.9, 95% CI 0.7 – 0.9, p<0.001) (Figure 2). One patient (with a baseline trough concentration of 51µg/ml) chose not to extend the interval further at a trough concentration of 17µg/ml. Further extension of the interval to seven weeks might have been feasible in this patient. See figure 3 for the course of natalizumab trough concentrations in the patients with different final treatment intervals.

Body weight was associated with baseline natalizumab trough concentration (*r* -0.26, 95% CI -0.5 – -0.04, p=0.043) as well as with the final treatment interval (*rho* -0.2, 95% CI -0.5 – 0.05, p=0.068).

Intra-individual natalizumab concentration stayed stable after one year of personalized extended interval dosing; median concentration after 12 months was 10µg/ml (IQR 8.5–13.0) (n=48), median difference of the concentration after 18 months was 0.3µg/ml (IQR-2.8–1.6) (n=25) and median difference of the concentration after 24 months in comparison to 12 months was -0,7µg/ml (IQR -3.9–1.7) (n=24). However, there was one outlier. One patient had a baseline natalizumab trough concentration of 33µg/ml and extended the interval to 6 weeks until a trough concentration of 5.6µg/ml at 12 months. After 18 months, natalizumab trough concentration was 0.5µg/ml. The dosing interval was shortened to 4 weeks and natalizumab trough concentration rose to 3.9µg/ml after one 4 week interval. CD49d expression at that time (after one four week interval) was 8.3 MFI compared to 3.5 MFI just after baseline. The patient remained clinically and radiologically stable during the extension phase but dosing was kept on a 4 week interval.

In all patients included at the Amsterdam UMC (n=36) trough α4ß1-integrin receptor saturation on CD8 cells was measured at different time points. α4ß1-integrin saturation was lower in extended intervals compared to standard intervals (mean difference 14.6, 95% CI 9.4 – 19.6, p<0.001) and CD49d expression was comparable in extended intervals and in standard intervals (mean difference -0.2, 95% CI -0.8 – 0.4, p=0.51) (Figure 4).

Longitudinal serum NfL (n=34) did not increase after one year of personalized extended interval dosing, mean difference of NfL at one year follow-up was -0.8 (95% CI -2.2 – 0.6) pg/ml compared to baseline.

Questionnaires

The score of the MSIS-29 and SF-36 questionnaire did not significantly differ after 12 months compared to baseline. The mean difference of the MSIS-29 questionnaire between baseline and 12 months was 0.1 (95% CI -3.1 – 3.4) . The different subscales of the SF-36 did not significantly differ at baseline and after 12 months of extended interval dosing. Mean difference of baseline and after 12 months were 1.0 (95% CI -2.8 – 4.8) for physical function; 4.0 (95% CI -4.3 – 12.3) for role limitations due to physical health; 0.7 (95% CI -10.1 – 11.6) for role limitations due to emotional health; 2.9 (95% CI -2.9 – 8.6) for social function; -1.9 (95% CI -7.6 – 3.9) for bodily pain; 0.2 (95%

CI -3.3 – 3.8) for vitality; 0.6 (95% CI -3.3 – 4.6) for mental health; and 1.8 (95% CI -2.6 – 6.3) for general health.

After one year, all but two patients chose to remain on personalized extended interval dosing. The first patient chose to return to a four week interval because she missed the social contacts with other standard interval patients. Another patient returned to a 4 week interval 15 months after inclusion because of an increase of wearing-off effect (end of cycle recurrence of MS symptoms) without new MRI lesions or increase of EDSS. The patient reported to feel slightly better after returning to the 4-week interval.

Discussion

Our study provides the first prospective data regarding the efficacy of natalizumab in extended interval dosing. Natalizumab retained its efficacy as none of the patients experienced disease activity when extending the interval based on natalizumab trough concentrations.

Several retrospective studies have reported on natalizumab extended interval dosing ranging from four weeks and three days to eight weeks and five days.[3, 5, 6] In these studies, the treatment interval was chosen at the discretion of the treating neurologist and systematic follow-up was lacking. None of these studies used biomarkers to assign the treatment intervals. In all three studies, natalizumab efficacy was maintained in different extended dosing regimens. The main motivator for extended dosing of natalizumab is the reduction of PML risk. The underlying hypothesis is based on a decrease of natalizumab resulting in an equilibrium of increasing immune surveillance in the CNS to maintain JCV suppression but sufficient immune suppression to halt MS disease activity. Excitingly, a study was recently published showing a drastic reduction of PML in extended interval dosing versus standard dosing using data from the TOUCH Prescribing Program.[4] In our study, no patient developed PML. Still, PML has been described in several cases of patients on extended interval dosing and thus the risk of PML is not obliterated by extended intervals.[4, 19] As Scarpazza et al. suggested, the course of PML in extended interval dosing might be less severe than PML in standard intervals of natalizumab.[19] Our previous small retrospective study (n = 5) suggested that the risk of PML is not

related to high natalizumab concentrations as three of five patients had longitudinal pre-PML trough natalizumab levels below 15µg/ml.[20] Therefore, it can be considered that trough concentrations should drop lower than 10µg/ml before being protective of PML.

Natalizumab serum concentration is associated with α4ß1-integrin saturation; when the natalizumab concentration rises from 1-10µg/ml, the α4ß1-integrin saturation increases rapidly from approximately 20 to 80%.[9] However, above a concentration of 10µg/ml the saturation curve flattens and an excess of natalizumab is mainly reflected by a high serum concentration.[9] A 70-80% α4ß1-integrin saturation was assumed to be necessary for optimal drug efficacy but recent studies show a much lower threshold of approximately 20% saturation before the return of significant disease activity.[8, 11] In our study, all patients remained without radiological and clinical disease activity with the aim of 10µg/ml natalizumab trough concentration and in a subgroup tested for α4ß1-integrin saturation, none of the patient dropped below 30% saturation. It is plausible that a lower aim of natalizumab trough concentration (e.g. 5µg/ml) would also be sufficient to suppress disease activity.

Natalizumab decreases the expression of CD49d,[21] which increases again after discontinuation or during extended interval dosing.[11, 22] Increase of expression of CD49d could reflect a lower percentage of α4ß1-integrin saturation levels and lower natalizumab concentration. This phenomenon was seen in the one patient with a low natalizumab trough concentration in the extension phase with a double CD49d expression compared to the start of the study. However, in all remaining patients intra-individual natalizumab trough concentrations were stable during the extension phase suggesting a new formed equilibrium between CD49d expression, receptor saturation and natalizumab concentration in the large majority of patients receiving personalized extended interval dosing.

NfL is released in the cerebrospinal fluid (CSF) after axonal injury in various neurological disorders including MS.[23] There is a strong positive association with NfL in CSF and serum using the Simoa assay and NfL in serum is associated with both MS disease activity and natalizumab associated PML lesion load.[24-26] Our study showed low levels of serum NfL at start of extended dosing and after one

year of personalized extended dosing, which is to be expected as all patients were stable at baseline and did not experience disease activity during follow-up.

Natalizumab treated patients report a sustained improvement of physical and psychological health after natalizumab initiation.[27] In our study, quality of life was comparable between start of extended dosing and after one year of personalized extended dosing. Although the natalizumab wearing-off effect is not associated with natalizumab trough concentrations,[28] one patient reported an increase of wearing-off symptoms after extending the interval to 5 weeks. The large majority stayed on personalized extended interval dosing, indicating their content with the new interval. Besides the benefit of decreased hospital visits and the decreased risk of PML, personalized extended interval dosing could lead to great financial benefits. Taking into account Dutch healthcare costs (‘*Dutch Healthcare Authority’*) for one natalizumab infusion and accessory infusion costs, introducing personalized extended dosing of natalizumab could lead to over one million euros/dollars reduction of annual healthcare costs per 100 treated patients.

This study has possible limitations. We exclusively included patients with stable disease in the year prior of starting the study, which may limit extrapolating the results to the larger patient population. However, most natalizumab patients are stable under natalizumab treatment. We are currently setting up a national observational study to implement personalized extended interval dosing in the Netherlands, funded by both the Brain Foundation Netherlands and the Dutch MS Research Foundation.

In conclusion, this study shows continued optimal efficacy of natalizumab in stable RRMS patients after switching to personalized extended interval dosing based on natalizumab trough concentrations.

Appendix 1: Authors

Name	Location	Contribution
Zoé L.E. van Kempen, MD	Amsterdam University Medical Centers, Vrije Universiteit, Amsterdam, The Netherlands	Conception and design of the study, acquisition of the data, interpretation of data and drafting of the manuscript, statistical analysis, revision of the manuscript critically for intellectual content.
Erwin L.J. Hoogervorst, PhD	St. Antonius Hospital, Utrecht, the Netherlands	Conception and design of the study, acquisition of the data, interpretation of data and drafting of the manuscript, revision of the manuscript critically for intellectual content.
Mike P. Wattjes, PhD	Amsterdam UMC, The Netherlands. Hannover Medical School, Hannover, Germany.	Conception and design of the study, acquisition of the data, interpretation of data and drafting of the manuscript, revision of the manuscript critically for intellectual content.
Nynke F. Kalkers, PhD	OLVG hospital, Amsterdam, The Netherlands	Conception and design of the study, acquisition of the data, interpretation of data and drafting of the manuscript, revision of the manuscript critically for intellectual content.
Jop P. Mostert, PhD	Rijnstate Hospital, Arnhem, the Netherlands	Conception and design of the study, acquisition of the data, interpretation of data and drafting of the manuscript, revision of the manuscript critically for intellectual content.
Birgit I. Lissenberg-Witte, PhD	Amsterdam University Medical Centers, Vrije Universiteit, Amsterdam, The Netherlands	Interpretation of data and drafting of the manuscript, statistical analysis, revision of the manuscript critically for intellectual content.
Annick de Vries, PhD	Sanquin Research, Amsterdam, The Netherlands	Acquisition of the data, interpretation of data and drafting of the manuscript, revision of the manuscript critically for intellectual content.
Anja ten Brinke, PhD	Sanquin Research, Amsterdam, The Netherlands	Acquisition of the data, interpretation of data and drafting of the manuscript, revision of the manuscript critically for intellectual content.
Bob W. van Oosten, PhD	Amsterdam University Medical Centers, Vrije Universiteit, Amsterdam, The Netherlands	Conception and design of the study, acquisition of the data, interpretation of data and drafting of the manuscript, revision of the manuscript critically for intellectual content.
Frederik Barkhof, PhD	Amsterdam University Medical Centers, Vrije Universiteit,	Conception and design of the study, acquisition of the data, interpretation of data and drafting of the manuscript, revision of the manuscript critically for intellectual

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Charlotte E. Teunissen, PhD	Amsterdam University Medical Centers, Vrije Universiteit, Amsterdam, The Netherlands	Acquisition of the data, interpretation of data and drafting of the manuscript, revision of the manuscript critically for intellectual content.
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Theo Rispens, PhD	Sanquin Research, Amsterdam, The Netherlands	Conception and design of the study, acquisition of the data, interpretation of data and drafting of the manuscript, revision of the manuscript critically for intellectual content.
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Tables

Table 1. Baseline patient characteristics

	Extended interval dosing (n=51)	Standard interval dosing (n=10)	Total (n=61)
Age, years	40.7 (10.4)	41.6 (10.2)	40.8 (10.3)
Women	38 (75)	6 (60)	44 (72)
Body weight, kg	76.3 (14.0)	77.1 (17.6)	76.4 (14.5)
Time since diagnosis, years	11.0 (7.0-18.0)	6.5 (3.8-14.3)	10.0 (5.5-18.0)
Duration of NTZ treatment, years	4.0 (2.0-7.0)	5.0 (1.8-7.3)	4.0 (2.0-7.0)
Duration of radiological stability, years*	4.0 (2.0-7.0)	4.0 (2.0-6.3)	4.0 (2.0-7.0)
JCV positivity	17 (33)	3 (30)	20 (33)
JCV index**	1.1 (0.5-1.8)	1.6	1.1 (0.5-1.9)
Baseline NTZ trough concentration, µg/ml	27.0 (20.0-34.0)	9.3 (6.9-10.6)	24.0 (18.0-33.0)
Included patients per center			
Amsterdam UMC	39 (77)	4 (40)	43 (71)
OLVG	3 (6)	1 (10)	4 (7)
St. Antonius hospital	6 (12)	2 (20)	8 (13)
Rijnstate hospital	3 (6)	3 (30)	6 (10)

Patients are divided in extended interval dosing (with baseline natalizumab trough concentration ≥15µg/ml and standard interval dosing with baseline natalizumab trough concentration of <15µg/ml).

Data are n (%), mean (SD), or median (IQR).

kg=kilogram. NTZ=natalizumab. JCV=John Cunningham virus.

* radiological stability is defined as no new/enlarging T2 lesions and gadolinium enhancing lesions

**JCV index is reported of the JCV positive patients

Table 2. Clinical and radiological endpoints of patients on extended interval dosing

n 48	Baseline	3 months	6 months	9 months	12 months
Gd+ lesions	0	0	0	0	0
Active T2 lesions	0	0	0	0	0
Relapse	0	0	0	0	0
EDSS score	3.0 (2.0-5.0)	3.0 (2.0-5.3)	3.5 (2.4-5.3)	3.5 (2.5-5.4)	3.0 (2.0-5.0)
T25FW	4.3 (3.4-5.2)				4.1 (3.4-5.5)
9HPT, dominant hand	21.6 (19.2-24.2)				20.6 (18.7-22.0)
9HPT, non-dominant hand	21.6 (19.2-27.4)				22.9 (19.9-26.6)
PASAT	90.8 (73.3-96.7)				88.3 (66.7-96.7)

Clinical and radiological endpoints showing patients completing follow-up (n=48). Data are n or median (IQR). The MSFC (T24FW, 9HPT and PASAT) were assessed at baseline and 12 months follow-up. T25FW and 9HPT is reported in seconds. PASAT is reported in percentage of correct answers.

Gd+=gadolinium enhancing lesions. EDSS=Expanded disability status scale. T25FW=Timed 25 foot walk. 9HPT=9 hole peg test. PASAT=Paced auditory serial addition test.

Figures legends

Figure 1. Trial profile

Figure 1 shows the inclusion and follow-up at time. Ten patients stayed on the 4 week interval due to a baseline natalizumab trough concentration of <15µg/ml, these patients did not receive further follow-up. When the last patient completed the final visit of one year follow-up, the database was closed. Up to that time 26 patients completed the extension phase.

Abbreviations: NTZ; natalizumab, JCV; John Cunningham virus

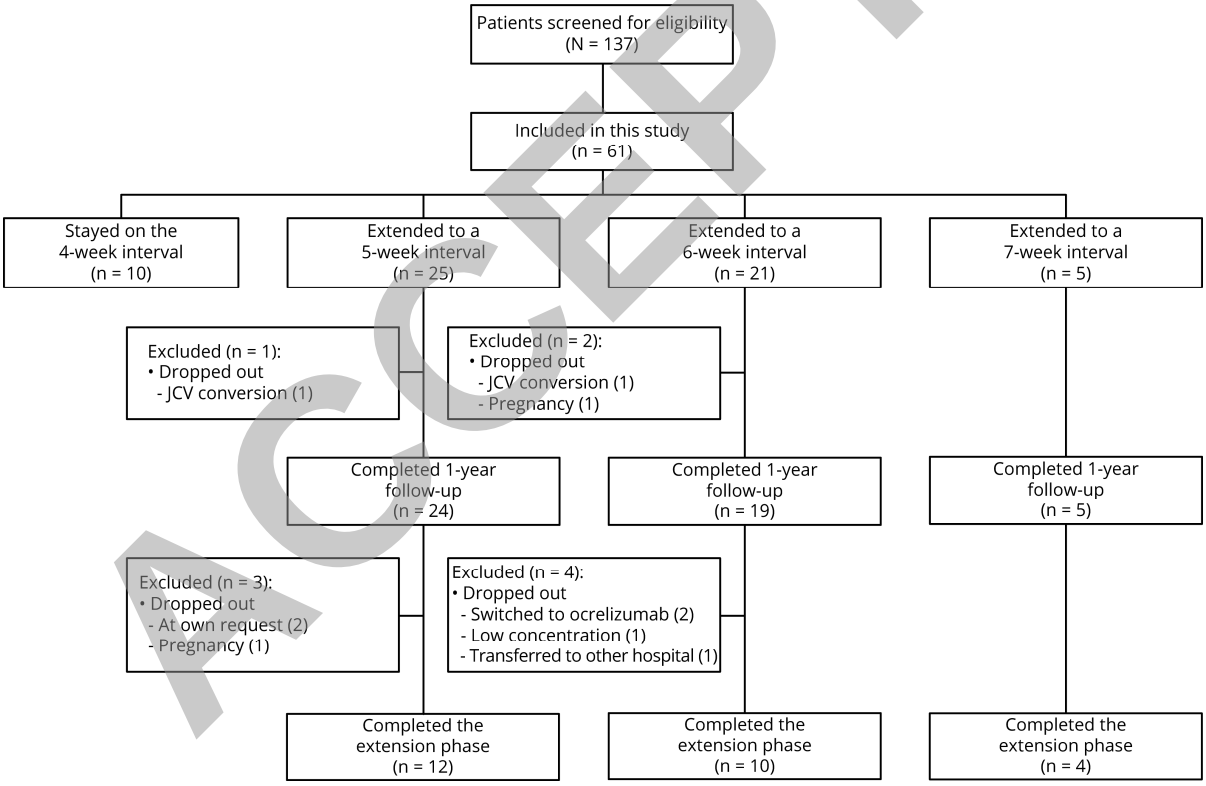


Figure 2. Baseline natalizumab trough concentration and final treatment interval

Boxplot showing the association of baseline natalizumab concentration with the final treatment interval (*rho* 0.9, 95% CI 0.7 – 0.9, p<0.001). The figure shows the median, interquartile range, range (within 1.5 IQR) and outliers.

*The patient with a concentration >50 µg/ml in the 6 week interval group chose not want to extend the interval at a trough concentration of 17 µg/ml. It is feasible that this patient could have extended the interval to seven weeks.

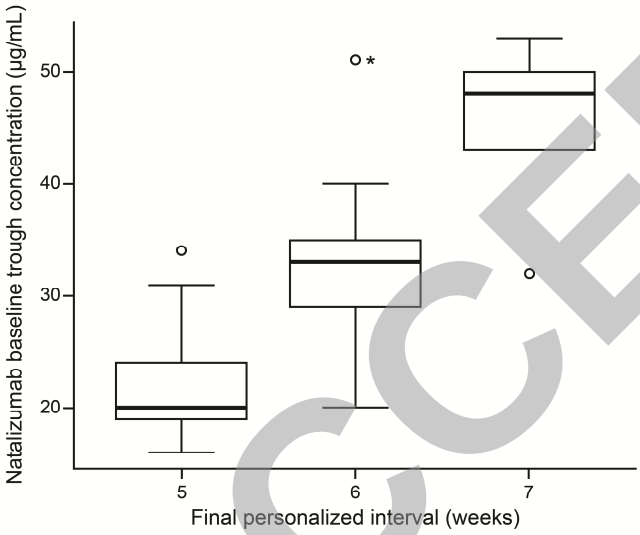


Figure 3. Mean natalizumab trough concentration in patients with different final treatment intervals.

Graph showing natalizumab trough concentration of consecutive infusions during 1 year follow-up in four patient categories based on final treatment interval (4, 5, 6 and 7 weeks). The figure shows a mean and 95% CI.

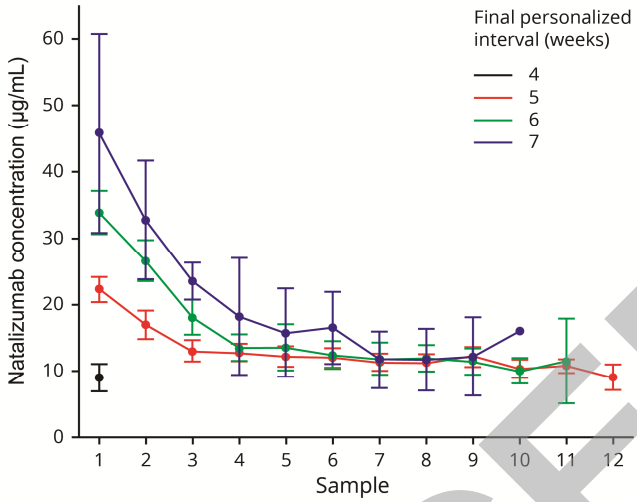
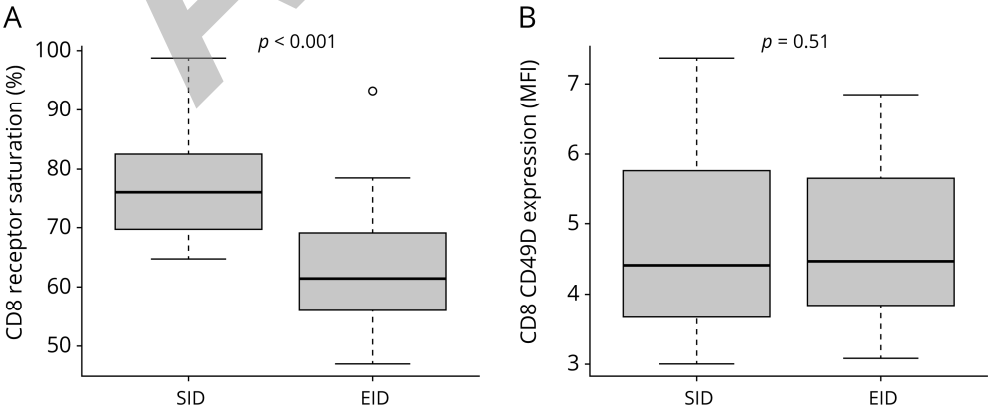
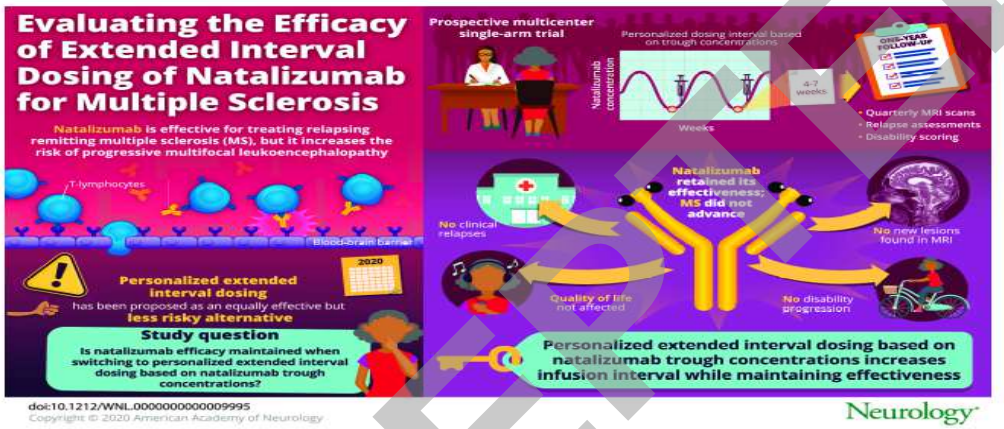


Figure 4. Natalizumab α4ß1-integrin receptor saturation and CD49d expression

Figure showing the natalizumab α4ß1-integrin saturation (A) and CD49d expression (B) (n=36) of patients in standard interval dosing (SID) and personalized extended interval dosing (EID).





doi:10.1212/WNL.0000000000009995

Neurology

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Neurology published online July 20, 2020
DOI 10.1212/WNL.0000000000009995