



# Comparison of switching to 6-week dosing of natalizumab versus continuing with 4-week dosing in patients with relapsing-remitting multiple sclerosis (NOVA): a randomised, controlled, open-label, phase 3b trial

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## Summary

*Lancet Neurol* 2022; 21: 608–19

Published Online

April 25, 2022

[https://doi.org/10.1016/S1474-4422\(22\)00143-0](https://doi.org/10.1016/S1474-4422(22)00143-0)

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**Background** Treatment with natalizumab once every 4 weeks is approved for patients with relapsing-remitting multiple sclerosis, but is associated with a risk of progressive multifocal leukoencephalopathy. Switching to extended-interval dosing is associated with lower progressive multifocal leukoencephalopathy risk, but the efficacy of this approach is unclear. We aimed to assess the safety and efficacy of natalizumab once every 6 weeks compared with once every 4 weeks in patients with relapsing-remitting multiple sclerosis.

**Methods** We did a randomised, controlled, open-label, phase 3b trial (NOVA) at 89 multiple sclerosis centres across 11 countries in the Americas, Europe, and Western Pacific. Included participants were aged 18–60 years with relapsing-remitting multiple sclerosis and had been treated with intravenous natalizumab 300 mg once every 4 weeks with no relapses for at least 12 months before randomisation, with no missed doses in the previous 3 months. Participants were randomly assigned (1:1), using a randomisation sequence generated by the study funder and contract personnel with interactive response technology, to switch to natalizumab once every 6 weeks or continue with once every 4 weeks. The centralised MRI reader, independent neurology evaluation committee, site examining neurologists, site backup examining neurologists, and site examining technicians were masked to study group assignments. The primary endpoint was the number of new or newly enlarging T2 hyperintense lesions at week 72, assessed in all participants who received at least one dose of assigned treatment and had at least one postbaseline MRI, relapse, or neurological examination or efficacy assessment. Missing primary endpoint data were handled under prespecified primary and secondary estimands: the primary estimand included all data, regardless of whether participants remained on the assigned treatment; the secondary estimand classed all data obtained after treatment discontinuation or study withdrawal as missing. Safety was assessed in all participants who received at least one dose of study treatment. Study enrolment is closed and an open-label extension study is ongoing. This study is registered with EudraCT, 2018-002145-11, and ClinicalTrials.gov, NCT03689972.

**Findings** Between Dec 26, 2018, and Aug 30, 2019, 605 patients were assessed for eligibility and 499 were enrolled and assigned to receive natalizumab once every 6 weeks (n=251) or once every 4 weeks (n=248). After prespecified adjustments for missing data, mean numbers of new or newly enlarging T2 hyperintense lesions at week 72 were 0·20 (95% CI 0·07–0·63) in the once every 6 weeks group and 0·05 (0·01–0·22) in the once every 4 weeks group (mean lesion ratio 4·24 [95% CI 0·86–20·85]; p=0·076) under the primary estimand, and 0·31 (95% CI 0·12–0·82) and 0·06 (0·01–0·31; mean lesion ratio 4·93 [95% CI 1·05–23·20]; p=0·044) under the secondary estimand. Two participants in the once every 6 weeks group with extreme new or newly enlarging T2 hyperintense lesion numbers ( $\geq 25$ ) contributed most of the excess lesions. Adverse events occurred in 194 (78%) of 250 participants in the once every 6 weeks group and 190 (77%) of 247 in the once every 4 weeks group, and serious adverse events occurred in 17 (7%) and 17 (7%), respectively. No deaths were reported. There was one case of asymptomatic progressive multifocal leukoencephalopathy (without clinical signs) in the once every 6 weeks group, and no cases in the once every 4 weeks group; 6 months after diagnosis, the participant was without increased disability and remained classified as asymptomatic.

**Interpretation** We found a numerical difference in the mean number of new or newly enlarging T2 hyperintense lesions at week 72 between the once every 6 weeks and once every 4 weeks groups, which reached significance under the secondary estimand, but interpretation of statistical differences (or absence thereof) is limited because disease activity in the once every 4 weeks group was lower than expected. The safety profiles of natalizumab once every 6 weeks and once every 4 weeks were similar. Although this trial was not powered to assess differences in risk of progressive multifocal leukoencephalopathy, the occurrence of the (asymptomatic) case underscores the importance of monitoring and risk factor consideration in all patients receiving natalizumab.

**Funding** Biogen.

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## Introduction

Natalizumab, a monoclonal antibody against the  $\alpha 4\beta 1$  integrin receptor, which is approved for dosing once every 4 weeks as a 300 mg intravenous infusion,<sup>1</sup> is a high-efficacy treatment for patients with relapsing forms of multiple sclerosis, as shown by clinical trials<sup>2–4</sup> and real-world studies.<sup>5–7</sup> The once every 4 weeks regimen was based on phase 2 and preliminary phase 3 dose-

finding studies that showed greater than 80% saturation of  $\alpha 4\beta 1$  integrin receptors that was maintained for 3–4 weeks after a single 300 mg dose of natalizumab.<sup>8</sup> Natalizumab treatment is associated with a risk of progressive multifocal leukoencephalopathy—a rare but serious opportunistic infection of the CNS.<sup>9,10</sup> Risk factors for progressive multifocal leukoencephalopathy include the presence of anti-JC virus (JCV) antibodies in serum,

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See Online for appendix

## Research in context

### Evidence before this study

We searched PubMed for papers published in English from Jan 1, 1966, to Aug 14, 2021, using the terms “multiple sclerosis” AND (“natalizumab” AND “extended” OR “dosing frequency” OR “dosing interval”), to identify studies on the effect of a natalizumab dosing schedule of approximately once every 6 weeks in patients with multiple sclerosis. The search identified nine studies that assessed clinical or radiological efficacy of natalizumab extended-interval dosing compared with dosing once every 4 weeks. Eight of these studies were retrospective, observational, cohort studies that used variable definitions of extended-interval dosing (intervals ranging from 33 days to 8 weeks). The duration of natalizumab once every 4 weeks dosing received by patients before switching to extended-interval dosing was also variable, ranging from 3 to 24 months. All eight studies suggested that natalizumab effectiveness, as assessed by clinical and radiological outcome measures, was maintained in patients who switched to extended-interval dosing. The ninth study was a prospective study comparing the occurrence of new gadolinium-enhancing lesions in 51 patients who switched to extended-interval dosing (dosing interval range from once every 5 weeks to once every 7 weeks) with ten patients who continued to receive natalizumab every 4 weeks. No new gadolinium-enhancing lesions were observed in patients receiving extended-interval dosing or standard-interval dosing in up to 2 years of follow-up. Additionally, we identified a retrospective cohort study based on a large US safety database that assessed the risk of progressive multifocal leukoencephalopathy in anti-JC virus antibody-positive patients with multiple sclerosis who were treated with natalizumab extended-interval dosing or standard-interval dosing (n=35 521). Three definitions of extended-interval dosing were used to encompass variations in natalizumab dosing intervals observed in real-world clinical practice; for all definitions, the risk of progressive multifocal leukoencephalopathy was significantly lower with extended-interval dosing than with standard-interval dosing.

### Added value of this study

To our knowledge, this is the first randomised controlled trial to provide efficacy data for natalizumab 6-week dosing in patients with relapsing-remitting multiple sclerosis. Assessment of the primary endpoint with one of two prespecified analysis methods

(the secondary estimand) showed a significantly greater number of new or newly enlarging T2 hyperintense lesions at week 72 in the natalizumab once every 6 weeks group than in the once every 4 weeks group. The difference was primarily due to two participants in the once every 6 weeks group who had atypically large numbers of lesions. Distributions of participants with none, one, or two new or newly enlarging T2 hyperintense lesions were similar in the two groups, as were secondary clinical and MRI outcomes over 72 weeks. Proportions of participants who reached the exploratory endpoint of no evidence of disease activity were similar in the two groups, indicating that disease activity was well controlled in both groups. Safety findings were consistent with the known profile of natalizumab, and the incidences of adverse events and serious adverse events were similar between the two groups. Overall, despite small numerical differences in the primary endpoint between the groups, after taking all study efficacy endpoints into account (including the effect of the two participants with extreme lesion numbers in the once every 6 weeks group), the results of this study suggest that most patients who are stable on natalizumab once every 4 weeks can switch to once every 6 weeks with no clinically meaningful loss of efficacy.

### Implications of all the available evidence

The efficacy observations from this trial and previous real-world studies strongly support the hypothesis that multiple sclerosis disease activity is well controlled in patients who switch to natalizumab once every 6 weeks after at least 12 months of stable treatment with once every 4 weeks dosing. Although the US safety database analysis showed a significant reduction in progressive multifocal leukoencephalopathy risk with natalizumab extended-interval dosing (approximately once every 6 weeks) compared with standard-interval dosing (once every 4 weeks), the occurrence of the single (asymptomatic) case in the once every 6 weeks group in our study underscores the importance of vigilant monitoring and risk factor consideration in all patients receiving natalizumab. Overall, these data contribute substantially to the understanding of natalizumab efficacy with 6-week dosing and provide important information for physicians to consider when making treatment decisions with natalizumab on the basis of individual patient benefit and risk.

previous immunosuppressant use, and longer duration of natalizumab treatment, especially beyond 2 years.<sup>10</sup>

A retrospective cohort analysis of the Tysabri Outreach: United Commitment to Health (TOUCH) Prescribing Program safety database (n=35 521) showed that natalizumab extended-interval dosing with an average interval of approximately 6 weeks was associated with a significantly lower risk of progressive multifocal leukoencephalopathy than dosing once every 4 weeks in anti-JCV antibody-positive patients with relapsing-remitting multiple sclerosis (RRMS).<sup>11</sup> However, because efficacy measures are not captured in the TOUCH database, these analyses provide no information on the effectiveness or risk–benefit profile of extended-interval dosing.

Extension of the natalizumab dosing interval to longer than 4 weeks has been suggested as a possible means to reduce risk of progressive multifocal leukoencephalopathy, potentially by allowing limited CNS immune surveillance to return without the disease activity associated with treatment interruption.<sup>12</sup> An evaluation of multiple sclerosis disease activity during a planned 24-week interruption of natalizumab treatment showed an absence of radiological disease activity for up to 12 weeks after the last dose.<sup>13</sup> However, in a study of patients who switched to natalizumab once every 12 weeks after at least 12 months of dosing once every 4 weeks, radiological and clinical disease activity were not adequately controlled with the longer dosing interval.<sup>14</sup> Population pharmacokinetic and pharmacodynamic modelling studies have suggested that initiation of natalizumab treatment with extended-interval dosing might result in inadequate protection from clinical and radiological disease activity<sup>15</sup> and that disease activity is more likely to return with a switch to dosing intervals of longer than 6 weeks.<sup>16</sup> However, a precise relationship between natalizumab serum concentration and progressive multifocal leukoencephalopathy remains to be shown.<sup>17</sup>

Real-world retrospective<sup>12,18–22</sup> and prospective<sup>23</sup> studies have compared the effectiveness of natalizumab extended-interval dosing with standard-interval dosing in patients who switched to extended-interval dosing after a period of stable dosing once every 4 weeks. Although these studies have used variable definitions of extended-interval dosing and did not use well-matched treatment cohorts, they found that the effectiveness of natalizumab on clinical or radiological outcomes was not diminished with extended-interval dosing.

Taken together, the previous studies suggest that the effectiveness of natalizumab extended-interval dosing and 4-week dosing are similar if patients switch to extended-interval dosing (approximately once every 6 weeks) after 1–2 years of stable treatment once every 4 weeks. We aimed to assess the safety and efficacy of natalizumab once every 6 weeks compared with once every 4 weeks in patients with RRMS.

## Methods

### Study design

We did a randomised, controlled, open-label, rater-blinded, phase 3b trial (NOVA) at 89 multiple sclerosis centres across 11 countries in the Americas, Europe, and Western Pacific (appendix pp 9–12). This study was carried out in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice Guideline. The study protocol was approved by each centre's ethics committee or institutional review board, and all participants provided written informed consent before screening. The assessment of the efficacy, safety, and tolerability of natalizumab once every 6 weeks versus once every 4 weeks comprised part 1 of NOVA, and is reported here. An open-label extension study in participants who completed part 1 of this trial is ongoing and is not included in this publication. Changes to the original study protocol are detailed in the appendix (pp 6–7).

### Participants

Eligible participants were aged 18–60 years with a diagnosis of RRMS, an Expanded Disability Status Scale (EDSS) score of 5·5 or lower at screening, and no relapses in the 12 months before randomisation (as determined by the enrolling investigator), and had received at least 11 doses of natalizumab monotherapy consistent with the approved dosing (300 mg by intravenous infusion once every 4 weeks) for at least 12 months before randomisation, with no missed doses in the previous 3 months. Key exclusion criteria were a diagnosis of primary progressive or secondary progressive multiple sclerosis and the presence of gadolinium-enhancing lesions on MRI at screening. A full list of inclusion and exclusion criteria is provided in the appendix (pp 3–5).

### Randomisation and masking

Eligible participants were randomly assigned (1:1) to receive intravenous natalizumab 300 mg once every 6 weeks or to continue with once every 4 weeks. The randomisation sequence was generated by the study funder and contract personnel, using interactive response technology. Participants were randomly assigned at each site using block randomisation within each stratum: country or region (North America, UK, Europe and Israel, or Australia), baseline bodyweight ( $\leq 80$  kg vs  $> 80$  kg), and duration of natalizumab exposure at baseline ( $\leq 3$  years vs  $> 3$  years). The centralised MRI reader, independent neurology evaluation committee, site examining neurologist, site backup examining neurologist, and site examining technician were masked to participant treatment group assignment, infusion dates, and laboratory data, to maintain rater blinding. Treatment group assignment was known to the participants, other investigators, the study funder, and the contract research organisation responsible for administrative aspects of the study, including study initiation, monitoring, management of serious adverse event reports, and data management.

## Procedures

Duration of part 1 of the study for each participant was up to 102 weeks, consisting of a 6-week screening period, 72 weeks of randomly assigned treatment, a 12-week follow-up period, and a follow-up safety phone call 24 weeks after the last dose of study treatment (roughly 12 weeks after the end of the 12-week follow-up period; appendix p 2).

According to the investigator's decision, participants in either group with evidence of disease activity could receive rescue therapy consisting of a switch to an alternative disease-modifying therapy per local standard of care, or could revert to dosing once every 4 weeks (participants in the once every 6 weeks group only). Evidence of disease activity was shown by the presence of one or more of the following: two or more new or newly enlarging T2 hyperintense lesions of any size (compared with a previous MRI scan); confirmed disability worsening (defined as an increase, confirmed after at least 24 weeks, of  $\geq 1.0$  in EDSS score from a previous score of  $1.0$  to  $\leq 5.5$ , an increase of  $\geq 1.5$  if the previous score was 0, or an increase of  $\geq 0.5$  if the previous score was  $\geq 6.0$ ); clinical relapse (defined as new or recurrent neurological symptoms, not associated with fever or infection, with a duration of  $\geq 24$  h) accompanied by a worsening of EDSS score; or an increase of  $\geq 1$  grade in two or more functional scales of the EDSS or an increase of  $\geq 2$  grades in one functional scale. Rescue treatment decisions were made within 4 weeks from either the date of MRI that showed the disease activity or the date of confirmation that disability worsening had persisted for at least 24 weeks.

Proton density-weighted, T2-weighted, and three-dimensional (3D) T1-weighted MRI scans, and axial or oblique two-dimensional diffusion-weighted images, were acquired before gadolinium injection. A fluid-attenuated inversion recovery scan was done immediately after gadolinium injection, and a 3D T1-weighted scan was performed 10 min after the end of the injection. MRI scans were performed within the period of 2 days before to 5 days after the regularly scheduled visits at weeks 24, 48, and 72. MRI scans were also performed at early termination or in the event of on-study neurological worsening or relapse. All MRI scans were read and interpreted by a central MRI reader who was masked to treatment allocation. EDSS assessments were performed at screening and at study weeks 24, 48, 72, and 84, and in the event of radiological evidence of disease progression or potential relapse.

## Outcomes

The primary endpoint was the number of new or newly enlarging T2 hyperintense lesions at week 72. Secondary clinical endpoints were assessment of the time to first relapse, annualised relapse rate at week 72, and time to 24-week confirmed disability worsening. Relapses were adjudicated by the independent neurology evaluation

committee. Secondary MRI endpoints were the numbers of participants with new gadolinium-enhancing and T1 hypointense lesions at weeks 24, 48, and 72, and with new or newly enlarging T2 hyperintense lesions at weeks 24 and 48. Safety was a secondary outcome and included assessment of all adverse events and serious adverse events. The proportion of participants with no evidence of disease activity at 72 weeks was an exploratory endpoint.

## Statistical analysis

The planned sample size was based on historical data on multiple sclerosis treatments, including from a meta-analysis of the relationship between new or newly enlarging T2 lesions and relapses, which suggested little or no clinical relevance of a difference of  $0.2$ – $0.3$  in mean lesion numbers over 72 weeks.<sup>24</sup> With the planned sample size of 200 participants per group, the precision of the estimate was sufficient to provide greater than 80% probability to observe a 95% CI lower limit of greater than 1 for the ratio of estimated mean lesion numbers of the 6-week dosing group to the 4-week dosing group, if the true mean was 0.5 in the once every 6 weeks group and 0.3 in the once every 4 weeks group. In the other direction, the planned sample size provided a precision that gave approximately 90% probability to observe a 95% CI upper limit of 2 or lower if the true mean lesion numbers in both groups was 0.3. The trial was powered so that a clinically meaningful difference in the primary outcome would be detected with a p value of less than 0.05, under the assumption that the mean number of new or newly enlarging T2 lesions in the once every 4 weeks group was 0.3. Enrolment of about 480 participants accounted for an expected dropout rate of approximately 17%. Changes to the original statistical analysis plan are described in the appendix (p 8).

Primary and secondary efficacy outcomes were evaluated in the modified intention-to-treat population, defined as all participants who received at least one dose of assigned study treatment and had at least one postbaseline MRI scan, relapse, neurological examination, or EDSS efficacy assessment. The primary endpoint was analysed using negative binomial regression models with treatment as the classification variable and the same covariates as used for randomisation (country or region, baseline bodyweight, and natalizumab exposure duration at baseline). In case of non-convergence of the negative binomial regression model, a Poisson regression model with the same covariates was used. If the total number of observed new or newly enlarging T2 hyperintense lesions in both groups at week 72 was fewer than 15, neither the negative binomial model nor Poisson regression model would be conducted, and efficacy would be assessed on the basis of descriptive summary statistics. Ratios of adjusted mean numbers of lesions for once every 6 weeks versus once every 4 weeks were derived from the model with a 95% CI and associated p value. A 95% CI lower limit of greater than 1 was considered as evidence of a difference between groups.

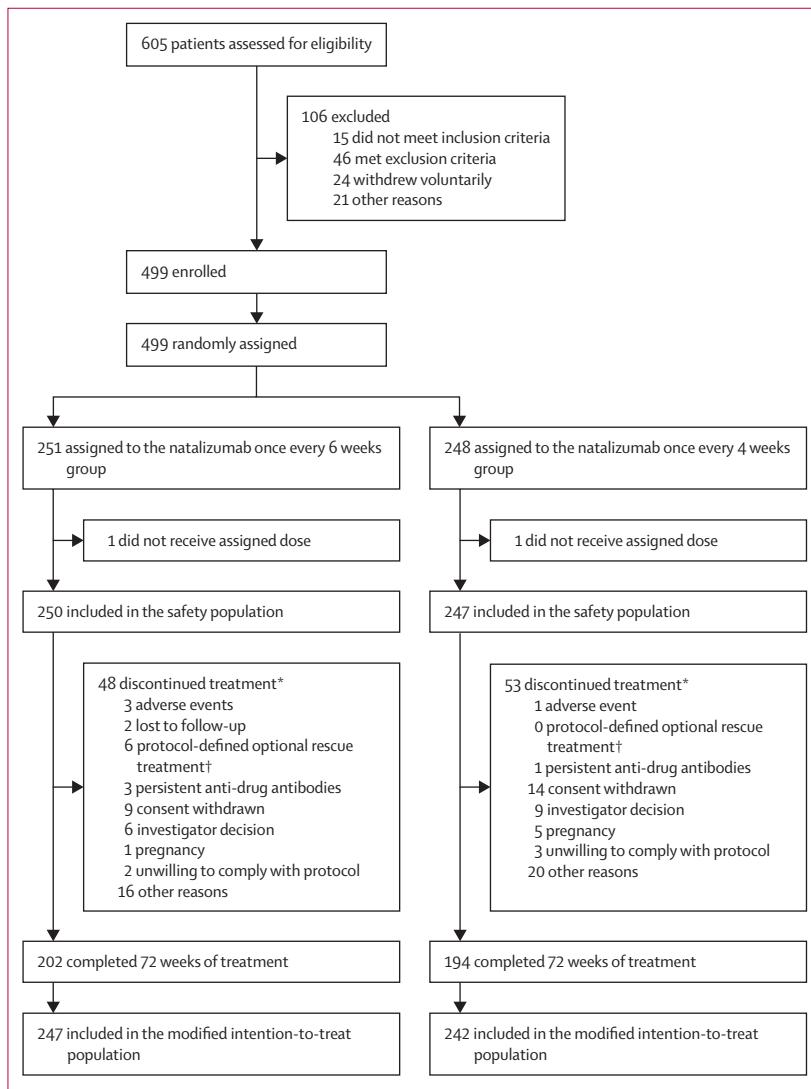
A secondary inference considered that the possibility of a two-fold increase in the mean lesion number in the once every 6 weeks group compared with the once every 4 weeks group was excluded if the 95% CI upper limit was 2 or lower. Proportions of participants with no new or newly enlarging T2 lesions were assessed using logistic regression models with the same covariates as used for randomisation.

Missing data due to intercurrent events (events occurring after randomisation that affected observation or interpretation of an outcome of interest—eg, early discontinuation of study treatment, early withdrawal from study, or treatment switching) were handled using worst-case replacement or a multiple imputation approach under prespecified primary and secondary estimands.<sup>25,26</sup>

The primary estimand used a treatment policy strategy<sup>27</sup> for estimation of differences in switching to natalizumab once every 6 weeks or remaining on once every 4 weeks in a clinical setting where changing dosing regimens or switching to different medication might occur, and utilised all measurements regardless of whether participants remained on the assigned treatment. Under the treatment policy estimand, missing data due to intercurrent events related or possibly related to efficacy or an adverse event were imputed by the worst observed value of participants on study treatment in the same group at the given timepoint (ie, worst-case replacement). For reasons classified as no information, or as no information or not related, the missing values were treated as missing at random and were addressed using a multiple imputation approach.

The secondary estimand used a hypothetical strategy<sup>27</sup> for estimation of differences as if no intercurrent events occurred. The hypothetical estimand therefore treated all data obtained after an intercurrent event as missing. Under the hypothetical strategy estimand, missing data due to intercurrent events resulting from treatment discontinuation or switching due to efficacy, or from withdrawals related to or possibly related to efficacy, were imputed by worst-case replacement. For intercurrent events due to all other reasons, missing data were addressed using a multiple imputation approach. Withdrawals and discontinuations were classified on the basis of all available key information (including but not limited to disposition, adverse events, laboratory findings, MRI, EDSS assessments, and relapses), by independent study funder personnel who were masked to treatment group assignment.

Analysis of time to first relapse was based on proportions of participants who were relapse-free over 72 weeks, estimated using the Kaplan-Meier method. Between-groups comparisons were based on Cox regression models with the same covariates as used in the analysis of the primary endpoint. Adjusted annualised relapse rate at week 72 was analysed using Poisson regression models with treatment as the classification variable and the same covariates as used in the analysis of the primary endpoint. Only relapses confirmed by the independent neurology evaluation committee were included in the analysis of annualised relapse rate. Time to 24-week confirmed disability worsening was analysed as the proportion of participants who were free of 24-week confirmed disability worsening over 72 weeks using Kaplan-Meier estimates and Cox regression models similar to the method described for time to first relapse. Secondary MRI endpoints (numbers of new gadolinium-enhancing and new T1 hypointense lesions at weeks 24, 48, and 72, and new or newly enlarging T2 hyperintense lesions at weeks 24 and 48) were analysed using the same approach as described for the primary endpoint. Missing secondary MRI values were handled under the hypothetical estimand. Suspected cases of progressive



**Figure:** Trial profile

\*Includes three participants in the once every 6 weeks group and five participants in the once every 4 weeks group who discontinued treatment before having any post-baseline efficacy assessments. †Seven participants in the once every 6 weeks group and five participants in the once every 4 weeks group met the rescue criteria for relapse.

multifocal leukoencephalopathy were confirmed by PCR testing of CSF (Unilabs, Geneva, Switzerland).

Adverse events were analysed in the safety population, defined as all participants who received at least one dose of assigned study treatment. Information on adverse events and serious adverse events was collected throughout the study and summarised using descriptive statistics.

No evidence of disease activity was defined as no gadolinium-enhancing lesions, no new or newly enlarging T2 lesions, no relapses, and no 24-week confirmed disability worsening at week 72; this endpoint was assessed using a logistic regression model adjusted for the same covariates as used in the assessment of the primary endpoint. Participants with one or more missing assessments were counted as having not reached no evidence of disease activity. As defined, the exploratory no evidence of disease activity endpoint is a composite endpoint related to both the primary and secondary endpoints.

A post-hoc analysis was performed to assess whether differences in relapse frequency or severity between the two groups were associated with an imbalance between the numbers of participants in the once every 6 weeks and once every 4 weeks groups who met relapse criteria for optional rescue therapy and who opted to receive rescue therapy.

All statistical analyses were performed using SAS (version 9.4). This study is registered with EudraCT, 2018-002145-11, and ClinicalTrials.gov, NCT03689972.

### Role of the funding source

The funder of the study was involved in study design, data collection, data analysis, and data interpretation, and provided medical writing and editorial support in the development of the manuscript.

### Results

Between Dec 26, 2018, and Aug 30, 2019, 605 participants were assessed for eligibility and 499 were enrolled and randomly assigned to receive natalizumab once every 6 weeks ( $n=251$ ) or once every 4 weeks ( $n=248$ ). Approximately equal proportions of participants in both groups completed 72 weeks of treatment (figure). 247 participants in the once every 6 weeks group and 242 in the once every 4 weeks group were included in the modified intention-to-treat population; baseline demographics and disease characteristics were well balanced between the groups, including similar median multiple sclerosis disease duration, median number of relapses in the past year, and median duration of natalizumab exposure before randomisation (table 1). Mean compliance (calculated as [number of infusions actually received during the randomised treatment dosing window divided by the number of infusions the participant was expected to receive by the last dose of randomised treatment]  $\times 100$ ) up to the last dose was 100% (SD 0·5) for the once every 6 weeks group and 100% (0·8) for the once

	Natalizumab once every 6 weeks group (n=247)	Natalizumab once every 4 weeks group (n=242)
Age, years	40·9 (9·66)	40·3 (9·94)
Sex		
Female	174 (70%)	176 (73%)
Male	73 (30%)	66 (27%)
Ethnicity		
Hispanic or Latino	9 (4%)	10 (4%)
Not Hispanic or Latino	220 (89%)	219 (90%)
Not reported*	18 (7%)	13 (5%)
Race		
White	208 (84%)	205 (85%)
Black or African American	14 (6%)	23 (10%)
Asian	4 (2%)	1 (<1%)
American Indian or Alaska Native	1 (<1%)	1 (<1%)
Other	5 (2%)	1 (<1%)
Not reported*	15 (6%)	11 (5%)
Region		
North America†	129 (52%)	130 (54%)
Europe‡ and Israel	101 (41%)	98 (40%)
Australia	12 (5%)	9 (4%)
UK	5 (2%)	5 (2%)
Bodyweight, kg		
Mean (SD)	79·70 (19·59)	78·62 (20·28)
≤80	146 (59%)	138 (57%)
Time since multiple sclerosis symptoms onset, years§	10·0 (6·0–15·0)	9·0 (5·0–15·0)
Time since RRMS diagnosis, years	8·0 (4·0–13·0)**	8·0 (4·0–12·0)††
Number of relapses in the 12 months before initiation of natalizumab	1·0 (0·0–2·0)††	1·0 (0·0–1·0)‡‡
Duration of natalizumab exposure at baseline, years	4·0 (2·1–6·6)	4·0 (2·2–6·1)
Participants with no missed natalizumab doses in the 3 months before screening	247 (100%)	241 (>99%)
Participants without dosing gap >3 months	227 (92%)	229 (95%)
EDSS score at baseline, mean (SD)	2·32 (1·3)	2·31 (1·3)
Previous disease-modifying therapy use§§	184 (74%)	175 (72%)
JC virus serostatus at baseline		
Positive	52 (21%)	46 (19%)
Negative	194 (79%)	195 (81%)
Missing	1 (<1%)	1 (<1%)
T2 hyperintense lesion volume, mL	10·0 (4·8–18·5)	9·6 (4·3–18·2)
T1 hypointense lesion volume, mL	0·6 (0·2–1·7)	0·6 (0·1–1·7)
Normalised brain volume, mL	1516·4 (1453·4–1572·7)	1532·5 (1459·1–1579·0)

Data are n (%), mean (SD), or median (IQR) unless otherwise stated. EDSS=Expanded Disability Status Scale. RRMS=relapsing-remitting multiple sclerosis. \*Not reported due to confidentiality regulations. †Includes USA and Canada. ‡Includes Belgium, France, Germany, Israel, Italy, Netherlands, and Spain. §Calculated as year of randomisation minus year of multiple sclerosis symptom onset. ||n=246. ||Calculated as year of randomisation minus year of RRMS diagnosis. \*\*n=245. ††n=241. ‡‡n=236. §§Previous treatment with daclizumab, dimethyl fumarate, fampridine, fingolimod, glatiramer acetate, human immunoglobulin, interferon  $\beta$  (included pegylated interferon  $\beta$ ), rituximab, or teriflunomide.

Table 1: Baseline characteristics in the modified intention-to-treat population

every 4 weeks group. The mean time between natalizumab doses was 6·0 weeks (SD 0·06) in the once every 6 weeks group and 4·0 weeks (0·08) in the once every 4 weeks group.

	Natalizumab once every 6 weeks group (n=247)	Natalizumab once every 4 weeks group (n=242)	Ratio of adjusted mean lesion numbers (95% CI)	p value
Number of lesions				
0	202 (82%)	189 (78%)	..	..
1	5 (2%)	7 (3%)	..	..
2	2 (1%)	1 (<1%)	..	..
3	0	0	..	..
4	0	0	..	..
≥5	2 (1%)*	0	..	..
Missing	36 (15%)	45 (19%)	..	..
Mean (SD)	0.3 (2.69)	0.0 (0.23)	..	..
Range	0–30	0–2	..	..
Adjusted mean number of lesions (treatment policy [primary] estimand)†‡	0.20 (0.07–0.63)	0.05 (0.01–0.22)	4.24 (0.86–20.85)	0.076
Adjusted mean number of lesions (hypothetical strategy [secondary] estimand)†§	0.31 (0.12–0.82)	0.06 (0.01–0.31)	4.93 (1.05–23.20)	0.044
Data are n (%) or mean (95% CI) unless otherwise stated. Proportions of participants with new or newly enlarging T2 lesions, gadolinium-enhancing lesions, or T1 lesions were calculated on the basis of the modified intention-to-treat populations. *One participant had 30 lesions and one had 25 lesions. †Estimated by negative binomial regression with treatment as classification and baseline bodyweight ( $\leq 80$ kg vs $> 80$ kg), duration of natalizumab exposure at baseline ( $\leq 3$ years vs $> 3$ years), and region (North America, UK, Europe and Israel, or Australia) as covariates. ‡Observed lesions are included for analysis, regardless of intercurrent events, and missing values for efficacy or safety (in the once every 6 weeks group, two participants switched to 4-week dosing and one discontinued treatment; in the once every 4 weeks group, one participant discontinued treatment) are imputed by the worst case among participants on treatment at the same visit in the same treatment group; otherwise via multiple imputation. §Observed lesions before intercurrent events are included for analysis, and missing data for efficacy (in the once every 6 weeks group, six participants switched to 4-week dosing and one discontinued treatment; in the once every 4 weeks group, one participant discontinued treatment) are imputed by the worst case among participants on treatment in the same group; otherwise via multiple imputation.				

**Table 2: New or newly enlarging T2 hyperintense lesions at week 72 in the modified intention-to-treat population (primary endpoint)**

Intercurrent events (events leading to missing data) were reported for 46 (19%) of 247 participants in the once every 6 weeks group and 51 (21%) of 242 in the once every 4 weeks group, and mean time to first intercurrent event was similar for the two groups (33.1 weeks [SD 18.75] and 30.3 weeks [21.83], respectively). Most intercurrent events were not related to treatment (35 [76%] of 46 events in the once every 6 weeks group and 44 [86%] of 51 in the once every 4 weeks group) or had no information on the reason for the event (four [9%] and four [8%, respectively]). Independent neurology evaluation committee-confirmed relapse events meeting rescue treatment criteria were reported for similar proportions of participants in the once every 6 weeks group (seven [3%] of 247) and the once every 4 weeks group (five [2%] of 242). However, the number of participants with relapse who opted for rescue treatment was imbalanced between the two groups (six of seven participants in the once every 6 weeks group chose to receive rescue therapy and switched to 4-week dosing after relapse; none of five participants in the once every 4 weeks group opted for rescue therapy).

With regard to the primary endpoint, the adjusted mean number of new or newly enlarging T2 hyperintense lesions at week 72 was 0.20 (95% CI 0.07–0.63) in the

once every 6 weeks group and 0.05 (0.01–0.22) in the once every 4 weeks group (ratio of adjusted mean lesion numbers 4.24 [95% CI 0.86–20.85]; p=0.076) with the primary (treatment policy strategy) estimand, and 0.31 (0.12–0.82) in the once every 6 weeks group and 0.06 (0.01–0.31) in the once every 4 weeks group (4.93 [1.05–23.20]; p=0.044) with the secondary (hypothetical strategy) estimand (table 2).

The distributions of participants with none, one, or two new or newly enlarging T2 lesions at week 72 were similar between the two groups. However, two participants in the once every 6 weeks group had more than two lesions, whereas no participants in the once every 4 weeks group had more than two lesions (table 2). One participant in the once every 6 weeks group with more than two lesions had similar baseline characteristics to the overall treatment group, except for a longer duration of natalizumab exposure (8.8 years vs median 4.0 years [IQR 2.1–6.6]). This participant had no new disease activity while receiving 6-week dosing but discontinued natalizumab after developing anti-JCV seropositive status at week 55 and did not initiate another disease-modifying therapy. At week 67, this participant had a relapse and a subsequent MRI scan showed 30 new or newly enlarging T2 hyperintense lesions. Because this increase in number of T2 lesions occurred while off treatment, the number of 30 lesions was not used for worst-case imputation for other participants in the once every 6 weeks group with missing data. The other participant with more than two lesions also had similar baseline characteristics to the overall treatment group, except for a shorter duration of natalizumab exposure (1.1 years vs median 4.0 years [IQR 2.1–6.6]) and greater T2 lesion volume (16.7 mL vs median 10.0 mL [IQR 4.8–18.5]). This participant was diagnosed with asymptomatic progressive multifocal leukoencephalopathy after completing 72 weeks of assigned treatment. Regularly scheduled MRI scans showed the presence of five new or newly enlarging T2 lesions at week 24, ten additional lesions at week 48, and ten more lesions at week 72 (25 lesions total). Because the lesion increase in this participant occurred during treatment, the week 72 lesion number of 25 was used for worst-case replacement for two participants who received rescue therapy in the once every 6 weeks group under the treatment policy strategy (primary) estimand and worst-case replacement for all six who received rescue therapy in the once every 6 weeks group under the hypothetical strategy (secondary) estimand. None of the six participants who received rescue therapy in the once every 6 weeks group had observable new or newly enlarging T2 lesions at 72 weeks.

Secondary MRI endpoints were generally similar between groups, and proportions of participants in the once every 6 weeks and once every 4 weeks groups with new or newly enlarging T2 hyperintense lesions, gadolinium-enhancing lesions, and T1 lesions were similar at all timepoints (table 3). Adjusted mean

	New or newly enlarging T2 hyperintense lesions		Gadolinium-enhancing lesions		T1 hypointense lesions	
	Natalizumab once every 6 weeks group (n=247)	Natalizumab once every 4 weeks group (n=242)	Natalizumab once every 6 weeks group (n=247)	Natalizumab once every 4 weeks group (n=242)	Natalizumab once every 6 weeks group (n=247)	Natalizumab once every 4 weeks group (n=242)
<b>Number of lesions at week 24</b>						
0	239 (97%)	226 (93%)	241 (98%)	226 (93%)	240 (97%)	226 (93%)
1	1 (<1%)	2 (1%)	0	1 (<1%)	1 (<1%)	0
2	1 (<1%)	1 (<1%)	0	0	0	1 (<1%)
3	0	0	0	0	0	0
4	0	0	0	0	0	0
≥5	1 (<1%)*	0	0	0	0	0
Missing	5 (2%)	13 (5%)	6 (2%)	15 (6%)	6 (2%)	15 (6%)
Median (IQR)	0 (0-0-0)	0 (0-0-0)	0 (0-0-0)	0 (0-0-0)	0 (0-0-0)	0 (0-0-0)
<b>Number of lesions at week 48</b>						
0	214 (87%)	200 (83%)	218 (88%)	202 (83%)	217 (88%)	200 (83%)
1	5 (2%)	5 (2%)	0	1 (<1%)	1 (<1%)	1 (<1%)
2	1 (<1%)	1 (<1%)	0	0	0	1 (<1%)
3	0	0	0	0	0	0
4	0	0	0	0	0	0
≥5	1 (<1%)*	0	0	0	0	0
Missing	26 (11%)	36 (15%)	29 (12%)	39 (16%)	29 (12%)	40 (17%)
Median (IQR)	0 (0-0-0)	0 (0-0-0)	0 (0-0-0)	0 (0-0-0)	0 (0-0-0)	0 (0-0-0)
<b>Number of lesions at week 72</b>						
0	..	..	209 (85%)	190 (79%)	206 (83%)	189 (78%)
1	..	..	0	1 (<1%)	1 (<1%)	1 (<1%)
2	..	..	0	0	1 (<1%)*	1 (<1%)
3	..	..	0	0	0	0
4	..	..	0	0	1 (<1%)†	0
≥5	..	..	1 (<1%)†	0	0	0
Missing	..	..	37 (15%)	51 (21%)	38 (15%)	51 (21%)
Median (IQR)	..	..	0 (0-0-0)	0 (0-0-0)	0 (0-0-0)	0 (0-0-0)

Data are n (%) unless otherwise stated. Proportions of participants with new or newly enlarging T2 lesions, gadolinium-enhancing lesions, or T1 lesions were calculated on the basis of the modified intention-to-treat populations. New or newly enlarging T2 hyperintense lesions at week 72 was the primary endpoint so is not included in this table.  
\*This was the participant who had asymptomatic progressive multifocal leukoencephalopathy and 25 new or newly enlarging T2 lesions at week 72. †This was the participant who discontinued treatment at week 55 and had 30 new or newly enlarging T2 lesions at week 72.

Table 3: Secondary MRI endpoints by timepoint in the modified intention-to-treat population

numbers of new or newly enlarging T2 lesions at 24 weeks and gadolinium-enhancing and T1 lesions at all three timepoints were not conducted as in the primary analyses because the total number of observed lesions in both groups was fewer than 15. Also, adjusted mean numbers of new or newly enlarging T2 lesions at 48 weeks could not be modelled as neither the negative binomial regression model nor the Poisson regression model converged. At 72 weeks, one (<1%) of 247 participants in the once every 6 weeks group and one (<1%) of 242 in the once every 4 weeks group had gadolinium-enhancing lesions. The participant in the once every 6 weeks group with gadolinium-enhancing lesions at week 72 was the participant who had 30 new or newly enlarging T2 lesions. At the same timepoint, three (1%) participants in the once every 6 weeks group and two (<1%) in the once every 4 weeks group had new T1 hypointense lesions. One of the participants with T1

lesions in the once every 6 weeks group was the participant who had 30 new or newly enlarging T2 lesions, and another was the participant with asymptomatic progressive multifocal leukoencephalopathy and 25 new or newly enlarging T2 lesions. At 48 weeks, the proportion of participants with new or newly enlarging T2 hyperintense lesions was similar in the two groups (table 3). In the once every 6 weeks group, the participant who had 30 new or newly enlarging T2 lesions at week 72 had no lesions at week 48 and the participant who had 25 new or newly enlarging T2 lesions at week 72 had 15 lesions at week 48. Although the prespecified analyses treated all lesions as multiple sclerosis, the week 72 MRI scan of the participant who had 25 lesions at week 72 was confounded by progressive multifocal leukoencephalopathy, and the cause of the T2 lesions at weeks 24 and 48 in this participant is unknown.

	Natalizumab once every 6 weeks group (n=247)	Natalizumab once every 4 weeks group (n=242)	HR or OR	p value
Participants with confirmed relapse*	7 (3%)	5 (2%)	..	..
Total participant-years	306.52	292.73	..	..
Unadjusted annualised relapse rate†	0.0228	0.0171	..	..
Adjusted annualised relapse rate (95% CI)‡	0.00013 (0.00006-0.00027)	0.00010 (0.00004-0.00024)	..	0.63
Time to first relapse (proportion of participants who were relapse-free at 72 weeks)§	0.97	0.98	1.31 (0.42-4.13)	0.64
Time to 24-week confirmed disability worsening (proportion of participants who were free of 24-week confirmed disability worsening at 72 weeks)§	0.90	0.92	1.29 (0.71-2.34)	0.40
Participants with no evidence of disease activity at 72 weeks	173 (70%)	163 (67%)	1.1 (0.8-1.7)**	0.52

Data are n (%) unless otherwise stated. Unadjusted annualised relapse rate, adjusted annualised relapse rate, the proportion of participants relapse-free at 72 weeks, and the proportion of participants free of 24-week confirmed disability worsening at 72 weeks were secondary clinical endpoints; proportion of patients with no evidence of disease activity was an exploratory endpoint. HR=hazard ratio. OR=odds ratio. \*Relapses confirmed by the independent neurology evaluation committee. †Total number of relapses that occurred during the treatment period divided by the total number of participant-years in the period. ‡Estimated using a Poisson regression model adjusted for baseline bodyweight ( $\leq 80$  kg vs  $> 80$  kg), duration of natalizumab exposure at baseline ( $\leq 3$  years vs  $> 3$  years), and region (North America, UK, Europe and Israel, or Australia). §Estimated proportions based on the Kaplan-Meier product limit method. ||HR (95% CI) based on a Cox regression model, with treatment as the classification variable and baseline bodyweight ( $\leq 80$  kg vs  $> 80$  kg), duration of natalizumab exposure at baseline ( $\leq 3$  years vs  $> 3$  years), and region (North America, UK, Europe and Israel, or Australia) as covariates. ||Participants with one or more missing assessments were counted as having not reached no evidence of disease activity. \*\*OR (95% CI).

Table 4: Secondary clinical and exploratory endpoints in the modified intention-to-treat population

Secondary clinical endpoints were assessment of the time to first relapse, annualised relapse rate at week 72, and time to 24-week confirmed disability worsening.

Secondary clinical outcomes in the modified intention-to-treat population did not differ significantly between the once every 6 weeks group and the once every 4 weeks group. The time to first relapse was similar in the two groups (appendix p 13); at 72 weeks, 97% of participants in the once every 6 weeks group and 98% of participants in the once every 4 weeks group were relapse-free (table 4). Unadjusted and adjusted annualised relapse rates were also similar between groups. Also, the time to 24-week confirmed disability worsening was similar in the once every 6 weeks group and the once every 4 weeks group (appendix p 14). At 72 weeks, 90% of participants in the once every 6 weeks group and 92% of participants in the once every 4 weeks group were free of 24-week confirmed disability worsening (table 4).

The safety population included 250 participants in the once every 6 weeks group and 247 in the once every 4 weeks group. The incidences of adverse events and serious adverse events were similar between the two treatment groups (table 5). Adverse events leading to discontinuation of study treatment were reported in four (2%) of 250 participants in the once every 6 weeks group and one (<1%) of 247 in the once every 4 weeks group. Among

	Natalizumab once every 6 weeks group (n=250)	Natalizumab once every 4 weeks group (n=247)
Any adverse event	194 (78%)	190 (77%)
Any severe adverse event	19 (8%)	9 (4%)
Any treatment-related adverse event	29 (12%)	19 (8%)
Any serious adverse event	17 (7%)	17 (7%)
Any treatment-related serious adverse event	3 (1%)	0
Any adverse event leading to discontinuation of study treatment	4 (2%)	1 (<1%)
Any adverse event leading to withdrawal from study	2 (1%)	1 (<1%)
Any adverse event of special interest*	41 (16%)	31 (13%)
Death	0	0
Progressive multifocal leukoencephalopathy	1 (<1%)	0
Treatment-emergent adverse events by system organ class†		
Infections and infestations	106 (42%)	105 (43%)
Nasopharyngitis	27 (11%)	32 (13%)
Urinary tract infection	24 (10%)	19 (8%)
Upper respiratory tract infection	12 (5%)	17 (7%)
Nervous system disorders	62 (25%)	55 (22%)
Headache	26 (10%)	23 (9%)
Musculoskeletal and connective tissue disorders	60 (24%)	52 (21%)
Arthralgia	18 (7%)	9 (4%)
Pain in extremity	14 (6%)	7 (3%)
General disorders and administration site conditions	46 (18%)	28 (11%)
Fatigue	25 (10%)	8 (3%)
Injury, poisoning, and procedural complications	43 (17%)	40 (16%)
Fall	14 (6%)	14 (6%)
Respiratory, thoracic, and mediastinal disorders	30 (12%)	24 (10%)
Cough	14 (6%)	4 (2%)

Data are n (%). \*Defined as progressive multifocal leukoencephalopathy events that occurred before the end of the study, infusion reactions, opportunistic infections, drug-induced liver injury, or hypersensitivity reactions. †Treatment-emergent adverse events that occurred in at least 5% of participants in either group, as preferred terms (Medical Dictionary for Regulatory Activities version 23.1).

Table 5: Adverse events in the safety population

participants with available immunogenicity data, two of 244 participants in the once every 6 weeks group developed anti-natalizumab antibodies (one transiently at week 12, one persistently from week 12 to week 36). These participants were not the same two participants who had very high new or newly enlarging T2 lesion numbers in this group. No participants in the once every 4 weeks group (of 241 with immunogenicity data) developed anti-natalizumab antibodies. No deaths were reported in either group.

One case of progressive multifocal leukoencephalopathy occurred in the once every 6 weeks group (in the

participant who had 25 T2 lesions at week 72), and no cases occurred in the once every 4 weeks group. The affected participant had known risk factors for progressive multifocal leukoencephalopathy, including total natalizumab exposure of longer than 2 years (1·1 year as once every 4 weeks before study enrolment and on-study 6-week dosing through week 72) and an anti-JCV antibody index greater than the limit of assay detection (reported as  $>2\cdot35$ ) at enrolment and all subsequent assessments. The participant had not been treated with any disease-modifying therapy for the year before initiating natalizumab after 7 years of injectable or oral disease-modifying therapy. The patient was confirmed as having asymptomatic progressive multifocal leukoencephalopathy and was detected on the basis of MRI findings and confirmed by PCR testing of CSF that detected 351 copies of JCV DNA. On the basis of the asymptomatic presentation at 48 weeks, the participant remained on the assigned study treatment. 12 months after the progressive multifocal leukoencephalopathy diagnosis, the participant remained asymptomatic without clinical sequelae as evidenced by no increase in EDSS score, or in Karnofsky or Modified Rankin scores, which were assessed specifically as part of the progressive multifocal leukoencephalopathy follow-up. The progressive multifocal leukoencephalopathy event was considered recovered and the patient had commenced treatment with teriflunomide for RRMS.

The exploratory endpoint no evidence of disease activity was reached by similar proportions of participants in both groups (table 4).

Proportions of participants who met the rescue criteria and required steroid treatment or hospitalisation were similar between the two groups, as were EDSS score changes at the time of relapse, indicating that relapse events were of similar frequency and severity overall, despite the observed imbalance in the proportions of participants who received rescue therapy (appendix p 15).

## Discussion

In this randomised controlled trial in patients with RRMS who were receiving stable natalizumab 4-week dosing and then switched to 6-week dosing or continued with 4-week dosing, we found a numerical difference in the estimated number of new or newly enlarging T2 hyperintense lesions between the once every 6 weeks and once every 4 weeks groups. These differences reached significance under one (the hypothetical strategy) of two estimands prespecified to account for missing data due to intercurrent events. Neither prespecified estimand could exclude the possibility of a two-fold worsening with 6-week dosing compared with 4-week dosing on the basis of the secondary inference cutoff of 2 or lower for the mean lesion number ratio 95% CI upper limit.

Analysis of the distribution of participants with new or newly enlarging T2 hyperintense lesions indicated that the estimated difference between the groups were strongly

influenced by three factors. First, two participants (both in the once every 6 weeks dosing group) had extreme T2 lesion numbers. Second, there was an imbalance in the number of participants who received optional rescue therapy. Third, the difference might have been exaggerated by the imputation rules used in the prespecified estimands. The 25 new or newly enlarging T2 lesions in one participant in the once every 6 weeks dosing group (the participant who had asymptomatic progressive multifocal leukoencephalopathy) were used to impute data for two of the participants who received rescue therapy under the treatment policy strategy (primary) estimand and all six participants who received rescue therapy under the hypothetical strategy (secondary) estimand. The proportions of participants with new or newly enlarging T2 hyperintense lesions over 72 weeks of treatment were similar in both groups. Notably, most participants had no new or newly enlarging T2 lesions at 72 weeks (82% in the once every 6 weeks group and 78% in the once every 4 weeks group). The extreme T2 lesion numbers in one of the two participants might have been related to factors other than the efficacy of 6-week dosing, such as the prolonged period without disease-modifying therapy after discontinuation of study drug at week 55, and confounding from asymptomatic progressive multifocal leukoencephalopathy in the other participant.

There were no clinically meaningful differences in secondary clinical or MRI endpoints at week 72 between the once every 6 weeks and once every 4 weeks dosing groups. The exploratory finding that similar proportions of participants in both groups reached the endpoint of no evidence of disease activity further indicates that disease activity was well controlled in both groups. Examination of the week 24 MRI findings and the Kaplan-Meier analysis of time to first relapse shows that increased disease activity did not occur at earlier timepoints.

Taken together, the effect of the two participants with extreme lesion numbers, coupled with the secondary and exploratory findings, support the hypothesis that differences in the primary outcome are not clinically meaningful and were at least partially due to factors other than the efficacy of natalizumab 6-week dosing, such as the prespecified methods for handling missing data. Our efficacy findings are consistent with real-world studies on the effectiveness of natalizumab extended-interval dosing.<sup>12,18–23</sup>

The safety findings in this study were consistent with the known safety profile of 300 mg intravenous natalizumab once every 4 weeks, and the proportions of participants with serious adverse events or adverse events were similar in both groups. A single case of progressive multifocal leukoencephalopathy was observed during the study, in the once every 6 weeks dosing group. The case was classified as asymptomatic because it was detected by MRI in the absence of clinical symptoms and was confirmed by the presence of JCV DNA in CSF. The progressive multifocal leukoencephalopathy might have

confounded the primary endpoint assessment for this participant because diagnosis was based on lesions detected on the week 72 MRI scan. The occurrence of the single (asymptomatic) progressive multifocal leukoencephalopathy case in the once every 6 weeks group is consistent with previous observations that, although progressive multifocal leukoencephalopathy risk is reduced with 6-week dosing, it is not eliminated.<sup>11,28</sup> The presence of known risk factors for progressive multifocal leukoencephalopathy does not preclude treatment with natalizumab, nor were they an exclusion criterion for this study. The risk factors in this participant (total natalizumab exposure >2 years and anti-JCV index >1.5) underscore the importance of vigilant monitoring and risk factor consideration for all patients being treated with natalizumab, as early detection of progressive multifocal leukoencephalopathy, including asymptomatic presentations, is associated with more favourable outcomes.<sup>29</sup> Updated consensus guidelines for management of patients with multiple sclerosis recommend an MRI screening interval of 3–4 months for JCV seropositive patients being treated with natalizumab extended-interval dosing or 4-week dosing.<sup>30</sup>

This study has several limitations. Sample size calculations and prespecified inferences were established using the assumption, based on previous trials,<sup>14</sup> that the true mean number of new or newly enlarging T2 hyperintense lesions in the 4-week dosing group would be 0.3. Significant differences, or absence thereof, should therefore be interpreted with caution, as the level of disease activity in this group was lower than expected. Also, as there was no assumption of equivalence for 6-week and 4-week dosing, this trial was not designed to assess non-inferiority, thereby limiting interpretation of the efficacy results. Furthermore, the size and duration of the trial were insufficient to be informative on rare adverse events, such as progressive multifocal leukoencephalopathy.

The definition of natalizumab extended-interval dosing specified in this study, as once every 6 weeks following at least 1 year of once every 4 weeks dosing, is consistent with observations from real-world clinical practice, which suggests that these findings might be generalisable to a larger population of patients receiving natalizumab. In the large retrospective study of progressive multifocal leukoencephalopathy risk, the average dosing interval was approximately 42 days (range 35–43), and approximately 85% of the patients who switched to extended-interval dosing did so after receiving a median of 25 infusions before switching (ie, after approximately 2 years of 4-week dosing).<sup>11</sup> In a comparison of clinical outcomes in patients treated with natalizumab once every 6 weeks or once every 4 weeks, most patients who received dosing regimens other than once every 4 weeks were indicated as being intentionally dosed once every 6 weeks.<sup>31</sup> Furthermore, of the patients who switched to natalizumab once every 6 weeks, only 26 (9%) of 224 did so after receiving less than 1 year of 4-week dosing.

Overall, after taking all efficacy endpoints into account, and the effect of the two participants in the once every 6 weeks group who had high numbers of new or newly enlarging T2 lesions, our findings suggest that most patients who are stable on natalizumab 4-week dosing can switch to 6-week dosing without clinically meaningful loss of efficacy. These results, in conjunction with results of the retrospective safety analyses of the TOUCH Prescribing Program, contribute to a more comprehensive understanding of the natalizumab once every 6 weeks dosing regimen. These results could provide important information for physicians who are making natalizumab treatment decisions, and reinforce the need to balance these decisions with consideration of individual patient benefit and risk. Continued follow-up and monitoring of our well defined patient population in the open-label extension study should provide additional important information on the efficacy and safety of natalizumab in patients receiving 4-week dosing who switch to 6-week dosing.

#### Contributors

JFF, GD, LZR, JAC, DLA, HB, GC, GG, JK, HW, KS, and NC designed the study. JFF, GD, LZR, and JAC were study investigators. DLA collected data. JFF verified the data. SX and GK analysed and verified the data. All authors interpreted the data, reviewed and revised the manuscript, and had full access to all data in the study and provided final approval of all content in this Article. The corresponding author had final responsibility for the decision to submit the manuscript for publication. All named authors met the International Committee of Medical Journal Editors criteria for authorship for this manuscript and take responsibility for the integrity of the work as a whole.

#### Declaration of interests

JFF reports personal compensation for consulting activities from Biogen and Octave; and compensation (paid to institution) for data safety monitoring or advisory boards from Biogen, Genentech, and Novartis. GD reports personal compensation for scientific advisory boards and funding for travel or speaker honoraria from Biogen, Bristol-Myers Squibb, Merck Serono, Novartis, Sanofi Genzyme, and Teva Pharmaceuticals; and research grants (paid to institution) from Biogen, Merck Serono, Novartis, and Sanofi Genzyme. LZR reports personal compensation for advisory board activities from Biogen, Genentech, and Novartis; and research support from Biogen, Celgene, and Genentech. JAC reports personal compensation for consulting from Biogen, Bristol-Myers Squibb, Convelo, Genentech, Janssen, NervGen, Novartis, and PSI; for speaking from H3 Communications; and for serving as an Editor of *Multiple Sclerosis Journal*. DLA reports consulting fees from Albert Charitable Trust, Alexion Pharma, Biogen, Celgene, Frequency Therapeutics, Genentech, Med-Ex Learning, Merck, Novartis, Population Council, Receptos, Roche, and Sanofi Aventis; grants from Biogen, Immunotec, and Novartis; and equity interest in NeuroRx. HB reports personal compensation for consulting from Oxford Health Policy Forum; compensation (paid to institution) for advisory board membership or speaker bureaus from Biogen, Merck, Novartis, Roche, and UCB Pharma; research support (paid to institution) from Biogen, Merck, Novartis, and Roche; and honorarium (paid to institution) for serving on NOVA trial steering committee. GC reports serving on data and safety monitoring boards for AstraZeneca, Avaxis, Biolinex, Brainstorm Cell Therapeutics, Bristol-Myers Squibb-Celgene, CSL Behring, Galmed, GreenValley Pharma, Mapi, Merck, Merck-Pfizer, Opko Biologics, OncoImmune, Neurim, Novartis, Orphazyme, Sanofi, Reata, Teva Pharmaceuticals, VielaBio, Vivus, the National Heart, Lung, and Blood Institute (Protocol Review Committee), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (Obstetric Pharmacology Research Unit oversight committee); consulting or advisory boards for Biodelivery Sciences International, Biogen, Click

Therapeutics, Genzyme, Genentech, GW Pharmaceuticals, Immunic, Klein-Buendel, MedDay, MedImmune, Neurogenesis, Novartis, Osmotica, Perception Neurosciences, Recursion–Cerexis, Rekover, Roche, and TG Therapeutics; is employed by the University of Alabama at Birmingham, Birmingham, AL, USA; and is president of Pythagoras, a private consulting company located in Birmingham, AL, USA. GG reports consulting or speaker fees from AbbVie, Aslan, Atara Bio, Biogen, Bristol-Myers Squibb–Celgene, GlaxoSmithKline, GW Pharma, Janssen–Actelion, Japanese Tobacco, Jazz Pharmaceuticals, LifNano, Merck & Co, Merck KGaA–EMD Serono, Novartis, Sanofi Genzyme, Roche–Genentech, and Teva Pharmaceuticals. JK reports speaker and consulting fees from Biogen, Genzyme, Merck Serono, Novartis, Roche, and Teva Pharmaceuticals. HW reports honoraria for consulting or speaking from AbbVie, Actelion, Alexion, Argenx, Biogen, Bristol-Myers Squibb, Cognomed, EMD Serono, Evgen, F Hoffmann-La Roche, Idorsia, IGES, Immunic, Immunovant, Janssen, Johnson & Johnson, MedDay, Merck Serono, Novartis, Roche, Sanofi Genzyme, the Swiss Multiple Sclerosis Society, Teva Pharmaceuticals, and UCB; travel support from Alexion, Biogen, Biologix, Cognomed, F Hoffmann-La Roche, Gemeinnützige Hertie-Stiftung, Genzyme, Merck, Novartis, Roche Pharma, Teva Pharmaceuticals, and WebMD Global; and research support from Biogen, GlaxoSmithKline, Roche, and Sanofi Genzyme. KS, GK, RK, and NC report support from Biogen as employees; and hold stock or stock options in Biogen. SX is a former employee of Biogen.

#### Data sharing

The data described in this Article are not publicly available. The authors and Biogen are fully supportive of allowing independent assessment and verification of these results. Biogen has established processes to share protocols, analysis plans, clinical study reports, study-level data, and deidentified patient-level data with qualified scientific researchers (requests can be submitted via the Biogen Data Request Portal at <https://biogen-dt-external.pharmacm.com//DT/Home/Index/>).

#### Acknowledgments

The authors were assisted in preparation of the manuscript by John Watson (Ashfield MedComms [an Ashfield Health company], Middletown, CT, USA). Celia Nelson (Ashfield MedComms, Middletown, CT, USA) assisted in editing of the manuscript. The authors provided final approval of all content. This trial was funded by Biogen.

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