

Pharmacokinetics and Pharmacodynamics of Natalizumab 6-Week Dosing vs Continued 4-Week Dosing for Relapsing-Remitting Multiple Sclerosis

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Abstract

Background and Objectives

Exposure to natalizumab, an efficacious treatment for relapsing-remitting multiple sclerosis (RRMS), is associated with increased risk of progressive multifocal leukoencephalopathy (PML). Compared with every-4-week (Q4W) dosing, extended-interval dosing of natalizumab is associated with decreased risk of PML. Clinical efficacy was maintained in the majority of patients switched to every-6-week (Q6W) dosing in the phase 3b NOVA clinical trial. In this article, we report pharmacokinetics (PK) and pharmacodynamics (PD) of Q6W vs Q4W dosing in NOVA.

Methods

In NOVA study Part 1, participants with RRMS (aged 18–60 years) and Expanded Disability Status Scale score <5.5, who were stable on IV natalizumab Q4W dosing for ≥12 months, were randomized to continue IV Q4W dosing or switched to IV Q6W dosing of natalizumab and followed for 72 weeks. Exploratory outcomes were measurements of trough serum natalizumab concentration, α 4-integrin saturation, and soluble vascular cell adhesion molecule-1 (sVCAM-1) concentration. A mixed model of repeated measures was used to estimate mean treatment differences between groups. Patient-level PK and PD data were examined in those with relapse or radiologic disease activity.

Results

In NOVA, 486 (Q6W, n = 245; Q4W, n = 241) and 487 (Q6W, n = 246; Q4W, n = 241) participants were included in the PK and PD populations, respectively. Mean trough natalizumab concentrations ranged from 10 to 21 μ g/mL (Q6W) and 33–38 μ g/mL (Q4W), and mean α 4-integrin saturation remained above 65.5% (Q6W) and above 77.9% (Q4W). In the Q6W group, mean sVCAM-1 levels increased 23.6% by week 24 and remained elevated throughout the study, while mean sVCAM-1 levels remained generally stable in the Q4W group. Most participants with T2 lesion activity or relapse activity, in either treatment arm, maintained trough natalizumab levels >10 μ g/mL and trough α 4-integrin saturation >50%.

Discussion

Compared with Q4W dosing, Q6W dosing was associated with a 60%–70% decrease in mean trough natalizumab levels and a 9%–16% decrease in mean α 4-integrin saturation. At the patient level, neither natalizumab concentration nor α 4-integrin saturation was consistently predictive

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Glossary

EDSS = Expanded Disability Status Scale; **IgG4** = immunoglobulin G4; **JCV** = JC virus; **LLOQ** = lower limit of quantification; **mITT** = modified intent-to-treat; **MMRM** = mixed model of repeated measures; **MNCs** = mononuclear cells; **MS** = multiple sclerosis; **PD** = pharmacodynamics; **PK** = pharmacokinetics; **PML** = progressive multifocal leukoencephalopathy; **Q4W** = every-4-week; **Q6W** = every-6-week; **RRMS** = relapsing-remitting multiple sclerosis; **sVCAM** = serum vascular cell adhesion molecule; **VCAM-1** = vascular cell adhesion molecule-1; **VLA-4** = very late activation antigen 4.

of lesion or relapse activity, suggesting that trough natalizumab and α 4-integrin saturation measurements should be interpreted with caution in clinical practice.

Trial Registration Information

ClinicalTrials.gov, NCT03689972; EudraCT, 2018-002145-11. Submitted 2018-09-27. First patient enrolled: 2018-12-26. <https://clinicaltrials.gov/study/NCT03689972>.

Introduction

Natalizumab (TYSABRI), a humanized immunoglobulin G4 (IgG4) antibody targeting the adhesion molecule α 4-integrin, was the first high-efficacy disease-modifying therapy approved for patients with relapsing forms of multiple sclerosis (MS).¹ Natalizumab is believed to act by limiting access of activated leukocytes to the CNS.² Very late activation antigen 4 (VLA-4), a dimer of α 4-integrin and β 1-integrin, is expressed on the surface of leukocytes—including mononuclear cells (MNCs), T cells, B cells, and natural killer cells—but not neutrophils.³⁻⁵ Inflammatory cytokines induce expression of vascular cell adhesion molecule-1 (VCAM-1), a binding partner of α 4-integrin, on vascular endothelial cells.⁶ Activation of leukocyte VLA-4 permits binding of α 4-integrin to VCAM-1 and is necessary for transmigration of immune cells across the blood-brain barrier.^{7,8} Natalizumab noncompetitively inhibits binding of α 4-integrin to VCAM-1.⁹ In addition, treatment with natalizumab is associated with a decrease in VCAM-1 on endothelial cells and a decrease in soluble VCAM-1 (sVCAM-1).¹⁰⁻¹² Therefore, levels of α 4-integrin saturation and of serum sVCAM-1 may be reflective of the biological efficacy of natalizumab.

Natalizumab standard-interval dosing of 300 mg every 4 weeks (Q4W) maintains >80% saturation of α 4-integrin in more than 90% of patients.¹³ In both clinical trials and real-world data, natalizumab Q4W is associated with a lower annualized relapse rate and decreased radiologic lesion activity in patients with relapsing-remitting MS (RRMS).¹⁴⁻¹⁷ However, Q4W dosing of natalizumab is associated with an increased risk of progressive multifocal leukoencephalopathy (PML), a rare but serious opportunistic infection of the CNS caused by the JC virus (JCV).^{18,19} Natalizumab is believed to decrease immunosurveillance in the CNS,²⁰ which might normally prevent the development of PML.

Anti-JCV antibody index, duration of natalizumab exposure, and prior immunosuppressant use are 3 known risk factors associated with an increased risk of PML.¹⁸ In efforts to balance clinical benefit with PML risk, clinicians have used off-label

extended-interval dosing of natalizumab to decrease natalizumab exposure over the long term.²¹ Extended-interval dosing of natalizumab has been shown to reduce, but not eliminate, PML risk.²² Real-world studies of dosing intervals between 6 and 8 weeks have reported similar clinical efficacy to Q4W dosing.^{23,24} In previous observational studies, lower trough natalizumab concentration and α 4-integrin saturation have been observed in patients receiving extended-interval dosing compared with patients receiving standard-interval dosing.²⁵⁻²⁷

NOVA (NCT03689972) was the first randomized, controlled, phase 3b clinical trial comparing the efficacy of Q4W and every-6-week (Q6W) dosing of natalizumab in patients with RRMS.²⁸ Results of NOVA suggested that patients who are stable on Q4W natalizumab can switch to Q6W dosing without a meaningful loss of radiologic or clinical efficacy, as assessed by comparing mean number of new or newly enlarging T2 lesions and annualized relapse rates across groups.²⁸ In addition, there were no differences in changes in lesion volume between patients switched to Q6W and those remaining on Q4W natalizumab.²⁹ Patients receiving Q6W natalizumab have also reported similar improvements in physical and psychological function as have those continuing on Q4W.³⁰

In this article, we report pharmacokinetic (PK) and pharmacodynamic (PD)—including trough natalizumab concentration, α 4-integrin saturation, and concentration of sVCAM-1—for patients receiving Q6W or Q4W natalizumab in NOVA. We also report PK and PD data at the patient level for individuals who experienced clinical or radiologic disease activity, including a patient who subsequently developed asymptomatic PML.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

The study design for NOVA (part I) has been described previously.²⁸ The ethics committee or institutional review board of each study center approved the NOVA protocol. The

study was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice Guideline. All participants provided written consent before screening.

Participants

Inclusion and exclusion criteria for NOVA have been described previously.²⁸ Briefly, participants (18–60 years of age) with a diagnosis of RRMS, with Expanded Disability Status Scale (EDSS) score <5.5, who had been treated with natalizumab Q4W with no relapse for ≥1 year were included.^{28,30} Participants were randomized 1:1 to switch to natalizumab Q6W or to remain on natalizumab Q4W for 72 weeks,^{28,30} and randomization occurred within the following strata: country/region, body weight (≤ 80 kg vs >80 kg), and natalizumab exposure duration (≤ 3 years vs >3 years).^{28,30} Participants who received ≥1 dose of randomly assigned study drug and had ≥1 postbaseline outcome evaluation were included in the modified intent-to-treat (mITT) population in NOVA.³⁰ All participants who received at least 1 dose of study drug and had ≥1 postbaseline PK or PD assessment were included in PK or PD analysis populations, respectively.

Clinical Assessments

EDSS was assessed at screening and at weeks 24, 48, 72, and 84 (eTable 1), and if there was radiologic evidence of disease progression or potential relapse. T2-weighted MRI scans were performed between 2 days prior and 5 days after scheduled visits at baseline; weeks 24, 48, and 72; and at early termination or if on-study neurologic worsening or relapse occurred. A central MRI reading center (NeuroRx), blinded to treatment allocation, analyzed all MRI scans. The primary components of the MRI protocol were 2 scouts (fast localizer scan [3 PLANE] and true mid-line sagittal scan) and 6 sequences: before gadolinium injection a PD-weighted scan, T2-weighted scan, axial/oblique 2D diffusion-weighted imaging (with slices parallel to the callosal line), and 3D T1-weighted scan (followed by gadolinium injection), a FLAIR scan (immediately after gadolinium injection during 10-minute wait period), and a 3D T1-weighted scan (performed 10 minutes after the end of injection). Participants were considered to have had an MS relapse if they experienced onset of new or recurrent neurologic symptoms lasting ≥24 hours, accompanied by new objective abnormalities on a neurologic examination, that was not explained solely by non-MS processes such as fever, infection, severe stress, or drug toxicity. This definition of MS relapse did not include radiologic changes. An Independent Neurology Evaluation Committee was formed to review and confirm potential abnormalities related to suspected relapses.

Laboratory Assessments

Measuring Trough Serum Natalizumab Concentration

Serum natalizumab values were measured before study drug administration at the baseline visit and at weeks 6, 12, 18, 24, 36, 48, 60, and 72 for the Q6W group, and at the baseline visit and at weeks 12, 24, 36, 48, 60, and 72 for the Q4W group

(eTable 1). Serum natalizumab (unbound) was measured at a GxP contracted research organization (BioAgilitix) with a sandwich ELISA in which natalizumab is captured by anti-idiotype antibody and subsequently detected by anti-human antibody. The assay has a linear range of 0.5–32 µg/L (lower limit of quantification [LLOQ] = 0.5 µg/mL), an interassay precision range of 4.8%–7.8%, and was validated following good practice guidelines.

Measuring α4-Integrin Saturation on Peripheral Blood Leukocytes

The α4 integrin saturation was determined on fresh (24–30 hours postcollection) blood samples using a flow cytometry-based assay validated and performed by Labcorp.³¹ During assay validation, evaluation of the stability data demonstrated that the assay performance was stable for 3 days (72–78 hours). Binding of natalizumab to α4 integrin was detected using an anti-human IgG4-phycoerythrin (hIgG4-PE) antibody. Two aliquots of whole peripheral blood were washed with phosphate-buffered saline containing 1% bovine serum albumin (PBS-1% BSA) to eliminate any unbound drug. One of the aliquots was incubated with natalizumab (10 µg/mL) at 2–8°C for 30 minutes, and the remaining aliquot was kept at 2–8°C without natalizumab. After incubation, immunofluorescence was performed using the following panel of labeled antibodies: CD19-fluorescein isothiocyanate (CD19-FITC, used for CD19⁺ lymphocyte identification), CD3-peridinin-chlorophyll protein (CD3-PerCP, used for CD3⁺ lymphocyte identification), CD11c-allophycocyanin (CD11c-APC, used for CD11⁺ cell identification), and hIgG4-PE to measure trough α4-integrin saturation. An isotype control staining with mouse IgG4-PE was used to exclude unspecific antibody binding (negative control). Mononuclear cells, monocytes, and lymphocytes were gated according to forward and side-ward scatter properties. The mean fluorescence intensity (MFI) of the PE signal was measured with a flow cytometer. The percentage of natalizumab saturation for each individual cell type was calculated from the MFI in samples using this formula: [MFI-hIgG4-PE signal (without natalizumab)/MFI-hIgG4-PE signal (with natalizumab)] × 100.

Measuring Serum sVCAM-1

The sVCAM-1 level in the serum was determined with a double-antibody sandwich ELISA method. The assay was developed by Biogen Inc. and performed by Labcorp, using the human VCAM-1/CD106 Duo Set ELISA Development kit component from R&D Systems (catalog #DY809). All samples, including quality control (QC) samples, were analyzed in duplicate. Exploratory QC data analyses revealed systematic variation between batches of assay runs—that is, batch effects. The concentrations of endogenous matrix QC samples analyzed in each run were interrogated to estimate and correct for the batch effects, which were defined as differences between the estimated mean concentration of the endogenous QC samples on each run and the mean concentration in a reference run and were subtracted from the measured concentrations of the participant-level data. More

specifically, a bivariate hierarchical statistical model was fit to the endogenous QC data to estimate batch-level correction factors that were then applied to the participant sVCAM-1 concentrations. Repeat testing of a subset of participant samples confirmed the appropriateness of the batch-effect correction.

Statistical Analysis

Trough serum natalizumab concentrations (C_{trough}) were summarized using descriptive statistics (number of participants with data, mean, SD, median, 25th percentile, 75th percentile, minimum, maximum, geometric mean, and coefficient of variation) at each scheduled timepoint for all participants and by participant weight subgroups (<60, <80, <90 kg) selected based on modeling analyses that predicted return-of-disease activity with extended interval dosing at higher weights.³²

For $\alpha 4$ -integrin saturation, measurement results and the changes from baseline were summarized by treatment group and timepoint, and mean values plotted over time for each parameter. A mixed model of repeated measures (MMRM) was used for estimating mean treatment difference over time and the differences at each timepoint, adjusting for baseline body weight (≤ 80 kg vs > 80 kg), duration of natalizumab exposure at baseline (≤ 3 years vs > 3 years), region/country (North America [the United States and Canada], United Kingdom, Europe [Belgium, France, Germany, Italy, Netherlands, and Spain], Israel, and Australia), and baseline $\alpha 4$ -integrin saturation on the cell type analyzed. The 3-year cutoff for duration of natalizumab exposure was based on findings of the numerically largest increase in risk of PML after 3 years.¹⁹ An unstructured variance-covariance matrix structure was used in the MMRM. Parameters with non-normal distributions were analyzed in logarithmic scale. For both Q6W and Q4W dosing arms, the relationship between natalizumab C_{trough} and $\alpha 4$ -integrin saturation at week 24 (timepoint when natalizumab C_{trough} was expected to reach steady state) with baseline body weight was evaluated using a Pearson correlation.

The change from baseline of log-transformed sVCAM-1 concentrations was modeled using an MMRM approach. The model adjusted for the following covariates: visit, treatment arm, visit by treatment arm interaction, baseline body weight (≤ 80 kg vs > 80 kg), duration of natalizumab exposure at baseline (≤ 3 years vs > 3 years), region/country (North America [the United States and Canada], United Kingdom, Europe [Belgium, France, Germany, Italy, Netherlands, and Spain], Israel, and Australia), and baseline sVCAM-1 concentration (log-transformed). An unstructured variance-covariance matrix structure was used in the MMRM. Geometric means of the ratio of sVCAM-1 concentrations over the baseline concentration, with 95% CIs, are presented by the treatment arm and visit; geometric means of the ratio of sVCAM-1 levels were used as these concentrations were log-normally distributed.

Data Availability

Individual participant data collected during the trial may be shared after anonymization and on approval of the research proposal. Biogen commits to sharing patient-level data, study-level data, CSRs, and protocols with qualified scientific researchers who provide a methodologically sound proposal. Biogen reviews all data requests internally based on the review criteria and in accordance with our Clinical Trial Transparency and Data Sharing Policy. Deidentified data and documents will be shared under agreements that further protect against participant reidentification. To request access to data, please visit <https://vivli.org/>.

Results

Participants

Among the NOVA mITT population ($N = 489$; Q6W, $n = 247$; Q4W, $n = 242$), 486 (Q6W, $n = 245$; Q4W, $n = 241$) participants were included in the PK population and 487 (Q6W, $n = 246$; Q4W, $n = 241$) participants were included in the PD population. Baseline and demographic characteristics for the NOVA mITT population have been previously reported and are presented in Table 1. Demographics and disease characteristics were well-balanced between the groups.²⁸

Population-Level Data

Trough Natalizumab Concentration

At baseline, all participants had received a minimum of 11 Q4W doses of natalizumab, and mean natalizumab C_{trough} were similar between the Q6W (34.1 $\mu\text{g}/\text{mL}$) and Q4W (34.3 $\mu\text{g}/\text{mL}$) groups (Figure 1A). In Q6W participants, from week 12 to week 72, mean C_{trough} ranged from 10 $\mu\text{g}/\text{mL}$ (at week 72) to 21 $\mu\text{g}/\text{mL}$ (at week 6). In Q4W participants, mean C_{trough} from week 12 to 72 ranged from 33 $\mu\text{g}/\text{mL}$ (at weeks 6 and 72) to 38 $\mu\text{g}/\text{mL}$ (at weeks 24 and 36). Mean natalizumab C_{trough} following Q6W dosing were approximately 60%–70% lower than those observed for Q4W dosing. Natalizumab C_{trough} negatively correlated with participant body weight in both Q6W and Q4W groups, with Pearson correlation values of -0.3312 and -0.4310 , respectively ($p \leq 0.001$ for both). The mean (SD) natalizumab C_{trough} at week 24 in participants < 60 kg were 28.3 (27.42) $\mu\text{g}/\text{mL}$ in Q6W participants and 50.6 (21.73) $\mu\text{g}/\text{mL}$ in Q4W participants; in participants ≥ 90 kg, mean (SD) natalizumab C_{trough} at week 24 of dosing were 4.4 (4.64) $\mu\text{g}/\text{mL}$ in Q6W participants and 27.6 (16.43) $\mu\text{g}/\text{mL}$ in Q4W participants.

Mean (SD) between-visit variation (CV%) after reaching steady-state across all participants for trough concentration was 48.76% (40.07%) for participants on Q6W dosing and 31.02% (19.78%) for participants on Q4W dosing. The overall mean (SD) compliance rate (ratio of actual infusions to planned infusions) was 90.5% (22.34%) and 88.0% (26.14%) in the Q6W and Q4W groups, respectively.

Trough $\alpha 4$ -Integrin Saturation

At baseline, mean trough $\alpha 4$ -integrin saturation on MNCs, monocytes, lymphocytes, B cells, T cells, and dendritic cells

Table 1 Baseline Characteristics in the Modified Intent-to-Treat Population

	Natalizumab once every 6-wk group (n = 247)	Natalizumab once every 4-wk group (n = 242)
Age, y	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 ^a	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 ^a	15 (6%)	11 (5%)
Region		
North America ^b	129 (52%)	130 (54%)
Europe ^c and Israel	101 (41%)	98 (40%)
Australia	12 (5%)	9 (4%)
United Kingdom	5 (2%)	5 (2%)
Baseline body weight, kg		
Mean (SD)	79.70 (19.59)	78.62 (20.28)
≤80	146 (59%)	138 (57%)
Time since multiple sclerosis symptoms onset, y^d	10.0 (6.0–15.0) ^e	9.0 (5.0–15.0)
Time since RRMS diagnosis, y^f	8.0 (4.0–13.0) ^g	8.0 (4.0–12.0) ^h
No. of relapses in the 12 mo before initiation of natalizumab	1.0 (0.0–2.0) ^h	1.0 (0.0–1.0) ⁱ
Duration of natalizumab exposure at baseline, y	4.0 (2.1–6.6)	4.0 (2.2–6.1)
Participants with no missed natalizumab doses in the 3 mo before screening	247 (100%)	241 (>99%)
Participants without dosing gap >3 mo	227 (92%)	229 (95%)
EDSS score at baseline, mean (SD)	2.32 (1.3)	2.31 (1.3)
Previous disease-modifying therapy use^j	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)

Continued

Table 1 Baseline Characteristics in the Modified Intent-to-Treat Population (continued)

	Natalizumab once every 6-wk group (n = 247)	Natalizumab once every 4-wk group (n = 242)
Normalized brain volume, mL	1,516.4 (1,453.4–1,572.7)	1,532.5 (1,459.1–1,579.0)

Abbreviations: EDSS = Expanded Disability Status Scale; IQR = interquartile range; Q4W = every 4 wk; Q6W = every 6 wk; RRMS = relapsing-remitting multiple sclerosis.
Data are n (%), mean (SD), or median (IQR) unless otherwise stated.
^a Not reported because of confidentiality regulations.
^b Includes the United States and Canada.
^c Includes Belgium, France, Germany, Israel, Italy, Netherlands, and Spain.
^d Calculated as year of randomization minus year of multiple sclerosis symptom onset.
^e n = 246.
^f Calculated as year of randomization minus year of RRMS diagnosis.
^g n = 245.
^h n = 241.
ⁱ n = 236.
^j Previous treatment with daclizumab, dimethyl fumarate, fampridine, fingolimod, glatiramer acetate, human immunoglobulin, interferon β (including pegylated interferon β), rituximab, or teriflunomide.

Reprinted from *Lancet Neurology*, 21(7), Foley JF, et al., Comparison of switching to 6-wk dosing of natalizumab vs continuing with 4-wk dosing in patients with relapsing-remitting multiple sclerosis (NOVA): a randomised, controlled, open-label, phase 3b trial, Pages 608–619, Copyright 2022, with permission from Elsevier.²⁸

was similar for both treatment groups. Data for trough α4-integrin saturation on MNCs are presented in Figure 1B. In the Q6W group, mean (SD) trough α4-integrin saturation on MNCs was 75.4% (13.24) at baseline. It decreased to 65.5% at week 12, after 2 Q6W doses, and remained above this level throughout the study, with 71.2% saturation at week 72. For the Q4W group, mean α4-integrin saturation was 75.0% (13.96) at baseline, 77.9% at 12 weeks, and consistently remained above this level throughout the study (Figure 1B). Compared with the Q4W group, α4-integrin saturation was 9%–16% lower across time points in the Q6W group. A trend of increasing α4 saturation levels was observed in both groups over time; however, this increase was small (<5% compared with previous visit) and could be explained by the assay performance and variability (acceptable variability range is ±20%).

Trough α4-integrin saturation negatively correlated with participant body weight in the Q6W group, with a Pearson correlation value of −0.4441 ($p \leq 0.001$). A smaller correlation of α4-integrin saturation with body weight was seen in the Q4W group, with a Pearson correlation value of −0.1520 ($p = 0.03$). The mean trough α4-integrin in the Q6W and Q4W groups ranged from a mean (SD) of 73.6% (11.35) and 81.7% (9.42), respectively, in participants with body weight <60 kg to 56.9% (20.06) and 79.0% (8.88), respectively, in participants with body weight >90 kg.

Mean (SD) between-visit variation (CV%) after reaching steady-state across all participants for α4-integrin saturation was 15.46% (15.45%) in the Q6W group and 9.63% (12.03%) in the Q4W group.

Trough sVCAM-1 Concentration

At baseline, mean sVCAM-1 levels were similar for both treatment groups. In the Q6W group, mean sVCAM-1 levels increased 16.9% by week 12 (GMR to baseline = 1.169; 95% CI, 1.122–1.218) and 23.6% by week 24 (GMR to baseline =

1.236; 95% CI, 1.184–1.291). Mean sVCAM-1 levels appeared to stabilize by week 24: from week 24 to week 72, GMRs to baseline ranged from 1.235 (week 72) to 1.246 (week 60; Figure 1C). In the Q4W group, mean sVCAM-1 levels were relatively stable throughout the study, with minor postbaseline increases of 2.1%–6.4% in geometric means (Figure 1C).

Increased Lesion Activity and PK/PD

An increase in disease activity, as indicated by ≥ 1 new or newly enlarging T2 lesions, was observed in 9 participants in the Q6W group and in 8 participants in the Q4W group of the mITT population. The number of participants with radiologic lesion activity was evenly spread across the quartiles of natalizumab C_{trough} and quartiles of α4-integrin saturation at week 24 (Table 2).

Participant-Level PK/PD Data

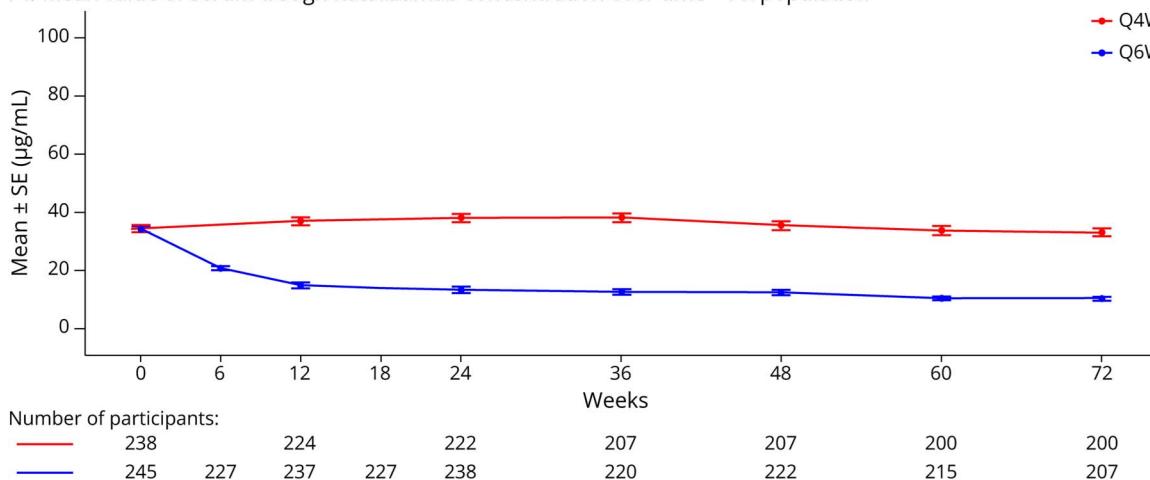
Participants With New or Newly Enlarging T2 Lesions

For the 9 participants who switched to Q6W dosing who showed increases in new or newly enlarging T2 lesions, natalizumab C_{trough} decreased over the first 12 weeks of treatment and stabilized by 24 weeks (Figure 2A). Relatively stable trough natalizumab concentrations were observed for the 8 participants continuing on Q4W who showed new or newly enlarging T2 lesions during the NOVA study. Natalizumab C_{trough} ranged from 1.56–124 µg/mL in Q4W participants and from 0–28.3 µg/mL in Q6W participants with new radiologic disease activity between week 12 and week 72 (Figure 2A).

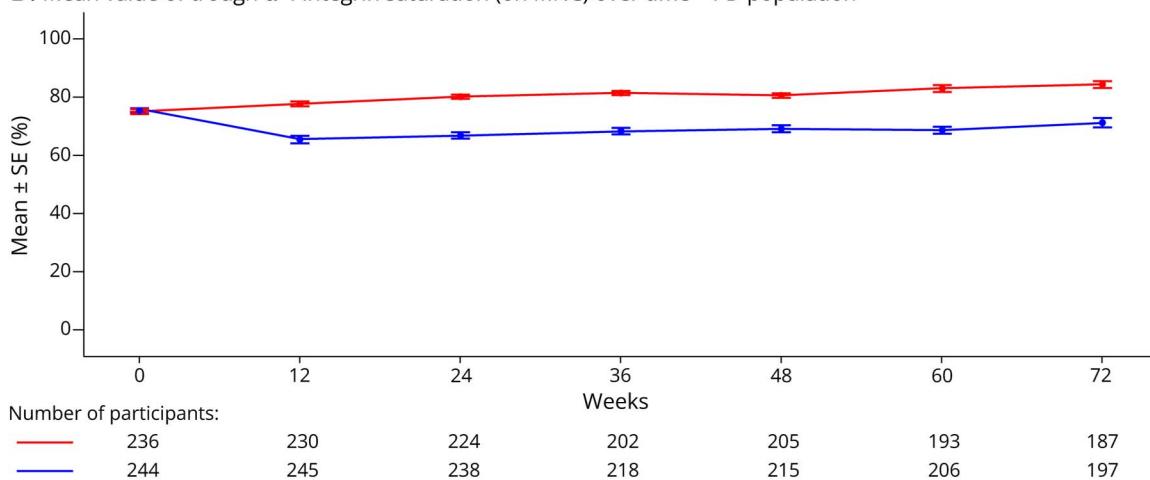
Natalizumab C_{trough} and MNC α4-integrin saturation were examined at times of increased lesion activity in individual participants in the Q6W and Q4W groups (Table 3). In 3 participants in the Q6W group, trough natalizumab levels decreased to 0 µg/mL and α4-integrin saturation decreased to 30.4%, 64.6%, and 23.5% at the time of disease activity. In 6 participants, natalizumab C_{trough} remained >7.5 µg/mL and α4-

Figure 1 Trough Natalizumab Concentration Over Time in the PK Population (A) and Trough $\alpha 4$ Integrin Saturation on MNCs Over Time in the PD Population (B) and GMRs to Baseline of sVCAM-1 (C) for Q6W and Q4W

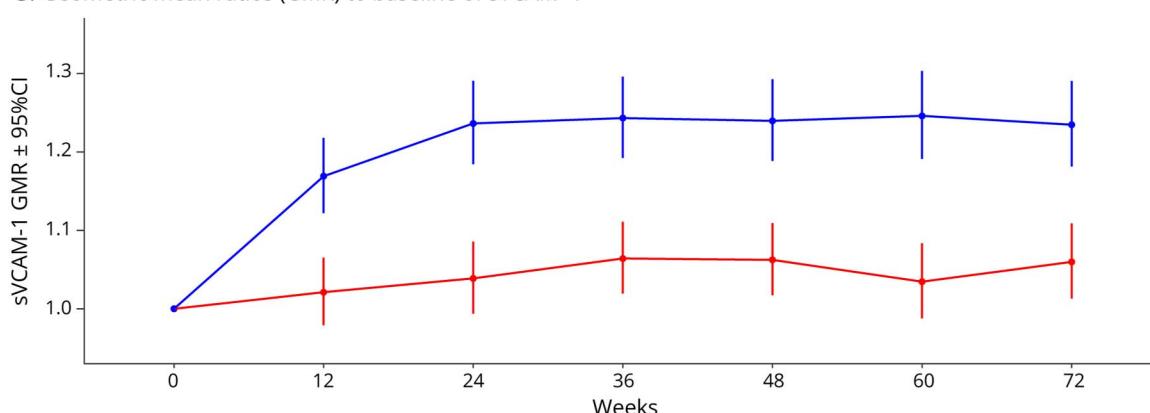
A. Mean value of serum trough natalizumab concentration over time – PK population



B. Mean value of trough $\alpha 4$ integrin saturation (on MNC) over time – PD population



C. Geometric mean ratios (GMR) to baseline of sVCAM-1



(A) Mean (SE) value of serum natalizumab C_{trough} over time for PK population. (B) Mean (SE) value of trough $\alpha 4$ integrin saturation (on MNC) over time for PD population. (C) GMR to baseline of sVCAM-1. Error bars represent 95% confidence intervals. Week 6 and 18 values were only available for the Q6W group. C_{trough} = trough concentrations; GMR = geometric mean ratio; MNC = mononuclear cells; N/NE = new/newly enlarging; PD = pharmacodynamic; PK = pharmacokinetic; Q4W = every 4 weeks; Q6W = every 6 weeks; SE = standard error; sVCAM = serum vascular cell adhesion molecule.

integrin saturation remained >69.1% at the time of lesion detection. In 1 participant in the Q4W group, natalizumab C_{trough} was 1.89 $\mu\text{g}/\text{mL}$ and $\alpha 4$ -integrin saturation was 53.1% at the

time of lesion detection. For 7 participants in the Q4W group, natalizumab C_{trough} remained >9.5 $\mu\text{g}/\text{mL}$ and $\alpha 4$ -integrin saturation remained >65.4% at the time of lesion detection.

Table 2 N/NE T2 Lesions by Natalizumab Concentration and α 4-Integrin Saturation Quartiles at Week 24

	No. of participants included (Q6W, Q4W)	No. of participants with N/NE T2 lesions (Q6W), n (% mITT population) ^a	No. of participants with N/NE T2 lesions (Q4W), n (% mITT population) ^b
Natalizumab concentration at week 24 (μ g/mL) ^c	0–9.64 (99, 10)	3 (1.2)	1 (0.4)
	>9.64–19.65 (74, 35)	3 (1.2)	1 (0.4)
	>19.65–37.65 (31, 78)	1 (0.4)	2 (0.8)
	>37.65 (11, 98)	0	4 (1.6)
% Saturation of α 4-integrin at week 24 ^c	0–65.50 (89, 23)	3 (1.2)	2 (0.8)
	>65.50–75.50 (71, 41)	3 (1.2)	1 (0.4)
	>75.50–82.60 (52, 59)	1 (0.4)	2 (0.8)
	>82.60 (18, 93)	1 (0.4)	3 (1.2)

Abbreviations: mITT = modified intent-to-treat; N/NE = new/newly enlarging; PD = pharmacodynamic; PK = pharmacokinetic; Q4W = every 4 weeks; Q6W = every 6 weeks.

^a n = 242.

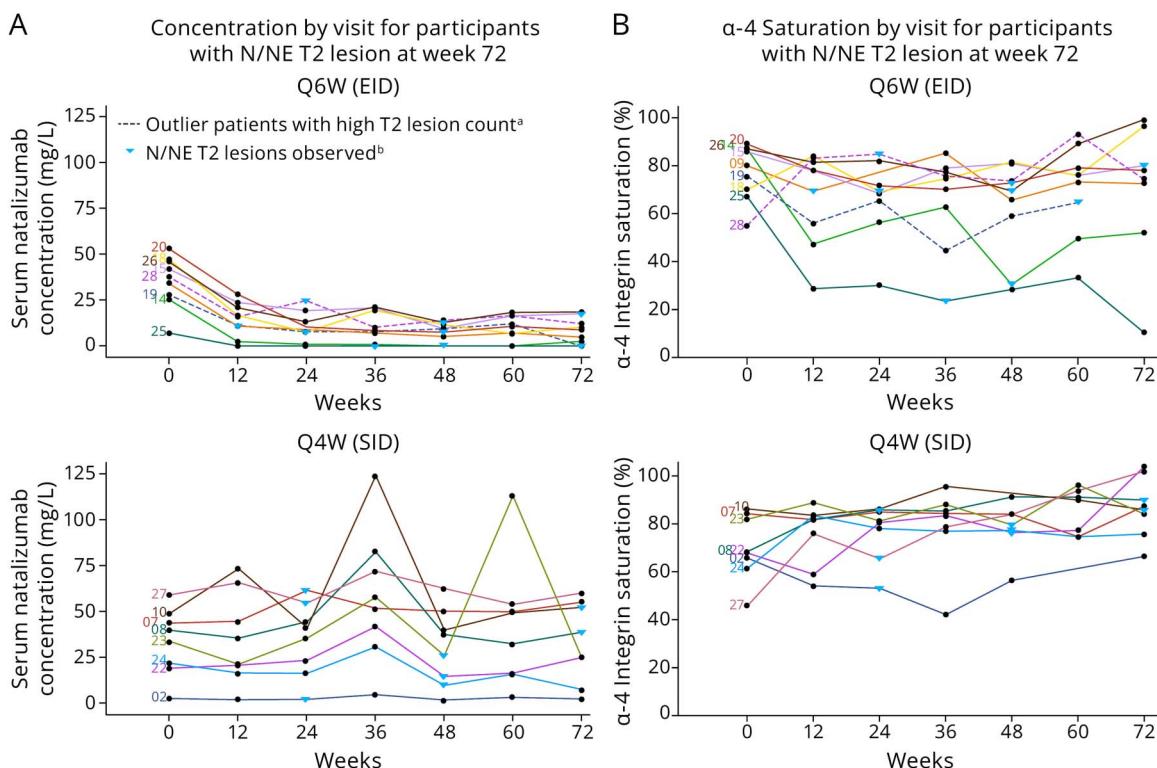
^b n = 247.

^c Quartiles include entire mITT population.

Participants With MS Relapses

Seven participants receiving Q6W dosing and 5 participants receiving Q4W dosing had an MS relapse during the NOVA study.

Trough natalizumab levels for the 7 participants in the Q6W group ranged from 0–70.9 μ g/mL from week 12 to week 72 of treatment, and in the 5 participants in the Q4W group, trough natalizumab

Figure 2 Natalizumab C_{trough} (A) and α 4-Integrin Saturation on MNCs (B) for Participants With N/NE T2 Lesions

^aPatients with high T2 lesion count; one of these participants developed asymptomatic progressive multifocal leukoencephalopathy (participant no: 28) and the other participant discontinued treatment before study completion (participant no: 19). ^bN/NE T2 lesions are shown at the closest timepoint for which serum PK natalizumab or α -4 integrin saturation measurements were made. In the overall population, mean serum natalizumab C_{trough} ranged from 10–21 μ g/mL for Q6W to 33–38 μ g/mL in Q4W participants. Mean trough % α -4 integrin saturation on MNCs remained above 65.5% in Q6W participants and above 77.9% in Q4W participants. C_{trough} = trough concentrations; EID = extended-interval dosing; MNC = mononuclear cells; N/NE = new/newly enlarging; PK = pharmacokinetic; Q4W = every 4 weeks; Q6W = every 6 weeks; SID = standard-interval dosing.

Table 3 PK/PD Levels at Time of Detection of N/NE T2 Lesions in Q6W and Q4W Groups

	Participant no.	Visit when N/NE T2 lesions identified	Natalizumab C _{trough} at time of N/NE T2 lesions (µg/mL)	α4-Integrin saturation on MNC at time of N/NE T2 lesions (%)
Q6W	09	Week 24	10.8 (week 12) ^a	69.3 (week 12) ^a
	14	Week 48	0	30.4
	15	Week 72	17.6	80.0
	18	Week 24	7.68	69.1
	19	Week 72	0	64.6 (week 60) ^a
	20	Week 48	7.5	72.8
	25	Week 48	0	23.5 (week 36) ^a
	26	Week 48	12.8	69.4
Q4W	28	Week 24	24.8	84.8
	02	Week 24	1.89	53.1
	07	Week 24	61.4	84.9
	08	Week 72	38.5	89.9
	10	Week 72	52.1	85.8
	22	Week 48	14.4	76.3
	23	Week 48	25.8	79.5
	24	Week 48	9.52	77.2
	27	Week 24	54.5	65.4

Abbreviations: C_{trough} = trough concentrations; MNC = mononuclear cell; N/NE = new/newly enlarging; PD = pharmacodynamic; PK = pharmacokinetic; Q4W = every 4 weeks; Q6W = every 6 weeks.

^aC_{trough} and/or α4-integrin saturation were not available at the time of lesion identification; data from the visit closest to lesion detection (week in parentheses) are shown.

levels ranged from 11.6–56.2 µg/mL (Figure 3A). Trough α4-integrin saturation ranged from 10.4%–89.8% in Q6W participants with MS relapse and from 54.5%–92.82% in the Q4W participants with relapse (Figure 3B). At the visit before relapse, trough natalizumab ranged from 0–50.8 µg/mL and α4-integrin saturation ranged from 33.2%–100% in these 12 participants (Table 4).

PK/PD in 2 Participants With High T2 Lesion Activity

Two participants receiving Q6W natalizumab had extremely high T2 lesion numbers. One of these participants developed asymptomatic PML (participant no: 28); trough natalizumab levels for this participant remained >10 µg/mL throughout the study (Figure 2A). Trough α4-integrin saturation remained >73.6% in this participant (Figure 2B). The other participant in NOVA with high T2 lesion numbers (participant no: 19) discontinued natalizumab at week 55; trough natalizumab levels for this participant remained >7.7 µg/mL and trough α4-integrin saturation remained >44.5% in this participant through week 60. No data were available at week 72 for this participant.

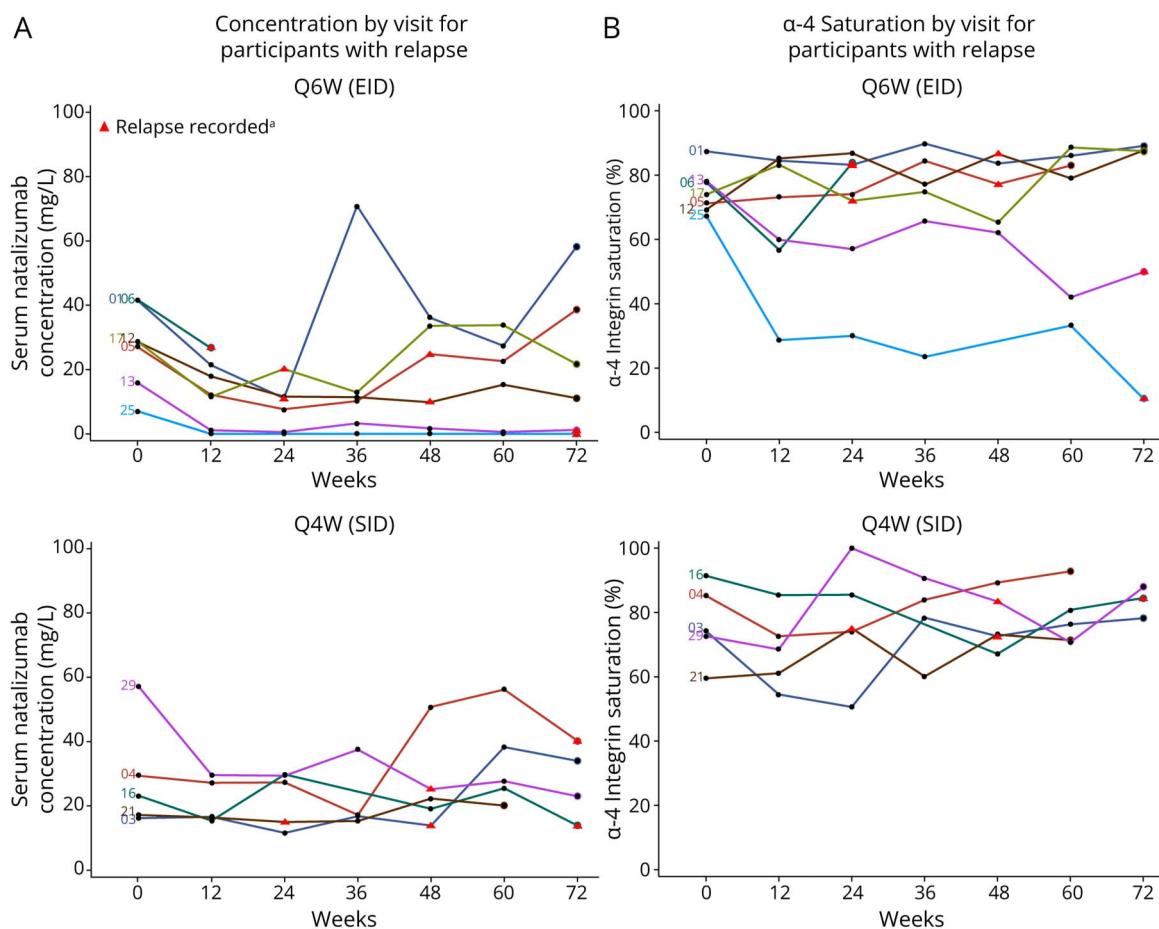
Discussion

By 12 weeks of treatment, PK and PD differences between Q6W and Q4W dosing were observed in the NOVA study. At

a population level, mean trough natalizumab concentrations were decreased by 60%–70% in those participants switched to Q6W dosing in comparison with participants continuing Q4W dosing; this is in line with data from the NEXT MS trial, which found that median natalizumab C_{trough} decreased to approximately 5 µg/mL during Q6W dosing.³³ In this study, mean trough natalizumab remained >10 µg/mL in the Q6W population through week 72 of treatment. Despite the large decrease in trough natalizumab concentration, only a 9%–16% decrease in mean trough α4-integrin saturation was observed in participants receiving Q6W in comparison with Q4W. Mean α4-integrin saturation throughout the study remained >65% in the Q6W group and >76% in the Q4W group. Consistent with previous reports, mean natalizumab C_{trough} negatively correlated with body weight in both Q6W and Q4W groups^{25,26} and mean α4-integrin saturation correlated more strongly with body weight in the Q6W group than in the Q4W group.^{25,26,34}

Clinical efficacy of natalizumab was maintained in the majority of participants in the Q6W dosing group in NOVA.²⁸ Participants in the Q6W group demonstrated mean trough natalizumab levels >10 µg/mL and α4-integrin saturation >65% throughout the NOVA study; thus, the efficacy of Q6W dosing

Figure 3 Natalizumab C_{trough} (A) and $\alpha 4$ -Integrin Saturation on MNCs (B) for All Participants Who Had an MS Relapse



^aRelapses are shown at the time of the visit closest to the recorded relapse. Five participants from the SID arm and 7 participants from the EID arm had relapse. Participant 06 from the EID arm had relapse at week 72, and there is no PD sample for this participant at this timepoint. In the overall population, mean serum natalizumab C_{trough} ranged from 10–21 µg/mL for Q6W and 33–38 µg/mL in Q4W participants. Mean trough % $\alpha 4$ -integrin saturation on MNCs remained above 65.5% in Q6W participants and above 77.9% in Q4W participants. C_{trough} = trough concentrations; EID = extended-interval dosing; MNC = mononuclear cells; PK = pharmacokinetic; Q4W = every 4 weeks; Q6W = every 6 weeks; SID = standard-interval dosing.

in the NOVA study is consistent with the reported half-maximal effective concentration (EC_{50}) of natalizumab of 2.51 µg/mL.³⁵

Previous studies have differed on the minimal level of $\alpha 4$ -integrin saturation required to suppress disease activity. In RESTORE, a phase 4 study of participants with RRMS who had been treated with Q4W natalizumab for ≥ 1 year, participants were randomized to continue on Q4W or to stop natalizumab. An increase in relapse activity was seen as early as 4–8 weeks in participants stopping Q4W natalizumab, and increases in lesion activity were observed at 12 weeks after participants stopped.³⁶ At the 12-week assessment in RESTORE, the mean $\alpha 4$ -integrin saturation remained $>86\%$ for patients continuing on Q4W natalizumab ($n = 39$), whereas mean $\alpha 4$ -integrin saturation had fallen to approximately 30% for patients who had stopped treatment ($n = 109$).³¹ In the TOFINGO study, increased lesion activity was not seen until 12 weeks after the last natalizumab dose, at which time $\alpha 4$ -integrin saturation fell below 20%.³⁷ Among the 17 participants in TOFINGO who experienced relapse activity between

6 and 18 weeks after discontinuation of natalizumab, 12 had $\alpha 4$ -integrin saturation below the time-matched population median, suggesting a relationship between $\alpha 4$ -integrin saturation and relapse activity.³⁷

In this study, neither natalizumab concentration nor $\alpha 4$ -integrin saturation was consistently predictive of clinical or MRI lesion activity at the participant level, and the number of patients with radiologic lesion activity was evenly spread across the quartiles of natalizumab C_{trough} and $\alpha 4$ -integrin saturation at week 24 (Table 2). Only 3 of 9 participants with lesion activity in the Q6W group had levels of natalizumab below the threshold of detection ($LLOQ = 0.5 \mu\text{g}/\text{mL}$) at the time of lesions, and $\alpha 4$ -integrin saturation in the other 6 participants was above the mean $\alpha 4$ -integrin saturation for the overall Q6W group (65.5% at week 12). In 4 of 8 participants with lesion activity in the Q4W group, $\alpha 4$ -integrin saturation was above the mean $\alpha 4$ -integrin saturation for the overall Q4W group (77.9%) at the time of lesion detection. Among participants with relapse activity, $\alpha 4$ -integrin saturation before

Table 4 PK/PD Levels at Time of Relapse in Participants Who Met Criteria for Relapse in Q6W and Q4W Groups

	Participant no.	Timing of relapse	Natalizumab C_{trough} before relapse (µg/mL)	α4-Integrin saturation on MNC before relapse (%)
Q6W	01	Day 126 (18 weeks)	21.4 µg/mL (<i>week 12</i>)	84.5 (<i>week 12</i>)
	05	Day 248 (=35 weeks)	7.65 µg/mL (<i>week 24</i>)	74.1 (<i>week 24</i>)
	06	Day 84 (12 weeks)	26.7 µg/mL (<i>week 12</i>)	56.7 (<i>week 12</i>)
	12	Day 274 (=39 weeks)	11.4 µg/mL (<i>week 36</i>)	77.1 (<i>week 36</i>)
	13	Day 425 (=60 weeks)	0.585 µg/mL (<i>week 60</i>)	42.0 (<i>week 60</i>)
	17	Day 149 (=21 weeks)	11.5 µg/mL (<i>week 12</i>)	83.2 (<i>week 12</i>)
	25	Day 477 (=68 weeks)	0 µg/mL (<i>week 60</i>)	33.2 (<i>week 60</i>)
Q4W	03	Day 197 (=28 weeks)	11.6 µg/mL (<i>week 24</i>)	50.6 (<i>week 24</i>)
	04	Day 416 (=59 weeks)	50.8 µg/mL (<i>week 48</i>)	89.3 (<i>week 48</i>)
	16	Day 354 (=50 weeks)	19.1 µg/mL (<i>week 48</i>)	67.1 (<i>week 48</i>)
	21	Day 64 (=9 weeks)	17.2 µg/mL (<i>baseline</i>)	59.5 (<i>baseline</i>)
	29	Day 223 (=31 weeks)	29.4 µg/mL (<i>week 24</i>)	100 (<i>week 24</i>)

Abbreviations: MNC = mononuclear cell; NTZ = natalizumab; PD = pharmacodynamic; PK = pharmacokinetic; Q4W = every 4 week; Q6W = every 6 weeks. Visit week for when samples were collected for natalizumab C_{trough} and α4-integrin saturation analysis are shown as italicized values in parentheses.

relapse remained above 65.5% in 4 of 7 participants in the Q6W group and above 77.9% in 2 of 5 participants in the Q4W group. It is of note that although 2 participants in NOVA (both in the Q6W arm) were found to have anti-drug antibodies, exhibited neither clinical nor MRI disease activity.

The nonlinear relationship between α4-integrin saturation and natalizumab concentration has been seen previously. A study in patients treated with natalizumab found decreases in α4-integrin saturation levels only in the 3 patients for whom natalizumab concentration fell to <1 µg/mL with plasma exchange. In 9 patients with natalizumab concentration ≥1 µg/mL, α4-integrin saturation remained >60%.³⁸

The main mechanism of action of natalizumab is believed to be the direct blocking of α4-integrin, thereby blocking leukocyte interaction with VCAM-1 on brain endothelium. Blocking of α4-integrin by natalizumab is also known to decrease levels of α4-integrin expression.^{10,25,39-42} Three studies have reported higher levels of trough α4-integrin expression with Q6W dosing than with Q4W dosing.^{25,27,43} The higher number of total surface α4-integrin molecules with Q6W compared with Q4W might lead to an apparent decrease in saturation. This would suggest that with Q6W dosing, more free surface α4-integrin molecules are available for binding to VCAM.

Consistent with this, 2 studies reported an increase in levels of unbound surface α4-integrin on T and B lymphocytes of patients treated with Q6W dosing as compared with those treated with Q4W natalizumab.^{27,43} Higher levels of free α4-integrin observed with Q6W dosing may facilitate better CNS

immunosurveillance and might mediate the decrease in PML risk seen with extended-interval dosing, with an overall maintained efficacy.²²

Natalizumab standard-interval dosing has been associated with decreased sVCAM-1.^{12,31,44} Levels of sVCAM-1 have been shown to negatively correlate with serum natalizumab, and sVCAM-1 is increased in the presence of anti-natalizumab antibodies.¹⁰ The finding that sVCAM-1 is elevated with Q6W dosing in comparison with Q4W dosing is consistent with other studies of extended-interval dosing and sVCAM-1.²⁷ Elevated sVCAM-1 expression with Q6W in comparison with Q4W suggests a higher potential for immunosurveillance with Q6W dosing. As found in other studies, high interindividual variability in sVCAM-1 levels precludes use of sVCAM-1 as a biomarker of disease activity.⁴⁴

The participant with asymptomatic PML (participant no: 28) had known risk factors for PML, including total natalizumab exposure of longer than 2 years (1.1 years Q4W before enrollment and Q6W dosing through week 72 of the study) and an anti-JCV antibody index greater than the limit of assay detection (reported as >2.35) at enrollment and all subsequent assessments. In addition, 25 new or newly enlarging lesions were detected during regularly scheduled MRI scans during the study; PML was confirmed by PCR testing of CSF that detected 351 copies of JCV DNA.²⁸ Twelve months after diagnosis of PML, 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 as part of the PML follow-up.²⁸ This participant had α4-integrin saturation ranging from 55.0% to 98.5% during

this trial, whereas the average α 4-integrin saturation for Q6W participants was approximately 70%. Notably, ≥ 1 other participant was found to have similarly high levels of α 4-integrin saturation without the development of PML.

Limitations of this study include a lack of data on the expression levels of α 4-integrin, which may affect the efficacy of natalizumab and the level of immunosurveillance in the CNS. Moreover, it remains unknown whether the degree of available α 4-integrin receptors or their saturation drives the selective immunosurveillance with the Q6W regimen. In addition, the assay for α 4-integrin saturation had a variability range of $\pm 20\%$. Moreover, the interpretation of the association between lesion activity and PK and PD parameters is limited by the timing of MRI scans and trough PK/PD samplings in NOVA. Because T2-weighted images were obtained at 12-week intervals, detected lesions may have been active at or before the time of scanning. Trough PK and PD values collected at the time of scans may therefore not correspond directly to lesion activity. Although intervisit variability in both C_{trough} and α 4-integrin saturation was relatively uniform for the majority of patients, there were participants who exhibited a higher degree of variability in natalizumab concentrations or saturations across time; this may have been due to PK and PD values being much lower in Q6W vs Q4W, which could be closer to the assay detection limit and therefore increase variability. Finally, patients in the NOVA study were not surveyed for a “wearing-off” effect, as has been reported previously at the end of the dosing interval.⁴⁵ Therefore, no conclusions can be drawn from this study regarding the PK and PD parameters and the wearing-off effect. Of note, trough natalizumab concentration and α 4-integrin saturation were comparable between participants with worsening on the Neuro-QoL fatigue scale and the overall population in both the Q6W and Q4W groups in the NOVA study.

Natalizumab Q6W dosing demonstrates PK and PD differences from Q4W dosing, as measured by trough natalizumab concentrations and α 4-integrin saturations, but maintains their therapeutic levels. The highly variable nature of natalizumab concentration and α 4-integrin saturation levels makes direct correlation with individual patient outcomes difficult. Moreover, the α 4-integrin saturation level did not consistently predict disease activity in the limited number of patients with clinical or MRI activity. In 3 of 9 participants treated with Q6W who demonstrated lesion activity, trough natalizumab levels fell below the level of detection of the assay, and α 4-integrin saturation levels fell below the mean level of α 4-integrin saturation in the Q6W group (65.5%). However, the variable relationship between trough natalizumab concentration or α 4-integrin saturation with clinical disease activity suggests that trough PK and PD measurements should be interpreted with caution in clinical practice. In conclusion, Q6W dosing of natalizumab is an effective treatment that may help mitigate the PML risk while maintaining clinical efficacy in the majority of patients, and this analysis provides an exploration of the pharmacokinetics and pharmacodynamics underlying the maintenance of efficacy with Q6W dosing.

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Continued

Appendix (continued)

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Douglas L. Arnold, MD	Montréal Neurological Institute, McGill University; NeuroRx Research, Montréal, Quebec, Canada	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
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Gary R. Cutter, PhD	University of Alabama at Birmingham, School of Public Health	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data
Gavin Giovannoni, MBBCh, PhD, FCP (Neurol., SA), FRCP, FRCPPath	Blizard Institute, Barts and The London School of Medicine and Dentistry; Queen Mary University of London, United Kingdom	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
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Continued

Appendix (continued)

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