

α 4-integrin receptor desaturation and disease activity return after natalizumab cessation

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ABSTRACT

Objective: To describe the time course of α 4-integrin receptor desaturation and disease activity return in patients with relapsing-remitting MS who discontinued natalizumab and to investigate baseline and on-study predictors for the recurrence of disease activity.

Methods: In the course of TOFINGO, a 32-week, patient- and rater-blinded multicenter, parallel-group study, we performed MRI, counted relapses, and measured α 4-integrin receptor occupancy (RO) at baseline and 8, 12, 16, 20, and 24 weeks. The relationship between RO and total number of new T1 gadolinium-enhancing (Gd+) lesions was modeled using Poisson linear regression.

Results: Patients (N = 142) were randomized (1:1:1) to 8-, 12-, or 16-week washout (WO) groups. At randomization, the median RO in the 8-, 12-, and 16-week WO groups was 94.5%, 92.4%, and 90.9%, which declined to 79.8%, 30.7%, and 8.7% after 8, 12, and 16 weeks of WO, respectively. The percentage of patients with new T1 Gd+ lesions increased with longer WO period before commencing fingolimod: 2.1% (8 weeks), 9.1% (12 weeks), and 50.0% (16 weeks). Overall, 71% of patients with first relapse between weeks 6 and 18 had RO values below the time-matched population median. Higher T2 lesion volume (LV) at baseline predicted a higher number of new T1 Gd+ lesions.

Conclusions: A faster decline in natalizumab RO, longer WO period, and higher T2 LV at baseline were associated with an increased risk for return of inflammatory disease activity. These results provide a mechanistic rationale and, together with the main outcomes of the TOFINGO study, support initiation of fingolimod within 8 weeks of natalizumab discontinuation.

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GLOSSARY

FACS = fluorescence-assisted cell sorter; **Gd+** = gadolinium enhancing; **LNI** = last natalizumab infusion; **LV** = lesion volume; **RO** = receptor occupancy; **RRMS** = relapsing-remitting MS; **WO** = washout.

Patients with relapsing-remitting MS (RRMS) often discontinue natalizumab treatment, most frequently due to the risk of developing progressive multifocal leukoencephalopathy.¹ Natalizumab discontinuation leads to recurrence of clinical and radiologic disease activity within 4 months of discontinuation.² Natalizumab primarily acts via blocking the α 4-integrin receptor and preventing potentially autoaggressive immune cell transition through the blood-brain barrier.² Hence, α 4-integrin receptor desaturation should be a direct measure of its therapeutic activity. No systematic studies have reported the relationship between α 4-integrin receptor occupancy (RO) and recurrence of disease activity following natalizumab discontinuation.

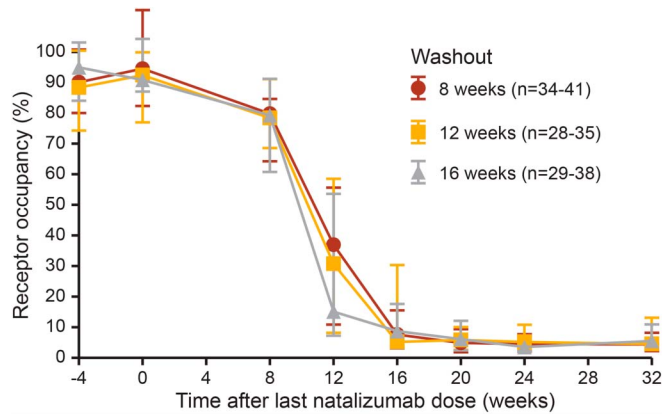
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Figure 1

Receptor desaturation after the last natalizumab dose. Median α 4-integrin receptor occupancy by the WO group at screening (–4 weeks), randomization (week 0), and during drug WO from weeks 8 to 32. Bars represent the IQR. A total of 137 patients (96.5%) provided at least 1 evaluable receptor occupancy measurement. IQR = interquartile range; WO = washout.

In daily practice, fingolimod 0.5 mg is frequently considered a suitable option for treatment of patients with RRMS after natalizumab discontinuation, but the optimal washout (WO) period remains unknown.³ TOFINGO, a randomized double-blind study, addressed the relationship between the duration of natalizumab WO before fingolimod initiation and recurrence of disease activity.⁴ The results of TOFINGO suggested that better control of disease activity is achieved if fingolimod is initiated within 8–12 weeks after natalizumab discontinuation, compared with 16 weeks.⁴ In the course of TOFINGO, blood samples were collected to determine α 4-integrin RO. We investigated the time course of α 4-integrin receptor desaturation and its temporal relation to MRI and clinical disease activity. Other possible baseline and on-study predictors for the recurrence of disease activity were also assessed. The analysis was aimed to provide a mechanistic rationale for the timing of switch from natalizumab to fingolimod in patients with RRMS.

METHODS Study design and patients. TOFINGO (ClinicalTrials.gov identifier: NCT01499667), a 32-week, patient- and rater-blinded, randomized, multicenter, parallel-group study, included 142 patients with RRMS who received their last natalizumab infusion (LNI; 300 mg IV) within ± 7 days of randomization. Details of the study have been published previously.⁴ Briefly, patients with RRMS aged 18–65 years who had received natalizumab treatment for ≥ 6 months immediately before screening were randomized 1:1:1 to one of the following 3

natalizumab WO groups before fingolimod therapy was initiated: 8-week WO (no treatment for 8 weeks since LNI followed by 24 weeks of treatment with oral fingolimod 0.5 mg); 12-week WO (no treatment for 8 weeks since LNI and placebo for 4 weeks followed by 20 weeks of treatment with fingolimod); or 16-week WO (no treatment for 8 weeks since LNI and placebo for 8 weeks followed by 16 weeks of treatment with fingolimod). All patients in the study population provided written informed consent. TOFINGO involved 44 study centers and was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Study assessments. Natalizumab serum concentration and α 4-integrin RO assessment. Patient visits were scheduled at screening, randomization, and weeks 8, 12, 16, 20, 24, and 32. At each visit, the serum concentration of natalizumab was determined using a validated ELISA method (lower quantification limit, 0.1 μ g/mL). Venous blood samples were collected at all visits to determine α 4-integrin RO (%) using a fluorescence-assisted cell sorter (FACS) analysis. Cells were stained with matched isotype control antibodies to determine the specificity of α 4 integrin and natalizumab staining. The signal obtained with matched isotype controls was used to set the threshold for CD49d-specific α 4-integrin staining and/or antinatalizumab staining with anti-IgG4 reagent. Natalizumab (final concentration, 10 μ g) was spiked in 150 μ L whole blood samples. The samples were incubated with detection antibodies, CD49d-specific α 4-integrin staining, CD3 staining, and/or antinatalizumab staining with anti-IgG4 reagent, for 15 minutes in the dark at 23°C. BD FACS lysing solution (2 mL) was added to the sample and incubated for 15 minutes in the dark at 23°C. The samples were then pelleted by centrifugation and washed twice with 2 mL of FACS Flow Sheath Fluid. One hundred thousand nucleated events were then analyzed on FACS Calibur, using FSC/CD3 staining for T-lymphocyte gating and determination of α 4-integrin and natalizumab-positive CD3 cells and median fluorescence intensity of α 4-integrin and natalizumab staining.

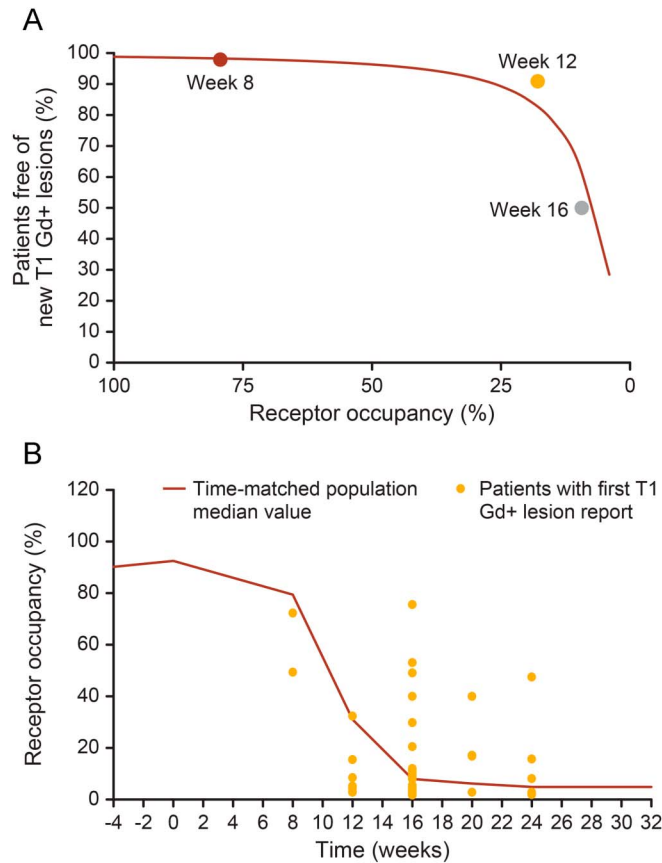
Assessment of disease recurrence. MRI assessments (T1 gadolinium-enhancing [Gd+] lesion counts; new T2 lesions) were conducted at baseline and at weeks 8, 12, 16, 20, and 24. All relapses during the study (both confirmed and not confirmed) were counted.

The association between RO and freedom from T1 Gd+ lesions was explored using median-effect analysis.⁵ The relationship between RO, patient-specific covariates, and total number of T1 Gd+ lesions was modeled using Poisson linear regression (e-Methods at Neurology.org/nn).

RESULTS Patient disposition and baseline characteristics.

Overall, 142 patients were randomized (8-week WO: n = 50; 12-week WO: n = 42; and 16-week WO: n = 50) and analyzed according to the WO group assigned at randomization. As reported previously, baseline demographic and MS characteristics were well balanced across the WO groups.⁴ Of the 142 patients, 137 (96.5%) provided ≥ 1 evaluable RO measurement. The percentage of patients providing evaluable RO values at each visit averaged 72% (range, 58%–83%). The median serum concentration of natalizumab at the end of WO for the 8-, 12-, and 16-week WO groups was 0 μ g/mL, with a range of 0–1.07, 0–0.383, and 0–0.308 μ g/mL,

Figure 2



(A) Median-effect modeling of receptor occupancy and freedom from T1 Gd+ lesions. Scatter plot of the percentage of patients free from T1 Gd+ lesions vs median α 4-integrin receptor occupancy for the 8-, 12-, and 16-week WO groups. Only patients not yet on fingolimod were included in the analysis. The curve represents the fit of the median-effect model to the data. (B) Median receptor occupancy and first reporting of T1 Gd+ lesions. Scatter plot showing the α 4-integrin receptor occupancy at the visit when first T1 Gd+ lesions were reported in the context of the population median receptor desaturation curve. Gd+ = gadolinium enhancing; WO = washout.

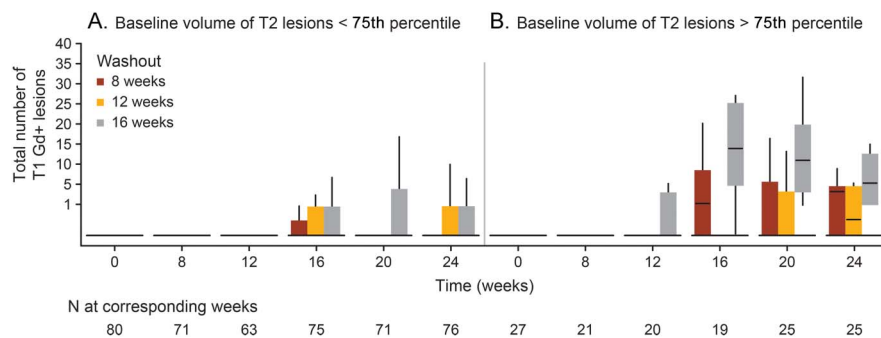
respectively (table e-1), indicating that in most patients, the drug was eliminated from blood in the first 2 months after LNI.

RO and first report of T1 Gd+ lesions or first relapse. At randomization, the median RO was 94.5%, 92.4%, and 90.9% in the 8-, 12-, and 16-week WO groups, respectively. Overall, the median RO declined to 79.8%, 30.7%, and 8.7% at weeks 8, 12, and 16, respectively (figure 1). MRI scans were available for 121 patients. As the length of the WO period before fingolimod initiation increased, the percentage of patients free from T1 Gd+ lesions decreased, although the relationship did not reach statistical significance (median-effect analysis, $r^2 = 0.891$, $p = 0.214$; figure 2A). Up to week 8, the median RO was relatively high (79.4%), and the incidence of new T1 Gd+ lesions was 2.1% ($n = 2$). Both these patients with new T1 Gd+ lesions had ROs below the population median. By week 12, the median RO had declined to 17.9%, and the percentage of patients with new T1 Gd+ lesions was 9.1%. At week 16, the median RO was 9.35%, and the percentage of patients with new T1 Gd+ lesions was the highest (50.0%; figure 2B). Similar results were observed with RO and the first reporting of new/enlarging T2 lesions (data not shown). Overall, 31 patients had at least 1 relapse, 26 of whom had RO data available. Of these, 17 had the first relapse between weeks 6 and 18. The majority ($n = 12$; 71%) of these 17 patients had RO values below the time-matched population median (figure e-1).

Model-based prediction of T1 Gd+ lesions and new/enlarging T2 lesions. High T2 lesion volume (T2 LV) at baseline was associated with a higher number of T1 Gd+ lesions after LNI. As shown in figure 3, patients with baseline T2 LV < 75th percentile experienced fewer new T1 Gd+ lesions than did those with a baseline T2 LV > 75th percentile. This was observed in all WO groups, most noticeable in the 16-week WO group (e-Results).

DISCUSSION Patients with RRMS frequently switch to fingolimod after natalizumab discontinuation to

Figure 3



Total number of T1 Gd+ lesions when the baseline volume of T2 lesions was less than (A) and greater than (B) the 75th quartile. Bottom of box is Q1 and the top of the box is Q3; upper whiskers are $1.5 \times$ IQR of the hinge; lower whiskers are $1.5 \times$ IQR of the hinge. Gd+ = gadolinium enhancing; IQR = interquartile range.

maintain adequate disease control.^{6,7} Several observational studies and the randomized rater- and patient-blinded TOFINO study have suggested that a natalizumab WO period of >12 weeks is associated with higher risk of disease reactivation,^{4,6} and that a WO period of <12 weeks is associated with lower MRI activity without being associated with increased risks.^{4,6,8} Our study also suggests that in ~80% of patients, disease recurrence (new T1 Gd+ lesions) is low up to week 12 during the WO period before fingolimod initiation. Significant disease return is observed between weeks 12 and 16 of the WO period at an RO of <20% (figure 2A). This might indicate that an RO lower than 90% is still sufficient to suppress disease activity. However, recent small studies have shown that some patients may relapse or have recurrence of MRI activity within 8 weeks of LNI, indicating possibilities of an early switch to fingolimod.^{9,10} While a long WO period between LNI and fingolimod initiation may increase the risk of disease recurrence, early initiation of fingolimod may result in additive effects on protective immune functions.

Despite a small sample size, the TOFINO study showed distinct numerical trends in pharmacodynamics, imaging, and clinical outcomes, which can help determine the risk of disease recurrence after natalizumab discontinuation in individual patients.

Our results showed that while natalizumab is cleared from the serum within 8–12 weeks after discontinuation, it still occupies $\alpha 4$ -integrin receptors, thus contributing to disease activity control. In view of the association of declining RO with recurring disease activity, RO may emerge as a more plausible measure for an individualized choice of the optimal WO period. Ideally, decisions regarding the optimal WO period would also take the individual risk profile into account. In addition to prenatalizumab relapse rate and MRI activity, which have previously been identified as risk factors, our study suggests that a high baseline T2 LV defines a patient population with an increased risk of recurrence after discontinuation.

AUTHOR CONTRIBUTIONS

The study was designed by the sponsor, Novartis Pharma AG, in collaboration with the steering committee. Data were collected by the investigators and analyzed by the sponsor. Novartis contributed to the interpretation of the study. All authors had full access to the data and had final responsibility for the contents and decision to submit for publication. T. Derfuss and J.M. Kovarik: design and conceptualization of the study, analysis and interpretation of the data, and drafting and revising the manuscript. L. Kappos: analysis and interpretation of the data and revising the manuscript. M. Savelieva: design and conceptualization of the study, statistical analysis and interpretation of the data, and revising the manuscript. H. Wiendl: design and conceptualization of the study, analysis and interpretation of the data, and drafting and revising the manuscript. Y. Zhang: design and conceptualization of the study, statistical analysis and interpretation of the data, and revising the manuscript. D. Tomic: design and conceptualization

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DISCLOSURE

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