

The incidence and significance of anti-natalizumab antibodies

Results from AFFIRM and SENTINEL



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ABSTRACT

Objective: To determine the incidence and clinical effects of antibodies that develop during treatment with natalizumab.

Methods: In two randomized, double-blind, placebo-controlled studies (natalizumab safety and efficacy in relapsing remitting multiple sclerosis [MS, AFFIRM] and safety and efficacy of natalizumab in combination with interferon β -1a [INFB1a] in patients with relapsing remitting MS [SENTINEL]) of patients with relapsing multiple sclerosis, blood samples were obtained at baseline and every 12 weeks to determine the presence of antibodies against natalizumab. Antibodies to natalizumab were measured using an ELISA. Patients were categorized as "transiently positive" if they had detectable antibodies ($\geq 0.5 \mu\text{g/mL}$) at a single time point or "persistently positive" if they had antibodies at two or more time points ≥ 6 weeks apart.

Results: In the AFFIRM study, antibodies were detected in 57 of 625 (9%) of natalizumab-treated patients: Twenty (3%) were transiently positive and 37 (6%) were persistently positive. Persistently positive patients showed a loss of clinical efficacy as measured by disability progression ($p \leq 0.05$), relapse rate ($p = 0.009$), and MRI ($p \leq 0.05$) compared with antibody-negative patients. In transiently positive patients, full efficacy was achieved after approximately 6 months of treatment, the time when patients were becoming antibody negative. The incidence of infusion-related adverse events was significantly higher in persistently positive patients. Results of SENTINEL were similar to AFFIRM, except with regard to sustained disability progression; differences between persistently positive and antibody-negative patients were not statistically significant.

Conclusions: The incidence of persistent antibody positivity associated with natalizumab is 6%. Reduced clinical efficacy is apparent in persistently positive patients. Patients with a suboptimal clinical response or persistent infusion-related adverse events should be considered for antibody testing. *Neurology*® 2007;69:1391-1403

GLOSSARY

BLQ = below the limit of quantification; **EDSS** = Expanded Disability Status Scale; **Gd+** = gadolinium enhancing; **IFN β 1a** = interferon β -1a; **MS** = multiple sclerosis; **MSFC** = multiple sclerosis functional composite; **OD** = optical density.

Natalizumab (TYSABRI; Biogen Idec and Elan Pharmaceuticals, Inc.) is the first α_4 -integrin antagonist in a new class of selective adhesion molecule inhibitors for the treatment of relapsing multiple sclerosis (MS). Natalizumab interferes with the pathogenesis of MS by selectively inhibiting α_4 -integrin-mediated adhesion of lymphocytes to the endothelium, thus preventing migration of leukocytes across the blood-brain barrier into the CNS.¹ Similar to other protein therapeutics agents,² antibodies to natalizumab may

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develop during treatment and increase drug clearance or hinder drug binding to α_4 -integrin, the target receptor. Both effects have the potential to diminish the efficacy of natalizumab.

Two randomized, double-blind, placebo-controlled, phase 3 clinical trials were conducted to evaluate the efficacy and safety of natalizumab as monotherapy (the AFFIRM study)³ and as add-on therapy to interferon β -1a (IFN β -1a; AVONEX; Biogen Idec) (the SENTINEL study)⁴ for the treatment of relapsing MS. In AFFIRM, natalizumab reduced the risk of sustained disability progression by 42% (hazard ratio = 0.58; 95% CI: 0.43, 0.77; $p < 0.001$) and reduced the annualized relapse rate by 68% ($p < 0.001$) over 2 years compared with placebo.³ In SENTINEL, natalizumab added to IFN β -1a reduced the risk of sustained disability progression by 24% (hazard ratio = 0.76; 95% CI: 0.61, 0.96; $p = 0.02$) and reduced the annualized relapse rate by 55% ($p < 0.001$) over 2 years compared with IFN β -1a alone.⁴

Herein we report the incidence and clinical effects of antibodies that developed over 2 years of natalizumab therapy among patients enrolled in the AFFIRM and SENTINEL studies.

METHODS Patients and study design. Details of the AFFIRM and SENTINEL studies have been previously published.^{3,4}

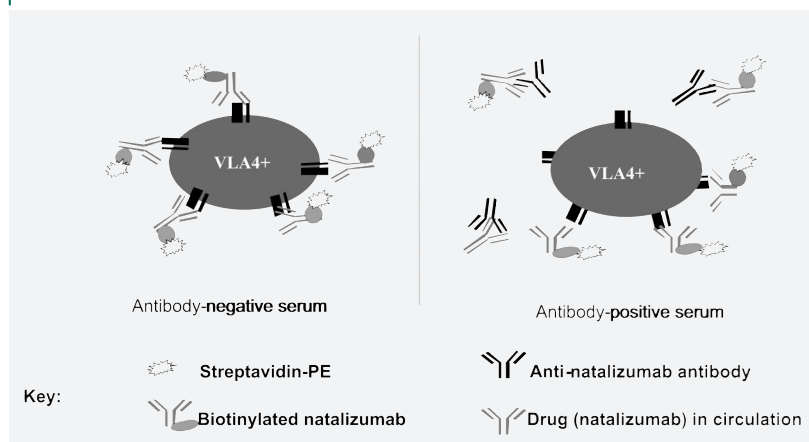
In both studies, blood samples were obtained at baseline before administration of the first dose of study medication and then every 12 weeks for pharmacokinetic analyses and determination of the immunogenicity of natalizumab. All serum samples were stored at -70°C until assayed. An ELISA measured serum concentrations of antibodies against natalizumab and a flow cytometric assay was used to determine the capacity of those antibodies to block natalizumab biologic activity. There was a high concordance of the results between the two assays. Based on the concordance between the assays and based on reduced interference by circulating drug in the ELISA, the results of the ELISA were used in the analyses reported in this article. On the basis of antibody measurements, patients were defined as being antibody negative (i.e., $<0.5\ \mu\text{g/mL}$ at all postbaseline visits), transiently positive ($\geq 0.5\ \mu\text{g/mL}$ at a single postbaseline visit prior to the final postbaseline visit), or persistently positive ($\geq 0.5\ \mu\text{g/mL}$ at two or more postbaseline visits that were at least 42 days apart or a single time point with no follow-up samples).

Natalizumab antibody detection by ELISA. A sandwich/bridging ELISA method was used to determine the concentration of natalizumab antibodies in serum relative to a monoclonal anti-natalizumab antibody calibrator (12C4). Wells of 96-well microtiter plates (Corning Laboratories, Corning, NY) were coated with $100\ \mu\text{L}$ /well of natalizumab diluted to $0.25\ \mu\text{g/mL}$ in coating buffer ($0.1\ \text{M}$ sodium phosphate, $2\ \mu\text{g/mL}$ bovine serum albumin [final concentration], pH 8.5) and blocked with Tris-sucrose blocking buffer ($0.1\ \text{M}$ Tris, pH 7.7, 20% sucrose) to reduce nonspecific protein binding. A murine monoclonal antibody against natalizumab (i.e., clone 12C4; Elan Pharmaceuticals, San Francisco, CA) was used for quality control and in the generation of the standard curve. The standard curve consisted of six points at 50, 100, 150, 200, 250, and 300 ng/mL (12C4 diluted in 10% serum). Addition of standards, controls, and serum samples (diluted 1:10) to the wells resulted in the capture of natalizumab antibodies by the coated natalizumab. Next, biotinylated natalizumab was added to bind the captured antibodies and was, in turn, bound by a streptavidin-alkaline phosphatase conjugate (Boehringer Ingelheim, Ridgefield, CT). Addition of a p -nitrophenyl phosphate substrate (Moss, Inc., Pasadena, MD) enabled detection of the captured antibodies through the generation of a colored reaction product, measured at a wavelength of 405 nm, using a plate reader (Molecular Devices, Sunnyvale, CA). The optical density (OD) value was proportional to the concentration of antibody in the standards, controls, and samples. The OD value of each standard point vs its concentration was plotted to fit the standard curve to a four-parameter logistic curve. The concentration of natalizumab antibodies in each quality control level was determined by interpolation from the standard curve. A sample was considered positive if its OD was greater than or equal to the minimum standard OD (standard 6 = 50 ng/mL), and the sample was considered negative if its OD was less than the minimum standard OD.

Positive samples were next evaluated in a titration assay. The final titer of total antibodies in serum samples was calculated from the dilution of the serum that matched the OD value of the monoclonal antibody calibrator set as the cut-point in the assay. Antibody concentration was calculated by multiplying the titer of the sample at the minimum standard by the calibrator concentration ($0.05\ \mu\text{g/mL}$). Analysis of samples from the AFFIRM and SENTINEL studies in the ELISA and titration assay was performed in the Clinical Science and Technology Laboratories of Biogen Idec, Inc. (Cambridge, MA, and Research Triangle Park, NC). All assays were performed by technicians masked to treatment assignment.

Flow cytometric assay to detect natalizumab-blocking antibodies. Serum samples positive for antibodies to natalizumab ($0.5\ \mu\text{g/mL}$), but negative for detectable natalizumab concentrations ($<0.25\ \mu\text{g/mL}$), were evaluated in a semiquantitative cell-based blocking bioassay. A pictorial representation of the blocking assay is depicted in figure 1. The blocking assay detected the ability of natalizumab-specific antibodies to block the binding of biotinylated natalizumab to its cognate α_4 receptor. The blocking assay utilized a two-tiered approach, a screening assay, using 1:10 diluted samples, and a titration assay, in which samples were diluted from 1:10 to 1:640 in normal human serum for analysis. When titers of ≥ 640 were found, higher initial dilutions were used for further analysis.

Figure 1 Pictorial representation of the semiquantitative cell-blocking bioassay



For the assay, biotinylated natalizumab was incubated with a positive quality control (normal human serum), negative (normal human serum) serum, or patient samples, followed by addition of K562 α 4 cells. Washed and resuspended cells were then incubated with streptavidin-phycoerythrin (PE) conjugate. After incubation, cells were washed and the cell-associated fluorescence was measured by flow cytometry. Cell-bound biotinylated natalizumab was bound by phycoerythrin-conjugated streptavidin and the cell-bound fluorescence was detected and quantified by flow cytometry. VLA = very late activation antigen.

For the assay, biotinylated natalizumab was incubated for 30 minutes at 4 °C with either positive quality control (normal human serum [Biochemed, Winchester, VA], spiked with 12C4 mAb at the IC₅₀ level and 100% inhibition level) or negative (normal human serum) serum or patient samples, followed by addition of K562 α 4 cells (2×10^4 cells/well in 100 μ L) expressing the $\alpha_4\beta_1$ integrin and incubated for 30 minutes at 4 °C. Washed and resuspended cells were then incubated with streptavidin-phycoerythrin conjugate (Becton Dickinson, San Jose, CA) for 15 minutes at 4 °C in the dark. After incubation, cells were washed and the cell-associated fluorescence was measured by flow cytometry. Cell-bound biotinylated natalizumab was bound by phycoerythrin-conjugated streptavidin and the cell-bound fluorescence was detected and quantified by flow cytometry. Natalizumab-positive serum samples that reduced the fluorescence intensity below a predetermined lower limit were considered to be positive for natalizumab-specific blocking antibodies.

Statistical analysis. Patients were assigned to one of four categories for analyses: placebo or IFN β -1a alone, antibody negative (<0.5 μ g/mL at all postbaseline visits), transiently positive (≥ 0.5 μ g/mL at a single postbaseline visit prior to the final postbaseline visit), or persistently positive (≥ 0.5 μ g/mL at two or more postbaseline visits that were at least 42 days apart or a single time point with no follow-up samples). Primary and secondary end points were analyzed by antibody status to assess the effect of antibodies on efficacy. Sustained disability progression was defined as a ≥ 1.0 -point increase in Expanded Disability Status Scale (EDSS) score, relative to baseline EDSS score of at least 1.0, which was sustained for 12 weeks, or alternatively, as a ≥ 1.5 -point increase in EDSS score from a baseline EDSS score of 0.0, which was sustained for 12 weeks. The cumulative proportion of patients with sustained disability progression over 2 years was analyzed using the Cox proportional hazards model adjusted for baseline EDSS and age. Annualized relapse rate was analyzed using Poisson regression adjusted for

baseline relapse rate, age, baseline presence/absence of gadolinium-enhancing (Gd+) lesions, and baseline EDSS. MRI lesion numbers were analyzed using logistic regression and lesion volumes were analyzed using Friedman analysis of covariance adjusted for the baseline volume. Spearman correlation coefficients were calculated between maximum antibody titer and serum natalizumab concentration. The incidence of adverse events by antibody status was analyzed using the Fisher exact test to assess the effects of antibodies on safety.

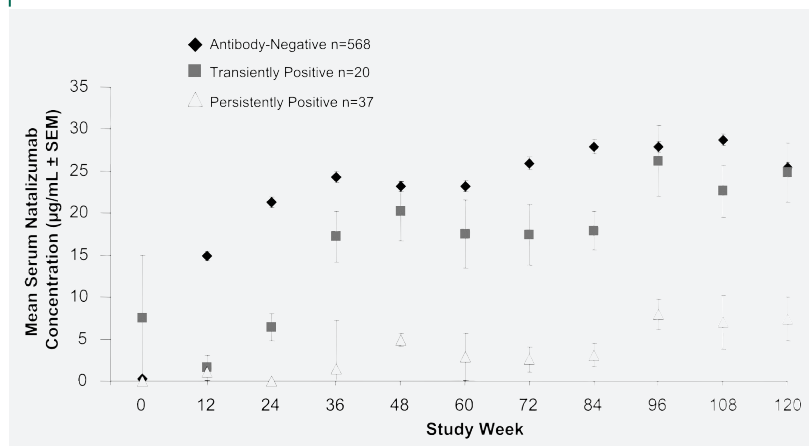
RESULTS Patients. In the AFFIRM study population of 942 patients, 627 were randomly assigned to natalizumab and 315 to placebo.³ In the SENTINEL study, 589 patients received add-on therapy (natalizumab plus IFN β -1a) and 582 received placebo plus IFN β -1a.⁴ The treatment groups were generally well matched on baseline characteristics within each study.^{3,4}

All but two natalizumab-treated patients in the AFFIRM study ($n = 625$) and four in the SENTINEL study ($n = 585$) had at least one post-baseline test for antibodies to natalizumab and were included in the analyses.

Incidence of antibody development during natalizumab therapy. In AFFIRM, 568 of the 625 natalizumab-treated patients (91%) were antibody negative throughout the 2-year study. Fifty-seven of the 625 natalizumab patients (9%) tested positive for antibodies at any time during the study; of these 57 patients, 20 (3%) were transiently positive (titers ranged from 0.59 to 9.18 μ g/mL) and 37 (6%) were persistently positive (titers ranged from 1.01 to 600 μ g/mL). Of all patients in AFFIRM who developed antibodies, 50 (88%) exhibited detectable antibodies by week 12, an additional 5 (9%) had detectable antibodies by week 24, and 1 (2%) more had antibodies by week 36. One patient had a single antibody-positive test at week 60, was antibody negative at week 72, and remained negative thereafter for the rest of the study. Of the patients who were transiently antibody positive at 24 weeks, all had reverted to antibody-negative status by the end of the study. Nineteen persistently positive patients were treated for the full 2 years of the study, at which time, 10 (53%) of those patients had reverted to antibody-negative status. All patients exhibiting antibodies by ELISA were also antibody positive in the blocking assay.

The incidence and timing of development of antibodies in SENTINEL were consistent with AFFIRM. In SENTINEL, 515 of the 585 natalizumab-treated patients (88%) were antibody negative throughout the 2-year study. Seventy of the 585 natalizumab patients (12%) tested

Figure 2 Trough natalizumab concentrations in sera of antibody-negative, transiently positive, and persistently positive patients over 2 years in the AFFIRM study



The value observed in transiently positive patients at baseline (week 0) was due to one patient who had natalizumab concentrations above the limit of quantification.

positive for antibodies at any time during the study; of these 70 patients, 32 (5%) were transiently positive (titers ranged from 0.81 to 33.6 µg/mL) and 38 (6%) were persistently positive (titers ranged from 0.93 to 2200 µg/mL). Detection of antibodies was apparent within the first 12 weeks of treatment in 96% of patients and by 24 weeks in an additional 4%. Twenty-four persistently positive patients were treated for the full 2 years of the study, at which time 10 (42%) of those patients had reverted to antibody-negative status.

In SENTINEL, the incidence of neutralizing antibodies to IFNβ-1a at any time during the study was 3% in each treatment group. The incidence of new neutralizing antibodies to IFNβ-1a was 1% (7/549) for add-on therapy (natalizumab plus IFNβ-1a) and <1% (4/545) for IFNβ-1a alone.⁴

Effect of antibodies on serum natalizumab concentrations. The presence of antibodies was correlated with a reduction in trough serum concentrations of natalizumab (figure 2). At week 12, mean serum natalizumab concentration was 14.9 µg/mL in antibody-negative patients compared with 1.3 µg/mL in the antibody positive patients; there was no difference between transiently positive and persistently positive patients at this timepoint, as natalizumab concentrations were below the limit of quantification (BLQ) in most patients. At week 24, all persistently positive patients remained BLQ, whereas mean natalizumab concentrations increased to 6.4 µg/mL in transiently positive patients and 21.3 µg/mL in antibody-negative patients. By week 36, mean na-

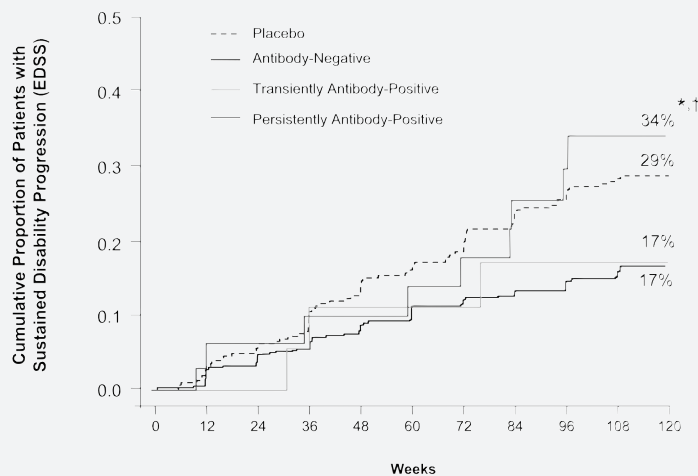
talizumab concentration was <2 µg/mL in persistently positive patients, 17.2 µg/mL in transiently positive patients, and 24.3 µg/mL in antibody-negative patients. The results at all subsequent timepoints to week 120 were consistent with those at week 36. However, there was an increase in the proportion of persistently positive patients with detectable levels of natalizumab by the end of the study, which likely reflects the group of persistently positive patients (16/37) who seroconverted to antibody-negative status prior to the end of the study.

Effect of natalizumab antibodies on sustained disability progression. Figure 3 shows cumulative probability of sustained disability progression (the primary end point at 2 years) over 2 years by antibody status in the AFFIRM study. The estimated proportions of patients with sustained disability progression over 2 years were identical (17%) for antibody-negative and transiently antibody-positive patients. Thus, patients who only transiently expressed antibodies did not experience a sustained loss of natalizumab clinical efficacy. In contrast, 34% of persistently positive patients had sustained disability progression, which was twofold higher than that of the antibody-negative patients (17%) (persistently positive vs antibody-negative, $p \leq 0.05$) (figure 3). Similarly, an analysis of mean change from baseline in multiple sclerosis functional composite (MSFC) Z scores showed that persistently positive patients had a worsening in MSFC score (−0.18) compared with antibody-negative patients (−0.18 vs 0.06; $p < 0.001$) (figure 4A). Transiently positive patients had a mean change in MSFC of −0.14, which was not significantly different from antibody-negative patients (mean MSFC change of 0.06).

In the SENTINEL study, persistently positive patients did not show a reduced effect of natalizumab on disability progression compared with that for antibody-negative patients ($p = 0.503$). The cumulative proportion of patients with sustained disability progression over 2 years (estimated based on Kaplan–Meier analysis) was 24% in antibody-negative patients, 19% in transiently positive patients, and 20% in persistently positive patients. Disability progression as measured by the MSFC worsened in persistently positive patients (Z score = −0.08) compared with antibody-negative patients (increase of 0.06) (persistently positive vs antibody negative; $p = 0.072$) (figure 4B).

Effect of natalizumab antibodies on relapse rate. Adjusted annualized relapse rate by antibody status is shown in 3-month intervals during the first

Figure 3 Kaplan-Meier estimates of the cumulative probability of patients with sustained disability progression, as measured by the EDSS scores, by antibody status over the 2-year AFFIRM study

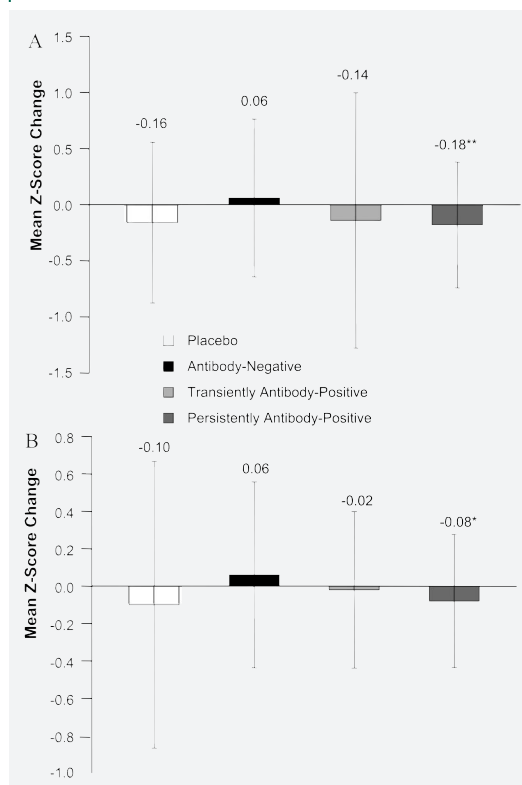


	Placebo	Antibody-Negative	Transiently Positive	Persistently Positive
Number of Patients at Risk				
0	315	568	20	37
12	296	550	19	32
24	283	538	18	26
36	264	526	16	25
48	248	506	16	24
60	240	487	16	22
72	229	480	15	22
84	216	470	14	19
96	208	460	14	16
108	200	449	14	15
120				

* $p \leq 0.05$ vs. antibody-negative patients
 $^{\dagger}p = 0.66$ vs. placebo

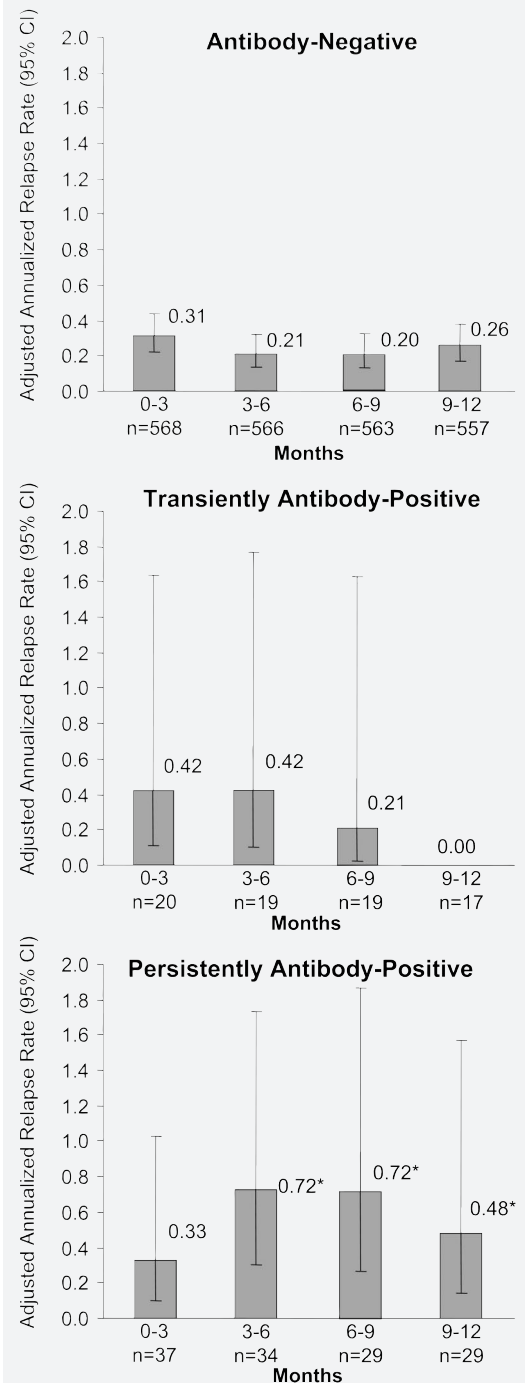
EDSS = Expanded Disability Status Scale.

Figure 4 Mean change from baseline in multiple sclerosis functional composite Z score at 2 years in the AFFIRM study (A) and the SENTINEL study (B)



* $p = 0.072$ vs antibody-negative patients; ** $p < 0.001$ vs antibody-negative patients.

Figure 5 Adjusted annualized relapse rates (95% CI) by antibody status in 3-month intervals from baseline through the first 12 months of natalizumab treatment



* $p < 0.05$ vs antibody negative.

year of natalizumab treatment (the time period during which antibodies develop) in AFFIRM (figure 5). Transiently positive patients experienced a delayed therapeutic effect on annualized relapse rate during the first 6 months of natalizumab therapy (relapse rate = 0.42) compared

Table 1 MRI end points by antibody status in AFFIRM

	Antibody negative, n = 568	Transiently positive, n = 20	Persistently positive, n = 37
No. of Gd+ lesions at y 2, mean $\pm$ SEM	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.6 $\pm$ 0.2
p Value vs antibody negative		0.371	<0.001
No. of new or enlarging T2-hyperintense lesions over 2 y, mean $\pm$ SEM	1.6 $\pm$ 0.4	1.4 $\pm$ 0.4	6.4 $\pm$ 1.8
p Value vs antibody negative		0.407	<0.001
Change from baseline* in T2 lesion volume, mm ³ median (min, max)	-641.5 (-26, 645, 186, 161)	-58.0 (-137, 441, 32, 877)	569.0 (-20, 208, 16, 861)
p Value vs antibody negative		0.006	< 0.001
No. of new T1-hypointense lesions over 2 y, mean $\pm$ SEM	1.0 $\pm$ 0.1	0.6 $\pm$ 0.2	2.5 $\pm$ 0.6
p Value vs antibody negative		0.760	< 0.001

*To 2 years.

Gd+ = gadolinium enhancing.

with antibody-negative patients (0.26) ($p = 0.38$), which was similar to the relapse rate observed in persistently positive patients (0.52) ($p = 0.73$). After 6 months, relapse rate in transiently positive patients declined (relapse rate = 0.0 to 0.21) with continued dosing and concomitant with reversion to antibody-negative status. Persistently positive patients showed an increase in relapse rate starting 6 months after initiation of therapy (0.72). Over the 2-year study (figure 6), transiently positive patients and antibody-negative patients had similar relapse rates (0.16 transiently positive vs 0.22 antibody negative). In contrast, persistently positive patients had a higher relapse rate than antibody-negative patients (0.48 persistently positive vs 0.22 antibody negative, $p = 0.009$), indicating a loss of natalizumab efficacy in this group.

Similar results were observed in SENTINEL. The annualized relapse rate was 0.31 in antibody-negative patients, 0.37 in transiently positive patients, and 0.65 in persistently positive patients; relapse rate was higher in persistently positive patients compared with antibody-negative patients ($p < 0.001$), indicating a loss of natalizumab efficacy in persistently positive patients.

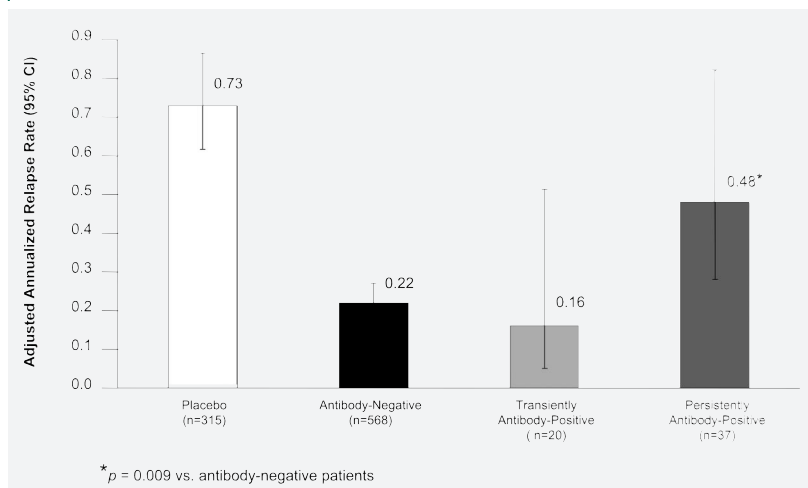
Effect of natalizumab antibodies on MRI outcomes. Table 1 shows MRI results by antibody status in AFFIRM. Over 2 years, persistently positive patients had higher numbers of Gd+ lesions ($p < 0.001$), new or enlarging T2-hyperintense lesions ($p < 0.001$), and new T1-hypointense lesions ($p < 0.001$) compared with antibody-negative patients (table 1); there were no significant differences between transiently positive and antibody-negative patients in numbers of lesions. The median T2-hyperintense lesion volume was decreased by 641.5 mm³ in antibody-negative patients and by

58.0 mm³ in transiently antibody-positive patients and increased by 569.0 mm³ in persistently positive patients over 2 years compared with baseline (persistently positive vs antibody negative, $p < 0.001$; transiently positive vs antibody negative, $p = 0.006$).

Results of MRI measures by antibody status in SENTINEL were similar to those observed in AFFIRM. Patients persistently positive were more likely than antibody-negative patients to develop Gd+ lesions ($p < 0.001$) and new or enlarging T2-hyperintense lesions ($p < 0.001$) on MRI at 2 years. There was no difference between persistently positive patients and antibody-negative patients for numbers of new T1-hypointense lesions ($p = 0.960$). The median change in T2 lesion volume from baseline to 2 years was -74.4 mm³ for antibody-negative, -50.1 mm³ for transiently positive, and 143.1 mm³ for persistently positive patients (persistently positive vs antibody negative, $p = 0.029$). There were no significant differences between transiently positive and antibody-negative patients on any of the measures of MRI lesions.

Relationship between antibody titer and loss of efficacy. Table 2 shows serum natalizumab concentration by antibody titer quartile in the 19 AFFIRM patients who were persistently positive and received at least 26 natalizumab infusions. As shown in the table, when antibodies were detected, there was essentially no detectable drug. There was an insignificant negative correlation between antibody titer and maximal serum natalizumab concentration ($r_s = -0.22$). However, this analysis is confounded because many persistently positive patients showed a decrease in titer over time with a loss of detectable antibodies and

Figure 6 Adjusted annualized relapse rates (95% CI) by antibody status over 2 years of natalizumab treatment



a concurrent rise in serum natalizumab concentrations.

The relationship between antibody titer and loss of clinical efficacy was difficult to evaluate because there were so few clinical events in the persistently positive patients who received at least 26 natalizumab infusions ($n = 19$); 9 of 19 patients had relapses and 3 of 18 patients had Gd+ lesions. The mean serum natalizumab concentration was $9.6 \mu\text{g/mL}$ in the 10 patients who had no relapses compared with $14.4 \mu\text{g/mL}$ in the 9 patients with relapses. The mean serum natalizumab concentration was $19 \mu\text{g/mL}$ for the 1 patient with more than two Gd+ lesions, $0 \mu\text{g/mL}$ for the 2 patients with one Gd+ lesion, and $13.8 \mu\text{g/mL}$ for the 15 patients with no Gd+ lesions.

Effect of natalizumab antibodies on safety. Although the incidence and types of adverse events were generally similar among groups categorized by antibody status, rates of infusion-related adverse events (defined as any event that occurred within 2 hours of the start of the 1-hour infusion) were higher in persistently positive patients. In AFFIRM, infusion-related reactions were experienced over the 2 years by 76% of persistently positive patients compared with 20% of antibody-negative patients, 25% of transiently positive patients, and 18% of placebo-treated patients. In SENTINEL, infusion-related reactions were experienced by 79% of persistently positive patients compared with 19% of antibody-negative patients, 31% of transiently positive patients, and 20% of IFN β -1a alone-treated patients. The most frequent infusion-related adverse events among persistently positive, transiently positive, and antibody-negative patients in

Table 2 Serum concentration of natalizumab by quartiles of antibody titer in AFFIRM ($n = 19$)

Visit	Quartiles of antibody titer	No. of patients	Serum concentration, mean (median)
Wk 12	≤ 2.83	4	0 (0)
	$> 2.83-37.7$	4	0 (0)
	$> 37.7-146.5$	4	0 (0)
	> 146.5	4	0 (0)
Wk 24	≤ 15.6	5	0 (0)
	$> 15.6-84.6$	4	0 (0)
	$> 84.6-229$	5	0 (0)
	> 229	4	0 (0)
Wk 36	≤ 23.9	5	3.78 (0.91)
	$> 23.9-120.5$	4	0 (0)
	$> 120.5-226$	5	0 (0)
	> 226	4	0 (0)
Wk 48	≤ 2.7	5	5.87 (1.10)
	$> 2.7-115$	5	0 (0)
	$> 115-267$	5	3.80 (0.00)
	> 267	4	0 (0)
Wk 60	≤ 1.3	5	8.30 (2.50)
	$> 1.3-66.6$	5	0.24 (0.00)
	$> 66.6-267$	5	0 (0)
	> 267	4	0 (0)
Wk 72	0	6	7.78 (6.85)
	$> 0-11.2$	3	0 (0)
	$> 11.2-188$	5	0 (0)
	> 188	4	0 (0)
Wk 84	0	9	6.22 (0.42)
	$> 0-114$	5	0 (0)
	> 114	4	0 (0)
Wk 96	0	10	15.02 (7.95)
	$> 0-84.1$	5	0 (0)
	> 84.1	4	0 (0)
Wk 108	0	9	13.19 (11.00)
	$> 0-165$	4	0 (0)
	> 165	4	0 (0)
Wk 120	0	9	13.24 (7.70)
	$> 0-60.8$	4	0 (0)
	> 60.8	4	0 (0)

the AFFIRM and SENTINEL studies are shown in table 3.

In AFFIRM, hypersensitivity reactions were experienced by 17 (46%) persistently positive patients, 3 (15%) transiently positive patients, and 4 (0.7%) antibody-negative patients (the antibody status for 1 patient who experienced a hypersensitivity reaction was missing). In SENTINEL, eight

Table 3 Incidence of common infusion-related adverse events by antibody status in AFFIRM and SENTINEL

	Antibody negative, n = 568	Transiently positive, n = 20	Persistently positive, n = 37
Natalizumab monotherapy			
Headache	4 (24)*	0*	19 (7)
Urticaria	<1 (4)*	5 (1)	14 (5)
Nausea	1 (8)*	0	16 (6)
Rigors	<1 (1)*	0	11 (4)
Natalizumab add-on therapy			
	n = 515	n = 32	n = 38
Rigors	<1 (1)*	0*	29 (11)
Nausea	1 (7)*	0*	18 (7)
Headache	4 (20)*	3 (1)	13 (5)
Pruritus	≤1 (4)*	3 (1)	11 (4)
Flushing	≤1 (3)*	0	13 (5)
Urticaria	≤1 (2)*	0	13 (5)
Tachycardia	0*	0	11 (4)
Dizziness	3 (15)	0	8 (3)
Pyrexia	<1 (3)*	0	8 (3)

Values are % (n).

* $p < 0.05$ vs persistently positive.

* $p < 0.01$ vs persistently positive.

* $p < 0.001$ vs persistently positive.

(21%) persistently positive patients, no transiently positive patients, and three (0.6%) antibody-negative patients experienced hypersensitivity reactions.

DISCUSSION The occurrence of antibodies to therapeutic proteins is a known biologic response to treatment.⁵ Antibodies against these proteins lead to loss of efficacy and can often be associated with infusion/injection-related reactions to the product. This article is the first report of the incidence and clinical significance of antibodies that develop during natalizumab therapy in patients with relapsing MS.

In AFFIRM, persistent antibody positivity reduced trough serum natalizumab concentrations and had an adverse effect on treatment response as evidenced by significantly reduced benefits on disability progression, relapse rate, and disease activity measured by MRI in persistently antibody-positive patients compared with antibody-negative patients. In addition to the patients who developed persistently positive antibodies, there was a small group of patients who developed antibodies that were transient in nature (transiently positive patients). Although these antibodies led to a delay in the full therapeutic response to natalizumab, efficacy was restored to that of the antibody-negative population once antibodies were no longer detectable after 6 months of continued treatment. The results of

SENTINEL were generally consistent with those of AFFIRM. The one exception to this is the analysis of EDSS progression, where persistently positive patients in SENTINEL had similar efficacy to antibody-negative patients. Given the overwhelming evidence from AFFIRM and SENTINEL that persistent antibodies to natalizumab lead to a loss of efficacy across multiple endpoints, this result is likely spurious and due to one or more of the following factors. First, the EDSS is a relatively insensitive measure. As such, the study was not powered to show a difference given the small number of patients who developed persistent antibodies. In addition, the fact that the comparison in SENTINEL was to an active control group further reduces the ability to discern differences between the groups. Finally, a proportion of patients in SENTINEL eventually became antibody negative by the end of the study, and it is probable that these patients began to respond to natalizumab once antibodies were no longer present.

A higher incidence of infusion-related adverse events was generally observed in persistently antibody-positive patients, including hypersensitivity reactions, in both the AFFIRM and the SENTINEL studies. Hypersensitivity reactions were more common in persistently positive patients, occurring in 17 of 37 (46%) patients in AFFIRM. However, one could not predict which

persistently positive patients would develop a hypersensitivity reaction based upon antibody status. These reactions generally occurred early in the course of treatment, before determination of persistent positivity could be made. In patients experiencing hypersensitivity, allergic, or anaphylactic reactions (including symptoms of urticaria or bronchospasm) to natalizumab infusion, therapy should be discontinued and not reintroduced. To our knowledge, no untoward safety effects of neutralizing antibodies to IFN β therapy have been reported.

The results reported herein are also consistent with the literature regarding the effects of antibodies to therapeutic proteins on efficacy and safety. In rheumatoid arthritis, antibodies to infliximab have been shown to reduce efficacy and lead to an increase in infusion-related reactions.⁶⁻⁸ In MS, neutralizing antibodies have been shown to develop to IFN β products. These neutralizing antibodies occasionally resolve spontaneously; however, persistent high-titer antibodies significantly interfere with the beneficial effects of treatment as measured by clinical and MRI outcomes.⁹⁻¹² Our definitions of transiently positive and persistently positive are similar to those used in studies of the development of neutralizing antibodies to IFN β .¹³⁻¹⁵

Antibodies that develop during natalizumab therapy were effectively detected by using an ELISA. Binding antibodies detected in the ELISA assay were further characterized in a flow cytometric-based blocking assay. The high concordance of the results between the two assays suggests that most or all of the antibodies that are generated against natalizumab are directed toward the VLA4 binding domain. Patients were considered positive for antibodies if they had a titer of ≥ 0.5 $\mu\text{g/mL}$. This low titer level was used as a cut-off for antibody positivity to ensure that all patients with antibodies were detected.

Results of the AFFIRM and SENTINEL studies suggest that antibody positivity may be a predictor of clinical response to natalizumab therapy for relapsing MS. Because most patients (94%) were not persistently positive for antibodies to natalizumab, routine monitoring of antibody titers in all patients may not be warranted. The presence of persistent antibodies is associated with continued MS disease activity and persistence of certain infusion-related events such as flushing, fever, and rigors. These symptoms, as well as continued disease activity (e.g., relapses), should serve as an indicator of the patients for

whom testing should be considered. Another factor, however, is the timing of testing. Early testing of patients (before 6 months of treatment) may lead to an erroneous determination of clinically meaningful antibody status given the proportion of patients in whom antibodies are only transient. Conclusions regarding antibody status during this timeframe could lead to patients being discontinued from treatment when they may shortly become antibody negative with continued infusions. Therefore, in the unusual circumstance where a patient needs to be tested within the first 6 months after the initiation of therapy, physicians should determine persistent positivity by retesting at least 3 months after the first positive test. Thus, given the current knowledge, for the majority of patients, routine antibody testing is not necessary. However, if, after 6 months of natalizumab therapy, patients continue to have clinical activity or have persistent infusion reactions (not hypersensitivity, which mandates discontinuation of the drug), they should be tested for antibodies. If positive, patients should be retested in 3 months and those with confirmed persistent antibody positivity on retesting should be discontinued from natalizumab treatment. Testing for antibodies to natalizumab is commercially available from Focus Diagnostics, Inc. (Cypress, CA) and Athena Diagnostics, Inc. (Worcester, MA).

Natalizumab is an effective therapy for the treatment of MS. Although the rate of persistent antibodies during treatment was low (6%), they were associated with a substantial reduction in the benefits of natalizumab treatment and were associated with an increase in certain infusion-related risks, altering the benefit-risk profile. Therefore, physicians should be aware of the possibility of persistent antibodies in patients who experience disease activity or persistent infusion-related adverse events on natalizumab and, when indicated, incorporate testing for such antibodies into their clinical practice.

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REFERENCES

1. Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. Prevention of experimental autoimmune encephalomyelitis by antibodies against $\alpha 4 \beta 1$ integrin. *Nature* 1992;356:63–66.
2. Schellekens H. Bioequivalence and the immunogenicity of biopharmaceuticals. *Nat Rev* 2002;1:457–462.
3. Polman CH, O'Connor PW, Havrdova E, et al. A randomized placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2006;354:899–910.
4. Rudick RA, Stuart WH, Calabresi PA, et al. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. *N Engl J Med* 2006;354:911–923.
5. Schellekens H, Casadevall N. Immunogenicity of recombinant human proteins: causes and consequences. *J Neurol* 2004;251(suppl 2):II4–II9.
6. Anderson PJ. Tumor necrosis factor inhibitors: clinical implications of their different immunogenicity profiles. *Semin Arthritis Rheum* 2005;34(suppl 1):19–22.
7. Haraoui B, Cameron L, Ouellet M, White B. Anti-infliximab antibodies in patients with rheumatoid arthritis who require higher doses of infliximab to achieve or maintain a clinical response. *J Rheumatol* 2006;33:31–36.
8. Wolbink GJ, Vis M, Lems W, et al. Development of anti-infliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006;54:711–715.
9. The IFNB Multiple Sclerosis Study Group, the University of British Columbia MS/MRI Analysis Group. Neutralizing antibodies during treatment of multiple sclerosis with interferon beta-1b: experience during the first three years. *Neurology* 1996;47: 889–894.
10. PRISMS (Prevention of Relapses and Disability by Interferon β -1a Subcutaneously in Multiple Sclerosis)

- Study Group, the University of British Columbia MS/MRI Analysis Group. PRISMS-4: long-term efficacy of interferon- β -1a in relapsing MS. *Neurology* 2001;56:1628–1636.
11. Sorensen PS, Ross C, Clemmesen KM, et al. Clinical importance of neutralising antibodies against interferon beta in patients with relapsing-remitting multiple sclerosis. *Lancet* 2003;362:1184–1191.
 12. Kappos L, Clanet M, Sandberg-Wollheim M, et al. Neutralizing antibodies and efficacy of interferon β -1a. A 4-year controlled study. *Neurology* 2005;65:40–47.
 13. Bertolotto A, Gilli F, Sala A, et al. Persistent neutralizing antibodies abolish the interferon β bioavailability in MS patients. *Neurology* 2003;60:634–639.
 14. Malucchi S, Sala A, Gilli F, et al. Neutralizing antibodies reduce the efficacy of β IFN during treatment of multiple sclerosis. *Neurology* 2004;62:2031–2037.
 15. Solberg Sorensen P, Koch-Henriksen N, Ross C, Clemmesen KM, Bendtzen K. and the Danish Multiple Sclerosis Study Group. Appearance and disappearance of neutralizing antibodies during interferon-beta therapy. *Neurology* 2005;65:33–39.

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