

# Natalizumab reduces loss of gray matter and thalamic volume in patients with relapsing-remitting multiple sclerosis: A post hoc analysis from the randomized, placebo-controlled AFFIRM trial

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## Abstract

**Background:** Loss of brain gray matter fractional volume predicts multiple sclerosis (MS) progression and is associated with worsening physical and cognitive symptoms. Within deep gray matter, thalamic damage is evident in early stages of MS and correlates with physical and cognitive impairment. Natalizumab is a highly effective treatment that reduces disease progression and the number of inflammatory lesions in patients with relapsing-remitting MS (RRMS).

**Objective:** To evaluate the effect of natalizumab on gray matter and thalamic atrophy.

**Methods:** A combination of deep learning-based image segmentation and data augmentation was applied to MRI data from the AFFIRM trial.

**Results:** This *post hoc* analysis identified a reduction of 64.3% ( $p=0.0044$ ) and 64.3% ( $p=0.0030$ ) in mean percentage gray matter volume loss from baseline at treatment years 1 and 2, respectively, in patients treated with natalizumab versus placebo. The reduction in thalamic fraction volume loss from baseline with natalizumab versus placebo was 57.0% at year 2 ( $p<0.0001$ ) and 41.2% at year 1 ( $p=0.0147$ ). Similar findings resulted from analyses of absolute gray matter and thalamic fraction volume loss.

**Conclusion:** These analyses represent the first placebo-controlled evidence supporting a role for natalizumab treatment in mitigating gray matter and thalamic fraction atrophy among patients with RRMS.

**ClinicalTrials.gov identifier:** NCT00027300

URL: <https://clinicaltrials.gov/ct2/show/NCT00027300>

**Keywords:** Natalizumab, atrophy, gray matter, thalamus, MRI outcomes, U-net analysis

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## Introduction

Multiple sclerosis (MS), the most common central nervous system chronic, inflammatory, and demyelinating disease, results in progressive physical and cognitive disability due to neuronal-axonal demyelination, degeneration, and loss.<sup>1,2</sup> Axonal demyelination, caused by inflammatory autoimmune processes, can be detected as localized areas of hyperintensity (lesions) in white matter on magnetic resonance imaging (MRI).<sup>1,3</sup>

Although the number of lesions seen on MRI does not necessarily predict functional manifestations of MS disease activity,<sup>4</sup> loss of brain gray matter fractional volume is a predictor of disease progression<sup>5</sup> and has been associated with worsening of physical and cognitive symptoms in people with MS.<sup>6,7</sup> Within deep gray matter, thalamic damage is evident in early stages of MS<sup>8,9</sup> and correlates with physical and cognitive impairment.<sup>10,11</sup> As such, the thalamus has been proposed as a candidate imaging marker for neurodegeneration in MS.<sup>12</sup>

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Natalizumab (TYSABRI®), a highly effective treatment for patients with relapsing forms of MS, has demonstrated the ability to reduce the pace of disease progression and the number of inflammatory lesions in patients with relapsing-remitting MS (RRMS) in clinical trials<sup>13,14</sup> and real-world studies.<sup>15–17</sup> However, the effects of natalizumab on gray matter volume atrophy are still unclear. While some studies have suggested a benefit of natalizumab treatment on gray matter atrophy rates, others have not, and placebo-controlled evidence is lacking.<sup>18–21</sup>

Acquisition and quantification of gray matter volume poses technical challenges, including volume underestimation caused by the presence of white matter lesions, confounding effects due to localized and diffuse gray matter lesions, measurement distortions caused by gray matter atrophy itself, and intra- and interscanner variability in acquisition, analysis, and reproducibility.<sup>22–24</sup> Recently, image segmentation analysis using a convolutional neural network with deep machine learning has greatly advanced the speed and precision of automated image classifiers.<sup>25</sup> Such techniques have the potential to provide greater clarity of the effects of treatment on gray matter volume changes associated with MS.

The purpose of this *post hoc* analysis was to determine whether loss of gray matter volume and thalamic volume in patients with RRMS differed between those treated with natalizumab and those given placebo in the pivotal trial AFFIRM (NCT00027300). A combination of deep learning-based image segmentation and data augmentation was developed to make efficient use of limited annotated samples.<sup>25</sup>

## Patients and methods

### Patients

Proton density/T2-weighted MRI scans from AFFIRM—a multisite, randomized, double-blind, placebo-controlled trial of natalizumab efficacy and safety in patients with RRMS<sup>13,14</sup>—were used for this analysis. Patients enrolled in AFFIRM had a mean (SD) of 1.52 (0.86) relapses in the past year, a mean (SD) Expanded Disability Status Scale (EDSS) score of 2.3 (1.2) at study baseline, and a median duration of disease of 5 years (range, 0–34 years).

Longitudinal MRI scans for gray matter and thalamic volumetric determination were available for 565 of 627 (90.1%) patients randomized to receive natalizumab and 276 of 315 (87.6%) patients randomized to receive placebo at baseline and year 1. Of these, 513

(82.6%) natalizumab-treated patients and 247 (78.7%) placebo-treated patients also had MRI scans for volumetric determination at year 2. We conducted a sensitivity analysis based on gadolinium-enhancing (Gd+) status at baseline.

Written informed consent was obtained from all patients prior to enrollment, and the AFFIRM study protocol was approved by central and local ethics committees for each site in accordance with the Declaration of Helsinki. Further details of ethics committee approvals are provided in Supplemental Table 1.

### Brain volume assessments

**Deep learning-based segmentation.** Brain gray matter volume and thalamic fractional volume at baseline, year 1, and year 2 were measured using a deep learning-based segmentation method.<sup>25</sup> A modified, deep convolutional network (2-dimensional U-net, original version is available at <http://lmb.informatik.uni-freiburg.de/people/ronneber/u-net>) was used to classify tissue volume into gray matter (including cerebral, cerebellar, and deep gray matter), white matter, ventricular cerebrospinal fluid, hippocampus, thalamus, outer cerebrospinal fluid, and MS lesions. The U-net network is based largely on a standard convolutional network with a major difference: the up-sampling steps include a large number of feature channels, enabling the propagation of context information to higher-resolution layers and resulting in roughly symmetric contraction and expansion paths.

The gold standard segmentation for gray matter and thalamus was derived from FreeSurfer 6.0 on 3D T1 FLASH or MPRAGE images and manually corrected. Two experienced reviewers manually segmented white matter hyperintensity. The mean interrater Dice score was 0.671 with a Pearson correlation of 0.962 from 12 scans. The U-net was trained to segment tissue using 239 training cases and 24 validation cases, resulting in a validation Dice score of 0.85 for gray matter volume and 0.86 for thalamic volume. During the training of the algorithm, data augmentation procedures were applied to account for variations related to noise level, intensity inhomogeneity, intensity levels, and contrast levels.

All images were preprocessed using a standard pipeline. A denoising filter based on nonlocal means was used to correct for noise. Intensity inhomogeneity was corrected with an iterative application of N3 with varying parameters. Intersite variances in image intensity and contrast were mitigated by applying image scaling based on the intensity levels of the brain and cerebrospinal fluid.

Furthermore, to reduce within-subject variance, longitudinal image intensity scaling was applied to normalize the intensity within subjects. Each patient's baseline scan was used to nonlinearly register to the International Consortium for Brain Mapping (ICBM) normal brain template using Advanced Normalization Tools (ANTs). Tissue probability maps for gray matter, white matter, cerebrospinal fluid, lateral ventricles, and MS lesions were inversely transformed back to the native proton density/T2 image space. Two experienced reviewers blinded to treatment visually checked the analyzed segmentation images. Tissue volumes were normalized by the outer contour of the brain, resulting in the gray matter and thalamic fractions reported here.

### Statistical analysis

R version 4.2.1 was used for the analysis. Comparisons of percentage and absolute change in gray matter volume and thalamic fraction volume from baseline at years 1 and 2 for natalizumab versus placebo were assessed using a linear mixed model (random effect with patients), adjusted for sex and the corresponding baseline fractional volume. The mixed effect model for GMF and THF are:

$$Y_{it} = \beta_0 + \alpha_i + \beta_{\text{baseline}} Y_{i0} + \beta_{\text{gender}} \text{GENDER}_i + \beta_{T\_12} I(t = 12)_{it} + \beta_{T\_24} I(t = 24)_{it} + \beta_{NTZ} I(\text{GROUP} = \text{NTZ})_i + \beta_{NTZ*T\_12} I(t = 12) * I(\text{GROUP} = \text{NTZ}) + \beta_{NTZ*T\_24} I(t = 24) * I(\text{GROUP} = \text{NTZ}) + \epsilon_{it}$$

where  $Y_{it}$  is the observation of the  $i$ th patient at time  $t$ , and  $I(\cdot)$  is the identity function.

The natalizumab treatment effect was defined as the percentage reduction in the loss of gray matter fraction or thalamic fraction for natalizumab in comparison with placebo.

## Results

### Patients

This analysis included 565 natalizumab-treated patients and 276 placebo-treated patients from AFFIRM with sufficient volumetric data at baseline and year 1, and 518 natalizumab-treated patients and 248 placebo-treated patients with sufficient data at baseline and year 2. Five natalizumab-treated patients and 1 placebo-treated patient had sufficient data at baseline and year 2, but not year 1 (Supplemental Figure S2). At AFFIRM baseline, demographic and

disease characteristics of analyzed patients were well balanced between treatment groups, with the exception of sex (Table 1 and Supplemental Table 2).

### Changes in gray matter fraction volume

At years 1 and 2, the mean percentage volume change from baseline in gray matter fraction was significantly reduced for patients treated with natalizumab in comparison with those treated with placebo (Figure 1(a) and Supplemental Figure 1A). At year 1, the mean (SE) percentage change from baseline in gray matter volume was  $-0.10\%$  (0.04) for natalizumab and  $-0.27\%$  (0.05) for placebo. A similar difference in mean (SE) gray matter volume change for natalizumab versus placebo was observed at year 2 (natalizumab:  $-0.13\%$  (0.04); placebo:  $-0.36\%$  (0.05)). The natalizumab treatment effect (percentage reduction in gray matter fraction loss from baseline for natalizumab vs. placebo) was maintained from year 1 (64.3%;  $p = 0.0044$ ) to year 2 (64.3%;  $p = 0.0030$ ).

At years 1 and 2, the mean absolute volume change from baseline in gray matter fraction was also significantly reduced for patients treated with natalizumab in comparison with those treated with placebo (Figure 1(b) and Supplemental Figure 1B). The mean (SEs) absolute change from baseline in gray matter volume fraction was  $-0.000465$  (0.000170) for natalizumab and  $-0.001227$  (0.000243) for placebo at year 1, and  $-0.000618$  (0.000175) for natalizumab and  $-0.001665$  (0.000251) for placebo at year 2. Natalizumab treatment effects for absolute gray matter fraction reduction from baseline at years 1 and 2 were 62.1% ( $p = 0.0102$ ) and 62.9% ( $p = 0.0006$ ), respectively.

### Changes in thalamic fraction volume

At years 1 and 2, the mean (SE) percentage volume change from baseline in thalamic fraction volume was also significantly lower for the natalizumab group than for the placebo group (year 1:  $-0.78\%$  (0.12) vs.  $-1.32\%$  (0.18); year 2:  $-0.96\%$  (0.13) vs.  $-2.23\%$  (0.18)) (Figure 2(a)). For years 1 and 2, the natalizumab treatment effect for reduction of thalamic fraction loss from baseline was 41.2% ( $p = 0.0147$ ) and 57.0% ( $p < 0.0001$ ), respectively.

The mean (SE) absolute volume change from baseline in thalamic fraction volume was also significantly lower for the natalizumab group than for the placebo group at both time points (year 1:  $-0.000088$  (0.000013) vs.  $-0.000138$  (0.000018); year 2:

**Table 1.** Demographic and disease characteristics of patients included in the MRI analysis at AFFIRM baseline.

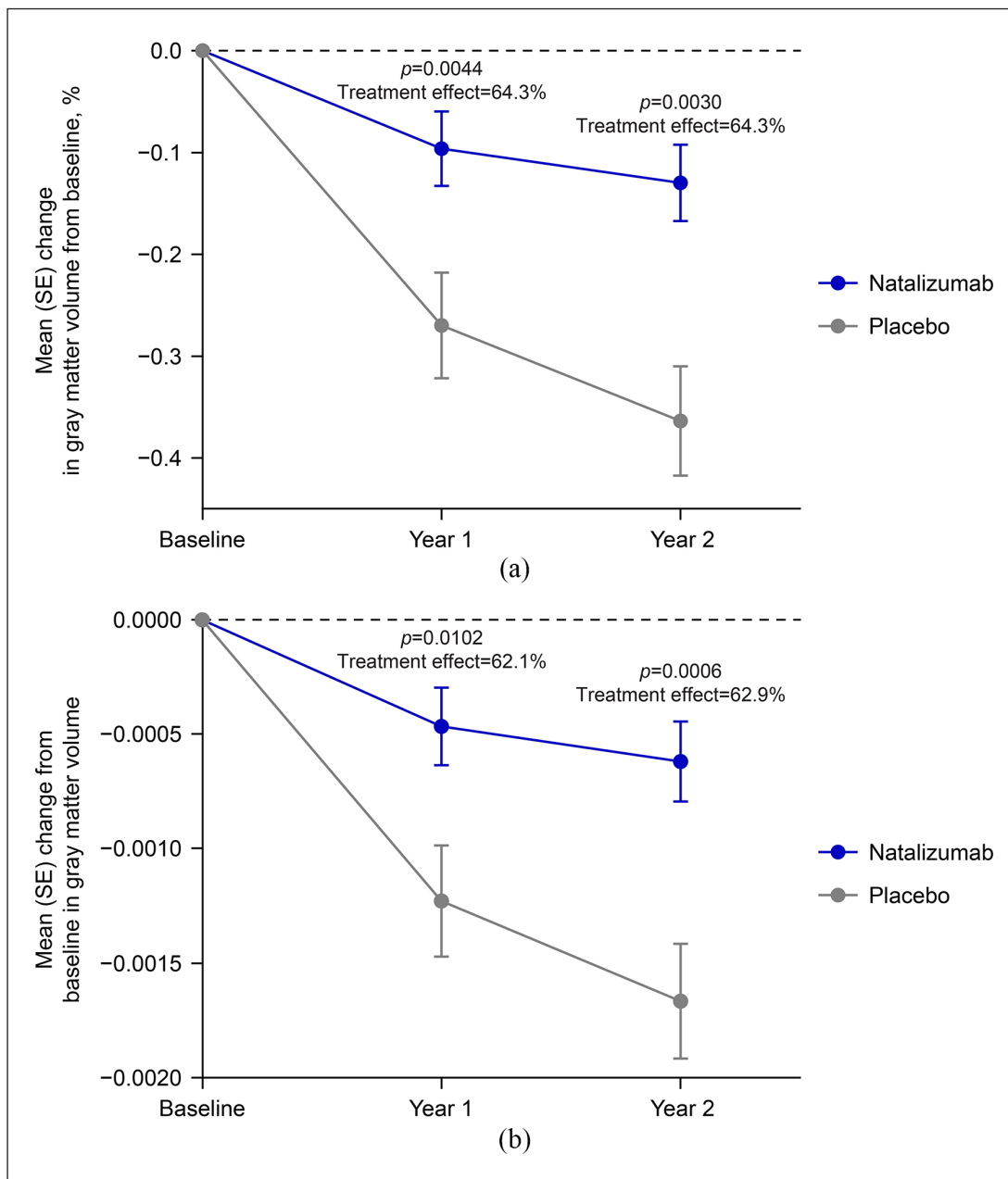
Characteristic	Natalizumab ( <i>n</i> =565)	Placebo ( <i>n</i> =276)	<i>p</i> value
Age, mean (SD), years	35.7 (8.52)	36.5 (7.76)	0.196
Female, <i>n</i> (%)	405 (71.7)	178 (64.5)	0.041
Race, <i>n</i> (%)			0.501
Asian	3 (0.5)	2 (0.7)	
Black/African American	5 (0.9)	6 (2.2)	
Hispanic	6 (1.1)	6 (2.2)	
Other	5 (0.9)	2 (0.7)	
White	543 (96.1)	259 (93.8)	
Missing	3 (0.5)	1 (0.4)	
Disease duration, mean (SD), years	7.45 (6.51)	7.49 (6.54)	0.937
Number of relapses in the prior year, mean (SD)	1.53 (0.92)	1.47 (0.73)	0.360
Number of relapses in the prior year, <i>n</i> (%)			
0	4 (0.7)	4 (1.4)	
1	336 (59.5)	165 (59.8)	
2	174 (30.8)	85 (30.8)	
≥3	51 (9.0)	22 (8.0)	
EDSS score, mean (SD)	2.31 (1.16)	2.26 (1.15)	0.523
EDSS score, <i>n</i> (%)			
0	26 (4.6)	16 (5.8)	
1.0–1.5	168 (29.7)	85 (30.8)	
2.0–2.5	184 (32.6)	89 (32.2)	
3.0–3.5	118 (20.9)	57 (20.7)	
4.0–4.5	51 (9.0)	24 (8.7)	
5.0	16 (2.8)	4 (1.4)	
≥5.5	2 (0.4)	1 (0.4)	
T1 hypointense lesion volume, mm <sup>3</sup> , mean (SD)	5764 (8101.1)	5927 (7959.0)	0.783
T2 hyperintense lesion volume, mm <sup>3</sup> , mean (SD)	15833 (17284.4)	15328 (16076.1)	0.684
Number of T2 hyperintense lesions, <i>n</i> (%)			
<9	28 (5.0)	15 (5.4)	0.897
≥9	537 (95.0)	261 (94.6)	
Gd+ lesion volume, mm <sup>3</sup> , mean (SD)	371 (795.9)	308 (681.1)	0.257
Number of Gd+ lesions, mean (SD)	2.30 (4.75)	1.91 (4.28)	0.256
Number of Gd+ lesions, <i>n</i> (%)			
0	270 (47.8)	148 (53.6)	
1	108 (19.1)	48 (17.4)	
2	59 (10.4)	23 (8.3)	
3	34 (6.0)	16 (5.8)	
≥4	94 (16.6)	41 (14.9)	
Gray matter fraction, au, mean (SD)	0.45 (0.02)	0.45 (0.02)	0.611
Thalamic fraction, au, mean (SD)	0.01 (0.00)	0.01 (0.00)	0.688

au: arbitrary unit; EDSS: Expanded Disability Status Scale; Gd+: gadolinium-enhancing; MRI: magnetic resonance imaging; SD: standard deviation.

−0.000103 (0.000013) vs −0.000227 (0.000019) (Figure 2(b)). The natalizumab treatment effect for absolute thalamic fraction reduction from baseline was 36.5% ( $p=0.0250$ ) for year 1 and 54.6% ( $p<0.0001$ ) for year 2.

## Discussion

The analysis of MRI results from AFFIRM presented here provides the first placebo-controlled evidence for a natalizumab treatment effect in mitigating gray matter fraction and deep gray (thalamic)



**Figure 1.** (a) Mean (SE) percentage change, and (b) mean (SE) absolute change from baseline in gray matter volume over 2 years in AFFIRM patients.

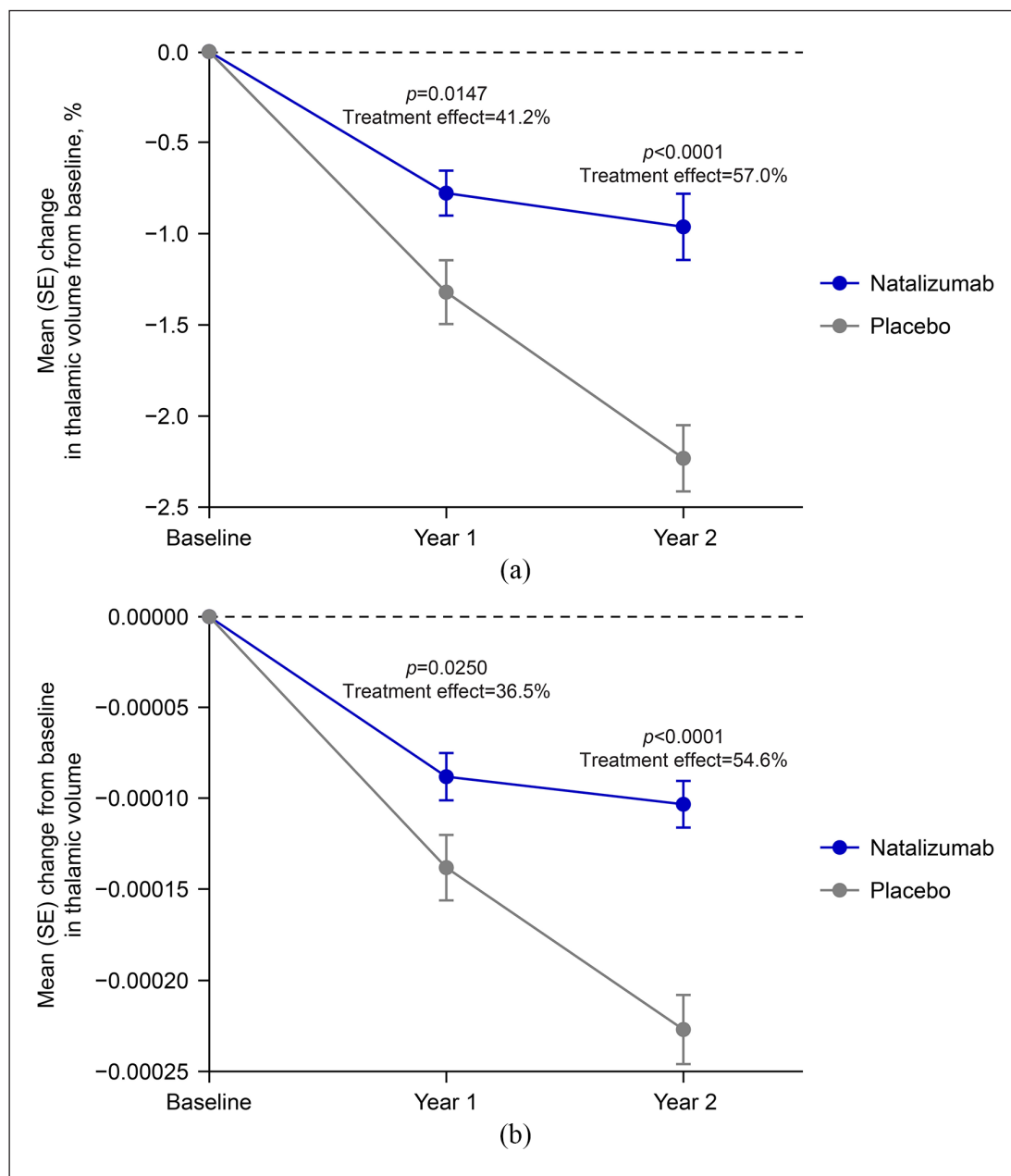
The analysis included 565 natalizumab-treated patients and 276 placebo-treated patients at both baseline and year 1 and, among these, 513 natalizumab-treated patients and 247 placebo-treated patients at year 2. The  $p$  values for mean change from baseline in gray matter fraction for natalizumab versus placebo were assessed using a linear mixed model, adjusted for baseline gray matter fraction and sex (random effect with patients). The treatment effect was calculated as the percentage reduction in gray matter volume loss for natalizumab in comparison with placebo.

SE = standard error.

fraction volume loss. There was significantly less gray matter and thalamic fraction atrophy in patients who received natalizumab after 1 and 2 years of treatment in comparison with those who received placebo. The natalizumab treatment effect for reducing gray matter volume loss was relatively

stable over 2 years, whereas the treatment effect for thalamic fraction volume loss increased from year 1 to year 2.

These results lend further support to results from prior studies that have suggested a beneficial effect



**Figure 2.** (a) Mean (SE) percentage change and (b) mean (SE) absolute change from baseline in thalamic fraction volume over 2 years in AFFIRM patients.

The analysis included 565 natalizumab-treated patients and 276 placebo-treated patients at both baseline and year 1 and, among these, 513 natalizumab-treated patients and 247 placebo-treated patients at year 2. The  $p$  values for mean change from baseline in thalamic fraction for natalizumab versus placebo were assessed using a linear mixed model, adjusted for baseline thalamic fraction and sex (random effect with patients). The treatment effect was calculated as the percentage reduction in thalamic volume loss for natalizumab in comparison with placebo.

SE=standard error.

of natalizumab treatment on brain volume atrophy in patients with MS. A prospective study among 110 patients found that the percentage of brain volume loss among patients with MS during the second year of treatment with natalizumab was similar to that of matched healthy controls.<sup>26</sup> A retrospective study of

20 patients treated with natalizumab for 4 years found that, while cortical gray matter atrophy occurred over years 3 and 4 of treatment, subcortical gray matter volume was stable, and thalamic volume significantly increased during that time period.<sup>19</sup>

In AFFIRM, natalizumab treatment was associated with significant reductions in the number of Gd+, new or newly enlarging T2 hyperintense, and new T1 hypointense lesions at year 1 and year 2 in comparison with placebo.<sup>14</sup> Natalizumab-treated patients experienced a greater loss of BPF in year 1 and a reduced loss of BPF in year 2 versus placebo-treated patients.<sup>14</sup> In this *post hoc* analysis, gray matter and thalamic volumes decreased to a nominally greater extent in year 1 than year 2, which may have been influenced by an early resolution of inflammation and may represent a potential secondary neuroprotective effect. This pattern of exaggerated brain volume loss early after treatment initiation in AFFIRM and other studies has been suggested to reflect pseudoatrophy, an apparent decrease in brain volume caused by a reduction in inflammation and resolution of associated edema, primarily localized to white matter.<sup>14,20,27</sup> Some evidence suggests that gray matter pseudoatrophy, through a reduction of diffuse inflammation in gray matter, can also occur early during treatment, though this effect has not been consistently observed.<sup>28,29</sup> In this study, we conducted a sensitivity analysis stratified by baseline Gd+ status (data not shown) and did not observe greater volume loss in the first year of treated patients who had Gd+ lesions at baseline. While the core AFFIRM study showed a significant pseudoatrophy effect in whole brain atrophy, there was no evidence of significant pseudoatrophy effect in gray matter or thalamic atrophy in this study. In addition, data were rebaselined at month 12 as another method to control for potential pseudoatrophy effect; treatment effects were significant in year 2 for thalamic fraction but not for gray matter fraction, suggesting there was not a large effect of pseudoatrophy in this study. Further studies are needed to understand gray matter pseudoatrophy.

Normal age-dependent atrophy of whole brain and gray matter fractional volume in patients with MS can also confound understanding of the impact of MS disease and disease-modifying therapy (DMT) on brain volume measurements.<sup>30</sup> In AFFIRM, natalizumab- and placebo-treated patients had similar mean ages (35.6 and 36.7 years, respectively) with similar ranges (18–50 and 19–50 years, respectively), suggesting that the gray matter volume differences observed were not influenced by age-related factors.<sup>13</sup>

Implementation of brain volumetric analyses for MS into routine clinical practice presents a number of challenges.<sup>22</sup> The annual rate of whole brain atrophy in patients with RRMS has been estimated at between 0.5% and 1.35%, which, though greater than rates in

people without neurodegenerative disease (estimated at 0.1%–0.3%),<sup>31</sup> is still small enough to require techniques with very high reproducibility for reliable detection and accurate quantification. To use brain volume changes in the clinic, it would be useful to have a cutoff for pathological rates of atrophy. One such proposed estimate is a 0.4% annual whole brain atrophy rate, based on data from 206 patients with MS (87% with RRMS) and 35 healthy controls.<sup>30</sup> However, uncertainty in longitudinal volumetric assessments can be introduced by physiological (i.e., within-patient) variability, interpatient variability, interscanner and intrascanner variability, and by differences in image acquisition and analysis.<sup>22</sup> Indeed, different analysis techniques have been demonstrated to produce different brain volume atrophy cutoff estimates based on the same data source.<sup>22,32</sup>

This analysis of natalizumab-treated patients with RRMS highlights the utility of applied data augmentation and a deep learning convolutional neural network analysis in the investigation of gray matter and thalamic fraction atrophy. Data augmentation was combined with a U-net analysis stream to improve the ability to analyze multicenter MRI data. The combination of preprocessing and data augmentation to correct for image-processing issues related to noise, intensity inhomogeneity, and intensity and contrast levels resulted in a robust algorithm that likely enabled quantification of differences between groups in this study. Applying this type of analysis to future cohorts could help clarify the differences between normal brain atrophy, treatment-related pseudoatrophy, and gray matter fraction atrophy associated with MS progression.

This analysis has some limitations. The *post hoc* nature of the study is hypothesis finding and therefore definitive conclusions are limited. In addition, AFFIRM was a 2-year study, and further data are needed to determine whether the beneficial effects observed with natalizumab would be maintained over the longer term. The deep learning algorithm applied in this analysis used automatic random augmentation both in intensity and spatial domains and therefore is unlikely to cause bias. However, there could be unknown factors which may introduce hidden bias, though without explicit investigation we are unable to determine if such bias exists in the model. Other potential sources of bias are the different MS diagnostic criteria in use at the time of this study, as well as the lack of alternative high-efficacy therapies at that time. Another limitation of this analysis is that clinical correlations, such as EDSS, PASAT, and T25FW, were not reported in

this analysis. In a separate analysis, no strong correlations of the MRI outcomes with disability progression were observed during the 2-year study; however, patient populations with disability progression were too small for the correlation analysis to be robust (Biogen data on file).

In summary, this analysis of AFFIRM clinical trial data provides the first placebo-controlled evidence to support the beneficial effects of natalizumab treatment on gray matter atrophy in patients with RRMS. This treatment benefit was also observed in the thalamus, with a stronger treatment effect in the second year, in agreement with recent findings of a transient decrease followed by an increase in thalamic volume with long-term natalizumab treatment.<sup>19</sup> These findings were greatly enabled by the application of data augmentation with a U-net analysis stream. Further development and application of robust methods for longitudinal gray matter volumetric assessments will contribute to an increased understanding of the impact of DMTs for MS and other neurodegenerative diseases on the long-term maintenance of brain volume and also may help integrate these analyses into routine clinical practice.

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### Data Availability Statement

Requests for the data supporting this manuscript should be submitted to <https://vivli.org/>

### Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: KN received licensing fees from Biogen; research funding to institution from Biogen, National Institutes of Health, National Multiple Sclerosis Society, Novartis, Patient-Centered Outcomes Research Institute (PCORI), Sanofi Genzyme, US Department of Defense. ZS, CH-C, KB, and RLA are employees of and own stock and/or stock options in Biogen. KB was an employee of and owned stock and/or stock options in Biogen at the time the research was conducted.

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### Supplemental Material

Supplemental material for this article is available online.

### References

1. Trapp BD, Peterson J, Ransohoff RM, et al. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998; 338(5): 278–285.
2. Ferguson B, Matyszak MK, Esiri MM, et al. Axonal damage in acute multiple sclerosis lesions. *Brain* 1997; 120(Pt 3): 393–399.
3. Brück W, Bitsch A, Kolenda H, et al. Inflammatory central nervous system demyelination: Correlation of magnetic resonance imaging findings with lesion pathology. *Ann Neurol* 1997; 42(5): 783–793.
4. University of California San Francisco MS-EPIC Team, Cree BAC, Hollenbach JA, et al. Silent progression in disease activity-free relapsing multiple sclerosis. *Ann Neurol* 2019; 85(5): 653–666.
5. Dalton CM, Chard DT, Davies GR, et al. Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes. *Brain* 2004; 127(Pt 5): 1101–1107.
6. Eshaghi A, Prados F, Brownlee WJ, et al. Deep gray matter volume loss drives disability worsening in multiple sclerosis. *Ann Neurol* 2018; 83(2): 210–222.
7. Grothe M, Lotze M, Langner S, et al. Impairments in walking ability, dexterity, and cognitive function in multiple sclerosis are associated with different regional cerebellar gray matter loss. *Cerebellum* 2017; 16(5–6): 945–950.
8. Henry RG, Shieh M, Okuda DT, et al. Regional grey matter atrophy in clinically isolated syndromes at presentation. *J Neurol Neurosurg Psychiatry* 2008; 79(11): 1236–1244.
9. Azevedo CJ, Overton E, Khadka S, et al. Early cns neurodegeneration in radiologically isolated syndrome. *Neurol Neuroimmunol Neuroinflamm* 2015; 2(3): e102.

10. Sandroff BM, Motl RW, Román CAF, et al. Thalamic atrophy moderates associations among aerobic fitness, cognitive processing speed, and walking endurance in persons with multiple sclerosis. *J Neurol* 2022; 269(10): 5531–5540.
11. Schoonheim MM, Hulst HE, Brandt RB, et al. Thalamus structure and function determine severity of cognitive impairment in multiple sclerosis. *Neurology* 2015; 84(8): 776–783.
12. Azevedo CJ, Cen SY, Khadka S, et al. Thalamic atrophy in multiple sclerosis: A magnetic resonance imaging marker of neurodegeneration throughout disease. *Ann Neurol* 2018; 83(2): 223–234.
13. 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(9): 899–910.
14. Miller DH, Soon D, Fernando KT, et al. MRI outcomes in a placebo-controlled trial of natalizumab in relapsing ms. *Neurology* 2007; 68(17): 1390–1401.
15. Horakova D, Uher T, Krasensky J, et al. Long-term effectiveness of natalizumab on mri outcomes and no evidence of disease activity in relapsing-remitting multiple sclerosis patients treated in a czech republic real-world setting: A longitudinal, retrospective study. *Mult Scler Relat Disord* 2020; 46: 102543.
16. Perumal J, Balabanov R, Su R, et al. Natalizumab in early relapsing-remitting multiple sclerosis: A 4-year, open-label study. *Adv Ther* 2021; 38(7): 3724–3742.
17. Butzkueven H, Kappos L, Wiendl H, et al. Long-term safety and effectiveness of natalizumab treatment in clinical practice: 10 years of real-world data from the tysabri observational program (top). *J Neurol Neurosurg Psychiatry* 2020; 91(6): 660–668.
18. Khalid F, Tauhid S, Chua AS, et al. A longitudinal uncontrolled study of cerebral gray matter volume in patients receiving natalizumab for multiple sclerosis. *Int J Neurosci* 2017; 127(5): 396–403.
19. Rekik A, Aissi M, Rekik I, et al. Brain atrophy patterns in multiple sclerosis patients treated with natalizumab and its clinical correlates. *Brain Behav* 2022; 12(5): e2573.
20. Vidal-Jordana A, Sastre-Garriga J, Pérez-Miralles F, et al. Early brain pseudoatrophy while on natalizumab therapy is due to white matter volume changes. *Mult Scler* 2013; 19(9): 1175–1181.
21. Sotirchos ES, Gonzalez-Caldito N, Dewey BE, et al. Effect of disease-modifying therapies on subcortical gray matter atrophy in multiple sclerosis. *Mult Scler* 2020; 26(3): 312–321.
22. Amiri H, de Sitter A, Bendfeldt K, et al. Urgent challenges in quantification and interpretation of brain grey matter atrophy in individual ms patients using mri. *Neuroimage Clin* 2018; 19: 466–475.
23. de Sitter A, Burggraaff J, Bartel F, et al. Development and evaluation of a manual segmentation protocol for deep grey matter in multiple sclerosis: Towards accelerated semi-automated references. *Neuroimage Clin* 2021; 30: 102659.
24. Nakamura K and Fisher E. Segmentation of brain magnetic resonance images for measurement of gray matter atrophy in multiple sclerosis patients. *Neuroimage* 2009; 44(3): 769–776.
25. Ronneberger O, Fischer P and Brox T. U-net: Convolutional networks for biomedical image segmentation. In: Navab N, Hornegger J, Wells WM, et al. (eds) *Medical Image Computing and Computer-assisted Intervention—Miccai 2015*. Cham: Springer, 2015, pp. 234–241.
26. Alvarez E, Nair KV, Hoyt BD, et al. Brain atrophy rates in patients with multiple sclerosis on long term natalizumab resembles healthy controls. *Mult Scler Relat Disord* 2021; 55: 103170.
27. Sastre-Garriga J, Tur C, Pareto D, et al. Brain atrophy in natalizumab-treated patients: A 3-year follow-up. *Mult Scler* 2015; 21(6): 749–756.
28. De Stefano N, Giorgio A, Gentile G, et al. Dynamics of pseudo-atrophy in rms reveals predominant gray matter compartmentalization. *Ann Clin Transl Neurol* 2021; 8(3): 623–630.
29. Fisher E, Nakamura K, Lee JC, et al. Effect of intramuscular interferon beta-1a on gray matter atrophy in relapsing-remitting multiple sclerosis: A retrospective analysis. *Mult Scler* 2016; 22(5): 668–676.
30. De Stefano N, Stromillo ML, Giorgio A, et al. Establishing pathological cut-offs of brain atrophy rates in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2016; 87(1): 93–99.
31. De Stefano N, Airas L, Grigoriadis N, et al. Clinical relevance of brain volume measures in multiple sclerosis. *CNS Drugs* 2014; 28(2): 147–156.
32. Andorra M, Nakamura K, Lampert EJ, et al. Assessing biological and methodological aspects of brain volume loss in multiple sclerosis. *JAMA Neurol* 2018; 75(10): 1246–1255.

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