

CD49d antisense drug ATL1102 reduces disease activity in patients with relapsing-remitting MS

OPEN  

Volker Limmroth, MD
Frederik Barkhof, MD,
PhD
Nuket Desem, MBA
Mark P. Diamond, MBA
George Tachas, PhD
For the ATL1102 Study
Group

Correspondence to
Dr. Tachas:
george.tachas@antisense.com.au

ABSTRACT

Objective: This study evaluated the efficacy and safety of ATL1102, an antisense oligonucleotide that selectively targets the RNA for human CD49d, the α subunit of very late antigen 4, in patients with relapsing-remitting multiple sclerosis (RRMS).

Methods: In a multicenter, double-blind, placebo-controlled randomized phase II trial, 77 patients with RRMS were treated with 200 mg of ATL1102 subcutaneously injected 3 times in the first week and twice weekly for 7 weeks or placebo and monitored for a further 8 weeks. MRI scans were taken at baseline and weeks 4, 8, 12, and 16. The primary endpoint was the cumulative number of new active lesions (either new gadolinium-enhancing T1 lesions or nonenhancing new or enlarging T2 lesions) at weeks 4, 8, and 12.

Results: A total of 72 patients completed the study and 74 intention-to-treat patients were assessed. ATL1102 significantly reduced the cumulative number of new active lesions by 54.4% compared to placebo (mean 3.0 [SD 6.12] vs 6.2 [9.89], $p = 0.01$). The cumulative number of new gadolinium-enhancing T1 lesions was reduced by 67.9% compared to placebo ($p = 0.002$). Treatment-emergent adverse events included mild to moderate injection site erythema and decrease in platelet counts that returned to within the normal range after dosing.

Conclusions: In patients with RRMS, ATL1102 significantly reduced disease activity after 8 weeks of treatment and was generally well-tolerated. This trial provides evidence for the first time that antisense oligonucleotides may be used as a therapeutic approach in neuroimmunologic disorders.

Classification: This study provides Class I evidence that for patients with RRMS, the antisense oligonucleotide ATL1102 reduces the number of new active head MRI lesions. *Neurology*[®] 2014;83:1780-1788

GLOSSARY

ALAT = alanine aminotransferase; **CI** = confidence interval; **EDSS** = Expanded Disability Status Scale; **Gd** = gadolinium; **ITT** = intention-to-treat; **MS** = multiple sclerosis; **PML** = progressive multifocal leukoencephalopathy; **PP** = per-protocol; **RRMS** = relapsing-remitting multiple sclerosis; **SC** = subcutaneously; **TEAE** = treatment-emergent adverse event; **VLA-4** = very late antigen 4.

Relapsing-remitting multiple sclerosis (RRMS) is an immune-mediated disease that damages the myelin in CNS, causing neurologic impairment and frequently severe disability.¹ Currently most treatments act as immunosuppressors or immunomodulators. The monoclonal antibody natalizumab that targets the adhesion molecule very late antigen 4 (VLA-4), thought to interfere with the transmigration of leukocytes into the CNS, significantly reduces brain lesions,² relapse frequency, and the progression of disability in patients with RRMS.³ Natalizumab, however, can cause progressive multifocal leukoencephalopathy⁴ with a high lethality, which has impacted on its use.

ATL1102 is a second-generation antisense oligonucleotide to CD49d RNA, the α chain of VLA-4. ATL1102 binds CD49d RNA by Watson-Crick base pairing and recruits intracellular

Editorial, page 1776

Supplemental data
at Neurology.org

From the Department of Neurology (V.L.), Cologne City Hospitals, University of Cologne, Germany; the Department of Radiology (F.B.), VU Medical Centre, Amsterdam, the Netherlands; and Antisense Therapeutics Ltd. (N.D., M.P.D., G.T.), Melbourne, Australia.

Coinvestigators are listed on the *Neurology*[®] Web site at Neurology.org.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article. The Article Processing Charge was paid by Antisense Therapeutics Ltd.

This is an open access article distributed under the terms of the Creative Commons Attribution-Noncommercial No Derivative 3.0 License, which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially.

RNase H leading to degradation of the RNA strand of the RNA:DNA duplex.^{5,6} ATL1102 selectively reduces CD49d RNA and VLA-4 expression in primary human cells and in several human cell lines and inhibits cell adhesion. ATL1102 is rapidly cleared from the blood after administration and distributes to tissues including lymphoid organs that contain lymphocytes that express VLA-4. The aim of this proof-of-concept trial was to evaluate whether ATL1102 treatment was able to reduce brain lesion activity and to determine its safety profile in patients with RRMS.

METHODS ATL1102. ATL1102 is a single-stranded second-generation antisense oligonucleotide designed to hybridize to the 3'-untranslated region of human CD49d RNA. ATL1102 is 20 bases in length, with a molecular weight of 7230 Da. It is the 19-sodium salt of a 3' → 5' phosphorothioate oligonucleotide 20-mer with a 3-9-8, 2'-O-(2-methoxyethyl) gapmer design to support an RNase H antisense mechanism of action. The ATL1102 sequence is 5'-MeC^{Me}UG AGT^{Me}CTG TTT^{Me}U^{Me}C^{Me}C^{Me}A^{Me}U^{Me}U^{Me}C^{Me}U-3', with the first 3 and last 8 bases 2'-O-(2-methoxyethyl) modified and cytosine and uracil bases 5' methylated (Me).

Study protocol approvals, registrations, and patient consents. The trial is registered with the Australian New Zealand Clinical Trials Registry as trial number ACTRN12608000226303. Written informed consent was obtained from all patients before initiating study-related procedures. The conduct of the trial was approved by each country's regulatory authority and independent ethics committees of each participating center.

Trial design. This phase II trial in patients with RRMS was conducted as a randomized, multicenter, double-blind, placebo-controlled study. The study comprised 3 periods as follows:

1. 2-week screening period (visit V1, day -14 to 0), during which 95 patients were screened;
2. 8-week treatment period involving a 1-week induction phase (visit V2, day 1; visit V3, day 2) where 200 mg of ATL1102 was administered on days 1, 4, and 7 followed by a 7-week maintenance phase (visit V4, day 14; V5, day 28; V6, day 56) with 2 doses of 200 mg ATL1102 administered weekly on days 4 and 7; and an
3. 8-week follow-up period (visits V7, day 84; V8, day 112).

At visit V2, 77 eligible patients were randomly allocated in a ratio of approximately 1:1 to either the ATL1102 group (n = 36) or placebo group (n = 41) as outlined in appendix e-1 on the *Neurology*[®] Web site at Neurology.org. Study medication was administered subcutaneously (SC) by the investigator or the patient, blinded to treatment assignment. Each dose of ATL1102 contained 200 mg of ATL1102 in water for injection adjusted to pH7.4 and was administered as 2 100-mg SC injections.

Patient inclusion and exclusion criteria. Male and female patients were eligible for inclusion in the study if they were aged 18–55 years and had RRMS; at least 9 T2 lesions or at least 4 T2 lesions if one was gadolinium (Gd)-enhancing, with at least one relapse in the previous 12 months, but no relapses in the previous 4 weeks; and Expanded Disability Status Scale (EDSS) score 0–6.0.

For patients previously treated with immunosuppressive drugs or immunomodulating drugs, there was a 6- or 2-month prestudy washout period, respectively.

Patients were excluded if they were HIV-positive or had detectable levels of JC virus in the blood as measured by quantitative PCR.

Detailed eligibility criteria are available on the Australian New Zealand Clinical Trials Registry, number ACTRN12608000226303.

MRI of T1 and T2 lesions. MRI sites were selected based on successful performance of a dummy run and all in-study scans were subjected to a quality control procedure at the Image Analysis Centre in Amsterdam for central blinded assessment. The MRI protocol included T2-weighted images and T1-weighted images before and after standard-dose Gd. Five MRI scans were performed per patient and were taken at baseline (between day -14 and -7), week 4 (after 9 doses), week 8 (after 17 doses), week 12, and week 16. In case of relapse, an additional MRI scan was performed.

The following MRI efficacy assessment was done by an experienced reader blinded to treatment allocation:

- Number of new T1 Gd-enhancing lesions indicating blood–brain barrier disruption
- Number of new/enlarging T2 hyperintense lesions
- Total volume of enhancing lesions

Scans were also reviewed by a board-certified neuroradiologist for progressive multifocal leukoencephalopathy (PML) independently of the team assessing the scans for efficacy.

Efficacy assessments. The primary objective of this trial was to evaluate whether ATL1102 treatment was able to reduce brain lesion activity compared to placebo in patients with RRMS and Class I evidence is provided. The primary efficacy endpoint was the cumulative number of new active lesions (either new T1 Gd lesions or nonenhancing, new, or enlarging T2 lesions) on MRIs at weeks 4, 8, and 12 in ATL1102 compared to placebo-treated patients.

A secondary efficacy endpoint was the cumulative volume of all T1 Gd lesions on MRIs at weeks 4, 8, and 12 in the ATL1102 compared to placebo-treated patients.

Additional assessments included the cumulative number of T1 Gd lesions on MRIs taken at weeks 4, 8, and 12, and in a post hoc analysis, the cumulative number of new T1 Gd lesions at weeks 4, 8, and 12 in the ATL1102-treated patients compared to placebo. Also assessed were the total number of multiple sclerosis (MS) relapses, the total number of patients with no relapse, and the EDSS.

Safety data. Safety was evaluated on the basis of adverse events, laboratory data, vital signs, MRI assessment for PML, physical examination, 12-lead ECG, and local tolerance. Adverse events were reported by the patient or noted by the investigator over the entire study period.

Statistical procedures. Efficacy analyses were carried out for the intention-to-treat (ITT) population (randomized patients who received at least one injection of study medication, had a valid baseline MRI scan, and at least one valid postbaseline MRI scan), the ITT subset population (patients with valid postbaseline MRI brain lesion counts at weeks 4, 8, and 12), and the per-protocol (PP) population (patients with no major protocol violations).

The primary efficacy variable (cumulative number of new active lesions on MRI at weeks 4, 8, and 12) was analyzed using a negative binomial regression model. The secondary efficacy

Table 1 Patient demographic and MS history data of the randomized population

Characteristic	Placebo (n = 41)	ATL1102 (n = 36)	Total (n = 77)
Age, y, mean (SD)	38.0 (9.90)	39.6 (8.78)	38.8 (9.37)
Sex, n (%)			
Female	25 (61.0)	26 (72.2)	
Male	16 (39.0)	10 (27.8)	
Duration of MS, y, mean (SD)	3.76 (4.14)	5.67 (6.48)	
Median (range)	1.8 (0.0–15.7)	3.0 (0.0–25.8)	2.5 (0.0–25.8)
MS relapses within previous year, n (%)			
1	32 (78.0)	22 (61.1)	54 (70.1)
2	8 (19.5)	10 (27.8)	18 (23.4)
3	0	4 (11.1)	4 (5.2)
4	1 (2.4)	0	1 (1.3)
Mean ^a	1.27	1.50	1.38
Time since most recent prestudy relapse, d, median (range)	129.0 (59–365)	126.0 (53–357)	128.0 (53–365)
EDSS score at screening, mean (SD)	2.83 (1.42)	2.49 (1.17)	
Median (range)	2.5 (1.0–6.0)	2.0 (0.0–5.5)	2.5 (0.0–6.0)
T1 Gd lesions at baseline, mean (SD)	1.1 (2.28)	1.2 (2.58)	
T1 Gd lesion volume at baseline, mean (SD)	121 (305.7)	151 (370.6)	
Previous medication, n (%) ^b	n = 37	n = 34	n = 71
Glucocorticoids	34 (91.9)	31 (91.2)	65 (91.5)
Interferons	5 (13.5)	7 (20.6)	12 (16.9)
Other centrally active	4 (10.8)	5 (14.7)	9 (12.7)
Other immunosuppressive	4 (10.8)	4 (11.8)	8 (11.3)

Abbreviations: EDSS = Expanded Disability Status Scale; MS = multiple sclerosis.

^aMean numbers of MS relapses within past year.

^bMost common previous medications for MS taken by 8 or more patients. Includes all medications stopped prior to first injection of study medication. Percentage is based on the total number of patients with previous medication.

variable (cumulative volume of T1 Gd lesions on MRI at weeks 4, 8, and 12) was analyzed using a nonparametric rank analysis of covariance model. The primary and secondary efficacy analyses used treatment, sex, and country (pooled centres) as factors, and age and number or volume of enhancing lesions on screening MRI as covariates, respectively. One-sided *p* values, point estimates, and 2-sided 90% confidence intervals (CIs) for the differences between treatment groups were determined. For the primary efficacy analysis, a 1-sided significance level of 0.05 was specified in the study protocol. The sample size justification and powering are outlined in appendix e-1.

Missing MRI data due to causes other than relapse were replaced by the median lesion count of all new active lesions on MRIs taken at the same planned week for all patients within the same treatment group. Of the patients in the ITT population, 2 patients in the placebo group had one missing postbaseline scan each and one patient in the ATL1102 group had 3 missing scans postbaseline scan, all for reasons other than relapse.

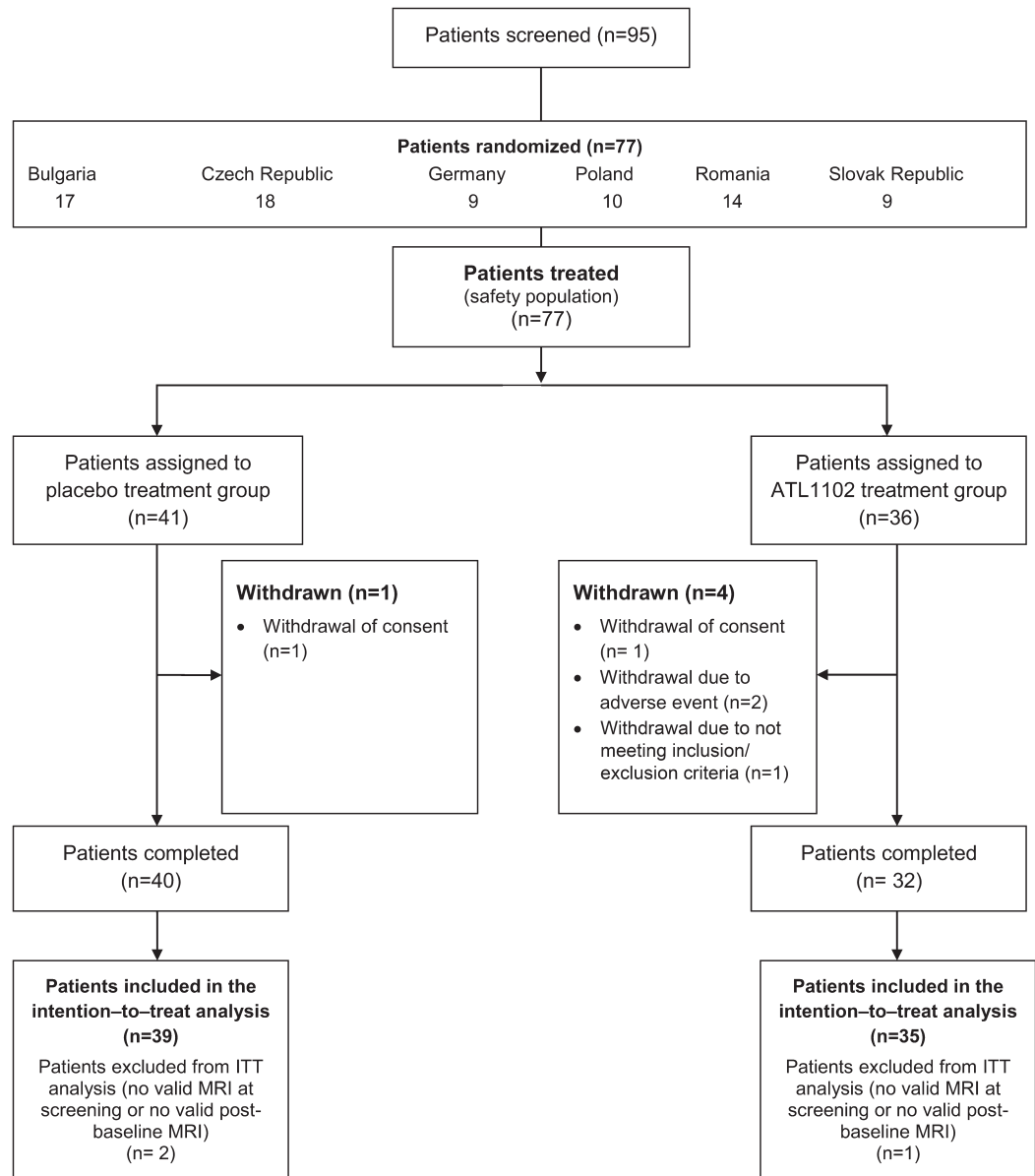
RESULTS Study patients and conduct. Of the 95 patients screened, 77 were randomized and treated with study medications. The patient demographic data were summarized for each treatment group (table 1). Overall, the treatment groups were well-balanced with

respect to demographic data. There were no clinically relevant differences with previous or concomitant medical conditions or medications. The median duration of history of MS prior to enrollment was lower in the placebo group (1.8 years, range 0.0–15.7) than in the ATL1102 group (3.0 years, range 0.0–25.8).

Five patients withdrew prematurely and 72 completed the study. Patients who discontinued the study still underwent the final examinations. The ITT population consisted of 74 patients, the ITT subset 71 patients, and the PP population 68 patients (figure 1). The mean (SD) duration of study medication in the randomized population was slightly higher in the placebo group, 55 (7.06) days, compared to ATL1102 group, 53.3 (9.32) days.

Efficacy. The cumulative number of new active lesions for weeks 4, 8, and 12 (primary outcome measure) was 54.4% lower in the ATL1102 group than in the placebo group (figure 2A); mean (SD) number of new active lesions 3.0 (6.12) vs 6.2 (9.89), 1-sided

Figure 1 Number of patients in each stage of the study starting from all screened patients



ITT = intention-to-treat.

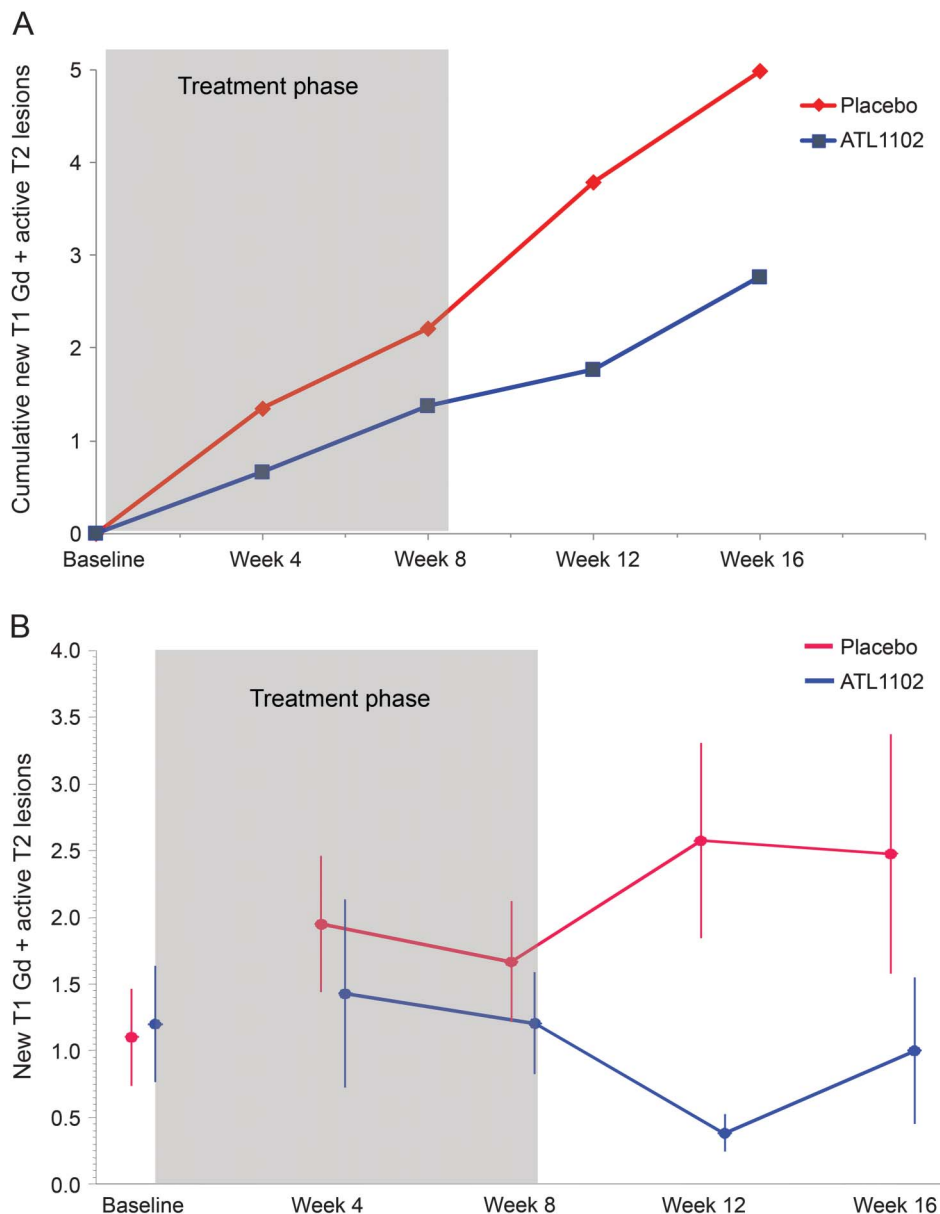
p value 0.0050 (90% CI 1.2855, -0.2840) (ITT population). A significant difference between the 2 treatment groups was present at the end of the active treatment phase (week 8); mean (SD) number of new active lesions 2.6 (5.75) vs 3.6 (5.49), 1-sided *p* value 0.0456 (90% CI -1.0005, -0.0133) (ITT population). Similar results were found in the ITT subset and PP populations.

The cumulative T1 Gd lesion volume for weeks 4, 8, and 12 (secondary outcome measure) was lower in the ATL1102 group than the placebo group: 358 (1,028.4) mm³ vs 589 (1,107.6) mm³, with a clear trend towards significance (1-sided *p* = 0.0534) (90% CI -0.129, 12.863). ATL1102 significantly reduced T1 Gd lesion volume by 84.4% at week 12

compared to placebo (2-sided *p* = 0.0172; Wilcoxon 2-sample test) (figure 3A).

ATL1102 reduced the cumulative number of T1 Gd lesions for weeks 4, 8, and 12 by 66.7% compared to placebo, mean (SD) 2.9 (7.92) vs 6.1 (11.13), 1-sided *p* value 0.0010 (ITT) (figure 3B), and reduced the cumulative number of new T1 Gd lesions for weeks 4, 8, and 12 by 67.9% compared to placebo, mean (SD) 2.1 (5.55) vs 5.2 (9.40), 1-sided *p* value 0.0008 (ITT) (figure e-1A). Notably, ATL1102 significantly reduced T1 Gd and new T1 Gd lesion numbers by 88.5% and 90.5%, respectively, at week 12 compared to placebo (2-sided *p* value = 0.010 and 0.005, respectively; Wilcoxon 2-sample test) (figure 3B and e-1B).

Figure 2 (A) Cumulative number of new active lesions and (B) number of new active lesions (mean \pm SEM)

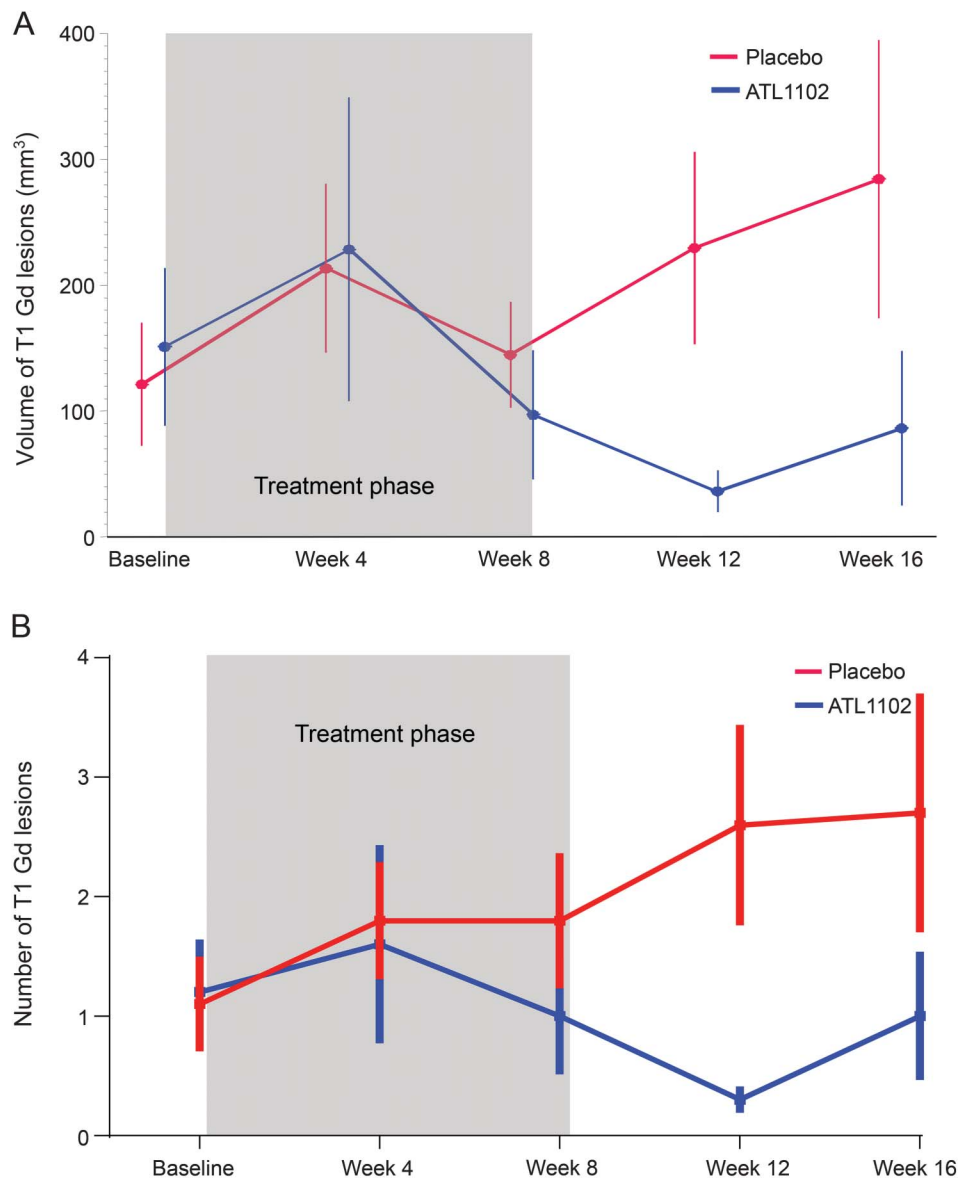


ATL1102 treatment led to an absolute increase of 24.8% ($p = 0.0332$) and relative increase of 51% (risk reduction = $73.5/48.7 = 1.51$) in the percentage of T1 Gd lesion-free patients compared to placebo measured at 8 and 12 weeks combined (χ^2 , figure 4).

The number of patients with MS relapses in the 2 treatment groups were 8 (19.5%) patients in the placebo group vs 6 (16.7%) patients in the ATL1102 group; however, the difference did not reach significance. Mean EDSS scores decreased slightly during the study in both groups but the difference between the groups was not statistically significant. Frequencies of patients with increases or decreases in EDSS were comparable in the 2 treatment groups.

Safety. The most common treatment-emergent adverse events (TEAEs) in the ATL1102 group were injection site erythema (25.0% patients), alanine aminotransferase (ALAT) increases (19.4%), MS relapses (16.7%), aspartate aminotransferase increases (11.1%), headache (11.1%), and thrombocytopenia (22.2%) (table e-1). MS relapse was the most frequent TEAE in the placebo group (19.5% patients). Injection site erythema, ALAT increases, and thrombocytopenia were more frequent in the ATL1102 group than in the placebo group (difference $\geq 5\%$). Serious TEAEs were MS relapses and one case of grade 2 thrombocytopenia in the ATL1102 group. No JC virus in the blood or PML was observed.

Figure 3 (A) T1 gadolinium (Gd) lesion volume and (B) T1 Gd lesion number (mean \pm SEM)



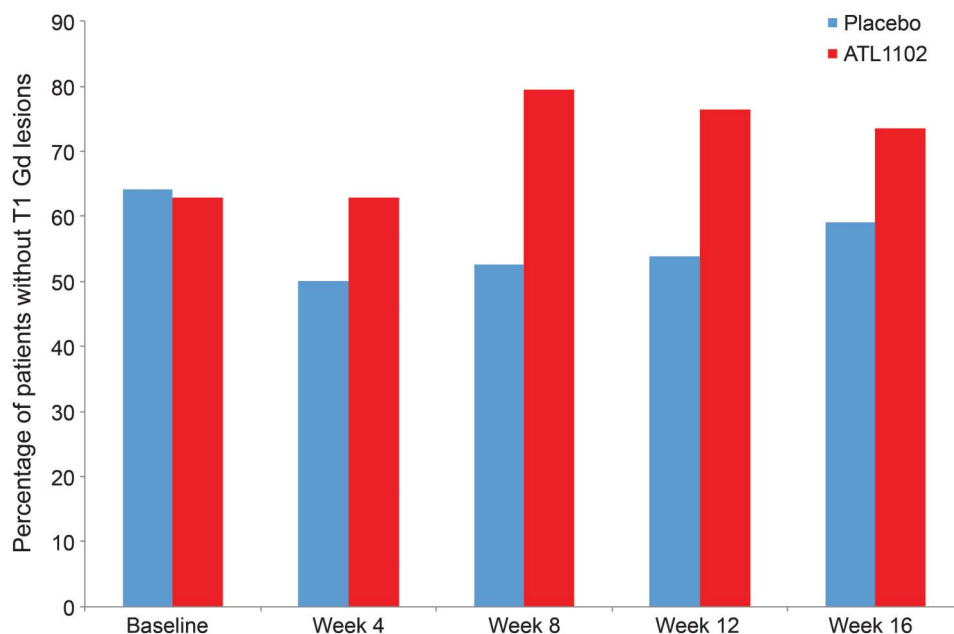
DISCUSSION The ATL1102 trial conducted in patients with RRMS met its primary efficacy endpoint, reducing cumulative number of new active lesions at weeks 4, 8, and 12 by 54% vs placebo after 8 weeks of dosing and the cumulative number of new T1 Gd lesions by 67.9% vs placebo.

Treatment of patients with RRMS with natalizumab with 2 injections over 2 months resulted in reductions in the cumulative number of new active lesions over 12 weeks of 50% vs placebo and the cumulative number of new T1 Gd lesions by 52% vs placebo.² Treatment of patients with MS with other drugs registered for RRMS have shown reductions in brain lesions over the first 12-week treatment period,^{7–12} albeit with different, generally lower, levels of activity. Pairwise meta-analysis comparisons between marketed

treatments in longer randomized controlled trials indicate that natalizumab may have better relative effectiveness on the parameters of “patients without MRI progression” and “patients free of relapse.”¹³

ATL1102 demonstrated an increasing effect over time with T1 Gd lesion reductions by week 8 and the greatest T1 Gd lesion reductions observed at week 12, 4 weeks after the last dose. This extended duration of activity postdosing of ATL1102 was potentially related to the time course for the formation-turnover of new enhancing lesions and the drug’s long (>3 weeks) tissue half-life.¹⁴ The extended duration of action supports the proposition of less frequent or lower dosing in longer-term studies than the twice-weekly 200-mg dosing employed in the current trial. Pharmacometric modeling suggests 200 mg once

Figure 4 Percentage of patients without T1 gadolinium (Gd) lesions at screening and weeks 4, 8, 12, and 16



weekly, every other week, and every 3 weeks over 6 months has the potential to significantly reduce MRI brain lesions and to minimize side effects including platelet reductions.¹⁵ This dosing schedule could be employed in longer-term clinical trials.

TEAEs with a frequency of more than 10% in the ATL1102 group vs a lower frequency (difference of >5%) in the placebo group were mild to moderate injection site erythema, mild increases in liver enzyme ALAT, and a decrease in platelet count that was reversible after treatment interruption and not accompanied by any clinical consequences.

There were fewer patients with relapses in the ATL1102 group; however, this study was not powered to detect significant differences in relapses or neurologic disability as assessed by the EDSS, the view being that longer duration and larger studies are needed to see changes in these clinical parameters.^{2,7-11} MRI brain lesion reductions in longer-term studies are associated with reductions in these clinical parameters.^{16,17}

ATL1102, like other antisense oligonucleotides of the same class, has a short half-life of 4.8 hours in the blood.¹⁴ There was no significant change in blood leukocyte CD49d RNA levels with ATL1102,¹⁴ which may reflect the short exposure of these cells to ATL1102 in the blood and poor cellular uptake. Animal studies have shown that like other antisense drugs,¹⁸ ATL1102 distributes rapidly to the bone marrow, spleen, and lymph nodes in relatively high drug concentrations.¹⁴ An antisense fully complementary to murine CD49d RNA reduced VLA-4

expression in inflamed lymph nodes and spleen. Accordingly, ATL1102 may be reducing CD49d RNA and VLA-4 expression on immune cells in these lymphoid tissues in patients with RRMS. Supporting this hypothesis, ATL1102 treatment produced a ~10% reduction in the number of CD19+ (pre) B cells with detectable levels of VLA-4 expression in the blood at 8 weeks¹⁴ (appendix e-2). A small number of leukocytes are known to migrate from the secondary lymphoid tissues via the blood,¹⁹ which may be the source of these VLA-4-negative CD19+ cells in the blood.

ATL1102 treatment reduced the number of circulating CD19+ (pre) B cells (53%) and granulocytes (43%) at 8 weeks compared to treatment with placebo; T cells were less significantly reduced (~25%), but ATL1102 treatment had no effect on monocyte or NK lymphocyte numbers¹⁴ (appendix e-2). VLA-4 has a role in the maturation, apoptosis, activation, adhesion, and migration of B and T cells.²⁰⁻²⁵ ATL1102 may be having an effect on one or more of these activities on CD19+ (pre) B and T cells within the lymphoid tissues of patients with RRMS, thereby reducing leukocyte number and activity in blood, and in turn the CNS, and subsequently reducing the number and volume of MS brain lesions in this RRMS study.

Natalizumab interferes with transmigration of VLA-4+ leukocytes and disproportionately increases circulating B cells more than other lymphocytes and monocytes in blood of patients with RRMS.^{26,27} The 44% of disease-free natalizumab-treated patients with

RRMS are characterized by a substantial reduction of CD19+ B cells, particularly the CD5+ subset, and plasmablasts in the CNS.²⁸ The therapeutic effects of CD20 antibodies that deplete CD20+ blood B cells also point to the importance of reducing proinflammatory B cells in CNS in RRMS.²⁹

More analysis is required to characterize the pharmacologic and pharmacodynamic action of ATL1102. This also extends to ascertaining the profile of ATL1102 with respect to the risk of PML. Natalizumab increases the release of CD34+ hematopoietic stem/progenitor cells and CD19+ pre B cells and CD20+ B cells into the blood, which carry latent low copy JC virus,^{30,31} including in individuals who are seronegative.³¹ Latent JC virus activation is theorized to involve B-cell differentiation, including B-cell DNA-binding protein Spi-B, which increases JCV transcription.³⁰ Natalizumab has a long half-life in the blood (6 days), and a broad VLA-4 antagonist effect, and can impair JC virus immunosurveillance, leading to PML.^{4,30}

Preliminary data have shown ATL1102 treatment increases CD34+ RNA 50% at week 8 vs baseline, though its effects at the CD34+ cell level need to be explored¹⁴ (appendix e-2). The reduction of CD19+ (pre) B cells with ATL1102 treatment suggests that release of lymphoid precursors into the blood may be low or they do not survive, potentially reducing the pool of cells that may carry latent virus. ATL1102 does not bind cell surface VLA-4. Natalizumab binding to VLA-4 induces intracellular signaling-associated proinflammatory effects, leading to poor outcomes in patients with PML following treatment suspension.^{30,32} The short ATL1102 half-life in plasma of 4.8 hours¹⁴ potentially limits exposure of circulating leukocytes to drug, which may better preserve blood leukocyte VLA-4-mediated immunosurveillance and therefore be at less risk of causing PML. IFN- β 1 treatment of RRMS reduces blood mononuclear cell CD49d RNA³³ and VLA-4 expression on CD8+ lymphocytes³⁴ and CD4+CD45RO+ primed memory T cells, while preserving other blood leukocyte VLA-4 function.³⁵ IFN- β 1 has not been associated with PML.

ATL1102, which employs a unique antisense mechanism to reduce VLA-4 expression, has in this study substantially reduced disease activity in RRMS at doses that are generally well-tolerated. Longer-term trials are required to confirm its potential as a valuable additional therapeutic option in the treatment of RRMS.

AUTHOR CONTRIBUTIONS

V. Limmroth was the study Principal Investigator and a member of the protocol development team, took primary responsibility for the safety oversight of the study, and revised the manuscript. F. Barkhof was a member of the protocol development team, advised on statistical aspects of the study, and revised the manuscript. N. Desem was a member of the protocol development team, managed day-to-day activities of the study,

participated in the safety monitoring process, oversaw the analysis of the data, including the statistical work on the primary and secondary efficacy endpoints carried out by the Accovion GmbH Marburg Germany statistical analysis team and reporting of the study, and contributed to the writing of the manuscript. M.P. Diamond was a member of the protocol development team and contributed to the analysis of the data and the writing of the manuscript. G. Tachas was a member of the protocol development team, contributed to the analysis and interpretation of the data, oversaw the analysis of the statistical work on the additional and post hoc analyses carried out by the statistical analysis team of the McCloud Consulting Group, Sydney, Australia, and performed the primary writing of the manuscript.

ACKNOWLEDGMENT

The authors thank (1) Prof. Krzysztof for sharing his medical expertise in Polish regulatory and ethics approval; (2) Prof. Hass, Dr. Lensch, Dr. Scholz, and Dr. Strangel for information on patient eligibility selection criteria; (3) Prof. Zetl for logistic support to the trial investigators in Germany; (4) Accovion GmbH, Marburg, Germany, for the statistical work on the primary and secondary efficacy endpoints; (5) McCloud Consulting Group, Sydney, Australia, for the statistical work on the additional and post hoc analyses; and (6) Isis Pharmaceuticals, including Dr. S.T. Crooke, Dr. R.S. Geary, Dr. S.P. Henry, Dr. T.J. Kwok, Dr. J. Grundy, Dr. A.N. Scozzari, Dr. C.F. Bennett, and Dr. D.C. Capaldi, for sharing their expertise in antisense.

STUDY FUNDING

The study is industry-sponsored by Antisense Therapeutics Ltd., Melbourne, Australia.

DISCLOSURE

V. Limmroth has received payment for services from the sponsor for consultancy. The Department of Neurology (Dr. Limmroth), Cologne City Hospitals, University of Cologne, Germany, has received compensation for participation in the clinical trial. F. Barkhof has received payment for services from the sponsor for consultancy. The Department of Radiology (Dr. Barkhof), VU Medical Centre, Amsterdam, the Netherlands, has received compensation for participation in the clinical trial. N. Desem holds an equity interest in the sponsor. M. Diamond holds an equity interest in the sponsor. G. Tachas holds an equity interest in the sponsor. Go to Neurology.org for full disclosures.

Received December 30, 2013. Accepted in final form July 2, 2014.

REFERENCES

1. Goodin DS, Frohman EM, Garmany GP, et al. Disease modifying therapies in multiple sclerosis: report of the Therapeutics and Technology Assessment subcommittee of the American Academy of Neurology and the MS Council for Clinical Practice Guidelines. *Neurology* 2002;58:169–178.
2. Tubridy N, Behan PO, Capildeo R, et al. The effect of anti- α 4 integrin antibody on brain lesion activity in MS. *Neurology* 1999;53:466–472.
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. Clifford DB, De Luca A, Simpson DM, Arendt G, Giovannoni G, Nath A. Natalizumab-associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: lessons from 28 cases. *Lancet Neurol* 2010;4:438–446.
5. Crooke ST. Molecular mechanisms of antisense drugs: RNase H. *Antisense Nucleic Acid Drug Dev* 1998;8:133–134.
6. Baker F, Monia BP. Novel mechanisms for antisense-mediated regulation of gene expression. *Biochim Biophys Acta* 1999;1489:3–18.

7. Moharreggh-Khiabani D, Linker RA, Gold R, Stangel M. Fumaric acid and its esters: an emerging treatment for multiple sclerosis. *Curr Neuroparmacol* 2009;7:60–64.
8. Polman C, Barkhof F, Sandberg-Wollheim M, et al. Treatment with laquinimod reduces development of active MRI lesions in relapsing MS. *Neurology* 2005;64:987–991.
9. Cohen JA, Rovaris M, Goodman AD, et al. Randomized, double-blind dose comparison study of glatiramer acetate in relapsing-remitting MS. *Neurology* 2007;68:939–944.
10. Frank JA, Richert N, Bash C, et al. Interferon- β -1b slows progression of atrophy in RRMS. *Neurology* 2004;62:719–725.
11. Gold R, Giovannoni G, Selmaj K, et al. Daclizumab high-yield process in relapsing-remitting multiple sclerosis (SELECT): a randomised, double-blind, placebo-controlled trial. *Lancet* 2013;381:2167–2175.
12. O'Connor PW, Li D, Freedman MS, et al. Phase II study of the safety and efficacy of teriflunomide in multiple sclerosis with relapses. *Neurology* 2006;66:894–900.
13. Hadjigeorgiou GM, Doxani C, Miligkos M, et al. A network meta-analysis of randomized controlled trials for comparing the effectiveness and safety profile of treatments with marketing authorization for relapsing multiple sclerosis. *J Clin Pharm Ther* 2013;38:433–439.
14. Tachas G; Antisense Therapeutics Ltd. assignee. Method of mobilizing stem cells. PCT application PCT/AU2011/001205 (published as WO2012/034194). September 19, 2011.
15. Guzy S, Bauer R. Pharmacometrics in drug development: concepts and applications. In: Faltin FW, Kenett RS, Ruggeri F, eds. *Statistical Methods in Healthcare*. Chichester, UK: John Wiley & Sons; 2012:56–77.
16. Sormani MP, Bonzano L, Roccatagliata L, Cutter GR, Mancardi GL, Bruzzi P. Magnetic resonance imaging as a potential surrogate for relapses in multiple sclerosis: a meta-analytic approach. *Ann Neurol* 2009;65:268–275.
17. Sormani MP, Bonzano L, Roccatagliata L, Mancardi GL, Uccelli A, Bruzzi P. Surrogate endpoints for EDSS worsening in multiple sclerosis: a meta-analytic approach. *Neurology* 2010;75:302–309.
18. Geary RS, Yu RZ, Siwkowski A, Levin AA. Pharmacokinetics/pharmacodynamic properties of phosphorothioate 2'-O-(2-methoxyethyl)-modified antisense oligonucleotides in animals and man. In: Crooke ST, ed. *Antisense Drug Technology: Principles, Strategies, and Applications*, 2nd ed. Boca Raton, FL: CRC Press, Taylor & Francis Group; 2008:305–326.
19. Matsuno K, Ueta H, Shu Z, et al. The microstructure of secondary lymphoid organs that support immune cell trafficking. *Arch Histol Cytol* 2010;73:1–21.
20. Arroyo AG, Yang JT, Rayburn H, Hynes RO. Differential requirements for alpha4 integrins during fetal and adult hematopoiesis. *Cell* 1996;85:997–1008.
21. Carrasco YR, Batista FD. B-cell activation by membrane-bound antigens is facilitated by the interaction of VLA-4 with VCAM-1. *EMBO J* 2006;25:889–899.
22. Lo CG, Lu TT, Cyster JG. Integrin-dependence of lymphocyte entry into the splenic white pulp. *J Exp Med* 2003;197:353–361.
23. Alter A, Duddy M, Hebert S, et al. Determinants of human B-cell migration across brain endothelial cells. *J Immunol* 2003;170:4497–4505.
24. Tchilian EZ, Owen JJ, Jenkinson EJ. Anti-alpha 4 integrin antibody induces apoptosis in murine thymocytes and staphylococcal enterotoxin B-activated lymph node T-cells. *Immunology* 1997;92:321–327.
25. Niino M, Bodner C, Simard ML, et al. Natalizumab effects on immune cell responses in multiple sclerosis. *Ann Neurol* 2006;59:748–754.
26. Krumbholz M, Meinl L, Kumpfel T, Hohlfeld R, Meinl E. Natalizumab disproportionately increases circulating pre-B and B-cells in multiple sclerosis. *Neurology* 2008;71:1350–1354.
27. Putzki N, Baranwal MK, Tettenborn B, Limmroth V, Kreuzfelder E. Effects of natalizumab on circulating B-cells, T regulatory cells and natural killer cells. *Eur Neurol* 2010;63:311–317.
28. Villar LM, García-Sánchez MI, Costa-Frossard L, et al. Immunological markers of optimal response to natalizumab in multiple sclerosis. *Arch Neurol* 2012;69:191–197.
29. Krumbholz M, Derfuss T, Hohlfeld R, Meinl E. B-cells and antibodies in multiple sclerosis pathogenesis and therapy. *Nat Rev Neurol* 2012;8:613–623.
30. Bellizzi A, Anzivino E, Rodio DM, Palamara AT, Nencioni L, Pietropaolo V. New insights on human polyomavirus JC and pathogenesis of progressive multifocal leukoencephalopathy. *Clin Dev Immunol* 2013;2013:1–17.
31. Frohman EM, Monaco MC, Remington G, et al. JC virus in CD34⁺ and CD19⁺ cells in patients with multiple sclerosis treated with natalizumab. *JAMA Neurol* 2014;71:596–602.
32. Benkert TF, Dietz L, Hartmann EM, et al. Natalizumab exerts direct signaling capacity and supports a pro-inflammatory phenotype in some patients with multiple sclerosis. *Plos One* 2012;7:1–14.
33. Muraro PA, Liberati L, Bonanni L, et al. Decreased integrin gene expression in patients with MS responding to interferon-beta treatment. *J Neuroimmunol* 2004;150:123–131.
34. Calabresi PA, Pelfrey CM, Tranquill LR, Maloni H, McFarland HF. VLA-4 expression on peripheral blood lymphocytes is downregulated after treatment of multiple sclerosis with interferon beta. *Neurology* 1997;49:1111–1116.
35. Muraro PA, Leist T, Bielekova B, McFarland HF. VLA-4/CD49d downregulated on primed T lymphocytes during interferon-beta therapy in multiple sclerosis. *J Neuroimmunol* 2000;111:186–194.

Neurology[®]

CD49d antisense drug ATL1102 reduces disease activity in patients with relapsing-remitting MS

Volker Limmroth, Frederik Barkhof, Nuket Desem, et al.

Neurology 2014;83;1780-1788 Published Online before print September 19, 2014

DOI 10.1212/WNL.0000000000000926

This information is current as of September 19, 2014

Neurology® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright © 2014 American Academy of Neurology. All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.



Updated Information & Services	including high resolution figures, can be found at: http://n.neurology.org/content/83/20/1780.full
Supplementary Material	Supplementary material can be found at: http://n.neurology.org/content/suppl/2014/09/19/WNL.000000000000926.DC2 http://n.neurology.org/content/suppl/2014/09/19/WNL.000000000000926.DC3 http://n.neurology.org/content/suppl/2014/09/19/WNL.000000000000926.DC1
References	This article cites 32 articles, 12 of which you can access for free at: http://n.neurology.org/content/83/20/1780.full#ref-list-1
Citations	This article has been cited by 1 HighWire-hosted articles: http://n.neurology.org/content/83/20/1780.full##otherarticles
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): MRI http://n.neurology.org/cgi/collection/mri Multiple sclerosis http://n.neurology.org/cgi/collection/multiple_sclerosis
Errata	An erratum has been published regarding this article. Please see next page or: /content/84/1/105.4.full.pdf
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://www.neurology.org/about/about_the_journal#permissions
Reprints	Information about ordering reprints can be found online: http://n.neurology.org/subscribers/advertise

Neurology® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright © 2014 American Academy of Neurology. All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.



3. Sturm W, Fimm B, Cantagallo A, et al. Specific computerised attention training in stroke and traumatic brain-injured patients: a European multicenter efficacy study. *Z Neuropsychol* 2003;14:283–292.
4. Diener HC, Weimar C, eds. *Leitlinien für Diagnostik und Therapie in der Neurologie*. Stuttgart: Thieme; 2012.

INFLUENZA VACCINATION AND CARDIOVASCULAR RISK IN PATIENTS WITH RECENT TIA AND STROKE

Bayzidur Rahman, Anita Heywood, Aye Moa, C. Raina MacIntyre, Sydney, Australia: Lavallée et al.¹ found no effect of influenza vaccination on risk of cardiovascular disease in recent TIA or stroke patients. Vaccination status was determined by baseline self-report, which has poor validity and is subject to recall bias.² This can result in misclassification of vaccination status and bias of the reported effect.

Vaccination timing was not presented in the 3 component studies. Survival analysis was conducted on a baseline vaccination status for any cardiovascular event occurring during the 2-year follow-up. Without annual vaccination data, it is unknown whether participants were protected by vaccination at the time of subsequent cardiovascular events.

The pooling of data from 3 separate studies for the main analyses is not ideal. The analyses should appropriately consider between-study variation (e.g., individual patient data meta-analysis).³ Two of the component studies (OPTIC and PERFORM) were multicentered (clustered), which also warrants consideration. This study failed to show an unbiased effect of vaccination on cardiovascular events, which conflicts with data showing such an effect.^{4,5}

© 2014 American Academy of Neurology

1. Lavallée PC, Labreuche J, Fox KM, et al. Influenza vaccination and cardiovascular risk in patients with recent TIA and stroke. *Neurology* 2014;82:1905–1913.
2. Rolnick SJ, Parker ED, Nordin JD, et al. Self-report compared to electronic medical record across eight adult vaccines: do results vary by demographic factors? *Vaccine* 2013;31:3928–3935.
3. Riley RD, Lambert PC, Abo-Zaid G. Meta-analysis of individual participant data: rationale, conduct, and reporting. *BMJ* 2010;340:c221.
4. Udell JA, Zawi R, Bhatt DL, et al. Association between influenza vaccination and cardiovascular outcomes in high-risk patients: a meta-analysis. *JAMA* 2013;310:1711–1720.
5. Warren-Gash C, Smeeth L, Hayward AC. Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. *Lancet Infect Dis* 2009;9:601–610.

CORRECTIONS

MRI measurement of brain iron in patients with restless legs syndrome

In the article “MRI measurement of brain iron in patients with restless legs syndrome” by R.P. Allen et al. (*Neurology*[®] 2001;56:263–265), there is an error in the author byline. The third author’s name should read “F.W. Wehrli, PhD.”

Child Neurology: PRRT2-associated movement disorders and differential diagnoses

In the article “Child Neurology: PRRT2-associated movement disorders and differential diagnoses” by D. Ebrahimi-Fakhari et al. (*Neurology*[®] 2014;83:1680–1683), there is an error in the footnote under table 1. Table 1 is not reproduced from Gupta and Lang but was created by the authors. Supplemental table e-2 was modified from Gupta and Lang (Gupta A, Lang AE. Psychogenic movement disorders. *Curr Opin Neurol* 2009;22:430–436), with permission. The authors regret the error.

CD49d antisense drug ATL1102 reduces disease activity in patients with relapsing-remitting MS

In the article “CD49d antisense drug ATL1102 reduces disease activity in patients with relapsing-remitting MS” by V. Limmroth et al. (*Neurology*[®] 2014;83:1780–1788), there is an error in the Acknowledgment section: “Prof. Krzysztof” should read “Prof. Selmaj” and “Dr. Strangel” should read “Dr. Stangel.” In addition, the first sentence in the Methods under “Safety data” should read: “Safety was evaluated by an independent data safety monitoring board on the basis of adverse events, laboratory data, vital signs, MRI assessment for PML, physical examination, 12-lead ECG, and local tolerance.” The authors regret the errors.

Author disclosures are available upon request (journal@neurology.org).