

# PHASE IIa TRIAL OF FINGOLIMOD FOR AMYOTROPHIC LATERAL SCLEROSIS DEMONSTRATES ACCEPTABLE ACUTE SAFETY AND TOLERABILITY

JAMES D. BERRY, MD, MPH,<sup>1,2</sup> SABRINA PAGANONI, MD, PhD,<sup>1,2</sup> NAZEM ATASSI, MD, MSc,<sup>1,2</sup> ERIC A. MACKLIN, PhD,<sup>1,2</sup> NAMITA GOYAL, MD,<sup>3</sup> MICHAEL RIVNER, MD,<sup>4</sup> ERICKA SIMPSON, MD,<sup>5</sup> STANLEY APPEL, MD,<sup>5</sup> DANIELA L. GRASSO, BA,<sup>1</sup> NICTE I. MEJIA, MD,<sup>1</sup> FARRAH MATEEN, MD, PhD,<sup>1</sup> ALAN GILL, PhD,<sup>6</sup> FERNANDO VIEIRA, MD,<sup>6</sup> VALERIE TASSINARI, BS,<sup>6</sup> and STEVEN PERRIN, PhD<sup>6</sup>

<sup>1</sup>Neurological Clinical Research Institute, Massachusetts General Hospital, Harvard Medical School, 165 Cambridge Street, Suite 600, Boston, Massachusetts 02114, USA

<sup>2</sup>Department of Neurology, Harvard Medical School, Boston, Massachusetts, USA

<sup>3</sup>Department of Neurology, University of California, Irvine, Orange, California, USA

<sup>4</sup>Department of Neurology, Augusta University Medical Center, Augusta, Georgia, USA

<sup>5</sup>Department of Neurology, Methodist Hospital, Houston, Texas, USA

<sup>6</sup>ALS Therapy Development Institute, Cambridge, Massachusetts, USA

Accepted 25 June 2017

Additional supporting information can be found in the online version of this article.

**Abbreviations:** AE, adverse event; ALS, amyotrophic lateral sclerosis; ALSFRS-R, ALS Functional Rating Scale—Revised; ALSTDI, ALS Therapy Development Institute; AV, atrioventricular; BMI, body mass index; CNS, central nervous system; CSF, cerebrospinal fluid; ECG, electrocardiogram; FDA, U.S. Food and Drug Administration; FEV<sub>1</sub>, forced expiratory volume in 1 second; FOXP3, forkhead box P3; HR, heart rate; MGH, Massachusetts General Hospital; MS, multiple sclerosis; PFT, pulmonary function test; PET, positron emission tomography; SAE, serious adverse event; S1P, sphingosine 1-phosphate; SOD1, superoxide dismutase 1; SVC, slow vital capacity

**Key words:** circulating lymphocytes; clinical trial; FOXP3; neuroinflammation; RNA profiling; target engagement

**Funding:** This work was supported by the ALS Therapy Development Institute (to T.D.I.).

Study drug and placebo were provided by Novartis Pharmaceuticals.

**Disclosures:** J.D.B. has consulted with Biogen-IDEC, Denali Therapeutics, and NeuralTus Pharmaceuticals, and has received research support from Voyager Therapeutics, GSK, Cytokinetics, Brainstorm Cell Therapeutics, Novartis, ALS Therapy Development Institute, ALS Association, Muscular Dystrophy Association, and National Institutes of Health (NIH). S.P. has received funding from the NIH (Career Development Award 2K12HD001097-16), Target ALS, the Salah Foundation, and the Spastic Paraplegia Foundation, and has provided consulting for Roche and Pison Technology. N.A. is funded by an NIH Career Development Award and has research grants from the Harvard NeuroDiscovery Center, ALS Association, ALS Finding a Cure, Spastic Paraplegia Foundation, and Voyager Pharmaceuticals. He provides consulting for Biogen IDEC, Mitsubishi Tanabe, and Denali Therapeutics. E.M. has served on Data and Safety Monitoring Boards for Acorda Therapeutics and Shire Human Genetic Therapies and has received research funding from the Adolph Coors Foundation, ALS Association, ALS Therapy Alliance, ALS Therapy Development Institute, Autism Speaks, Biotie Therapies, California Institute of Regenerative Medicine, Michael J. Fox Foundation, Muscular Dystrophy Association, Food and Drug Administration, Health Resources and Services Administration, Patient-Centered Outcomes Research Institute, and NIH. N.G. has received research funding as a principal investigator or subinvestigator from Alexion, Alnylam, Amicus, Baxter, Bio-Blast, Biogen Idec, Biomarin, CSL Behring, Cytokinetics, Genzyme, Grifols, GSK, Idera, Ionis Pharmaceuticals, Myositis Association, Novartis, and Ultragenyx. She has received travel subsidies and honoraria as an advisory board member for Mallinckrodt and Novartis. M.R. is on the speakers bureau for Allogene Pharmaceuticals. He is currently funded by the NIH as a co-investigator (1R01NS090083: "Characterization of Agrin/LRP4 Antibody-positive Myasthenia Gravis"). S.A. is a member of the ALSTDI board, Avamir Speaker's Bureau, NeuralTus Scientific Advisory Board, and is a scientific consultant for Mitsubishi Tanabe Pharma America. N.J.M. currently receives funding from the NIH (NIH-NINDS 5U01NS077179-S), and previously received funding from NIH (T32 NS048005-08, 2T32 MH019733-19), the National Parkinson Foundation, Rappaport Foundation, and American Academy of Neurology. A.G., F.V., V.T. and S.P. are employees of ALSTDI.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

**Correspondence to:** J.D. Berry; e-mail: jdberry@partners.org

© 2017 The Authors Muscle & Nerve Published by Wiley Periodicals, Inc.  
Published online 29 June 2017 in Wiley Online Library (wileyonlinelibrary.com).  
DOI 10.1002/mus.25733

**ABSTRACT:** *Introduction:* Immune activation has been implicated in progression of amyotrophic lateral sclerosis (ALS). Oral fingolimod reduces circulating lymphocytes. The objective of this phase IIa, randomized, controlled trial was to test the short-term safety, tolerability, and target engagement of fingolimod in ALS. *Methods:* Randomization was 2:1 (fingolimod:placebo). Treatment duration was 4 weeks. Primary outcomes were safety and tolerability. Secondary outcomes included circulating lymphocytes and whole-blood gene expression. *Results:* Thirty participants were randomized; 28 were administered a drug (fingolimod 18, placebo 10). No serious adverse events occurred. Adverse events were similar by treatment arm, as was study discontinuation (2 fingolimod vs. 0 placebo, with no statistical difference). Forced expiratory volume in 1 second (FEV<sub>1</sub>) and FEV<sub>1</sub>/slow vital capacity changes were similar in the fingolimod and placebo arms. Circulating lymphocytes decreased significantly in the fingolimod arm ( $P < 0.001$ ). Nine immune-related genes were significantly downregulated in the fingolimod arm, including forkhead box P3 ( $P < 0.001$ ) and CD40 ligand ( $P = 0.003$ ). *Discussion:* Fingolimod is safe and well-tolerated and can reduce circulating lymphocytes in ALS patients.

*Muscle Nerve* 56: 1077–1084, 2017

**A**myotrophic lateral sclerosis (ALS) is a degenerative disorder primarily affecting motor neurons, which results in progressive wasting and paralysis of voluntary muscles.<sup>1</sup> Fifty percent of ALS patients die within 3 years of onset of symptoms and 90% within 10 years.<sup>2,3</sup> Riluzole, the only U.S. Food and Drug Administration (FDA)-approved disease-modifying drug to treat ALS, demonstrates a modest survival benefit.<sup>4,5</sup> An urgent need for novel treatments is clear.

Neuroinflammation is increasingly implicated in ALS pathogenesis.<sup>6–11</sup> Mouse models of ALS show that activation of microglia and influx of T lymphocytes into the central nervous system (CNS) occur early in the disease or before symptom onset<sup>6,11,12</sup> and colocalize with disease symptoms.<sup>13</sup> Human postmortem tissue analysis shows lymphocytic infiltrates in the CNS,<sup>14</sup> positron emission tomography (PET) imaging reveals increased microglial activation,<sup>9,10</sup> and analysis of circulating monocytes shows

activation.<sup>11,15–18</sup> The interplay among monocytes, T cells, and microglia facilitates a pro-inflammatory state, suggesting that targeted reduction of inflammation could alter ALS progression.

Although immune activation appears to be important, trials of broadly active immunosuppressants have failed to show benefit,<sup>19–22</sup> perhaps because these agents suppress all immune function. However, although one part of the immune response activates neuroinflammation, another part, namely the regulatory T-cell population, holds neuroinflammation in check.<sup>7</sup> A decrease in number and function of regulatory T cells correlates with faster disease progression, and increased numbers and function of regulatory T cells correlate with slowing of progression.<sup>23–25</sup> Regulatory T-cell function can be measured using expression of forkhead box P3 (FOXP3), and decreased FOXP3 expression also correlates with more rapid disease progression.<sup>25</sup> Thus, in ALS, more focused immune modulation that spares regulatory immune function may be required. In a screen of immune modulators at the ALS Therapy Development Institute (ALSTDI), anti-CD40 ligand antibody<sup>11</sup> and fingolimod improved survival in superoxide dismutase 1 (SOD1<sup>G93A</sup>) mice. Furthermore, an independent study demonstrated the benefit of fingolimod in SOD1 mice.<sup>26</sup>

Fingolimod is an immunomodulatory drug that antagonizes the sphingosine 1-phosphate (S1P) receptors (primarily S1P1), blocking egress of lymphocytes from secondary lymph organs and reducing circulating lymphocytes. Thus, it provides a more focused immune modulation than the broad immunosuppressive regimens previously tested in ALS. It has been used in neuroinflammatory conditions and is an FDA-approved therapy for multiple sclerosis (MS). At the time of this study, there was concern about the short-term safety profile of fingolimod, and this needed to be explored before long-term testing in ALS patients. Known side effects include first-dose bradycardia, which peaks approximately 6 hours after administration, and small and clinically insignificant decreases in forced expiratory volume in 1 second (FEV<sub>1</sub>), beginning within 1 month of drug initiation. Macular edema generally occurs after at least 3 months of administration, and progressive multifocal leukoencephalopathy has been reported in patients on the drug for many months, particularly those with a history of taking other immunosuppressive therapies.

The primary objective of this study was to determine the short-term safety and tolerability of oral administration of the FDA-approved fingolimod dose of 0.5 mg/day for 4 weeks, relative to placebo, in a phase IIa, randomized, placebo-controlled,

blinded trial. Secondary objectives were to quantify the effect of fingolimod on circulating lymphocyte populations in those with ALS, to confirm this pharmacodynamic marker of target engagement, and to evaluate the effect of fingolimod on gene expression profiles in whole blood to determine whether it may be used as a pharmacodynamic marker and provide further biological rationale for future studies of fingolimod use for ALS patients.

## METHODS

This study was coordinated through the Massachusetts General Hospital (MGH) Neurological Clinical Research Institute (NCRI), and included 4 participating sites: University of California, Irvine; MGH; Augusta University Medical Center; and Methodist University. The local institutional review board at each site approved the study. Whole-blood RNA analysis was performed at the ALSTDI. The trial has been registered at [clinicaltrials.gov](http://clinicaltrials.gov) (NCT01786174).

**Participant Selection Criteria.** At screening, eligible participants had a diagnosis of possible, probable laboratory-supported, probable, or definite ALS, according to El Escorial criteria<sup>27</sup>; a slow vital capacity (SVC) of  $\geq 65\%$  of the predicted normal value for height, age, and gender; a symptom duration of  $<2$  years; an ability to swallow capsules; and were either not on riluzole or on a stable dose for  $\geq 30$  days. Exclusion criteria included use of mechanical ventilation or presence of a feeding tube, previous use of fingolimod, pregnancy, exposure to investigational agents within 30 days of screening, lymphopenia, active autoimmune disease or infection, stroke or myocardial infarction within the last 6 months, atrioventricular (AV) block, bradycardia or prolonged QTc ( $>450$  ms for women,  $>430$  ms for men) by history or on electrocardiogram (ECG), history of macular edema or uveitis, or clinically significant abnormal safety laboratory values. Potential participants were also excluded for concurrent treatment with immunosuppressants, class Ia or III antiarrhythmic medications, beta-blockers, calcium-channel blockers, or QT-prolonging medications.

**Randomization.** A permuted-block randomization schedule was prepared by a statistician at the MGH Biostatistics Center, stratified by site. Participants were randomly assigned in a 2:1 ratio to oral fingolimod 0.5 mg/day or matching placebo.

**Study Overview.** Participants signed an informed consent form before screening. Medical history, detailed ALS history, physical and neurological examinations, medication review, vital capacity testing, administration of the ALS Functional Rating Scale—Revised (ALSF<sub>RS</sub>-R), vital signs, ECG, and laboratory tests were performed to determine eligibility. For eligible participants, the baseline visit (day 0), when study drug was initiated, occurred within 21 days of screening. Follow-up visits occurred at day 1, week 2, and week 4. A final phone call occurred 4 weeks after participants stopped study drug (week 8) (Fig. 1).

**Study Drug.** Because extensive dosing studies of fingolimod had already been completed, the FDA-approved dose was used for this trial. The first dose was administered in the clinic at the baseline visit and the remaining supply for the study was dispensed in blister packs. Participants were instructed to take the study drug daily and filled out a drug

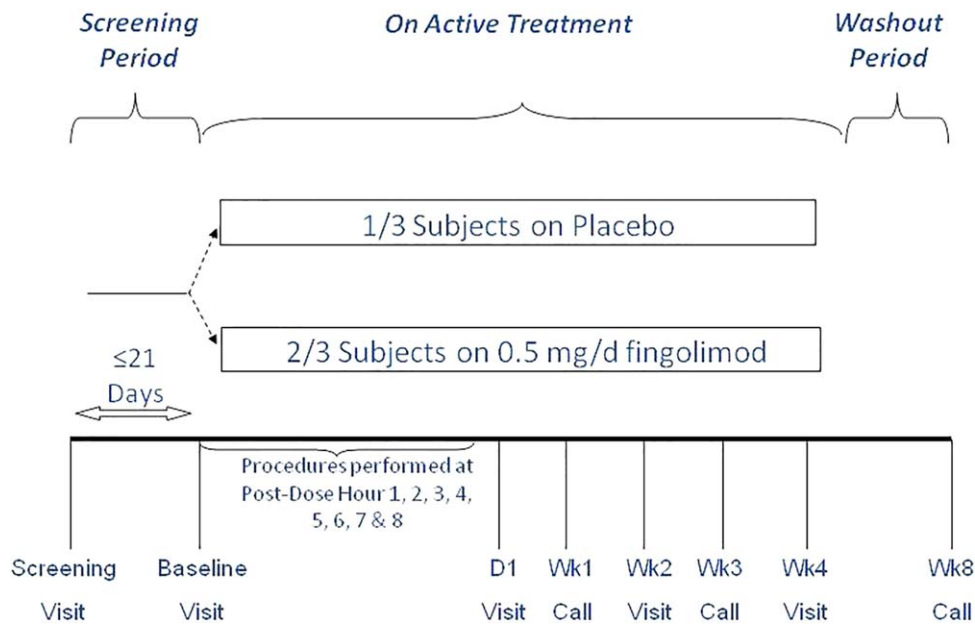


FIGURE 1. Trial design. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

diary for the duration of the study. They remained on study drug for 4 weeks.

**Study Procedures.** At the baseline visit, participants were monitored on-site for 8 hours with hourly vital signs and an ECG both before and after administration of drug. For safety, participants were not discharged from the study visit with symptomatic bradycardia or if their final heart rate measurement was the lowest of the day. Participants returned the day after the baseline visit (day 1) for evaluation of vital signs and for general health status.

Clinical measurements at all visits included SVC, FEV<sub>1</sub>, ALSFRS-R, and visual acuity using a Snellen chart.

Safety labs were collected at the baseline, week 2, and week 4 visits and analyzed at a central lab (University of Rochester, Rochester, New York). Safety labs were monitored by the site investigator and available to the study principal investigator for safety review. The lymphocyte counts in peripheral blood acted as a marker of peripheral target engagement. Therefore, after initiation of study drug, study staff at the site and coordination center were blinded to lymphocyte count. An independent reviewer monitored lymphocyte counts to maintain blinding of study staff while ensuring safe follow-up. The independent reviewer was to notify the site investigator if absolute lymphocyte counts dropped to <200/ $\mu$ l. Counts <500/ $\mu$ l were tolerated without stopping the drug.

For whole-blood RNA analysis, blood was collected into 3 RNase-free PAXgene tubes at the baseline and week 4 visits. The blood was shipped ambient overnight to a central lab (ALSTDI, Cambridge, Massachusetts) for RNA extraction using a blood total RNA extraction kit (Agencourt RNAdvance; Beckman Coulter, Porterville, California). Gene expression analysis was performed at the ALSTDI at the conclusion of the study, after all samples had been collected, but before unblinding.

**Whole-Blood Gene Expression Analysis.** After RNA extraction, 2 gene expression arrays were run to measure gene expression levels by quantitative polymerase chain

reaction. A human immune panel (Applied Biosystems TaqMan Array; Thermo Fisher Scientific, Waltham, Massachusetts) was used to measure 90 immune-related genes plus 6 housekeeping genes. A second custom panel containing FOXP3 plus 4 housekeeping genes was used to measure FOXP3 gene expression. Gene expression was normalized following the geNorm methodology<sup>28</sup> with levels for 18S, beta-actin (ACTB), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and phosphoglycerate kinase 1 (PGK1) used to normalize the pre-configured panel and ACTB, GAPDH, and PGK1 used to normalize FOXP3 expression levels. Mean log-transformed fold-change in relative expression from baseline to week 4 was compared between arms by the 2-sample Student *t*-test for each gene. For purposes of this exploratory analysis, differences with a nominal *P* < 0.01 were considered statistically significant.

**Statistical Analysis.** Safety was assessed with respect to incidence of bradycardia, general adverse events (AEs), lab abnormalities, and changes in FEV<sub>1</sub> and FEV<sub>1</sub>/SVC. AEs were coded to preferred terms from the MedDRA library (version 16.1) and compared using the Fisher exact test. Heart rate immediately posttreatment (days 0 and 1) was analyzed in repeated-measures analysis of variance models to detect treatment-dependent bradycardia, an AE of particular interest. Intolerance for an individual participant was defined as discontinuing study drug or dropping out from the study before 4 weeks after drug initiation at the baseline visit. Fingolimod was considered tolerable if the rate of intolerance was <40% with 80% confidence; that is, an upper 1-tailed 80% confidence bound on the proportion of fingolimod-treated participants determined intolerant that was <40%.

Target engagement was assessed by analyzing circulating lymphocyte counts. Changes in ALSFRS-R and SVC were monitored, but the trial did not have power to test efficacy.

Lymphocyte counts, ALSFRS-R, and pulmonary function testing (PFT) measurements were analyzed in shared-baseline, random-slope mixed models. Models for ALSFRS-R and PFT measurements adjusted for bulbar onset and

baseline SVC and their interactions with time. Four-week changes from baseline and their 95% confidence intervals were estimated using linear contrasts. All participants who initiated treatment were analyzed.

**Power Calculations.** *Tolerability.* Assuming 20 participants receiving active study drug, observing 5 or fewer intolerant participants would satisfy the criterion for judging fingolimod tolerable. The study had 80% power to declare fingolimod tolerable if the expected rate of intolerance was  $\leq 20\%$ .

*Safety.* Using the calculation  $\text{power} = 1 - (1 - P)^n$ , where  $P$  is probability of an event and  $n$  is the number of subjects in a treatment arm, and assuming 20 participants in the fingolimod arm, the study had  $>80\%$  power to detect any adverse event caused by fingolimod for which the expected frequency was  $\geq 8\%$ .

## RESULTS

Thirty-nine subjects were screened for this trial, 30 were randomized, and 28 initiated treatment and completed the study (Fig. 2). Two subjects were randomized but did not receive study drug: 1 subject developed a urinary tract infection and decided to withdraw from the study; another subject was found to have a prolonged QTc interval at the baseline visit and was not administered study drug. Under the pre-specified modified intention-to-treat analysis plan, data from these 2 subjects were not included in the final analysis. One subject on fingolimod discontinued treatment after 2 weeks due to fatigue and progressive weakness interfering with trial commitments. Another subject on fingolimod discontinued the study drug after 2 weeks due to prolonged QTc. Both subjects continued to be followed off study drug and were included in the intent-to-treat analysis as planned.

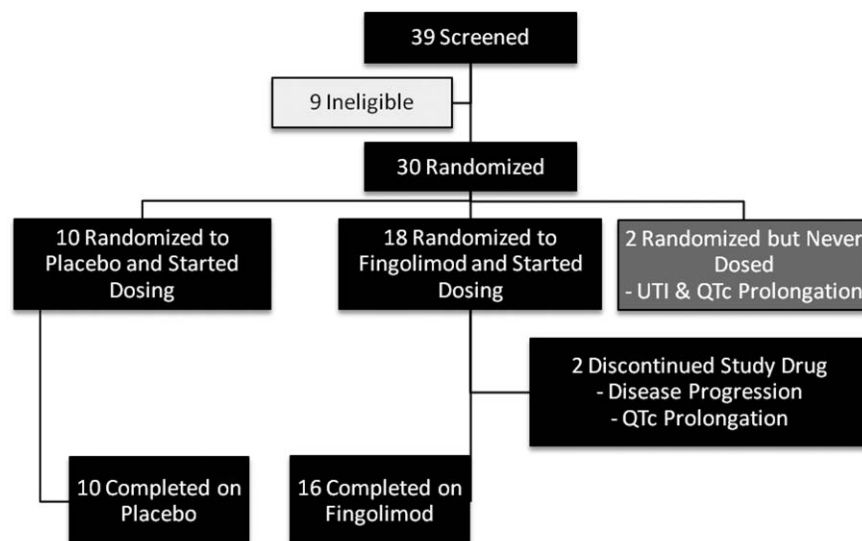
Treatment arms were similar on baseline characteristics. Bulbar onset was slightly overrepresented

in the placebo arm and SVC was slightly higher in the fingolimod arm, but the differences were not statistically significant. Although the differences between bulbar onset and baseline SVC were purely due to chance, their magnitude was potentially meaningful, so analyses of ALSFRS-R, FEV<sub>1</sub>, and SVC were adjusted for these variables, as described in the Methods section (Table 1).

Two major protocol violations related to eligibility occurred during the trial and were recognized in retrospect. Two participants were randomized despite being on QT-prolonging medications (1 on sertraline and 1 on amitriptyline). Both subjects completed the protocol.

**Safety and Tolerability.** The trial met its primary endpoints for short-term safety and tolerability. Eighty-nine percent (16 of 18) of the participants in the fingolimod arm and 100% (10 of 10) of those in the placebo arm completed the study on study drug (refer to Table S1 in the Supplementary Material, available online).

There were no serious adverse events (SAEs) during the trial. AEs occurring in at least 2 participants in the active arm are summarized in Table 2 by MedDRA preferred term; none were significantly more common in the fingolimod arm. Outside of the expected reductions in lymphocyte counts in participants receiving fingolimod, there were no clinically significant abnormalities on routine blood counts, chemistries, or urinalysis during the course of the study. Alkaline phosphatase increased by 8.4 U/L from baseline to week 4 in the fingolimod arm (vs. a decline of 2.8 U/L in the placebo arm,  $P = 0.02$ ). Liver function tests did not change significantly in the fingolimod arm or relative to the placebo arm.



**FIGURE 2.** CONSORT diagram. Participant enrollment, intervention allocation, and follow-up for the trial.



**Table 1.** Baseline characteristics of study participants

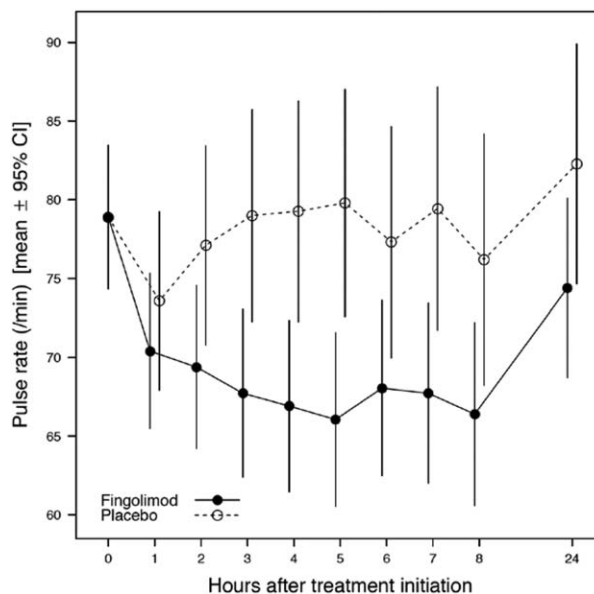
|                                       | Overall     | Fingolimod  | Placebo     | P-value |
|---------------------------------------|-------------|-------------|-------------|---------|
| Number                                | 30          | 18          | 10          |         |
| Male gender                           | 53.6%       | 55.6%       | 50.0%       | 1.0     |
| White race                            | 92.9%       | 100%        | 80%         | 0.12    |
| Age (years)                           | 55.9 ± 9.1  | 56.4 ± 8.0  | 55.1 ± 11.3 | 0.72    |
| Bulbar onset                          | 21.4%       | 11.1%       | 40.0%       | 0.15    |
| Riluzole use                          | 60.7%       | 61.1%       | 60.0%       | 1.0     |
| SVC (percent predicted)               | 89.2 ± 20.0 | 92.8 ± 18.5 | 82.8 ± 21.9 | 0.19    |
| FEV <sub>1</sub> (percent predicted)  | 82.8 ± 20.6 | 83.5 ± 16.1 | 81.4 ± 27.9 | 0.80    |
| FEV <sub>1</sub> /SVC                 | 75.3 ± 14.5 | 73.3 ± 10.0 | 78.8 ± 20.4 | 0.32    |
| ALSFRS-R                              | 38.5 ± 4.7  | 38.8 ± 4.1  | 37.9 ± 5.8  | 0.63    |
| Months since symptom onset            | 13.5 ± 5.3  | 12.6 ± 4.8  | 15.0 ± 6.2  | 0.25    |
| Months since diagnosis                | 5.7 ± 3.9   | 5.5 ± 3.9   | 6.0 ± 4.0   | 0.73    |
| Diagnostic delay (months)             | 7.8 ± 4.2   | 7.2 ± 4.2   | 9.0 ± 4.3   | 0.27    |
| EEC definite                          | 53.6%       | 61.1%       | 40.0%       | 0.27    |
| EEC probable                          | 32.1%       | 33.3%       | 30.0%       |         |
| EEC probable lab-supported            | 14.3%       | 5.6%        | 30.0%       |         |
| Lymphocyte count (10 <sup>9</sup> /L) | 1.86 ± 0.68 | 1.86 ± 0.74 | 1.86 ± 0.60 | 0.98    |
| Resting HR (bpm)                      | 74.6 ± 11.4 | 73.3 ± 11.0 | 76.9 ± 12.4 | 0.41    |
| BMI (kg/m <sup>2</sup> )              | 26.2 ± 3.7  | 26.1 ± 3.0  | 26.3 ± 4.9  | 0.88    |

Data are presented as either percentages or mean (± standard deviation). VC and FEV<sub>1</sub> are presented as percent of predicted values. Lymphocyte counts are measured as 10<sup>9</sup>/L. Diagnostic delay represents the time from symptom onset to diagnosis. ALSFRS-R, ALS Functional Rating Scale—Revised; BMI, body mass index (measured in kg/m<sup>2</sup>); EEC, El Escorial criteria; FEV<sub>1</sub>, forced expiratory volume in the first second; HR, heart rate (measured in beats per minute); SVC, slow vital capacity.

As noted, fingolimod is known to induce heart rate slowing in the hours after the first dose. In the hours after the first dose, participants in the fingolimod arm had a greater mean heart rate (HR) slowing than participants in the placebo arm, with a nadir at 5 hours (Fig. 3). Three participants were noted to have transient bradycardia, but there were no clinical symptoms associated with the heart rate slowing. By 24 hours after the first dose, the mean HR change did not differ between the fingolimod arm and the placebo arm (Fig. 3). Significant heart rate slowing was not seen in the fingolimod arm at 2 or 4 weeks.

Mean FEV<sub>1</sub> was 83% of predicted normal overall at baseline and did not differ significantly between the arms. FEV<sub>1</sub> declined modestly in both arms over the course of the study but did not decline more rapidly in the fingolimod arm (Fig. 4A). SVC and FEV<sub>1</sub>/SVC ratio also did not

decline more rapidly in the fingolimod arm (Fig. 4B). Finally, rate of decline in ALSFRS-R total score did not differ significantly between arms (Fig. 4C).

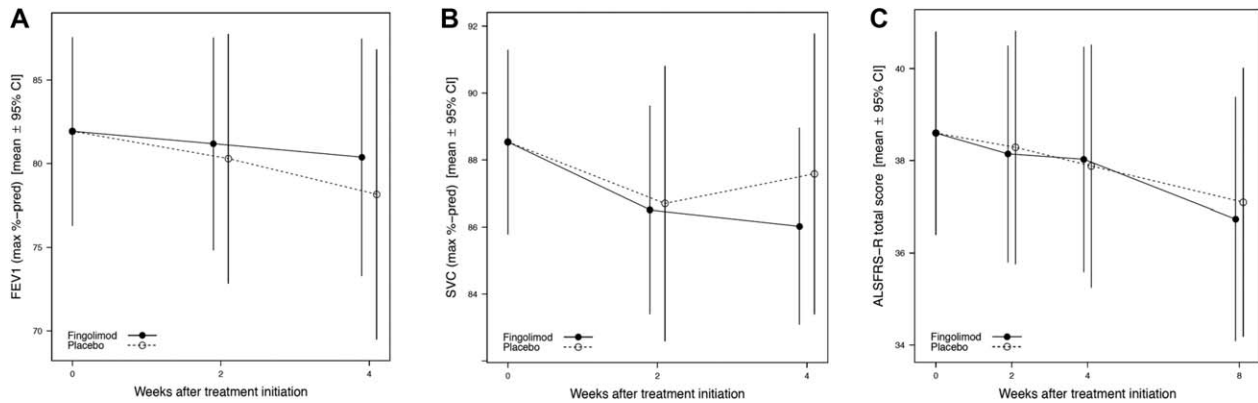


**FIGURE 3.** Hourly measurement of heart rate (HR) at the baseline visit in the active arm and placebo arm. Participants in the fingolimod arm had a mean HR decline of −12.8 beats per minute (bpm) (−7.0 to −18.7 bpm) from pre-dose to post-dose nadir, which occurred at 5 hours, on average. This change differed from placebo significantly ( $P = 0.002$ ). By 24 hours after the first dose, HR had largely returned to baseline and there was no statistically significant difference in HR between the 2 arms ( $P = 0.10$ ) [the mean HR change at 24 hours (relative to baseline) was −4.5 bpm (+28 to −11.7 bpm) in the fingolimod arm vs. +3.4 bpm (+12.2 to −5.5 bpm) in the placebo arm].

**Table 2.** Adverse events by treatment arm.

| Adverse event            | Fingolimod<br>(n = 18) | Placebo<br>(n = 10) | P-value |
|--------------------------|------------------------|---------------------|---------|
| Asymptomatic bradycardia | 17%                    | 0%                  | 0.53    |
| Fatigue                  | 22%                    | 30%                 | 0.67    |
| Fall                     | 33%                    | 10%                 | 0.36    |
| Muscular weakness        | 17%                    | 20%                 | >0.99   |
| Headache                 | 22%                    | 20%                 | >0.99   |
| Cough                    | 11%                    | 0%                  | 0.52    |

No adverse events were significantly more common in the fingolimod arm than the placebo arm. Adverse events occurring in at least 2 subjects in the treatment arm are shown.



**FIGURE 4.** FEV<sub>1</sub>, SVC, and ALSFRS-R at the baseline, week 2, and week 4 visits in the active and placebo arms. **(A)** FEV<sub>1</sub> did not decline more rapidly in the fingolimod arm (4-week change from baseline: fingolimod -1.6% vs. placebo -3.8%;  $P = 0.57$ ). **(B)** Fingolimod did not significantly affect rate of progression as measured by SVC, nor were clinically relevant benefits or harm ruled out in this small, short-duration trial. **(C)** Fingolimod did not significantly affect rate of progression as measured by ALSFRS-R (difference in 8-week change from baseline: -0.4; 95% confidence interval -2.1 to 1.4;  $P = 0.68$ ), nor were clinically relevant benefits or harm ruled out in this small, short-duration trial.

**Lymphocyte Counts.** Total lymphocyte counts in circulation declined from baseline in the fingolimod arm (Fig. 5A). The ratio of CD4<sup>+</sup> (helper) to CD8<sup>+</sup> (suppressor) lymphocytes in circulation declined, suggesting that CD4<sup>+</sup> lymphocytes were more effectively sequestered than CD8<sup>+</sup> lymphocytes (Fig. 5B). B-cells (CD19) decreased (week 4: -88% fingolimod vs. +1% placebo,  $P < 0.001$ ) as expected. Natural killer cells (CD16 + CD56) were the only lymphocyte subset that did not decrease.

Lymphocyte count did not drop below 200/ml in any of the participants, thus the independent monitor did not ever need to report counts to study sites, and no unblinding occurred.

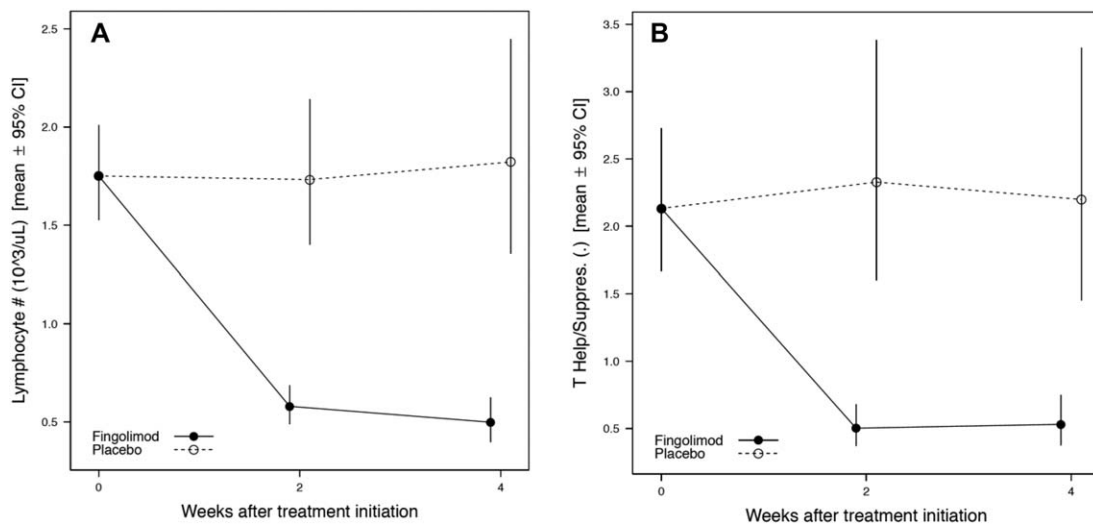
**Whole-Blood Gene Expression Profiling.** Whole-blood gene expression analysis investigated 91

immune-related RNAs (refer to Table S2 in Supplementary Material online) at baseline and week 4. Of these, 9 were significantly downregulated in the fingolimod arm relative to placebo after 4 weeks of treatment, including FOXP3 (Table 3).

#### DISCUSSION

The trial met its primary endpoint, demonstrating short-term safety and tolerability of oral fingolimod 0.5 mg/day in ALS patients. Given that the short-term safety profile had caused concern in early trials in multiple sclerosis, and hints of a deleterious effect on FEV<sub>1</sub> had been reported, this hurdle needed to be cleared before consideration of fingolimod for further study in those with ALS.

Circulating lymphocyte counts acted as a reliable peripheral biomarker of target engagement.



**FIGURE 5.** Total lymphocyte counts and lymphocyte subpopulations counts at baseline, week 2, and week 4 visits in the active and placebo arms. **(A)** Lymphocytes were dramatically reduced in the fingolimod arm relative to the placebo arm (week 2: -67% fingolimod vs. -1% placebo; week 4: -72% fingolimod vs. +4% placebo;  $P < 0.001$  for both). **(B)** T-helper lymphocytes were more affected than T suppressors (week 4: -75% fingolimod vs. +3% placebo;  $P < 0.001$ ).

**Table 3.** Immune-related genes with significantly different changes from baseline to week 4 in the fingolimod arm relative to the placebo arm

| Gene   | Fingolimod                   |                            |                      | Placebo                      |                            |                      | Treatment comparison |                            |
|--------|------------------------------|----------------------------|----------------------|------------------------------|----------------------------|----------------------|----------------------|----------------------------|
|        | Baseline relative expression | Week 4 relative expression | Week 0–4 fold-change | Baseline relative expression | Week 4 relative expression | Week 0–4 fold-change | Fold-change ratio    | Unadjusted <i>P</i> -value |
| BCL2   | 0.282                        | 0.076                      | 0.271                | 0.251                        | 0.360                      | 1.436                | 0.189                | <0.001                     |
| CCR4   | 0.336                        | 0.080                      | 0.024                | 0.289                        | 0.375                      | 1.295                | 0.183                | <0.001                     |
| CCR7   | 0.226                        | 0.004                      | 0.016                | 0.281                        | 0.264                      | 0.938                | 0.017                | <0.001                     |
| CD3E   | 0.169                        | 0.042                      | 0.246                | 0.129                        | 0.142                      | 1.097                | 0.224                | <0.001                     |
| CD8A   | 0.109                        | 0.044                      | 0.406                | 0.076                        | 0.083                      | 1.085                | 0.374                | 0.004                      |
| CD19   | 0.228                        | 0.021                      | 0.093                | 0.184                        | 0.263                      | 1.428                | 0.065                | <0.001                     |
| CD28   | 0.352                        | 0.025                      | 0.072                | 0.231                        | 0.322                      | 1.393                | 0.051                | <0.001                     |
| CD40LG | 0.229                        | 0.043                      | 0.188                | 0.261                        | 0.250                      | 0.995                | 0.189                | 0.003                      |
| FOXP3  | 0.422                        | 0.080                      | 0.189                | 0.426                        | 0.411                      | 0.965                | 0.196                | <0.001                     |

Of the 91 immune-related genes explored in whole blood using PCR analysis, 9 were significantly reduced from baseline to week 4 in the fingolimod arm relative to the placebo arm. CD40LG, cluster of differentiation 40 ligand.

Whole-blood gene expression profiling demonstrated a focused reduction of immune-related gene expression, including FoxP3 and CD40 ligand, both implicated in disease pathogenesis.

The well-described first-dose bradycardia of fingolimod seen in this study mimicked that previously described in patients with MS.<sup>29,30</sup> The current FDA recommendation for 6-hour observation seems to be appropriate in people with ALS.

To avoid unblinding effects of lymphocyte changes, we used an independent lymphocyte reviewer. The first-dose bradycardia could potentially have had an unblinding effect, although in this trial the risk was somewhat attenuated by substantial interparticipant variability in the heart rate changes. Future studies should incorporate surveys of participants and study staff to understand treatment assignment perceptions and quantify the unblinding effect. The incorporation of unblinded team members to monitor vital signs at the first visit could be considered to maintain blinding.

In multiple sclerosis, fingolimod produced a clinically insignificant decrease in FEV<sub>1</sub> within 1 month of therapy and was stable thereafter.<sup>29</sup> Likewise, there was no statistically significant reduction in FEV<sub>1</sub> or FEV<sub>1</sub>/SVC in those with ALS.

The preferential sequestration of CD4<sup>+</sup> lymphocytes over CD8<sup>+</sup> lymphocytes is notable in light of data suggesting slower disease progression in ALS patients with higher regulatory T-lymphocyte counts (CD4<sup>+</sup>/CD17<sup>+</sup>). One limitation of our pilot study is that it did not include direct quantification of CD17<sup>+</sup> cells, an aspect of the immune response that should be included in a follow-up study.

Our exploratory analysis of whole-blood gene expression provides evidence that fingolimod has a focused effect on immune function—only a small proportion of immune-related genes showed a significantly different change in the fingolimod arm.

The changes we did see may provide insight into the relevant action of the drug in ALS. For example, the reductions in costimulatory pathway genes (CD40 ligand and CD28) point to a beneficial effect on 1 pro-inflammatory pathway thought to be relevant in ALS. Previous studies in preclinical models of ALS suggest that blocking the costimulatory pathway may provide therapeutic benefit in ALS patients.<sup>11</sup> FoxP3 expression in whole blood may be a good proxy for regulatory T-cell activity. Given the correlation between increased regulatory T-cell activity and slow disease progression, an absolute reduction of FoxP3 in the absence of other immune-related gene changes may be cause for caution. In this case, the reduction in FoxP3 occurred in the context of a more elaborate immune modulation—for example, a reduction in total circulating lymphocytes—and must be interpreted with caution.

In this small trial, the addition of biomarkers to track inflammation in the CNS was impractical. The variability of cerebrospinal fluid cytokines, such as monocyte chemoattractant protein-1, would have meant that we would have had limited power to detect differences between the arms; the added complexity and expense of lumbar puncture would have been difficult to justify. Likewise, data regarding the peripheral benzodiazepine receptor (PBR28) PET ligand were limited, thus the additional complexity and high costs of PET were difficult to justify. In follow-up studies, these markers of inflammation in the CNS could be considered to supplement efficacy measures, although power calculations would be prudent to weigh potential benefit against known measurement variability.

The lack of an observable treatment effect of fingolimod on ALSFRS-R or SVC in this study is uninformative—the trial did not have statistical power to detect such a change. A larger study is

needed to evaluate clinical efficacy of oral fingolimod for the treatment of those with ALS.

Based on the rapid and robust decline in circulating lymphocytes and data from other studies, a 0.5-mg/day dose of fingolimod in an ALS patient may be too high. Given that the goal of fingolimod therapy in ALS is immune *modulation*, a higher-than-optimal dose may tip the balance from immune modulation toward overt (and potentially counterproductive) immune *suppression*. In preclinical studies of fingolimod in SOD1<sup>G93A</sup> mice at the ALSTDI, a substantially lower daily dose of 0.32 mg/kg had a maximal effect on lymphocyte depletion in the circulation by 14 days, and fingolimod accumulated in the spinal cord tissue over time (unpublished observations). Similar observations were noted in the FDA new drug application review of fingolimod for MS, where a daily dose of 0.5 mg was higher than the observed half-minimal inhibitory concentration (0.345 ng/ml).<sup>31</sup> Such an observation could suggest that a dose-optimization study would be warranted before further trials of fingolimod in ALS. A follow-up study in ALS could assess efficacy, directly quantify regulatory T-cell and monocyte populations, measure gene expression changes within specific cell populations, and possibly evaluate imaging-based markers of neuroinflammation.

Data suggesting a role for immune dysregulation in ALS are accumulating, and novel therapies aimed at suppressing the immune system entirely have not yet been successful at slowing disease progression. Fingolimod, an immune modulator, holds promise as a means to manipulate the immune system, creating a less inflammatory state. In this study we have demonstrated short-term safety and tolerability in this population, target engagement, and broad immune system function changes by whole-blood gene expression.

The authors thank Dr. Merit Cudkowicz for help with study design and guidance throughout the conduct of the study. Both study drug and placebo were provided by Novartis Pharmaceuticals.

## REFERENCES

1. Wijesekera LC, Leigh PN. Amyotrophic lateral sclerosis. *Orphanet J Rare Dis* 2009;4:3.
2. Kurtzke J, Kurland L. The epidemiology of neurologic disease. In: Joynt R, editor. *Clinical neurology*. Philadelphia: Lippincott; 1989. p 1–43.
3. Pupillo E, Messina P, Logroscino G, Beghi E, Group S. Long-term survival in amyotrophic lateral sclerosis: a population-based study. *Ann Neurol* 2014;75:287–297.
4. Bensimon G, Lacomblez L, Meininger V. A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *N Engl J Med* 1994;330:585–591.
5. Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst Rev* 2012;3:CD001447.
6. Alexianu ME, Kozovska M, Appel SH. Immune reactivity in a mouse model of familial ALS correlates with disease progression. *Neurology* 2001;57:1282–1289.
7. Appel SH, Zhao W, Beers DR, Henkel JS. The microglial-motoneuron dialogue in ALS. *Acta Myol* 2011;30:4–8.
8. Kano O, Beers DR, Henkel JS, Appel SH. Peripheral nerve inflammation in ALS mice: cause or consequence. *Neurology* 2012;78:833–835.
9. Zurcher NR, Loggia ML, Lawson R. Increased in vivo glial activation in patients with amyotrophic lateral sclerosis: assessed with [(11C)-PBR28]. *Neuroimage Clin* 2015;7:409–414.
10. Turner MR, Cagnin A, Turkheimer FE. Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [(11C)(R)-PK11195 positron emission tomography study. *Neurobiol Dis* 2004;15:601–609.
11. Lincecum JM, Vieira FG, Wang MZ. From transcriptome analysis to therapeutic anti-CD40L treatment in the SOD1 model of amyotrophic lateral sclerosis. *Nat Genet* 2010;42:392–399.
12. Henkel JS, Beers DR, Siklos L, Appel SH. The chemokine MCP-1 and the dendritic and myeloid cells it attracts are increased in the mSOD1 mouse model of ALS. *Mol Cell Neurosci* 2006;31:427–437.
13. Beers DR, Zhao W, Liao B. Neuroinflammation modulates distinct regional and temporal clinical responses in ALS mice. *Brain Behav Immun* 2011;25:1025–1035.
14. Engelhardt JI, Tajti J, Appel SH. Lymphocytic infiltrates in the spinal cord in amyotrophic lateral sclerosis. *Arch Neurol* 1993;50:30–36.
15. Vaknin I, Kunis G, Miller O. Excess circulating alternatively activated myeloid (M2) cells accelerate ALS progression while inhibiting experimental autoimmune encephalomyelitis. *PLoS One* 2011;6:e26921.
16. Butovsky O, Siddiqui S, Gabriely G. Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. *J Clin Invest* 2012;122:3063–3087.
17. Chiu IM, Phatnani H, Kuligowski M. Activation of innate and humoral immunity in the peripheral nervous system of ALS transgenic mice. *Proc Natl Acad Sci USA* 2009;106:20960–20965.
18. Chiu IM, Morimoto ET, Goodarzi H. A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. *Cell Rep* 2013;4:385–401.
19. Appel SH, Stewart SS, Appel V. A double-blind study of the effectiveness of cyclosporine in amyotrophic lateral sclerosis. *Arch Neurol* 1988;45:381–386.
20. Drachman DB, Chaudhry V, Cornblath D. Trial of immunosuppression in amyotrophic lateral sclerosis using total lymphoid irradiation. *Ann Neurol* 1994;35:142–150.
21. Werdelin L, Boysen G, Jensen TS, Mogensen P. Immunosuppressive treatment of patients with amyotrophic lateral sclerosis. *Acta Neurol Scand* 1990;82:132–134.
22. Meucci N, Nobile-Orazio E, Scarlato G. Intravenous immunoglobulin therapy in amyotrophic lateral sclerosis. *J Neurol* 1996;243:117–120.
23. Beers DR, Henkel JS, Zhao W. Endogenous regulatory T lymphocytes ameliorate amyotrophic lateral sclerosis in mice and correlate with disease progression in patients with amyotrophic lateral sclerosis. *Brain* 2011;134:1293–1314.
24. Zhao W, Beers DR, Liao B, Henkel JS, Appel SH. Regulatory T lymphocytes from ALS mice suppress microglia and effector T lymphocytes through different cytokine-mediated mechanisms. *Neurobiol Dis* 2012;48:418–428.
25. Henkel JS, Beers DR, Wen S. Regulatory T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival. *EMBO Mol Med* 2013;5:64–79.
26. Potenza RL, De Simone R, Armida M. Fingolimod: a disease-modifier drug in a mouse model of amyotrophic lateral sclerosis. *Neurotherapeutics* 2016;13:918–927.
27. Brooks BR, Miller RG, Swash M, Munsat TL. World Federation of Neurology Research Group on Motor Neuron Disease. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000;1:293–299.
28. Vandesompele J, De Preter K, Pattyn F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002;3:RESEARCH0034.
29. Cohen JA, Barkhof F, Comi G. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med* 2010;362:402–415.
30. Kappos L, Radue EW, O'Connor P. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N Engl J Med* 2010;362:387–401.
31. Fitter HD. New drug application number 22-527. Medical review. In: FDA CfDEaR, editor. Silver Spring, MD: FDA; 2010.