## Title

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## Journal

Annals of Neurology, 93(2)

## ISSN

0364-5134

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## Publication Date

2023-02-01

## DOI

10.1002/ana.26503

Peer reviewed

# Phase 1 Evaluation of Elezanumab (Anti–Repulsive Guidance Molecule A Monoclonal Antibody) in Healthy and Multiple Sclerosis Participants

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**Objective:** This study was undertaken to describe the safety, tolerability, pharmacokinetics, and immunogenicity of elezanumab (ABT-555), a fully human monoclonal antibody (mAb) directed against repulsive guidance molecule A (RGMa), in healthy and multiple sclerosis (MS) study participants.
**Methods:** The single-center, first-in-human, single ascending dose (SAD) study evaluated elezanumab (50–1,600mg intravenous [IV] and 150mg subcutaneous) in 47 healthy men and women. The multicenter multiple ascending dose (MAD; NCT02601885) study evaluated elezanumab (150mg, 600mg, and 1,800mg) in 20 adult men and women with MS, receiving either maintenance or no immunomodulatory treatment.
**Results:** No pattern of study drug-related adverse events was identified for either the SAD or MAD elezanumab regimens. Across both studies, the $T_{max}$ occurred within 4 hours of elezanumab IV infusion, and the harmonic mean of $t_{1/2}$ ranged between 18.6 and 67.7 days. Following multiple dosing, elezanumab $C_{max}$, area under the curve, and $C_{trough}$ increased dose-proportionally and resulted in dose-dependent increases in elezanumab cerebrospinal fluid (CSF) concentrations. Elezanumab CSF penetration was 0.1% to 0.4% across both studies, with CSF levels of free RGMa decreased by >40%. Changes in CSF interleukin-10 (IL-10) and free RGMa demonstrated dose/exposure-dependence.
**Interpretation:** The elezanumab pharmacokinetic profile supports monthly, or bimonthly, administration of up to 1,800mg with the option of a loading dose of 3,600mg. Elezanumab partitioning into CSF is within the range expected for mAbs. Reduced CSF levels of free RGMa demonstrate central nervous system target binding of elezanumab with an apparent maximal effect at 1,800mg IV. Exposure-associated increases in CSF IL-10, an anti-inflammatory cytokine with neuroprotective/neurorestorative properties, support potential pathway modulation in MS participants.

**ANN NEUROL 2023;93:285–296**

The major cause of irreversible disability in patients with multiple sclerosis (MS) is cumulative axon/neuronal and myelin/oligodendroglial damage over time.[1–3] Axonal damage, including transection, begins early in MS, correlates with inflammatory activity, and may also occur in areas with little or no evidence of inflammation.[4,5] Several mechanisms lead to axonal loss, including inflammatory mediators, loss of oligodendroglial-derived support, disruption of axonal ionic balance, energy failure, and calcium accumulation.[4,5] Axonal transection and loss described in postmortem studies are associated with factors inhibitory to remyelination and neuroregeneration.[6] In addition, brain and spinal cord atrophy are hallmark features in MS patients, and estimates of the total axonal loss in spinal cord lesions at end stage disease approach 70%.[1–3]

Despite major therapeutic advances over the past 2 decades in the development of more robust immunemodulatory, anti-inflammatory drugs, these treatments are

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.26503

Received Apr 21, 2022, and in revised form Aug 1, 2022. Accepted for publication Sep 6, 2022.

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only modestly effective in preventing or reversing the neurodegenerative components of axonopathy and oligodendroglial apoptosis that are the substrates of permanent neurological disability.[1–3,7,8] Existing therapeutic options only limit inflammatory relapses and slow disability progression.[1–3,7,8] Therefore, a major, emerging focus of MS research is the development of therapeutics that can promote remyelination and axonal repair.

Elezanumab (ABT-555) is a fully human monoclonal antibody (mAb) directed against repulsive guidance molecule A (RGMa). RGMa is a potent modulator of axonal growth, myelination, and downstream immunoregulatory molecules (eg, interlukin-10 [IL-10]) that are important factors for inhibiting neuronal and oligodendroglial regeneration and functional recovery after central nervous system (CNS) trauma or inflammation.[9–14] Postmortem evidence from MS patient brain sections showed upregulated RGMa at active and chronic CNS lesions.[15] RGMa neutralization is a novel approach that may provide neurorestoration/regeneration and functional recovery in a variety of degenerative CNS diseases.

In several nonclinical models of CNS demyelination, including targeted experimental autoimmune encephalomyelitis (EAE), disseminated EAE, and cuprizone-mediated demyelination, RGMa engagement via elezanumab administration promotes neuroregeneration, neuroprotection, and enhanced functional recovery of the affected animals.[16] Based on these encouraging preclinical efficacy signals, elezanumab treatment is being evaluated as an approach to mediate neural restoration leading to improvement in neurological disability in MS patients. This therapeutic strategy is distinct from the immunomodulatory and anti-inflammatory drugs currently approved for treatment of relapsing and progressive forms of MS and, if successful, could fulfill unmet medical needs of the MS patient population. Here, we outline the pharmacokinetic (PK), safety, and biomarker findings from 2 phase 1 studies.

## Subjects and Methods

### Study Design and Eligibility

The first-in-human single ascending dose (FIH-SAD) phase 1 study and the multiple ascending dose (MAD) phase 1b study (NCT02601885) were double-blind, placebo-controlled randomized studies, designed to evaluate the safety, tolerability, PK, and immunogenicity of elezanumab in healthy participants and participants with relapsing MS (RMS), respectively. Forty-seven adult men and women in general good health participated in the single center FIH-SAD study, and 20 adult men and women with RMS, who were receiving maintenance beta-interferon, glatiramer acetate, teriflunomide, fingolimod, dimethyl fumarate treatment, or no immunomodulatory treatment participated in the multicenter MAD study.

Key exclusion criteria included participants who could not complete the lumbar puncture due to lumbar scoliosis, coagulopathy, or infected skin at needle puncture site or if they had used a blood-thinning compound, including nonsteroidal anti-inflammatory agents (eg, aspirin, ibuprofen, naproxen), clopidogrel, warfarin, heparin (or heparainoids), fondaparinux (or related compounds), thrombin inhibitors (dabigatran), or factor Xa inhibitors (eg, rivaroxaban, apixaban) within 14 days of lumbar puncture. Participants were excluded if they could not undergo magnetic resonance imaging (MRI; ie, aneurysm clip, metal fragments, internal electrical devices such as a cochlear implant, spinal cord stimulator, or pacemaker), were allergic to gadolinium, or were claustrophobic. Participants who had a baseline brain MRI scan that showed the presence of an intracranial mass or other evidence that precluded the participant from undergoing a lumbar puncture and participants who were considered by the investigator, for any reason, to be an unsuitable candidate to receive elezanumab were excluded. Participants with a positive screen for drugs of abuse, alcohol, or cotinine were excluded. Participants with a history of suicidal ideation or an episode of clinically severe depression within 1 month prior to study drug administration as evidenced by answering "yes" to Questions 4 or 5 on the suicidal ideation portion of the Columbia-Suicide Severity Rating Scale (C-SSRS) completed at screening, or any history of suicide attempts, were also excluded.

The study protocol and informed consent form were approved by the institutional review board at each participating site prior to the initiation of any screening or study-specific procedures. Written informed consent was obtained from each individual participating in the study. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, as defined by the International Conference on Harmonization.

### Study Procedures

For the FIH-SAD study, eligible healthy participants were administered a single dose of elezanumab or placebo (0.9% saline) by intravenous (IV) infusion in 5 groups and by subcutaneous (SC) injection in one group. Each group consisted of 8 participants, 6 receiving elezanumab and 2 receiving placebo. In Group 6, only 5 participants received elezanumab. The elezanumab dose administration schematic is presented in Figure 1. A total of 124ml was infused IV over 2 hours with an infusion rate of ~1.0ml/min. Up to 2ml total, as two 1ml SC injections, was administered to participants in the SC group. A maximum of 2 participants were dosed per day, and the first 2 participants of each dosing group underwent sentinel dosing. The remaining participants were randomly assigned to placebo or elezanumab. Dose escalation was implemented only after available safety, tolerability, PK, and immunogenicity data from lower doses had been reviewed.

For the MAD study, eligible participants with RMS were administered multiple doses of elezanumab or placebo (0.9% saline) by IV infusion in 3 groups (150, 600, and 1,800mg) at approximately the same time in the morning on days 1, 29, 57, and 85 (every 28 days). A loading dose of 2 times the designated treatment dose was administered on day 1. The loading dose for the 1,800mg group was administered in 2 equally divided doses on days 1 and 2 (see Fig 1). All groups received doses via IV infusion at a constant rate over a 2-h duration in the morning. Participants in each group were dosed sequentially

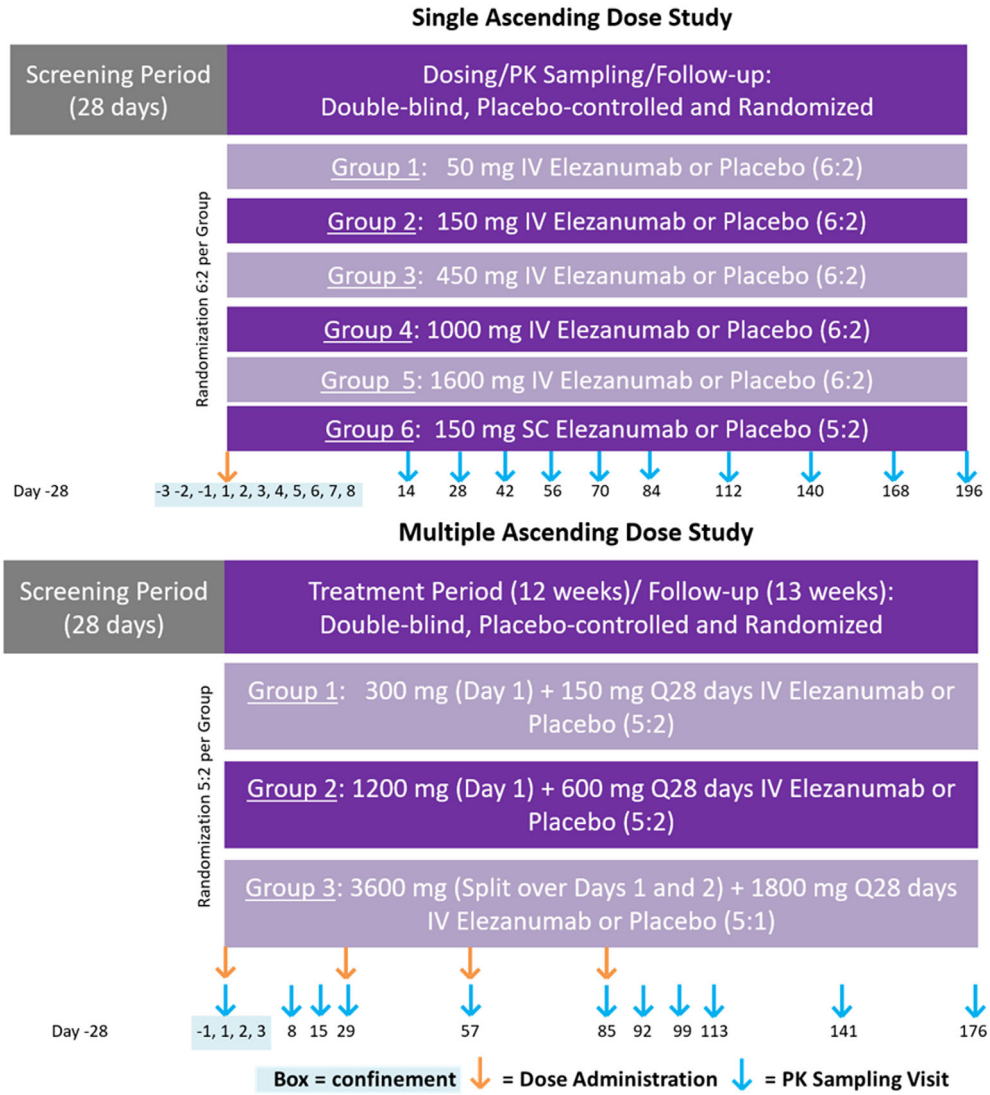


**FIGURE 1: Study dose and group assignments for first-in-human single ascending dose (SAD) and multiple ascending dose (MAD) studies. SAD: Visits 168 and 196 are optional. Participants could return for a final follow-up visit after day 196 at the investigator's discretion. MAD: Participants had the option of staying overnight at the study site or other local accommodation on days associated with the first dose (days 1–3); Group 3 loading dose of 3,600mg was given as 1,800mg on day 1 and 1,800mg on day 2. IV = intravenous; PK = pharmacokinetic.**

following review of available data after the last participant of the previous group received the second dose.

Adverse events (AEs) were defined as any untoward medical occurrence or clinical investigation in a participant administered a pharmaceutical product. All AEs reported from the time of study drug administration until 5 half-lives following discontinuation of study drug administration had elapsed were collected, whether solicited or spontaneously reported by the participant. In addition, serious AEs and protocol-related non-serious AEs were collected from the time the participant signed the study-specific informed consent. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA).

### *Selection of Doses*

A minimally biologically active dose was targeted as an FIH starting dose (50mg), which is 28-fold less than the maximum recommended starting dose and one third lower than the predicted efficacious dose (150mg). The planned maximal dose in the FIH study, 1,600mg, has a predicted exposure less than the rat and monkey no observed adverse effect level (NOAEL) exposure based on area under the curve (AUC).

Human PK and safety data from the FIH-SAD study and the predicted safety margins relative to the preclinical NOAEL were taken into consideration during selection of doses for the MAD study. The multiple dose exposure limits from the Good Laboratory Practice toxicology studies are larger and equivalent to the predicted steady-state AUC exposures at 9,600mg given every 28 days. The starting dose (150mg every 28 days) was predicted to be the minimal efficacious dose based on exposures observed in several preclinical studies. The maximum planned dose (1,800mg every 28 days) was predicted to result in exposures that are <15% of the steady-state AUC exposures at the NOAEL.

### Statistical Analysis

All participants were included in the analyses. The safety of elezanumab was assessed by evaluating the study drug exposure, AE monitoring, vital signs, physical examination, neurological examination, electrocardiogram (ECG), laboratory tests assessments, C-SSRS, and MRI scans. The number and percentage of participants reporting treatment-emergent AEs were tabulated by MedDRA preferred term and system organ class with a breakdown by route of study drug administration and dose level.

Dose proportionality of elezanumab was investigated using 1-way analysis of covariance (ANCOVA) for the natural logarithms of dose-normalized maximum serum concentration ($C_{max}$), and area under the concentration–time curve for the dosing interval at steady state ($AUC_{0\text{-}Tau,SS}$), or $AUC_{\infty}$. Estimates and tests on biomarker data were done using ANCOVA on log-transformed data. Tests were 1-sided for RGMa, and all *t* tests were performed with 8 degrees of freedom. The MRI count variables (number of new gadolinium-enhancing [Gd+] T1 lesions and number of new or newly enlarging T2 hyperintense lesions) were analyzed using a generalized linear model with the link for a negative binomial distribution. For each elezanumab dose level, the hypothesis of no difference between the dose level and placebo was tested against the alternative hypothesis that the mean was lower with the elezanumab dose level. The baseline value was included in the model as a covariate. For lesion volume of new and newly enlarging T2 hyperintense lesions at day 113, an analysis of variance (ANOVA) was performed.

### Determination of Sample Size

From the perspective of tolerability assessment, the probability that a given AE would not be observed in a group of 5 participants administered an assigned elezanumab regimen was estimated to be lower at 0.77, 0.59, 0.33, 0.17, 0.078, and 0.031 relative to true population incidence rates of 0.05, 0.10, 0.20, 0.30, 0.40, and 0.50, respectively. For doses other than the highest dose administered, both the data for the dose under consideration and the data for the next highest dose to be administered to another group of participants were accounted for in this estimation. Therefore, a sample size of 5 to 6 participants per group was used for each dose level of the SAD and MAD studies.

### Efficacy Assessments

MS disease activity was monitored during scheduled serial clinic visits and at unscheduled visits as needed. Clinical events that were captured and recorded included relapses, and disability was measured by the Expanded Disability Status Scale (EDSS), which was performed on days −1, 29, 57, 85, and 176 or upon participant discontinuation. Efficacy was assessed on day 85 because of uncertainty regarding durability of therapeutic effect following the last dose.

In addition, the participant-reported outcomes were measured using the Multiple Sclerosis Impact Scale (MSIS-29) and Multiple Sclerosis Quality of Life-54 (MSQOL-54) on the same days.

### Biomarker and MRI Assessments

Approximately 18.5ml of blood was collected for serum, plasma, and whole blood. Blood samples were collected on day 1 before dosing and on days 7 and 14 after dosing, before dosing on days 29, 57, and 85, and 14, 28, 56, and 91 days after dosing on day 85. Cerebrospinal fluid (CSF) samples for elezanumab and soluble RGMa (total and free) assays as well as for IL-10 and neurofilament light (NfL) among other exploratory biomarkers were collected by lumbar puncture within the same 2-hour period of the morning on days −1 and 113.

RGMa was quantitated using a validated liquid chromatography–tandem mass spectrometry technique with a lower limit of quantification (LLOQ) of ~1ng/ml. Analysis of CSF IL-10 was performed using the Quanterix digital immunoassay (LLOQ = 21fg/ml). CSF NfL was quantified using the UmanDiagnostics Immunoassay (LLOQ = 100pg/ml). MRI assessments using Gd+ T1 lesions and T2 hyperintense lesions were performed for participants with MS participating in the MAD study at screening and on days 57, 113, and 176.

### PK Sampling and Assessments

For the SAD study, serial blood samples for measurements of elezanumab concentrations in serum, assay of antidrug antibodies (ADAs) and neutralizing antibodies (Nabs) were collected before dosing and up to 336 hours after dosing on day 1; trough levels on days 28, 42, 56, 70, 84, 112, and 140; and Group 5 on days 168 and 196, and at a final follow-up visit after day 196 at the investigator's discretion. Intensive samples collected on day 1 were for elezanumab PK assessment only. CSF samples for assay of elezanumab were collected before dosing and on day 7.

For the MAD study, serial blood samples for measurements of elezanumab concentrations, ADAs, and Nabs were collected before dosing, at 2, 4, 6, 24, and 48 hours after the start of dosing on day 1, at 7 and 14 days after dosing on day 1, before dosing on days 29, 57, and 85, at 2, 4, and 6 hours after the start of dosing on day 85, and at 7, 14, 28, 56, and 91 days after dosing on day 85. Intensive samples collected on days 1 and 85 were for elezanumab PK assessment only. Group 3 had additional samples collected to characterize PK following infusions on days 1 and 2. CSF samples for assay of elezanumab were collected before dosing and on day 113.

The LLOQ for elezanumab was established at 31.3 and 15ng/ml in serum for SAD and MAD studies, respectively, and at 16.3 and 6.68ng/ml in CSF for SAD study and MAD studies, respectively.

The relative titers of serum elezanumab ADA for both SAD and MAD studies were determined using a validated titer-based electrochemiluminescence immunoassay in bridging format. $ADA^{+}$ samples were confirmed by adding 100μg/ml elezanumab into the assay. Positive confirmation was obtained by suppression ≥ 21.754% for SAD and ≥ 24.907% for MAD.

Elezanumab serum concentrations were quantified using a validated bioanalytical assay and analyzed using noncompartmental analysis in Phoenix WinNonlin (version 6.2; Pharsight Corporation, Mountain View, CA). $C_{max}$, time to peak concentration from the

beginning of infusion ($T_{max}$), and AUC were determined for elezanumab.

## Results

### Demographics

A total of 67 participants were enrolled across the phase 1 program: 47 healthy participants in the FIH-SAD study and 20 RMS participants in the MAD study. Table 1 summarizes the baseline characteristics, study drug exposure, and disposition of all 67 participants.

### Efficacy

Exploratory efficacy assessments were performed using the EDSS, MSIS-29, and MSQOL-54 scales in the MAD study, and no clinically meaningful changes were seen during the treatment period (4 dose intervals, 12 weeks).

### Safety

The safety and tolerability of elezanumab were evaluated in all 67 participants (50 participants who received elezanumab and 17 participants who received placebo). The most frequently reported treatment-emergent AEs in participants treated with elezanumab in SAD and MAD are presented in Table 2.

In the FIH-SAD study, no participant had an infusion reaction, systemic hypersensitivity reaction, injection site reaction, or concerning patterns of AEs or laboratory findings. No treatment-related medically significant AEs were identified; however, 2 treatment-unrelated serious adverse events (SAEs) resulted in death.

The first case involved a 34-year-old man who was assigned to active treatment in the 150mg IV dose group. All medical examinations at all visits before and after dosing (including ECG, laboratory, and physical examination) were normal. The participant was found dead in his residence 118 days after the dose. The individual PK analysis on this participant indicated that at the approximate time of death, an estimated 6.7 elimination half-lives of elezanumab had elapsed since elezanumab administration, meaning that approximately 1.0% of the drug remained in his system. All available evidence indicated that the event was not related to study drug. The postmortem blood analysis of this participant was positive for amphetamines, methocarbamol, cyclobenzaprine, fentanyl, and 5-MEO-MiPT (a psychedelic "designer drug"). The final autopsy and toxicology reports indicated the cause of death to be drug toxicity, combined with a preexisting severe stenosis of the left anterior descending coronary artery and alcoholism.

The second case involved a 27-year-old man with a reportedly uneventful medical history who was assigned to active treatment in the 1,600mg dose group. All baseline assessments, including laboratory results, vital signs, physical and neurological examinations, and MRI, were normal. The dose was well tolerated, and no AEs were reported. Follow-up assessments were normal, including vital signs, ECGs, physical and neurological examinations, clinical

**TABLE 1. Demographic Summary**

| Characteristic | Mean ± SD | Minimum–Maximum |
|---|---|---|
| Placebo healthy participants, n = 12 | | |
| Age, years | 38.2 ± 12.5 | 20–54 |
| Weight, kg | 78.3 ± 14.4 | 58.4–106 |
| Height, cm | 171 ± 11 | 154–188 |
| BMI, kg/m$^2$ | 26.7 ± 3.3 | 20.5–30.1 |
| Sex | 7 men (58%), 5 women (42%) | |
| Race | 4 White (33%), 8 Black (67%) | |
| Elezanumab healthy participants, n = 35 | | |
| Age, years | 38.7 ± 9.7 | 20–55 |
| Weight, kg | 81.9 ± 11.9 | 57.6–106 |
| Height, cm | 176 ± 9 | 157–200 |
| BMI, kg/m$^2$ | 26.4 ± 2.8 | 21.1–29.9 |
| Sex | 28 men (80%), 7 women (20%) | |
| Race | 19 White (54%), 14 Black (40%), 1 Asian (3%), 1 multirace (3%) | |
| Placebo multiple sclerosis participants, n = 5 | | |
| Age, years | 50.4 ± 9.4 | 36–59 |
| Weight, kg | 81.4 ± 23.4 | 51.3–106.5 |
| Height, cm | 168 ± 12 | 160–176 |
| BMI, kg/m$^2$ | 28.5 ± 6.4 | 19.3–34.4 |
| Sex | 1 men (20%), 4 women (80%) | |
| Race | 3 White (60%), 1 Black (20%), 1 multirace (20%) | |
| Elezanumab multiple sclerosis participants, n = 15 | | |
| Age, years | 47.1 ± 9.2 | 29–61 |
| Weight, kg | 80.5 ± 17.5 | 53.3–120 |
| Height, cm | 168 ± 13 | 150–183 |
| BMI, kg/m$^2$ | 28.7 ± 5.5 | 20.8–38.2 |
| Sex | 7 men (47%), 8 women (53%) | |
| Race | 9 White (60%), 6 Black (40%) | |

BMI = body mass index; SD = standard deviation.

**TABLE 2. Most Frequently Reported (>2%) Treatment-Emergent Adverse Events in 50 Participants Treated with Elezanumab (FIH-SAD and MAD)**

| System Organ Class Preferred Term (MedDRA version 22.1) | n (%) |
|---|---|
| Any adverse event | 31 (62.0) |
| Gastrointestinal disorders | 3 (6.0) |
| Nausea | 3 (6.0) |
| General disorders and administration site conditions | 2 (4.0) |
| Asthenia | 2 (4.0) |
| Infections and infestations | 4 (8.0) |
| Urinary tract infection | 2 (4.0) |
| Viral upper respiratory tract infection | 2 (4.0) |
| Injury, poisoning, and procedural complications | 10 (20.0) |
| Post–lumbar puncture syndrome | 3 (6.0) |
| Procedural headache | 4 (8.0) |
| Procedural pain | 6 (12.0) |
| Musculoskeletal and connective tissue disorders | 5 (10.0) |
| Back pain | 3 (6.0) |
| Pain in extremity | 2 (4.0) |
| Nervous system disorders | 15 (30.0) |
| Headache | 15 (30.0) |
| Skin and subcutaneous tissue disorders | 4 (8.0) |
| Pruritus | 2 (4.0) |
| Rash | 2 (4.0) |

FIH-SAD = first-in-human single ascending dose; MAD = multiple ascending dose; MedDRA = Medical Dictionary for Regulatory Activities.

laboratory results, and CSF analysis. The participant was found dead in a hotel under suspicious circumstances 22 days after dosing. The initial assessment of "possibly related" to study drug by the investigator and sponsor was subsequently changed to "not related" when the final autopsy and toxicology reports indicated the cause of death to be acute intoxication by the combined effects of cocaine and oxycodone.

Both deaths were investigated thoroughly, and the cause of death for both cases was determined to be unrelated to elezanumab treatment. Polysubstance abuse was determined to play a role in both fatalities. Neither deceased participant reported any history of alcohol or drug abuse at screening (a history of drug or alcohol use within 2 years of study participation was exclusionary), and both had negative drug and alcohol screens during screening in the days prior to study drug administration. Both SAEs were considered not to have impacted the overall risk/benefit profile of elezanumab. No other SAEs were reported in this study.

In the MAD study, 1 SAE, MS relapse, was reported among participants randomized to placebo, with an onset 66 days after the last dose of the study drug regimen, and was judged to unrelated to study drug. No deaths were reported. A total of 2 participants in the 1,800mg group discontinued from the study. One participant withdrew consent after receiving placebo on days 1, 2, and 30. Another participant was lost to follow-up after receiving elezanumab 1,800mg on days 1 and 2 (total dose of 3,600mg was received). These discontinuations were not secondary to AEs or considered to be related to elezanumab administrations.

### *Immunogenicity*

In the FIH-SAD study, 2 healthy participants had detectable ADA titers. One in the 450mg single IV dose group had ADA titers prior to dosing; the other, in the 150mg SC dose group, had ADA titers of 51 and 189 titer units on days 56 and 84. There was no apparent decrease in exposure when the positive titers occurred, suggesting immunogenicity did not change elezanumab PK. All participants in the MAD study tested negative for ADAs in serum. Nabs were not assayed per protocol, as there was no evidence of ADAs affecting the PK of elezanumab.

### *Pharmacokinetics*

Across the range of 50 to 1,600mg IV doses in the FIH-SAD study, elezanumab $C_{max}$ increased in a dose-proportional manner ($p > 0.4$), whereas $AUC_{\infty}$ showed a significant greater-than-dose-proportional increase ($p < 0.001$). Linear kinetics were also investigated in a 1-way ANOVA for β. β tended to decrease with dose ($p = 0.010$).

The $T_{max}$ was approximately 4 hours, and harmonic mean of the terminal phase elimination half-life ($t_{1/2}$) ranged from 19 to 50 days. Absolute bioavailability of 150mg SC elezanumab was estimated to be approximately 60% of IV administration. Elezanumab concentrations in CSF increased with dose (CSF/serum ratio = 0.1–0.3%).

Across the range of 150 to 1,800mg IV doses administered as 4 multiple doses every 28 days, elezanumab $C_{max}$, $AUC_{0\text{-}Tau,SS}$, and $C_{trough}$ following the fourth dose increased in a dose-proportional manner

($p > 0.8$). Linear kinetics were also investigated in a 1-way ANOVA for $\beta$, and $\beta$ was similar between 1,800mg and 150mg doses ($p > 0.2$). Across the 3 dose levels studied in the MAD, administration of loading dose helped achieve 70% of elezanumab concentrations at the end of the first dosing interval compared to the fourth dosing interval. The mean $T_{max}$ was approximately 2 to 4 hours, and harmonic mean $t_{1/2}$ ranged from 42 to 68 days following the fourth dose. Elezanumab concentrations in CSF increased with dose (CSF/serum ratio = 0.2–0.4%). All participants tested negative for elezanumab antibodies in serum.

Elezanumab serum concentration–time profiles from the FIH-SAD and MAD studies are provided in Figure 2, and PK parameters from this study are provided in Table 3.

### *Fluid Biomarkers from the MAD Study*

The geometric mean of free RGMa levels in CSF by day 113 following 1,800mg doses administered every 28 days was significantly lower than that of placebo ($p < 0.001$). Free RGMa levels in CSF decreased with increasing doses (with placebo considered a dose level) by day 113 ($p < 0.001$). The geometric mean of total RGMa levels in CSF by day 113 following 1,800mg doses administered every 28 days was significantly greater than that of placebo ($p < 0.001$). Total RGMa levels in CSF increased with increasing doses (compared to placebo) by day 113 ($p < 0.001$).

Overall, the CSF levels of elezanumab reduced free RGMa levels in CSF by >40% in a dose- and exposure-dependent manner, demonstrating CNS target binding with an apparent maximal effect at the 1,800mg IV dose level (Fig 3).

A dose-dependent increase in CSF IL-10 levels was observed when accounting for each participant's baseline levels, and a similar relationship was observed when comparing change from baseline in CSF IL-10 level and CSF elezanumab concentration at day 113 (Fig 4). There was

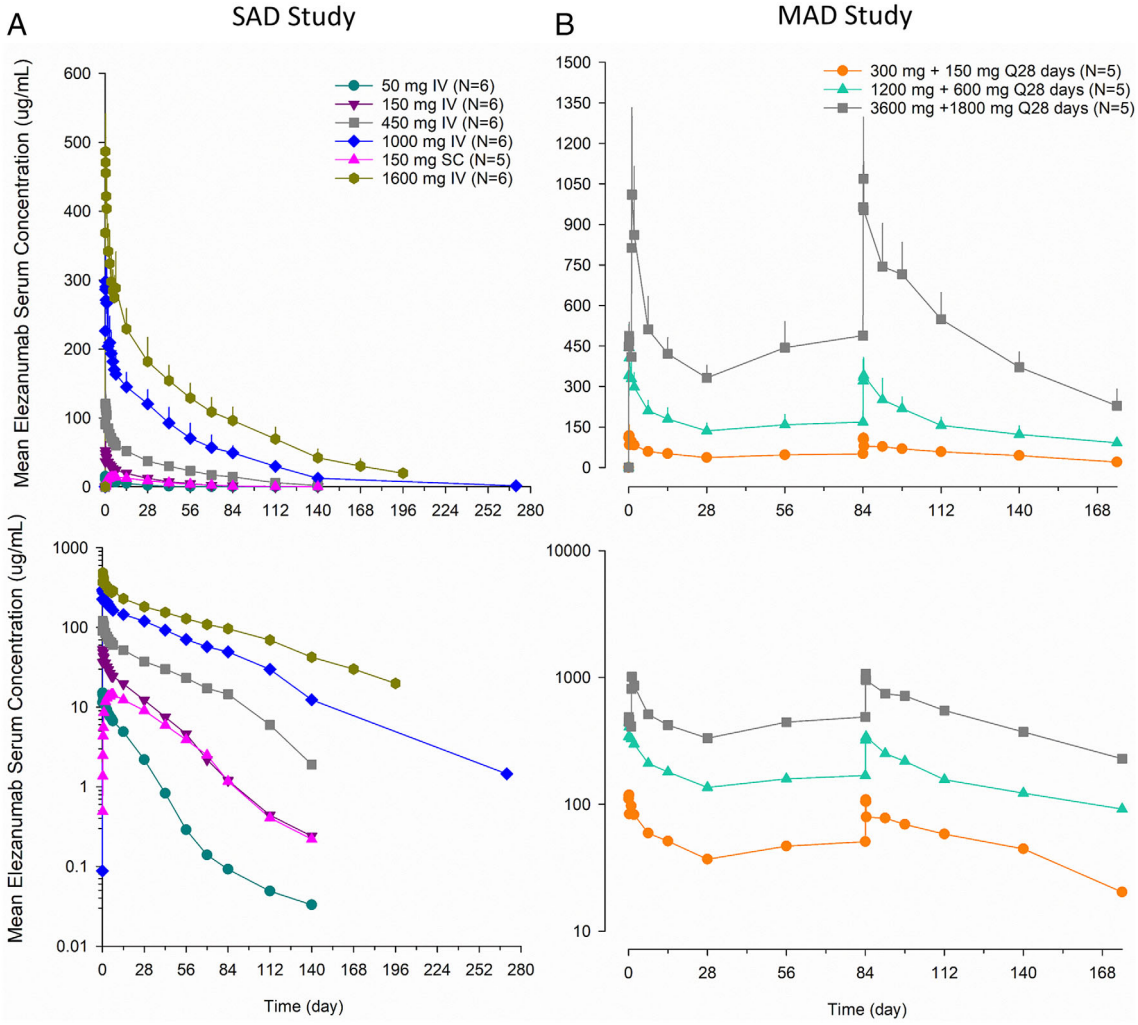


**FIGURE 2: Mean and standard deviation of elezanumab serum concentration versus time profiles for single doses in healthy participants from the single ascending dose (SAD) study and multiple doses in multiple sclerosis participants from the multiple ascending dose (MAD) study. IV = intravenous; Q = every; SC = subcutaneous.**

**TABLE 3. Pharmacokinetic Parameters of Elezanumab as Geometric Mean (Mean, %CV) Following Single or Multiple Doses**

**Single Dose**

| PK Parameters | Units | Elezanumab 50mg IV, n = 6 | Elezanumab 150mg SC, n = 5 | Elezanumab 150mg IV, n = 6 | Elezanumab 450mg IV, n = 6 | Elezanumab 1,000mg IV, n = 6 | Elezanumab 1,600mg IV, n = 5[a] |
|---|---|---|---|---|---|---|---|
| $C_{max}$ | µg/ml | 14.9 (15.1, 21) | 14.5 (15.0, 25) | 52.3 (53.7, 25) | 120 (121, 15) | 300 (304, 15) | 486 (488, 11) |
| $T_{max}$[b] | h | 4.0 (4.0–10.0) | 168 (72.0–168) | 4.0 (4.0–10.0) | 4.0 (4.0–4.0) | 4.0 (4.0–14.0) | 4.0 (4.0–6.0) |
| $AUC_t$ | µg•day/ml | 182 (186, 23) | 565 (586, 29) | 885 (903, 22) | 3,240 (3,280, 16) | 11,000 (11,100, 19) | 19,700 (19,900, 18) |
| $AUC_\infty$ | µg•day/ml | 185 (188, 22) | 572 (594, 29) | 892 (911, 22) | 3,300 (3,340, 16) | 11,000 (11,200, 20) | 21,100 (21,500, 20) |
| $t_{1/2}$[c] | Day | 35.2 (16.6) | 21.5 (4.12) | 18.6 (2.9) | 21.2 (3.91) | 35.1 (5.06) | 49.5 (9.36) |
| CL[d] | ml/h | 11.3 (11.5, 19) | 10.9 (11.4, 34) | 7.01 (7.1, 23) | 5.68 (5.7, 16) | 3.78 (3.8, 17) | 3.16 (3.2, 21) |
| $C_{max}$/dose | (µg/ml)/mg | 0.298 (0.303, 21) | 0.097 (0.100, 25) | 0.349 (0.358, 25) | 0.266 (0.268, 15) | 0.300 (0.304, 15) | 0.313 (0.314, 10) |
| $AUC_t$/dose | (µg•day/ml)/mg | 3.65 (3.72, 23) | 3.77 (3.91, 29) | 5.90 (6.02, 22) | 7.20 (7.28, 16) | 11.0 (11.1, 19) | 12.3 (12.5, 18) |
| $AUC_\infty$/dose | (µg•day/ml)/mg | 3.70 (3.76, 22) | 3.82 (3.96, 29) | 5.94 (6.07, 23) | 7.34 (7.42, 16) | 11.0 (11.2, 20) | 13.2 (13.4, 20) |

**Multiple Doses**

| PK Parameters | Units | Elezanumab 300mg IV, n = 5 | Elezanumab 1,200mg IV, n = 5 | Elezanumab 3,600mg IV, n = 5 | Elezanumab 150mg IV, n = 5 | Elezanumab 600mg IV, n = 5 | Elezanumab 1,800mg IV, n = 5 |
|---|---|---|---|---|---|---|---|
| | | First dose interval [days 1–28][e] | | | Fourth dose interval [days 85–113] | | |
| $C_{max}$ | µg/ml | 119 (122, 24) | 441 (446, 18) | 1,020 (1,060, 28) | 109 (111, 19) | 347 (351, 19) | 1,080 (1,090, 19)[f] |
| $T_{max}$[b] | h | 2.0 (2.0–6.0) | 4.0 (4.0–6.0) | 28.0 (28.0–48.0) | 4.0 (4.0–6.0) | 4.0 (2.0–6.0) | 4.0 (2.0–4.0)[f] |
| $AUC_{0\text{-}Tau,\ SS}$ | µg•day/ml | 1,530 (1,550, 18) | 5,440 (5,520, 19) | 11,700 (12,300, 32) | 2,000 (2,030, 18) | 6,300 (6,400, 20) | 19,600 (19,800, 15)[f] |
| $t_{1/2}$[c] | Day | — | — | — | 41.5 (7.11) | 67.7 (25.6) | 49.5 (8.44)[f] |
| CL | ml/h | — | — | — | 3.12 (3.17, 20) | 3.97 (4.03, 19) | 3.82 (3.86, 17)[f] |

%CV = percent coefficient of variation; AUC = area under the curve; CL = clearance; IV = intravenous; PK = pharmacokinetic; SC = subcutaneous; SS = steady state.

[a]6 participants were dosed with elezanumab 1600 mg; AUC, CL and half-life were estimated in only 5 subjects because one participant died. Only Cmax and Tmax were reported for this participant.

[b]Median (minimum through maximum).

[c]Harmonic mean (pseudo-standard deviation).

[d]CL/F was calculated for the 150 mg SC administration.

[e]Loading doses of 300 mg and 1200 mg was administered on Day 1 for Groups 1 and 2 respectively. For Group 3, the loading dose (3600 mg) was administered as two divided doses on Days 1 and 2.

[f]N = 4, CL, $t_{1/2}$ and steady-state AUC cannot be estimated from 28 days PK collection after first dose as elezanumab half-life is longer than 28 days.

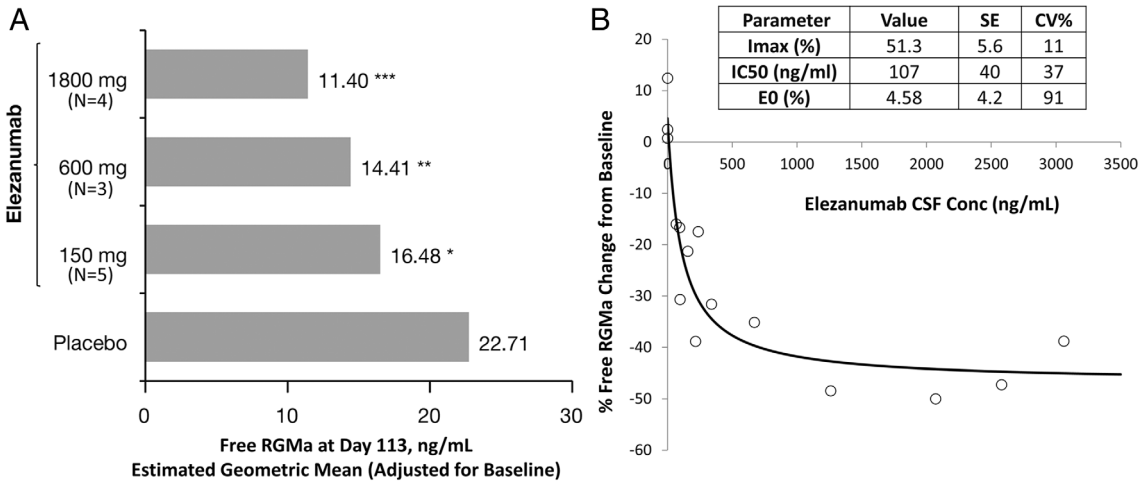


**FIGURE 3: Free repulsive guidance molecule A (RGMa) in cerebrospinal fluid (CSF) of multiple sclerosis participants (multiple ascending dose study) at day 113 by elezanumab dose levels (A) and elezanumab CSF levels (B). Estimates and tests were done in an analysis of covariance (4 groups) on log-transformed data. Tests were 1-sided for free RGMa, all *t* tests with 8 degrees of freedom. Estimated geometric mean value is the back transformation of the adjusted mean from the analysis of covariance for the transformed data. **p* < .05, ***p* < .001, ****p* < .0001. Conc = concentration; CV% = percent coefficient of variation; SE = standard error.**

also an apparent decrease in the CSF NfL levels, a measure of disease relevant neuronal loss and a maximal response to IL-10, in the 1,800mg dose group (see Fig 4).

### *MRI (MAD Study)*

The number of new Gd+ T1 lesions across day 57 and 113 following all dose groups was not significantly different from the placebo group. The mean number of new or newly enlarging hyperintense T2 lesions on day 113 following 600mg doses was significantly lower than that of placebo (1.37 vs 0.08, respectively, $p = 0.047$). Results following 150 and 1,800mg doses were not significantly different from the placebo group ($p > 0.05$). Overall, these exploratory findings do not suggest a consistent treatment effect of elezanumab on brain MRI lesions.

## Discussion

Clinical improvement in MS patients with permanent disabilities is an area of increasing emphasis in drug discovery and clinical research.[17] Despite promising evidence across a range of preclinical models with varied drug targets,

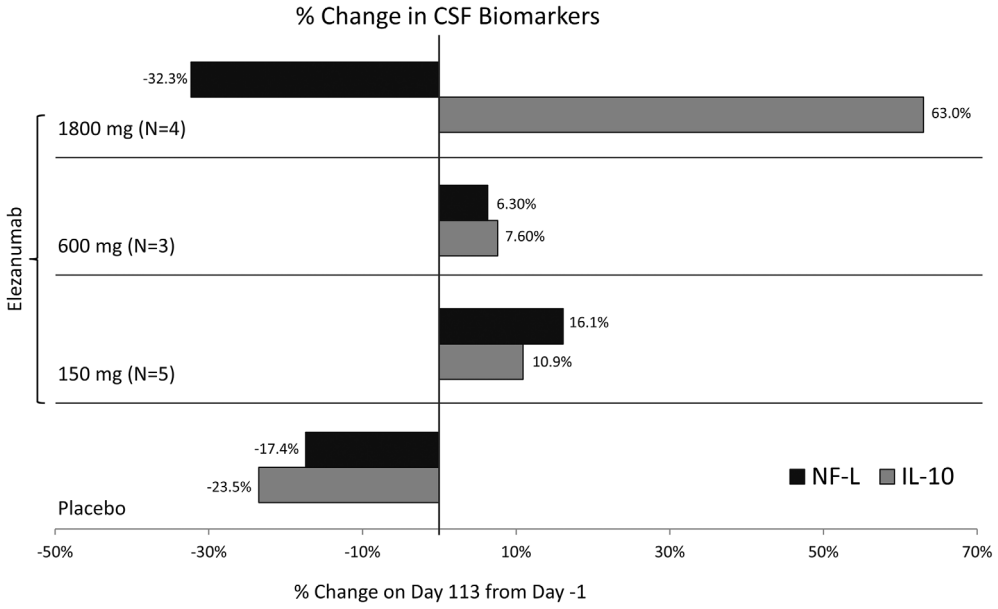


**FIGURE 4: Percent change in cerebrospinal fluid (CSF) interleukin-10 (IL-10) and neurofilament light (NF-L) levels by day 113 from baseline in multiple sclerosis participants (multiple ascending dose study) by elezanumab dose level.**

multiple attempts, including with drug candidates such as opicinumab[18] and high-dose biotin,[19] have not yielded consistent clinical improvement. Studies leveraging previously approved agents such as clemastine and MS disease-modifying treatments (DMTs) netted modest or inconsistent improvements, with potential benefits largely limited to myelination-associated biomarkers such as visual evoked potential conduction or MRI proxies of myelin content.[7,8,20,21] RGMa neutralization has the potential to promote axonal regeneration and remyelination following nervous system injury[15] and may promote neuroprotection from ongoing damage.[22–24] These phase 1 studies are the first to explore the PK and pharmacodynamic properties of the anti-RGMa mAb, elezanumab, in both healthy and RMS participants, along with other neurologic disorders.

Overall, elezanumab had favorable PK properties, with a long half-life and dose-proportional increases in systemic and CSF drug exposure. No safety concerns were identified, suggesting that elezanumab could be dosed intravenously as frequently as once per month and up to every other month. Use of a loading dose that is twice the monthly maintenance dose achieved steady-state exposure more rapidly and is expected to achieve desired target engagement faster. A low immunogenic response to elezanumab was observed at the level of quantitation in 2 of 47 (4%) participants in the SAD study; however, the response was not significant, and an immunogenic response was not believed to affect the PK disposition of elezanumab.

Appreciable and significant dose-dependent reductions in soluble free RGMa and dose-dependent increases in soluble total RGMa were observed in the CSF of MS participants, suggesting adequate blood–brain barrier penetration and engagement with the target of interest, thereby suggesting a range of doses that could be efficacious and tolerated in phase 2 studies. Because elezanumab engages with soluble RGMa, the elezanumab–RGMa complex is not expected to be eliminated as quickly as free RGMa, and therefore an increase in total RGMa levels is observed.

CNS injury is associated with increased RGMa expression in the tissue surrounding the injury.[25,26] As a consequence, RGMa signaling can activate the rho kinase pathway, which results in a cellular context-dependent response leading to the inhibition of neurite extension in neurons and the induction of proinflammatory cytokines.[27] IL-10 is an anti-inflammatory cytokine that is increased when the rho kinase pathway is inhibited in an inflammatory milieu as occurs in MS[28] and is associated with neural protection, repair, and remyelination.[29] An elezanumab dose and CSF exposure-dependent increase in CSF IL-10 levels was observed at day 113 post-treatment in MS participants when correcting for each participant's baseline CSF level, suggesting a potentially beneficial effect in MS patients. This is consistent with the blockade of RGMa signaling by elezanumab and acts as supporting evidence for elezanumab-mediated pathway modulation.

NfL has emerged as a leading fluid biomarker of CNS damage across multiple disease states.[30] NfL levels fluctuate in RMS patients, peaking following acute inflammation and then normalizing. Large trials studying anti-inflammatory DMT efficacy over a period of years found that DMTs reduce NfL levels, presumably through limiting inflammatory injury.[31] Multiple elezanumab doses administered every 28 days showed an apparent decrease in CSF NfL when corrected for individual baseline levels only in the MS participants who were administered 1,800mg elezanumab. A dose-dependent signal in CSF NfL was not seen in MS participants. Given that a reduction in NfL was also observed in placebo patients, in this study of limited duration, elezanumab therapy does not appear to reduce NfL levels. Due to the limited number of CSF samples in this study, these biomarker conclusions are considered preliminary. Therefore, elezanumab's potentially beneficial effects on IL-10 should be placed in context with a lack of effect on other damage-related markers such as NfL, as well as MRI parameters such as T2 lesions. Clinical scores such as the EDSS were also not impacted, although EDSS improvement was not anticipated in a phase 1b study following only 4 doses of elezanumab. Phase 2a studies in relapsing and progressive MS participants utilized a longer treatment duration (1 year), more comprehensive clinical endpoints, myelination- and axon-associated neuroimaging measures, and exploratory neurorestorative biomarkers.

Although the safety profile and PK properties of elezanumab appear favorable, in addition to appropriately modest phase 1 sample sizes, these data contain several limitations. Although elezanumab doses exceeding 450mg given IV reduced CNS free soluble RGMa levels by approximately 40%, it is not known what level of reduction in free soluble RGMa is necessary to achieve a beneficial effect. It is also unknown what the relative contributions of soluble versus membrane-bound RGMa are to human disease, because it is currently not possible to assess the magnitude of membrane-bound RGMa changes. All preclinical models assessed the degree of impairment and improvement when elezanumab was given in close temporal proximity to the injury. The ability of elezanumab to trigger repair and clinical improvement in patients with lesions ranging in age from months to decades remains unknown and will be evaluated further. It is also unclear whether patients across the range of MS phenotypes including RMS, active secondary progressive MS (SPMS), nonactive SPMS, and primary progressive MS will benefit from this therapy.

The MAD study in 20 MS participants with a 4-month treatment duration was likely not sufficient to detect beneficial or detrimental impacts on the MS disease process. Consistent with this, improvement on the EDSS (as assessed at day 85) and relevant MRI parameters such as T2 lesion volume were not observed. However, elevation of IL-10 hints at a potential impact on neuronal damage and the level of background inflammation, which will require additional exploration.

Given the favorable tolerability profile, coupled with preclinical efficacy models and preliminary fluid biomarker effects, elezanumab has advanced to phase 2 trials in multiple neuroscience indications including relapsing and progressive forms of MS, acute ischemic stroke, and acute traumatic spinal cord injury. Despite baseline recreational drug screening, 2 fatalities attributed to substance abuse occurred in the healthy participant study; therefore, phase 2 trials will exclude participants with a history of drug abuse within 2 years of trial participation. Drug and alcohol screening will be performed at each visit, and positive tests from recreational drug or alcohol abuse will trigger participant discontinuation. In addition to clinical efficacy scales, incorporation of innovative MRI and fluid biomarker assessments of neurorestoration, neuroprotection, and remyelination may further elucidate the mechanism(s) by which RGMa neutralization promotes neuronal repair following injury.

## Acknowledgments

This study was sponsored by AbbVie. AbbVie provided review and approval of the study design and this publication. Wesley Wayman, PhD, an employee of AbbVie, collated the manuscript and provided copyediting support.

## Author Contributions

A.Z., TP.M., M.R.R., W.L., and B.A.C.C. contributed to the conception and design of the study. H.V.K., M.R.R., and A.Z. acquired and analyzed the data. H.V.K., A.Z., and T.P.M. drafted a significant portion of the manuscript or figures.

## Conflict of Interest

H.V.K., M.R.R., A.Z., and W.L. are AbbVie employees and may hold stock or options. T.P.M. was an AbbVie employee at the time this work was conducted and may hold stock or options. B.A.C.C. has received personal compensation for consulting from Alexion, Atara, Autobahn, Avotres, Biogen, EMD Serono, Gossamer Bio, Horizon, Neuron23, Novartis, Sanofi, TG Therapeutics, and Therini and has received research support from Genentech.

### *Data Availability Statement*

AbbVie is committed to responsible data sharing regarding the clinical trials it sponsors. This includes access to anonymized, individual, and trial-level data (analysis data sets), as well as other information (e.g., protocols and Clinical Study Reports), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications. This clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and Statistical Analysis Plan and execution of a Data Sharing Agreement. Data requests can be submitted at any time and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.

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