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Safety, Pharmacokinetic, and Functional Effects of the Nogo-A Monoclonal Antibody in Amyotrophic Lateral Sclerosis: A Randomized, First-In-Human Clinical Trial

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Abstract

The neurite outgrowth inhibitor, Nogo-A, has been shown to be overexpressed in skeletal muscle in amyotrophic lateral sclerosis (ALS); it is both a potential biomarker and therapeutic target. We performed a double-blind, two-part, dose-escalation study, in subjects with ALS, assessing safety, pharmacokinetics (PK) and functional effects of ozanezumab, a humanized monoclonal antibody against Nogo-A. In Part 1, 40 subjects were randomized (3:1) to receive single dose intravenous ozanezumab (0.01, 0.1, 1, 5, or 15 mg/kg) or placebo. In Part 2, 36 subjects were randomized (3:1) to receive two repeat doses of intravenous ozanezumab (0.5, 2.5, or 15 mg/kg) or placebo, approximately 4 weeks apart. The primary endpoints were safety and tolerability (adverse events [AEs], vital signs, electrocardiogram [ECG], and clinical laboratory tests). Secondary endpoints included PK, immunogenicity, functional endpoints (clinical and electrophysiological), and biomarker parameters. Overall, ozanezumab treatment (0.01–15 mg/kg) was well tolerated. The overall incidence of AEs in the repeat dose 2.5 mg/kg and 15 mg/kg ozanezumab groups was higher than in the repeat dose placebo group and repeat dose 0.5 mg/kg ozanezumab group. The majority were considered not related to study drug by the investigators. Six serious AEs were reported in three subjects receiving ozanezumab; none were considered related to study drug. No study drug-related patterns were identified for ECG, laboratory, or vital signs parameters. One subject (repeat dose 15 mg/kg ozanezumab) showed a weak, positive anti-ozanezumab-antibody result. PK results were generally consistent with monoclonal antibody treatments. No apparent treatment effects were observed for functional endpoints or muscle biomarkers. Immunohistochemical staining showed dose-dependent co-localization of ozanezumab with Nogo-A in skeletal muscle. In conclusion, single and repeat dose ozanezumab treatment was well tolerated and demonstrated co-localization at the site of action. These findings support future studies with ozanezumab in ALS.

Trial Registration: ClinicalTrials.gov NCT00875446 GSK-ClinicalStudyRegister.com GSK ID 111330


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Competing Interests: AL, NW, PO, JP, SB, JH, DK, AB, BA and GM-T are employees of and hold stock in GlaxoSmithKline. JW was a full-time employee of GlaxoSmithKline at the time of study and holds shares in the company. He is now an employee of Novartis. GP was a full-time employee of GlaxoSmithKline at the time of study and holds shares in the company. He is now an employee of Hammersmith Medicines Research. VM has previously received a consultancy fee from GlaxoSmithKline. AC has previously acted as a consultant to GlaxoSmithKline at a single-day advisory board meeting. PNL received travel expenses and consultancy fees for his work on the advisory board of this development. JDR has previously received funding from NIH, MDA, Robert Packard Center for ALS research and has acted as a consultant for Psyadon Pharmaceutical, Biogen Pharma and Cytokinetics. MC is on the GlaxoSmithKline scientific advisory board. F-PP, BB, JBC, SJK, DL, TM, SM, SA-S and KEM have no conflicts of interest to declare. This does not alter the authors’ adherence to PLOS ONE policies on sharing data and materials.

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Introduction

Amyotrophic lateral sclerosis (ALS) is characterized by selective and progressive loss of upper motor neurons of the motor cortex and lower motor neurons of the brainstem and spinal cord.[1–3] The main manifestations of ALS are progressive widespread muscle weakness and atrophy, leading to severe motor disability that affects speech, swallowing, respiratory function, and the extremities.[4] Cognitive impairment, predominantly in the form of executive dysfunction, may be detected in around 50% of patients, with up to 15% experiencing frontotemporal dementia.[5] Most patients die within 5 years of onset.[1,4]

Excitotoxicity, i.e. an excessive drive of glutamate, is considered to be one of the mechanisms of neurodegeneration in ALS.[6] Riluzole, the only currently approved drug that alters survival in ALS, is thought to reduce excessive glutamatergic drive on neurons.[3,7] Although the exact mechanism of action of riluzole is unclear, it is likely to involve several components, including inhibition of glutamate release, blockade of calcium and sodium channels, modulation of γ-Aminobutyric acid (GABA) transmission, as well as effects on N-Methyl-D-aspartate (NMDA) or α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors.[7–9]

Nogo-A, a negative regulator of neuronal growth, is a potent neurite outgrowth inhibitor in the adult central nervous system and is expressed by oligodendrocytes.[10,11] Outside the central nervous system Nogo-A is overexpressed in the skeletal muscle of the superoxide dismutase 1 (SOD1) transgenic mouse model of ALS, as well as in human skeletal muscle, as demonstrated in biopsies taken from patients with ALS.[12] Nogo-A expression in skeletal muscle has been proposed as an early diagnostic biomarker of ALS, with the level of expression reported to correlate with disease severity.[12–14] This view is challenged by reports suggesting that Nogo-A is a marker of muscle denervation rather than ALS specifically, noted to be up-regulated in muscle in preclinical denervation models and in muscle biopsies from subjects with a range of myopathies and peripheral neuropathies.[15–18] In the SOD1 transgenic mouse genetic ablation of Nogo-A prolonged survival and reduced muscle denervation,[19] while overexpression of Nogo-A in muscle fibers of mice induced neuromuscular junction instability and promoted denervation.[19] There is therefore a strong rationale for testing antibodies against Nogo-A in ALS. It is anticipated that blockade of Nogo-A may inhibit neurite retraction and potentially slow the axonal degeneration pattern in lower motor neurons that begins at the neuromuscular junction.[20] This may enhance motor neuron-muscle coupling, leading to functional improvement and survival benefits in patients with ALS.

Ozanezumab (GSK1223249: GlaxoSmithKline) is a humanized monoclonal antibody against Nogo-A, which is currently being investigated for the treatment of ALS. Ozanezumab has two possible modes of action: preventing binding of Nogo-A to the Nogo-A receptor and/or Nogo-A down-regulation by antibody-induced internalization of cell surface Nogo-A.[21]

Given that the anticipated mechanism of action of ozanezumab is via Nogo-A, which is not appreciably expressed in skeletal muscle under physiological conditions but is overexpressed in ALS, it was felt that conduct of a study in healthy subjects would not adequately reveal the potential risks or effects of treatment. Therefore, the first-in-human, Phase I/IIa study presented here was performed in subjects with ALS to assess the safety, pharmacokinetic (PK), and functional and biomarker effects of ozanezumab.

Methods

Study design

This was a randomized, placebo-controlled, double-blind, single and repeat dose-escalation, two-part study in subjects with ALS, conducted at 11 sites in France, Italy, the UK, and the USA, between May 2009 and September 2011. Screening took place within 28 days of the first dose of investigational product. In Part 1, escalating single doses (SD) of ozanezumab (0.01, 0.1, 1, 5, or 15 mg/kg administered intravenously [IV]), were evaluated in five sequential subject cohorts (8 subjects per cohort, randomized 3:1 to receive ozanezumab or placebo). Part 2 was also of a sequential dose-escalating design: 36 subjects across three cohorts (12 subjects per cohort) were randomized (3:1) to receive two repeat doses (RD) of ozanezumab (0.5, 2.5, or 15 mg/kg administered IV) or placebo, approximately 4 weeks apart. IV infusions were given over 60 minutes except for the 0.01 mg/kg dose, which was given over 11.2 minutes. Key safety data were reviewed by a blinded Dose Escalation Committee (comprising GlaxoSmithKline staff and an external expert neurologist who was experienced in ALS) before proceeding to the next dosing cohort. To ensure tolerability before proceeding, the first four subjects in all cohorts of Part 1 and the first cohort of Part 2 received treatment on consecutive days, so that only one subject was randomized and dosed within any 24-hour period. Dosing of all other subjects was not staggered. The follow-up period was at least 12 weeks for all subjects.

Subjects in Part 2 were followed-up for 16 weeks and subjects receiving 15 mg/kg ozanezumab were followed-up for immuno- ninogenicity for 16–20 weeks.

Ethics statement

The study protocol, protocol amendments, and informed consent were approved by a national, regional or investigational center ethics committee or an institutional review board (IRB), at each of the participating sites: Comité de Protection des Personnes Ile-de-France VI, Hôpital La Pitié-Salpêtrière, Paris, France; Comitato Etico per la Sperimentazione, Azienda Ospedaliera Universitaria Integrata di Verona, Italy; Guy's Research Ethics Committee, St. Thomas Hospital, London, UK; Carolinas Healthcare System IRB, North Carolina; Wake Forest University.
Health Sciences, IRB, North Carolina; Johns Hopkins Medicine IRBs, Maryland; Western IRB, Washington; IRB, Weill Cornell Medical Center, New York; and IRB for the Protection of Human Subjects, SUNY Upstate Medical University, New York, USA. This study was conducted in accordance with Good Clinical Practice and the guiding principles of the Declaration of Helsinki, and all subjects provided written informed consent. This study is registered at clinicaltrials.gov/(NCT00875446) and at http://www.gsk-clinicalstudyregister.com (GSK ID 111330). The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1 and Protocol S1.

Randomization and masking
Subjects in each cohort were centrally randomized across all sites via an Interactive Voice Response System. The randomization schedule was computer-generated using the validated in-house RandAll system. Infusions were prepared by a non-blinded pharmacist at the study site and infusion lines were masked in order to maintain the study blind.

Patients
Eligible subjects were male or female of non-childbearing potential, 18–80 years of age, with a diagnosis of possible, laboratory-supported probable, probable or definite familial or sporadic ALS according to The Revised El Escorial diagnostic criteria,[22] and onset of muscle weakness within 60 months of study entry. Each subject was only allowed to participate in one part of the study. Subjects were also required to have a slow inspiratory vital capacity (SVC) ≥70% of predicted (changed by protocol amendment to include those with SVC <70% at the discretion of the investigator, as long as they did not show respiratory insufficiency). Medications (including riluzole) were required to have been stable within 28 days prior to dosing. Main exclusion criteria were: neuromuscular disorders (in addition to ALS, that could have impacted the study outcomes), dementia or psychiatric illnesses, that may have affected either outcome measures or patient understanding and/or compliance with the study requirements and procedures; positive alcohol or drugs tests at screening or a history of excessive alcohol consumption; vaccination within 3 weeks of study drug administration (originally 2 months; changed by protocol amendment); exposure to a clinical trial product within 6 months or exposure to four investigational products within 2 months; changed by protocol amendment); exposure to a clinical vaccination within 3 weeks of study drug administration (originally 2 weeks); and all subjects provided written informed consent. This study is registered at clinicaltrials.gov/(NCT00875446) and at http://www.gsk-clinicalstudyregister.com (GSK ID 111330). The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1 and Protocol S1.

Endpoints and assessments
The primary endpoint was the safety and tolerability of SD or RD ozanezumab in subjects with ALS. Secondary endpoints included PK, immunogenicity, functional (clinical and electrophysiological) and biomarker analyses. Assessment timings are provided in Tables S1 and S2. Safety. Adverse events (AEs), serious AEs (SAEs), electrocardiography (ECG), vital signs, and clinical laboratory tests (hematology and biochemistry) were monitored and assessed. ECG data for all subjects were centrally analyzed and reviewed by an independent cardiologist. Evaluation of safety signals also included any adverse effects on functional endpoints (clinical and electrophysiological) or immunogenicity.

Pharmacokinetics. Evaluation of plasma ozanezumab PK was performed at various time points in both parts of the study (Tables S1 and S2). PK parameters included: maximum observed plasma concentration (C max); area under the plasma concentration-time curve up to Week 4 and infinity (AUC 0–<Week 4 and AUC 0–inf, respectively); terminal phase half-life; and clearance.

An assessment of ozanezumab concentrations was performed on skeletal muscle biopsies from subjects in Cohorts 3 and 5 from Part 1 and from all cohorts in Part 2.

Immunogenicity. Immunogenicity was assessed at various time points (Tables S1 and S2) from serum samples using an immune-electrochemiluminescent assay.

Functional endpoints. Functional endpoints (clinical and electrophysiological) were: ALS functional rating scale-revised (ALSFRS-R) score,[23] % predicted SVC,[24] manual muscle strength test (MMT),[25] and two motor unit number estimation (MUNE) endpoints (estimated number of motor units and mean single motor unit potential amplitude).[26]

Biomarkers. Exploratory biomarker analyses were performed on muscle biopsies, taken from the weaker deltoid muscle (grade 3 or 4 on the MRC scale) and plasma samples at pre- and post-dose. Ribonucleic acid (RNA) expression was assessed in muscle biopsy samples using quantitative reverse transcriptase polymerase chain reaction/whole genome microarray, while the expression of Nogo-A protein in muscle and plasma was analyzed using enzyme-linked chemiluminescence.

Frozen sections of muscle biopsies were examined by immunohistochemistry (IHC) and laser scanning cytometry (LSC). Expression of Nogo-A protein, ozanezumab, and gamma sarcoglycan was measured to quantify the co-localization of Nogo-A and ozanezumab within the muscle plasma membrane using LSC. Detailed methods are available in Methods S1 and Table S3.

Pharmacokinetic/functional endpoint relationship. Graphical exploration of a potential exposure–response relationship for ozanezumab was performed for ALSFRS-R score (monthly rate of decline) at each post-baseline visit, using average plasma ozanezumab concentration over the dosing interval as a measure of exposure.

Statistical analyses
There was no formal calculation of power or sample size for this early phase clinical trial. The sample size was based on safety and feasibility. Safety data, drug concentration data, and PK parameters were presented in tabular and/or graphical format and summarized descriptively. All statistical analyses were performed in SAS software version 9.1.3 or higher. No formal hypotheses were tested. Point estimates and corresponding 95% confidence intervals were constructed for the difference between the mean of ozanezumab and the mean of placebo, calculated as μ(ozanezumab) - μ(placebo).

A mixed effects analysis of variance model was used to assess the dose proportionality of C max, AUC 0–<Week 4, and AUC 0–inf, for SD and RD ozanezumab. Estimates of slope with respect to log (dose) together with 90% confidence intervals were used to quantify the degree of non-proportionality.

For ALSFRS-R and MMT, the slope (monthly rate of decline) was modelled using a random coefficients regression model. For % predicted SVC and both MUNE endpoints, the percentage change from screening was modeled using a mixed effects repeated measures model. Planned comparisons were made between each dose and placebo.

Results
Study population
Of the 76 subjects who were enrolled, 71 completed the study (33 on ozanezumab, 18 on placebo). Subject disposition and
Baseline characteristics are presented in Figure 1. Across all subjects, the mean age was 58 years, mean body mass index was 26.2 kg/m², and the majority of subjects were white males (Table 1). The majority of subjects had sporadic ALS (69/76; 91%) with a mean time from diagnosis of 11.1 months and a mean time from onset of muscle weakness of 19.4 months; the mean time from onset of muscle weakness varied considerably between these cohorts (Table 1). Fifty-nine (78%) subjects (35/40 and 24/36 in the SD and RD cohorts, respectively) were taking riluzole.

Safety

Overall, ozanezumab was well tolerated. Forty-seven (62%) subjects across all cohorts (35/57 [61%] on active treatment, 12/19 [63%] on placebo) reported at least one AE (Table 2). The proportion of subjects who reported ≥1 AE in the ozanezumab groups was similar when compared with the placebo group (61% and 63%, respectively) although the overall incidence of AEs in the RD 2.5 mg/kg and 15 mg/kg ozanezumab groups (78% and 89% of subjects with any AE, respectively) was higher than in the RD placebo group (56%) and the RD 0.5 mg/kg ozanezumab group (44%). Most AEs were of mild or moderate intensity as judged by the investigator. Seven (9%) subjects (5/57 [8.8%] on active treatment, 2/19 [10.5%] on placebo) reported eight AEs of severe intensity. Among the severe AEs, three subjects experienced severe headache. Two of these were in placebo groups. The third subject reported onset of headache 9 days after receiving the first dose of ozanezumab (RD 2.5 mg/kg group); this was resolved after 6 days and was not considered related to treatment. Another subject experienced severe dysphagia (RD 15 mg/kg ozanezumab), which was unresolved at the end of the study and considered not related to study drug. The remaining four severe AEs in three subjects were classified as SAEs.

Thirty-two AEs (29 mild, one moderate, one severe, and one of unknown severity), that were considered possibly related to the study medication, were reported by eight subjects (Table 2). None resulted in withdrawal from the study. Most common AEs (reported in ≥4 subjects across all cohorts) were back pain, bronchitis, fall, headache and procedural pain at biopsy site (Table 2). AEs in the cardiac disorders category were reported in four subjects. One of these, sinus tachycardia of mild intensity, was considered possibly related to investigational product (RD 2.5 mg/kg ozanezumab). Other events, not considered related to study drug, included ventricular extrasystoles (SD 5 mg/kg ozanezumab), which resolved after 2 hours, with the subject remaining asymptomatic with no findings of clinical concern on Holter monitoring; second degree atrioventricular block (RD 2.5 mg/kg ozanezumab); and cardiac arrest and cardio-respiratory arrest, as described under SAEs below.

Six SAEs were reported in three subjects receiving ozanezumab, all considered by the investigators as unrelated to study medication. One subject (SD 15 mg/kg ozanezumab) suffered a head injury after an accidental fall, which led to hospitalization and ultimately resolved. The second subject (RD 5 mg/kg ozanezumab) experienced excess bronchial secretions resulting in hospitalization, and later died from respiratory failure, 17 weeks after the dosing. The third subject (RD 2.5 mg/kg ozanezumab) was hospitalized for abdominal pain, for which the etiology remained elusive; this subject later died from cardiac arrest and cardio-respiratory arrest 10.5 weeks after the second dose.

The events common to ALS showed no patterns of reporting to suggest an adverse drug effect on the underlying condition. The most frequently reported event common to ALS was weakness, occurring in 8/19 (42%) and 19/57 (33%) subjects in the placebo and ozanezumab groups, respectively.

![Figure 1. Subject disposition flow diagram for Parts 1 and 2.](https://example.com)
<table>
<thead>
<tr>
<th>Demographics</th>
<th>Placebo SD</th>
<th>Ozanezumab SD</th>
<th>Placebo RD</th>
<th>Ozanezumab RD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo SD n=10</td>
<td>0.01 mg/kg n = 6</td>
<td>0.1 mg/kg n = 6</td>
<td>1 mg/kg n = 6</td>
<td>5 mg/kg n = 6</td>
</tr>
<tr>
<td>Age in years, mean (STDV)</td>
<td>54.7 (11.38)</td>
<td>56.8 (9.47)</td>
<td>59.2 (6.82)</td>
<td>62.2 (6.15)</td>
</tr>
<tr>
<td>Sex (male), n (%)</td>
<td>7 (70)</td>
<td>4 (67)</td>
<td>5 (83)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>BMI in kg/m², mean (STDV)</td>
<td>24.69 (2.853)</td>
<td>27.70 (4.205)</td>
<td>25.90 (1.973)</td>
<td>25.81 (5.311)</td>
</tr>
<tr>
<td>Race; n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>10 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Baseline characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial ALS, n (%)</td>
<td>1 (10)</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Sporadic ALS, n (%)</td>
<td>9 (90)</td>
<td>5 (83)</td>
<td>5 (83)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Bulbar onset ALS, n (%)</td>
<td>0</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>0</td>
</tr>
<tr>
<td>Limb onset ALS, n (%)</td>
<td>10 (100)</td>
<td>5 (83)</td>
<td>5 (83)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Non bulbar/limb onset ALS, n (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean (STDV) time since onset of muscle weakness, months [min, max]</td>
<td>17.2 (14.37) [2, 51.8]</td>
<td>17.7 (7.28) [3, 18]</td>
<td>15.0 (3.95) [3, 9]</td>
<td>18.8 (7.31) [2, 24]</td>
</tr>
<tr>
<td>Mean (STDV) time since ALS diagnosis, months</td>
<td>8.6 (4.85)</td>
<td>6.2 (2.64)</td>
<td>9.7 (9.69)</td>
<td>17.7 (21.07)</td>
</tr>
<tr>
<td>Mean (STDV) ALSFRS-R score</td>
<td>35.0 (5.60)</td>
<td>34.0 (6.13)</td>
<td>38.3 (4.08)</td>
<td>38.8 (5.42)</td>
</tr>
</tbody>
</table>

*Two doses, received 4 weeks apart.
1Remaining patient was of African American/African heritage. ALS, amyotrophic lateral sclerosis; ALSFRS-R, ALS functional rating scale-revised; BMI, body mass index; n, number of subjects; STDV, standard deviation; SD, single dose; RD, repeated dose.

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Table 2. Summary of adverse events in Part 1 (SD) and Part 2 (RD).

<table>
<thead>
<tr>
<th>Preferred term</th>
<th>Placebo SD</th>
<th>Ozanezumab SD</th>
<th>Placebo RD</th>
<th>Ozanezumab RD^**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.01 mg/kg</td>
<td>0.1 mg/kg</td>
<td>1 mg/kg</td>
<td>5 mg/kg</td>
</tr>
<tr>
<td></td>
<td>n=10</td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
</tr>
<tr>
<td>Subjects with any AE, n (%)</td>
<td>7 (70)</td>
<td>2 (33)</td>
<td>3 (50)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>Back pain</td>
<td>0</td>
<td>0</td>
<td>1 (17)</td>
<td>0</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>2 (20)</td>
<td>0</td>
<td>1 (17)</td>
<td>0</td>
</tr>
<tr>
<td>Fall</td>
<td>2 (20)</td>
<td>1 (17)</td>
<td>2 (33)</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (10)</td>
<td>0</td>
<td>1 (17)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>Procedural pain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subjects with mild AEs</td>
<td>5 (50)</td>
<td>1 (17)</td>
<td>2 (33)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Subjects with moderate AEs</td>
<td>1 (10)</td>
<td>1 (17)</td>
<td>3 (50)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Subjects with severe AEs</td>
<td>1 (10)</td>
<td>0</td>
<td>0</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Subjects with any drug-related AE, n (%)^²</td>
<td>1 (10)</td>
<td>1 (17)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number of drug-related AEs</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asthenia</td>
<td>0</td>
<td>1 (17)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Paraesthesia</td>
<td>1 (10)</td>
<td>1 (17)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Procedural pain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subjects with serious AE, n (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Number of serious AEs</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*Only those occurring in ≥4 subjects across all cohorts are listed.

^Five unreported AEs were identified (in 3 subjects): 1 subject experienced severe diarrhea (2.5 mg/kg), 1 subject experienced shoulder pain, secondary to muscle biopsy (15 mg/kg), and 1 subject experienced some minor skin bruising, a fall and a hard swelling on the left hip (resulting from the fall) (15 mg/kg).

**Two doses, received 4 weeks apart.

doi:10.1371/journal.pone.0097803.t002
Functional endpoints (clinical and electrophysiological).

Peak plasma ozanezumab concentrations were generally observed at the end of infusion. Cmax and AUC typically increased in a linear fashion, with a terminal elimination half-life of approximately 20 days (Table 3 and Figure 2).

Pharmacokinetics

In one subject RD 15 mg/kg ozanezumab antibodies were detected in any other subject. Sera from ozanezumab antibodies were detected in any other subject. There were no apparent treatment effects on ALSFRS-R slope.

% of predicted SVC percentage change from screening or MMT varied. There were no apparent treatment effects on ALSFRS-R slope. Changes in heart rate were reported for a small number of subjects but none were considered clinically significant. Two laboratory findings raised no safety concerns. See Results S1 for additional details of laboratory findings.

Analysis of ECG data showed that the proportion of subjects with QTc >450 msec or QTcF >480 msec or QTcB >500 msec was not observed in any subject at any point of the study drug. There were no apparent treatment effects on ALSFRS-R slope. Changes in heart rate were reported for a small number of subjects but none were considered clinically significant. Two laboratory findings raised no safety concerns. See Results S1 for additional details of laboratory findings.

Table 3. Ozanezumab pharmacokinetic parameters.

<table>
<thead>
<tr>
<th>Ozanezumab SD</th>
<th>Ozanezumab RD*</th>
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</thead>
<tbody>
<tr>
<td>0.01 mg/kg n = 6</td>
<td>0.1 mg/kg n = 6</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>1.00 (0.98–1.00)</td>
</tr>
<tr>
<td>C_{max} (µg/mL)</td>
<td>0.265 (17.9)</td>
</tr>
<tr>
<td>AUC_{0–Week 4} (µg.h/mL)</td>
<td>17.6 (15.4)</td>
</tr>
<tr>
<td>AUC_{0–∞} (µg.h/mL)</td>
<td>-</td>
</tr>
<tr>
<td>Clearance (mL/h)</td>
<td>-</td>
</tr>
<tr>
<td>1/2 (days)</td>
<td>19.6 (6.0)</td>
</tr>
</tbody>
</table>

*Two doses received 4 weeks apart; AUC_{0–Week 4}, area under the plasma concentration-time curve up to Week 4; AUC_{0–∞}, area under the plasma concentration-time curve up to infinity; C_{max}, maximum observed plasma concentration; n, number of subjects; RD, repeated dose; SD, single dose; 1/2, apparent terminal phase half-life; T_{max}, time at which C_{max} was observed. T_{max} presented as median (range); all other values presented as geometric mean of the log-transformed data (coefficient of variation, CV%).

doi:10.1371/journal.pone.0097813.t003
placebo. However, a numerical difference in favor of ozanezumab versus placebo was observed in the RD 15 mg/kg group for each endpoint (Tables S5 and S6).

In MUNE data analysis, no trends between ozanezumab groups and placebo were observed (Table S6).

Biomarkers

There was no evidence of a pharmacological response with ozanezumab treatment on protein or RNA biomarkers. Measurement of Nogo-A and ozanezumab using IHC staining of muscle biopsies suggested co-localization of the drug at the site of action in skeletal muscle. Co-localization followed a similar trend to ozanezumab levels in muscle, suggesting that this was related to exposure. Greater than 90% co-localization was observed with the 15 mg/kg dose, 8 days after dosing, with levels dropping below 90% at 3–4 weeks post-dose (Figure 3).

Pharmacokinetic/functional endpoint relationship

Following graphical exploration of a potential exposure-response relationship of ozanezumab for ALSFRS-R score (monthly rate of decline) at each post-baseline visit, no PK/functional endpoint relationship was identified.

Discussion

The effort to develop new treatments for ALS has led to repeated failure since the demonstration that riluzole extended survival.[3] Despite initially encouraging results from a Phase II trial, which suggested beneficial effects on ALSFRS-R and survival,[27] the recent negative results of the dexpramipexole Phase III study is yet another disappointing example.[28] Thus, there is still an urgent need for new treatments for ALS. A number of compounds targeting different aspects of ALS pathogenesis are
Figure 3. Co-localization of membrane Nogo-A with ozanezumab in skeletal muscle of individual subjects. A. Triplicate readings are provided from biopsies in Cohort 7 and 8 (single reading from Cohort 5) dose 2 +D22–26, biopsy taken 22–26 days after the second dose; dose 1 +24H, biopsy taken 24 hours after first dose. B. Nogo-A (red), ozanezumab (green) and co-localization (yellow), in muscle biopsy, 24 hours post-dose. doi:10.1371/journal.pone.0097803.g003
The lack of emerging safety signals, support future studies of skeletal muscle, are encouraging; these observations, along with antibodies. The trends observed on functional endpoints in the current study may have been too short to detect such responses. In this, and despite subjects receiving only one or two doses each, a trend was observed for clinical endpoints such as ALSFRS-R, SVC, and MMT, which possibly suggested a response in the highest dose cohorts. Trends observed should be interpreted with great caution given the small sample size, and other studies will be required to confirm and further investigate these trends. The relationship between ozanezumab administration and pharmacological signal on protein or RNA requires further investigation; the current study may have been too short to detect such responses. In future studies, optimum time points, treatment times, and analysis methods will need to be established.

IHC staining of muscle biopsies suggested that there were dose-dependent changes in ozanezumab quantification and detection and that ozanezumab distributed and co-localized with Nogo-A at the site of action in skeletal muscle. Following the full distribution of ozanezumab to muscle tissue and peak co-localization approximately 1 week after dosing, the percentage of co-localisation appeared to be related to levels of ozanezumab, suggesting a possible relationship with exposure. Overall, ozanezumab was well tolerated and PK parameters were generally consistent with those of humanized monoclonal antibodies. The trends observed on functional endpoints in the present study, along with the co-localization of ozanezumab in skeletal muscle, are encouraging; these observations, along with the lack of emerging safety signals, support future studies of ozanezumab in this devastating disease, and a Phase II study of efficacy and safety of ozanezumab (NCT01753076) is currently underway.

Supporting Information

Table S1 Assessment schedules for Part 1. AE, adverse event; ALSFRS-R, amyotrophic lateral sclerosis functional rating scale-revised; ECG, electrocardiogram; FU, follow-up; MUNE, motor unit number estimation; PK, pharmacokinetic; SAE, serious adverse event. *The precise timing of safety, functional assessments, and PK blood sampling may have been altered during the course of the study based on emerging data. If the profile indicated that more sampling or assessments were needed, additional time points were to be added. Study assessments to follow PK sampling at end of infusion. (The 1-hour PK sample was collected directly at the end of the infusion, Cohorts 2–8). †Only SAEs related to study participation were collected prior to the start of the investigational product. Once the investigational product infusion began, all AEs and SAEs were collected until the last FU visit. ‡Continuous Lead II ECG commenced approximately 1 hour pre-dose on Day 1 until 24 hours post-dose. ‡Muscle biopsies, which were voluntary collections in Cohort 3 (1 mg/kg) and required collections in Cohort 5 (15 mg/kg) were collected pre-dose and at Week 4. The pre-dose muscle biopsy was only to be done when the subject had passed all screening assessments and eligibility had been reconfirmed. This meant the pre-dose biopsy could be done at any appropriate time before Day 1. Blood samples at pre-dose and at Week 4 were collected regardless of whether or not a muscle biopsy was to be taken. The number and schedule of FU visits after Week 12 for each subject was to vary depending on plasma concentrations of ozanezumab reaching a low enough level to allow a final blood sample to have been assayed for immunogenicity.

Table S2 Assessment schedules for Part 2. AE, adverse event; ALSFRS-R, amyotrophic lateral sclerosis functional rating scale-revised; ECG, electrocardiogram; FU, follow-up; MUNE, motor unit number estimation; PK, pharmacokinetic; SAE, serious adverse event. *The precise timing of safety, functional assessments and PK blood sampling may have been altered during the course of the study based on emerging data. If the profile indicated that more sampling or assessments were needed, additional time points were to be added. Study assessments to follow PK sampling at end of infusion. (The 1-hour PK sample was collected directly at the end of the infusion, Cohorts 2–8). †Only SAEs related to study participation were collected prior to the start of the investigational product. Once the investigational product infusion began, all AEs and SAEs were collected until the last FU visit. ‡Continuous Lead II ECG commenced approximately 1 hour pre-dose until 24 hours post-Dose 1 and for 6 hours post-Dose 2. In cohorts 6 and 7 the pre-dose muscle biopsy and blood sample were only done when the subject had passed all screening assessments and eligibility had been reconfirmed. This meant the pre-dose biopsy and blood sample could be done at any appropriate time before Day 1. The post-dose muscle biopsy and blood sample were scheduled for collection at Week 8 (unless emerging data suggested the post-dose muscle biopsy and blood sample should have been collected at an alternative week). In cohort 8, muscle biopsies and blood sample were collected from subjects at pre-dose and at one time point after the first dose.
Subjects were assigned for a post-dose muscle biopsy and blood sample collection at either Day 1 (+24 hours), Day 6 or Week 4 (Day 22–24) based on subject preference determined at screening (see Section 7.3). If the subject had the Day 1 (+24 hours) collection then the pre-dose muscle biopsy and blood sample were collected at least 8 days before Day 1. The number and schedule of FU visits after Week 16 for each subject varied depending on plasma concentrations of ozanezumab reaching a low enough level to allow a final blood sample to have been taken for immunogenicity assays.

Table S3 Primer and probe sets used in biomarker analyses.

Table S4 Summary of QTc values of potential clinical importance at any visit post-baseline. n, number of subjects; SD, single dose; RD, repeated dose. Two doses, received 4 weeks apart.

Table S5 Summary of ALSFRS-R and MMT analyses. n, number of evaluable subjects; RD, repeat dose; SD, single dose; SE, standard error. ALSFRS-R, ALS functional rating scale-revised; CI, confidence interval; MMT, manual muscle strength test; Measured at Week 12 for SD study, Week 16 for RD study. Two doses, received 4 weeks apart.

Table S6 Summary of SVC and MUNE analyses. CI, confidence interval; MUNE, motor unit number estimation; n, number of evaluable subjects; RD, repeat dose; SD, single dose; SE, standard error; SMUP, single motor unit potential; SVC, slow inspiratory vital capacity. Measured at Week 12 for SD study, Week 16 for RD study. Two doses, received 4 weeks apart.

References


Methods S1

Results S1

Checklist S1 CONSORT checklist.

Protocol S1 Redacted protocol.

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Author Contributions

Conceived and designed the experiments: VM P-FP JP SB NW AC JW BRB. Performed the experiments: VM P-FP AC GP BRB JBC DL TM SM KEM PNL. Contributed reagents/materials/analysis tools: NW JB AB DK SA-S. Wrote the paper: P-FP AL JP PB JDR BRB JBC SJK PNL DK. Performed data analysis and interpretation: VM P-FP AL PO SB NW JW BRB SJK AB. Undertook data collection: VM P-FP AC GP BRB JBC DL TM SM KEM RWO. Undertook ongoing medical monitoring during the study: JW BRB. Responsible for recruitment of patients: P-FP BRB KEM. Undertook a literature search: BA. Undertook data cleaning: NW. Provided pharmacokinetic data analysis and interpretation: JB AB. Provided immunohistochemistry and laser scanning cytometry analysis: DK. Provided histological examination and histochemical and immunohistochemistry assessment of muscle biopsies: SA-S.

Additional files

Table S6 Summary of SVC and MUNE analyses.

Table S5 Summary of ALSFRS-R and MMT analyses.

Table S4 Summary of QTc values of potential clinical importance at any visit post-baseline.

Appendix S1 Redacted appendix.

Figure S1 CONSORT flow diagram.

Figure S2 CONSORT checklist.

Figure S3 CONSORT flow diagram.

Figure S4 CONSORT checklist.

Figure S5 CONSORT flow diagram.

Figure S6 CONSORT checklist.

Figure S7 CONSORT flow diagram.

Figure S8 CONSORT checklist.

Figure S9 CONSORT flow diagram.

Figure S10 CONSORT checklist.

Figure S11 CONSORT flow diagram.

Figure S12 CONSORT checklist.

Figure S13 CONSORT flow diagram.

Figure S14 CONSORT checklist.

Figure S15 CONSORT flow diagram.

Figure S16 CONSORT checklist.

Figure S17 CONSORT flow diagram.

Figure S18 CONSORT checklist.

Figure S19 CONSORT flow diagram.


