**Safety and efficacy of ozanezumab in patients with amyotrophic lateral sclerosis: a randomised placebo-controlled phase 2 trial**

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**RESEARCH IN CONTEXT**

**Evidence before this study**

We searched PubMed on Feb 3, 2016, with the terms “(nogo‐a OR RTN4 OR "neurite outgrowth inhibitor") AND (ALS OR motor neurone disease)” and no language or date restrictions. Evidence from mouse models of amyotrophic lateral sclerosis (ALS), together with molecular analysis of skeletal muscle from patients with ALS suggested a role for the neurite outgrowth inhibitor protein, Nogo-A, in the pathophysiology of ALS. Results from a first-in-human clinical study showed that ozanezumab, a humanised monoclonal antibody against Nogo-A, was well tolerated in patients with ALS. We did not identify any randomised, controlled trials of efficacy of ozanezumab or other drugs with this mechanism of action.

**Added value of this study**

This study was the first randomised placebo-controlled clinical trial designed to assess the safety and efficacy of an anti-Nogo-A monoclonal antibody for the treatment of patients with ALS. This study did not show any evidence of efficacy of ozanezumab over placebo. The desired level of exposure to ozanezumab was achieved; therefore, the absence of efficacy was not thought to be related to suboptimal dosing. The results of this study suggest the futility of further clinical testing of an anti-Nogo-A monoclonal antibody for the treatment of ALS.

**Implications of all the available evidence**

Currently, only one approved medication, riluzole, has been shown to have a slight effect on survival of patients with ALS. Thus, there remains a high unmet need to identify new options for the treatment of patients with ALS.

**Summary**

**Background**: Nogo-A is a neurite outgrowth inhibitor protein that is thought to have a role in the pathophysiology of amyotrophic lateral sclerosis (ALS). A monoclonal antibody against Nogo-A showed a positive effect in the *SOD1*G93A mouse model of ALS, and a humanised form of this antibody (ozanezumab) was well tolerated in a first-in-human trial. We therefore assessed the safety and efficacy of ozanezumab in patients with ALS.

**Methods**: In a phase 2, double-blind study, patients with ALS were randomised in a 1:1 ratio with a computer-generated allocation schedule to receive ozanezumab (15 mg/kg) or placebo as intravenous infusions every 2 weeks for 46 weeks, followed by a week 48 assessment and a 12-week follow-up. Patients and study personnel were blinded to treatment assignment. The primary endpoint was a joint-rank analysis of function (ALSFRS-R) and survival. Analysis was by modified intent to treat. **Trial Registration**: ClinicalTrials.gov NCT01753076 GSK-ClinicalStudyRegister.com GSK ID 1223249

**Findings**: The first patient was enrolled into the study on Dec 20, 2012, and the last patient visit was on Jan 22, 2015. 303 patients were randomly assigned to the placebo group (n=151) or ozanezumab (n=152). The joint-rank score indicated a non-significant difference in favour of placebo (adjusted placebo mean 15·0[SE 13·58] *vs* ozanezumab mean –14·9 [SE 13·54], with least squares mean difference –30·0 [95% CI –67·9 to 7·9]; p=0·120). The incidences of dyspepsia (7% *vs* 3%), depression (7% *vs* 3%), and diarrhoea (16% *vs* 8%) in the ozanezumab group were almost twice those in the placebo group. A numerically higher incidence of fatal SAEs was observed with ozanezumab versus placebo (18 [12%] *vs* 13 [9%] deaths), driven mainly by respiratory failure events (ten [7%] *vs* five [3%]). Respiratory failure was the most common SAE, reported in 12 (8%) and seven (5%) patients in the ozanezumab and placebo arms, respectively.

**Interpretation**: Ozanezumab did not demonstrate efficacy compared with placebo in patients with ALS. Therefore, Nogo-A does not seem to be an effective therapeutic target in ALS.

**Funding**: GlaxoSmithKline.

**INTRODUCTION**

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterised by progressive degeneration of motor neurons in the brain and spinal cord.1 In most cases, ALS progressively involves muscles, leading to weakness and ultimately death, typically due to respiratory failure.2 Most patients die within 5 years after symptom onset.3 The pathophysiological mechanisms remain unconfirmed, but important pathogenic changes are considered to involve interference with normal protein degradation and defects in RNA processing.1 These changes lead to progressive cellular failure, disruption of axonal architecture and function and axonal retraction, and ultimately to denervation of neurons or muscles.1 The processes of axonal retraction and denervation might be further modulated by axonal attraction and repellent systems, which are responsible for the development and stabilization of the neuronal network.1 Oxidative stress, glutamate toxicity, mitochondrial dysfunction, autophagic dysfunction, and immune-inflammatory responses have also been implicated in the pathogenesis of ALS.2

Only one approved medication, riluzole, has an effect on survival of patients with ALS,2 but this effect remains small. Data from randomised, controlled trials suggest that riluzole extends survival by 2–3 months (median), whereas results from uncontrolled registry studies have suggested prolongation of survival by up to 21 months.4 Despite encouraging results from preclinical studies and many clinical trials, no other treatments have shown any effect on the disease course.5 Apart from riluzole, multidisciplinary palliative care remains the main management approach for ALS.2 The progressive course, fatal outcome, and lack of effective treatments present a high unmet medical need in ALS.

Neurite outgrowth inhibitor A (Nogo-A) is a high-molecular-weight transmembrane protein, initially identified as a potent myelin-associated inhibitor of axonal growth expressed mostly by oligodendrocytes, that has been suggested to have a role in the pathophysiology of ALS.6,7 Nogo-A is expressed at very low levels in healthy skeletal muscle, but is upregulated in the skeletal muscle of patients with ALS,6 where its expression seems to be associated with disease severity.8 The link between Nogo-A and ALS was strengthened by the finding that Nogo-A was associated with neuromuscular junction denervation and associated with faster functional decline in patients with ALS.9 Nogo-A expression is also upregulated in the skeletal muscle of the superoxide dismutase 1 (*SOD1*) transgenic mutant mouse, a widely used model for ALS.10 Exogenous overexpression of Nogo-A in the skeletal muscle of wild-type mice leads to denervation and instability of the neuromuscular junction,11 whereas deletion of the Nogo-A gene in *SOD1*G86R mice resulted in a moderate but statistically significant increase in lifespan and was associated with a neuroprotective effect.11 These findings suggested that Nogo-A expression in skeletal muscle could contribute to the pathology of ALS and that Nogo-A represented a potential novel therapeutic target for ALS.

Ozanezumab (GSK1223249) is a humanised monoclonal antibody against Nogo-A.12 In the first-in-man study, ozanezumab was well tolerated and although the study was not designed to assess efficacy, results for functional endpoints were numerically in favour of ozanezumab at the highest dose (two doses of 15 mg/kg given approximately 2 weeks apart.12

The aim in this phase 2 trial was to assess the effect of ozanezumab on the function and survival of patients with ALS.

**METHODS**

**Study design**

This was a randomised, placebo-controlled, parallel-group, double-blind repeat-dose phase 2 study in patients with ALS, done in 34 centres across 11 countries (Australia, Belgium, Canada, France, Germany, Italy, Japan, Korea, the Netherlands, the UK, and the USA). The protocol was amendedonce, before data analysis, on May 29, 2013 (documented and approved by the relevant ethics committees), when two substantial changes were made: the statistics section was changed to reflect refinements made to power calculations, and an additional secondary analysis of ALS Functional Rating Scale-Revised (ALSFRS-R) data to aid clinical interpretation of the results.13 At the time of protocol amendment, safety data from part A of the study had been reviewed and recruitment for part B was underway (see ‘Procedures’). The protocol for this trial is available at http://www.gsk-clinicalstudyregister.com/study/112264#ps.

The study was done and monitored in accordance with Good Clinical Practice and the guiding principles of the Declaration of Helsinki 2008.

**Participants**

Male and female patients aged 18–80 years with a diagnosis of familial or sporadic ALS (defined as meeting the possible, laboratory-supported probable, probable, or definite criteria for a diagnosis of ALS according to the revised World Federation of Neurology El Escorial criteria14) with onset of muscle weakness ≤30 months before the screening visit, and with slow vital capacity (SVC) of ≥65% at screening (predicted for gender, age, ethnicity, and height) were eligible for inclusion. Full study inclusion and exclusion criteria are listed in the Supplementary Materials. Eligible patients were identified in the clinic at each of the study sites and were recruited into the study according to the protocol. All patients provided written informed consent.

**Randomisation and masking**

After an initial screening period, patients were enrolled by study investigators and randomised 1:1 to receive ozanezumab or placebo at the second study visit (week 0). Patients were randomised centrally across all sites in accordance with a computer-generated, GSK-validated randomisation schedule. Anonymised patient numbers were provided to investigators via an Interactive Voice Response System. All investigators were blinded to the treatment allocation; the investigators took part in the other study procedures with the exception of the study treatment preparation. Infusions were prepared by a non-blinded pharmacist at the study site, and infusion bags were masked using orange tape adhered to the infusion bag and drip chamber. Patients and all study personnel administering the interventions, assessing outcomes, and analysing data were masked to treatment assignment.

**Procedures**

An ozanezumab dose regimen of 15 mg/kg once every 2 weeks was selected for this study based on analysis of drug biodistribution into muscle, co-localisation of Nogo-A at the target site (muscle cell membrane), plasma pharmacokinetic data, and the safety profile from clinical and non-clinical studies.12, 15–16 Patients received ozanezumab or placebo as intravenous infusions over 1 h. A total of 24 infusions were planned, starting at the baseline (week 0) visit and then every 2 weeks up to the last dose at week 46, followed by a week 48 assessment and 12-week follow-up. As the dosing regimen used in this study had not been tested in humans previously, a two-part study design (parts A and B) was used. In part A, an initial set of patients had more intensive safety monitoring during their first four antibody infusions than did patients in part B (see Supplementary Materials for more information).

**Outcomes**

The primary endpoint was a joint-rank analysis of function (ALSFRS-R) and 48-week survival. The ALSFRS-R questionnaire was administered at clinic visits but, for subjects not able to attend the clinic, it could also be administered by telephone, thus reducing risk of missing data and allowing collection from patients withdrawn from study treatment but not withdrawing consent.

Secondary endpoints included: change from baseline in ALSFRS-R total score at week 48, rate of decline in ALSFRS-R total score at week 48; progression-free survival (where progression is defined as at least a six-unit decrease on ALSFRS-R) at week 48, overall survival at week 60, Clinical Global Impression-Improvement Scale responders at week 48, respiratory function (SVC) at week 48; muscle power (measured by hand held dynamometry, HHD) at week 48; and overall survival at week 48.

Further secondary outcomes included: health outcomes measured up to week 48 based on the EuroQol-Short Form 5-level version [EQ-5D-5L], the Amyotrophic Lateral Sclerosis Assessment Questionnaire [ALSAQ-40], safety, immunogenicity, and pharmacokinetics (PK) of ozanezumab and riluzole (to assess any PK interaction with ozanezumab). Health resource utilisation and treatment satisfaction were exploratory outcomes. The key safety assessments were the monitoring of serious adverse events (SAEs), AEs, disease-related events (DREs), clinical laboratory tests, vital signs, and electrocardiograms (ECGs). AEs and DREs were coded using the Medical Dictionary for Regulatory Activities coding system.

Patients who were withdrawn or who withdrew voluntarily from the study medication, but who did not withdraw consent to continue in the study, were encouraged to continue to provide ALSFRS-R and safety data to week 48 via telephone contact, and mortality was checked up to week 60. They were also requested to return for a follow-up immunogenicity visit approximately 14 weeks after the last infusion.

**Statistical analysis**

The joint-rank analysis of function (ALSFRS-R) and 48-week survival was determined as follows: Briefly, each patient was assigned a summary score based on pairwise comparisons against all other patients in the study at week 48 (across both treatment groups). For each comparison, the patient scored +1 if they had a better outcome (higher functional score at the last common visit, or longer survival), 0 if there was no difference in outcome, or –1 if they had a worse outcome (lower functional score at the last common visit or shorter survival). Each patient's summary score was calculated based on the sum of each individual score. The mean total score of patients receiving ozanezumab was compared with that for patients receiving placebo. This analysis differs from the combined analysis of function and survival (CAFS), as used in the recent EMPOWER study17 in ALS (published after the current study had commenced), in which the joint rank score was ranked and then the mean rank was compared between groups.17,18

A sample size of 147 patients per arm was estimated to provide approximately 86% power to detect a significant statistical difference in the primary endpoint between treatment arms using a two-sided alpha of 5%, with 80% power to detect a 30% improvement in the rate of decline in ALSFRS-R and 31% power to detect a 5% improvement in survival. These estimates are based on the following assumptions: the mean weekly rate of decline in ALSFRS-R with placebo is 0·235 and with ozanezumab is 0·165; the weekly rate of decline in ALSFRS-R has a variance of 0·044 in both the placebo and ozanezumab arms; the within-subject change from baseline variance is 4; overall survival follows an exponential distribution in both arms, the 48-week mortality with placebo is 10%, and the absolute reduction in mortality with ozanezumab is 5%; the dropout rate (not due to death) of the ALSFRS-R is 20% in both arms; and the correlation between ALSFRS-R and overall survival for each subject is zero. Refinements made to power calculations as part of the protocol amendment indicated a slightly increased power (although the power was still within the range of 80%–90%) and no change in the sample size.

The modified intent-to-treat (ITT) population, used for efficacy analyses, comprised all randomised patients who received at least one dose of study drug. The safety population, used for the analysis of safety data, comprised all randomised patients who received at least one dose of study drug. The PK ozanezumab concentration population consisted of all patients in the modified ITT population who received at least one dose of ozanezumab, and from whom at least one plasma sample was analysed for ozanezumab. The PK riluzole concentration population consisted of all patients in the ITT population who took at least one dose of riluzole during the study, and from whom at least one plasma sample was analysed for riluzole.

Analysis of the primary endpoint used on-treatment data (defined as data collected up to 21 days after the patient's last infusion) for the ITT population. Additional, separate pre-specified analyses of joint-rank scores, ALSFRS-R data, and survival data were conducted, including retrieved follow-up data (where retrieved follow-up data are defined as data collected more than 21 days after the patient's last infusion, typically by telephone). Data were adjusted for baseline ALSFRS-R total score, riluzole use, country group, and treatment. AEs, SAEs, ECG, vital signs, and clinical laboratory evaluations (haematology and biochemistry) were monitored and assessed. Values for clinical laboratory evaluations were compared with both the appropriate normal ranges and ranges of potential clinical concern. Any abnormal test result or other safety assessment judged by the investigator to be clinically significant was recorded as an AE or SAE. Other safety measures included: primary cause of death; use of respiratory support (invasive or non-invasive); occurrence of tracheostomies and gastrostomies; assessment of suicidal ideation and behaviour (measured by the Columbia-Suicide Severity Rating Scale) and possible suicidality-related AEs; neurological examination; and immunogenicity.

An independent data-monitoring committee (IDMC) reviewed unblinded safety data, including AEs, laboratory results, ECGs, and other safety assessments at regular intervals throughout the study.

This study is registered at clinicaltrials.gov/ (NCT01753076) and at http://www.gsk-clinicalstudyregister.com (GSK ID 112264).

**Role of the funding source**

The funder’s role included study concept and design, funding of participating centres, analysis of data, and development of the final report and manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**RESULTS**

The first patient was enrolled into the study on Dec 20, 2012, and the last patient visit was on Jan 22, 2015. A total of 303 patients were randomised and received study drug—these patients constituted both the ITT and safety populations. Overall, 216 patients completed the study (defined as completion of the follow-up visit 14 weeks after the last dose, and provision of data for ≥60 weeks after baseline). A summary of patient disposition is presented in **Figure 1**. Patient demographics and baseline characteristics were well balanced between the two treatment arms (**Table 1**).

Analysis of the primary endpoint indicated a non-significant difference in joint-rank score in favour of placebo, with a difference in least squares means (ozanezumab minus placebo) of –30·0 (95% CI –67·9 to 7·9; p=0·120) (**Table 2**).

All secondary efficacy endpoints supported the primary endpoint, showing a small, non-significant difference in favour of placebo (**Table 2, Table S1**).The adjusted mean difference between ozanezumab and placebo in change from baseline in ALSFRS-R total score at week 48 was –1·3 (95% CI: –3·1 to 0·4; p=0·139; **Figure 2, Table 2**).

Survival endpoints were not significantly different between treatment arms. Week 48 survival (on-treatment data) is shown in **Figure 3**.At week 48, six deaths (4%) had occurred in the placebo group and eight deaths (5%) had occurred in the ozanezumab group (on-treatment period). Inclusion of retrieved follow-up data into week 48 survival analysis showed nine deaths (6%) on placebo and 15 deaths (10%) on ozanezumab with a hazard ratio (HR, 95% CI) of 1·30 (0·56 to 3·01; p=0·541). The progression-free survival at week 48 was 30·8% (95% CI: 23·0 to 38·6) in the placebo group and 28·5% (95% CI: 21·1 to 35·9) in the ozanezumab group with a HR (95% CI) of 1·07 (0·81 to 1·42; p=0·642). Analysis of survival at Week 60 showed 16 deaths (11%) in the placebo group and 20 deaths (13%) in the ozanezumab group with a HR (95% CI) of 1·03 (0·53 to 2·01; p=0·923; **Table S2).** As the proportionality assumptions are questionable for the Cox proportional hazard model, an ad hoc analysis using the Kolmogorov-Smirnov test was carried out, p-values are presented in **Table S3** and do not alter the interpretation of the results. There was no difference between placebo and ozanezumab for any of the health outcomes assessed (**Table S4**).

Plasma ozanezumab concentrations were consistent with pre-study predictions, in that concentrations increased steadily with each dose and steady-state was reached by Week 12 (dose 7). There was no evidence of a change in elimination or clearance (**Figure S1**). Plasma riluzole concentrations generally remained consistent with baseline values over the course of the study, with no evidence of a change in elimination or clearance (**Table S5**).

24 patients out of the total 303 were included in part A, of whom 12 received ozanezumab and 12 received placebo. After review of safety data from part A by the IDMC, no significant safety concerns were identified, and recruitment into part B (remaining study population) continued as planned.

Overall, reported AEs, SAEs, and AEs leading to permanent discontinuation of study drug or withdrawal from study were similar between the treatment groups (**Table 3**).A total of 36 deaths were reported. Five deaths (two in the ozanezumab group and three in the placebo group) occurred outside the timeframe specified for SAE collection. No associated SAEs were reported for these cases, and all five were judged by investigators to be due to ALS. 31 deaths occurred during the treatment or follow-up (18 [12%] in the ozanezumab group and 13 [9%] in the placebo group). Two deaths were considered related to the study medication (one each in the ozanezumab [bladder transitional cell carcinoma] and placebo arms [cerebrovascular accident]). The higher number of deaths in the ozanezumab group than in the placebo group was attributable to a higher incidence of respiratory failure events in the ozanezumab group (ten [7%] in the ozanezumab group vs five [3%] in the placebo group, **Table S6**). 46 (30%) patients in the placebo arm experienced an SAE compared with 47 (31%) in the ozanezumab arm(**Table 3**). Respiratory failure was the most common SAE reported, in 12 (8%) and seven (5%) patients in the ozanezumab and placebo arms, respectively. There were three cases of drug-related non-fatal SAEs reported in the ozanezumab group (anaemia, appendicitis, and pulmonary embolism) and two cases (unilateral blindness and thrombosis) in the placebo group. The most common AEs (reported by >10% of the overall population) were falls (125 [41%] overall), nasopharyngitis (67 [22%] overall), headache (55 [18%] overall), cough (37 [12%] overall), diarrhoea (37 [12%] overall), and constipation (36 [12%] overall) (**Figure S2**). The incidence of some AEs was roughly double in the ozanezumab arm that in the placebo arm, including dyspepsia (10 [7%] in ozanezumab arm vs four [3%] in placebo arm), depression (11 [7%] vs five [3%]), and diarrhoea (25 [16%] vs 12 [8%]). No SAEs related to depression were reported in either group.

No clinically significant safety findings for clinical laboratory parameters, vital signs, or ECG results (including corrected QT interval) were observed following dosing with ozanezumab. 111 (74%) patients in the placebo arm and 123 (81%) patients in the ozanezumab arm reported DREs. The difference in the incidence of DREs between arms was largely due to differences in the incidence of respiratory, thoracic, and mediastinal disorders (60 [39%] patients in the ozanezumab arm vs 44 [29%] in the placebo arm). There was a slightly higher incidence of psychiatric disorders reported in the ozanezumab arm (23 [15%] of patients vs 17 [11%] in the placebo arm), mainly due to differences in the frequency of insomnia (six [4%] in the ozanezumab arm vs one [<1%] in the placebo arm). 12 (8%) patients in the placebo arm and 20 (13%) patients in the ozanezumab arm reported DREs of weight loss. A higher incidence of possible suicidality-related AEs was observed in the ozanezumab arm (four patients, 3%) compared with the placebo arm (one patient, <1%).

15 (10%) patients in the ozanezumab group tested positive for anti-drug antibodies (ADA) post-baseline. One of these patients tested positive for neutralising ADA, but there was no evidence of AEs related to immunogenicity (eg, hypersensitivity, rash) or any impact on efficacy or PK in this patient.

**DISCUSSION**

In this phase 2 study, ozanezumab did not show any evidence of efficacy—instead, the primary and all secondary efficacy endpoints showed small, non-significant differences in favour of placebo. However, ozanezumab was generally well tolerated; rates of AEs, SAEs, and AEs leading to permanent discontinuation of study drug or withdrawal from the study were similar in the ozanezumab and placebo arms.

These findings are somewhat surprising because the results of previous studies had suggested a link between Nogo-A and ALS, illustrated by the upregulation of Nogo-A in the skeletal muscle of patients with ALS and its relation with disease severity.8,9,19 Although Nogo-A overexpression was also observed in skeletal muscle in other neuromuscular diseases, leading to suggestions that this might be a non-specific marker of denervation,20, 21 in patients with pure lower motor neuron syndrome, Nogo-A expression in skeletal muscle tissue predicted conversion to ALS with 91% accuracy, 94% sensitivity, and 88% specificity.19 In ALS, it has been suggested that neuromuscular junction destabilisation and neurite retraction might precede degeneration of spinal motor neurons (the “dying-back phenomenon”).22 Studies in mice supported a potential role for Nogo-A in this process. In the *SOD1*G86R mouse model of ALS, Nogo-A expression was upregulated in skeletal muscle before the onset of the phenotypic manifestations and was associated with an increase in markers of denervation, whereas genetic ablation of Nogo-A attenuated this denervation and extended the survival of the mice.11 In wild-type mice, ectopic overexpression of Nogo-A in the skeletal muscle was associated with degeneration of the neuromuscular junction and retraction of the nerve terminal.11 These findings led to the therapeutic hypothesis that blockade of Nogo-A signalling could prevent motor neuron loss in ALS.9,11 In the preclinical development programme, ozanezumab resulted in a dose-dependent decrease or reversal of neurite outgrowth inhibition in a rat post-natal cerebellar granular neuron culture.16 Furthermore, the murine parent antibody of ozanezumab had a positive effect in the *SOD1*G93A mutant transgenic mouse model of ALS, where it improved spinal motor neuron and motor unit survival and increased skeletal muscle force10 and statistically significantly delayed the time to symptom onset (assessed as magnitude of motor deficit compared with vehicle controls) and time to death (unpublished). In these studies, antibody treatment at an early symptomatic stage (70 days after birth) led to statistically significant functional benefits and a slight reduction in markers of muscle denervation at the late symptomatic stage of 90 days, although many of these differences were not maintained by day 120.10 In a subsequent first-in-human study, ozanezumab was well tolerated and although the study was not designed to assess efficacy, results for functional endpoints (such as ALSFRS-R and SVC) and manual muscle testing were numerically in favour of ozanezumab at the highest dose of 15 mg/kg.5 Taken collectively, the results of these studies were deemed sufficient to move to a phase 2 efficacy study.

The results of the current study, although negative, are robust and important. The study population was representative of patients with mild to moderate ALS, with concomitant medical disorders that were as expected for this population. Demographic and baseline disease characteristics were similar between the two treatment groups, and the functional decline observed with placebo was similar to that reported in other clinical studies of ALS.13 A further strength is that the study had an IDMC in place for the periodic review of safety and efficacy data, and was done in accordance with a two-part design to protect patient safety because the dosing regimen used had not been tested in humans previously.

Based on modelling done with preclinical and clinical data from previous studies, the dose regimen used in this study was predicted to achieve >90% co-localisation of ozanezumab with Nogo-A, which was anticipated to achieve a relevant pharmacodynamics effect.15 Plasma concentrations of ozanezumab observed during the study confirm that the targeted level of exposure to ozanezumab was achieved and maintained over the duration of dosing, suggesting that dosing was optimum. Furthermore, riluzole concentrations were consistent with those reported previously,,23,24 remained consistent over the duration of ozanezumab dosing, and were generally similar for both treatment groups, suggesting that there was no PK interaction between ozanezumab and riluzole.

The primary endpoint was a combined analysis of the two key aspects of ALS progression—namely, functional decline and survival, which is intended to address the limitations of these endpoints when used individually. Survival as an endpoint is robust and reliably determined, but potentially less sensitive than functional endpoints and so would require studies with longer duration or larger sample sizes.25 However, analysis of functional endpoints can be confounded by missing data due to deaths during the treatment period. The analysis of the combined survival and functional endpoint used in this study is based on methodology described by Finkelstein and Schoenfeld,25 and can be considered as an analysis of the ALSFRS-R with an adjustment for missing data due to mortality.18 Combined analysis overcomes problems with missing functional data owing to death or study dropouts that are not adequately addressed using standard techniques for the analysis of function.18

One limitation of this combined endpoint is that it is difficult to interpret clinically, and analyses of the data for function and survival components are required to understand the specific clinical effects of the study drug. Consequently, the component data were analysed individually as secondary endpoints. Another limitation of the current study is the absence of a pharmacodynamic marker to confirm engagement of ozanezumab at the target.

The lack of efficacy in this study contrasts with effects observed with the murine parent antibody of ozanezumab in the *SOD1* mutant mouse model of ALS.10 Although this is a commonly used animal model of ALS, no clinical translation has yet been demonstrated.26,27 Riluzole showed mixed results in this model, although this was subsequent to demonstration of efficacy in clinical studies.26,28 The main advantages of the *SOD1* mouse model are its pathological and phenotypic similarities with human ALS, its well-established endpoints, and the existing guidelines on experimental design and methods.29,30 The major limitation of the model relates to the inherent differences between the mouse model and human disease.1,29 For example, disease onset and progression are typically more heterogeneous and less aggressive in human ALS than in the *SOD1* mouse model.1,29 Furthermore, mutations in the *SOD1* gene account for a few cases and do not have the TDP-43 pathology associated with most forms of ALS.29,30 Highly penetrant human *SOD1* mutations (eg, A4V) do not induce the disease phenotype in the mouse model.29 Finally, unlike ALS clinical trials, treatment in many *SOD1* mouse experiments is often administered before symptom onset.1,29 Although preclinical models such as the *SOD1* mouse model can provide valuable information on the pharmacology of a novel investigational drug, findings have not translated to the clinical setting so far.26,27 One possible approach to address this could be, with appropriate justification by preclinical pharmacology and safety data, to conduct early small experimental medicine clinical studies in a well-defined patient subset (eg, in patients with predictors of fast disease progression), using robust endpoints with clear success criteria. It would also be crucial to demonstrate evidence of the therapeutic target engagement (preclinically and then in the early clinical programme) before progressing to a clinical efficacy study. Clearly, advancing our understanding of ALS pathophysiology and natural history including disease modelling, development, and validation of reliable biomarkers and more sensitive clinical endpoints would increase likelihood of a successful translation into the clinic.

In this study, there were more deaths in the ozanezumab arm than in the placebo arm (13% vs 11%at Week 60, respectively), and all other efficacy endpoints showed small, non-significant numerical differences in favour of placebo. These findings could reflect a possible negative effect of ozanezumab. The pathogenesis of ALS is still poorly understood, and although upregulation of Nogo-A has been associated with ALS, this finding may reflect a compensatory (rather than disease-causing) role for Nogo-A in human disease. If this is the case, it could explain the potential worsening effect observed with ozanezumab in this study. Alternatively, blockade of Nogo-A at the neuromuscular junction by ozanezumab could lead to enhanced sprouting by increasing the metabolic demand on the motor neuron.

However, as the effect in favour of placebo was not significant, it could be argued that targeting Nogo-A simply had no effect. The differences in survival observed between groups could reflect natural variability in the rate of disease progression among patients with ALS (although ALS onset, diagnosis characteristics and phenotype at entry were similar between groups). Mortality in both groups was predominantly driven by respiratory failure, which is a common complication associated with progression of ALS, and is the main cause of death among people with ALS.30 Furthermore, Nogo-A overexpression previously observed in patients with ALS could be a downstream event in the disease process. This is supported by reports suggesting that Nogo-A overexpression in skeletal muscle might be a non-specific marker of denervation present in a range of neuromuscular disorders.20, 21 In this scenario, blockade of Nogo-A would not be expected to translate into clinical benefit, which is consistent with the results observed in the current study.

Therefore this mechanism has been comprehensively tested for ALS. In our study, ozanezumab did not show any evidence of efficacy over placebo for the treatment of ALS, and the results suggest the futility of further clinical testing of an anti-Nogo-A monoclonal antibody for the treatment of ALS.

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**AUTHOR CONTRIBUTIONS**

VM, JMS, PNL, KEM, WR, PR, DB, JB, MD, RM, and A Lavrov were involved in the study conception and design; VM, JMS, PNL, KEM, MCK, WR, AG, CD, SP, A Ludolph, AC, SHK, and LHvB were study investigators; VM, JMS, KEM, MCK, SP, AC, LHvB, PR, DB, JB, MD, RM, JT, and A Lavrov contributed to the analysis and interpretation of data; all authors critically revised the manuscript.

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**DECLARATION OF INTERESTS**

VM reports non-financial support from Fishawack Indicia during the conduct of the study.

AG reports non-financial support from Fishawack Indicia during the conduct of the study.

LHvB reports non-financial support from Fishawack Indicia during the conduct of the study; personal fees from Biogen and Cytokinetics, grants and personal fees from Baxalta outside the submitted work.

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CD reports non-financial support from Fishawack Indicia during the conduct of the study; personal fees from Genzyme Sanofi Co and BioMarin Co outside the submitted work.

KEM reports non-financial support from Fishawack Indicia during the conduct of the study.

SP reports non-financial support from Fishawack Indicia and clinical trial conduct payment/services from GSK during the conduct of the study; clinical trial conduct, travel, investigator meeting and accommodation payments from BIOGEN, Cytokinetics, Inc. and Orion Pharma outside the submitted work.

DB reports non-financial support from Fishawack Indicia during the conduct of the study; GSK employment and stock ownership outside the submitted work.

JT reports non-financial support from Fishawack Indicia during the conduct of the study; GSK employment and stock ownership outside the submitted work.

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MD reports non-financial support from Fishawack Indicia during the conduct of the study GSK employment and stock ownership outside the submitted work.

JB reports non-financial support from Fishawack Indicia during the conduct of the study; GSK employment and stock ownership outside the submitted work.

PR reports non-financial support from Fishawack Indicia during the conduct of the study GSK employment and stock ownership outside the submitted work.

A Lavrov reports non-financial support from Fishawack Indicia during the conduct of the study; GSK employment and stock ownership outside the submitted work.

**FIGURE LEGENDS**

**Figure 1: Trial profile**

aThe patient did not receive the study drug bPatients were considered to have completed placebo/ozanezumab if they completed all doses up to week 46. cPatients who withdrew from study medication, but continued to provide ALSFRS-R data and any relevant safety information by telephone up to week 48 and completed their follow-up visit.

**Figure 2: Adjusted mean change (95% CI) from baseline in ALSFRS-R total score over 48 weeks in the intent-to-treat population**

**Figure 3: Survival over 48 weeks in the intent-to-treat population**

**Figure S1: Observed vs predicted plasma ozanezumab concentrations (PK ozanezumab population\*)**

\*PK ozanezumab population comprised patients in the ITT population who received at least one dose of ozanezumab and from whom at least one plasma sample was analysed for ozanezumab

**Figure S2: Summary of common (≥5% of patients in either treatment arm) AEs any time during on-treatment or follow-up phase**

**TABLES**

**Table 1: Demographics and baseline characteristics(ITT population)**

|  |  |  |
| --- | --- | --- |
|  | **Placebo**  **(n=151)** | **Ozanezumab**  **(n=152)** |
| Age, years | 55·5 (11·04) | 55·7 (10·40) |
| Age group, n (%) |  |  |
| 18–64 years | 115 (76) | 120 (79) |
| 65–74 years | 34 (23) | 28 (18) |
| ≥75 years | 2 (1) | 4 (3) |
| Sex, n (%) |  |  |
| Female | 54 (36) | 49 (32) |
| Ethnicity |  |  |
| Not Hispanic or Latino | 150 (>99) | 149 (98) |
| Hispanic or Latino | 1 (<1) | 3 (2) |
| Height, cm | 170·6 (9·21) | 171·2 (10·25) |
| Weight at baseline, kg | 72·8 (14·1) | 75·1 (16·4) |
| Age at muscle weakness onset, years | 54·4 (10·95) | 54·6 (10·28) |
| Site of disease onset, n (%) |  |  |
| Upper limb(s) | 69 (46) | 63 (41) |
| Lower limb(s) | 44 (29) | 46 (30) |
| Both upper and lower limb(s) | 5 (3) | 6 (4) |
| Bulbar | 32 (21) | 33 (22) |
| Other | 1 (<1) | 4 (3) |
| Time to diagnosis from onset of muscle weakness, months | 8·0 (5·77) | 8·8 (5·55) |
| Time since muscle weakness onset, months | 17·9 (6·57) | 18·5 (6·34) |
| Time since initial diagnosis, months | 9·8 (7·19) | 9·6 (6·72) |
| Age at initial diagnosis, years | 55·1 (11·10) | 55·3 (10·37) |
| Type of disease, n (%) |  |  |
| Sporadic | 139 (92) | 143 (94) |
| Familial | 12 (8) | 9 (6) |
| Level of certainty of diagnosis, n (%) |  |  |
| Possible | 11 (7) | 14 (9) |
| Laboratory-supported probable | 25 (17) | 22 (14) |
| Probable | 67 (44) | 72 (47) |
| Definite | 48 (32) | 44 (29) |
| Number of regions involved,  n (%) |  |  |
| One | 26 (17) | 22 (14) |
| Two | 68 (45) | 62 (41) |
| Three | 38 (25) | 51 (34) |
| Four | 19 (13) | 17 (11) |
| Percent predicted SVC, L | 95·7 (17·96) | 93·3 (17·53) |
| Riluzole use, n (%) | 132 (87) | 131 (86) |
| Riluzole plasma concentration, ng/mL | 122·4 (100·87) | 103·3 (99·49) |
| ALSFRS-R total score | 38·4 (5·12) | 37·7 (5·49) |

Data are mean (SD) unless otherwise specified. ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; ITT, intent-to-treat; SD, standard deviation; SVC, slow vital capacity.

**Table 2: Primary and secondary/exploratory efficacy endpoints, week 48 vs baseline (ITT population)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **n** | | **Adjusted mean (SE)** | |  |  |
|  | **Placebo**  **(N=151)** | **Ozanezumab**  **(N=152)** | **Placebo**  **(N=151)** | **Ozanezumab**  **(N=152)** | **Difference vs placebo (95% CI)** | **p-value for difference** |
| Joint rank scorea | 151 | 152 | 15·0 (13·58) | –14·9  (13·54) | –30·0 (–67·9 to 7·9) | 0·120 |
| Change in ALSFRS-R total scoreb | 104 | 101 | −9·1  (0·64) | −10·4  (0·64) | −1·3 (−3·1 to 0·4) | 0·139 |
| Change in ALSFRS-R total scorec | 120 | 111 | −9·5  (0·68) | −10·8  (0·68) | −1·3  (−3·2 to 0·6) | 0·172 |
| Monthly rate of decline in ALSFRS-R total scoreb | 149 | 150 | −0·84 (0·063) | −0·96  (0·062) | −0·12 (−0·30 to 0·05) | 0·173 |
| Change in SVC (L) | 96 | 98 | −0·899 (0·0804) | −1·026 (0·0804) | −0·127 (−0·351 to 0·097) | 0·265 |
| Average percent change in HHDd | 99 | 95 | −34·7 (3·77) | −42·9  (3·75) | −8·2  (−18·7 to 2·3) | 0·125 |

aData collected within 21 days of the patient's last infusion. bOn-treatment data. cIncludes retrieved follow-up data. dAcross the muscle groups that were non-missing/non-zero at baseline. SE, standard error; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; CI, confidence interval; HHD, Hand Held Dynamometry; ITT, intent-to-treat; SVC, slow vital capacity.

**Table 3: Summary of AEs (any time during on-treatment or follow-up phase, safety population)**

|  |  |  |
| --- | --- | --- |
|  | **Placebo (N=151)** | **Ozanezumab (N=152)** |
| All AEs | 139 (92) | 140 (92) |
| SAEs | 46 (30) | 47 (31) |
| Fatalities  Any time after randomisationa  During study period | 16 (11)  13 (9) | 20 (13)  18 (12) |
| Drug-related SAEs | 3 (2) | 3 (2) |
| Adverse events leading to permanent discontinuation of study drug or withdrawal from study | 18 (12) | 19 (13) |

Data are n (%). aThe investigators notified the study team of five additional deaths outside the protocol specified SAE collecting timeframe. No SAEs were reported in any of these five fatal cases.

AE, adverse events; SAE, serious AEs.

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