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

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See Comment page 175

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Summary

Background Neurite outgrowth inhibitor A (Nogo-A) 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. Therefore, we aimed to assess the safety and efficacy of ozanezumab in patients with ALS.

Methods This randomised, double-blind, placebo-controlled, phase 2 trial was done in 34 centres in 11 countries. Patients aged 18–80 years with a diagnosis of familial or sporadic ALS were randomly assigned (1:1), centrally according to a computer-generated allocation schedule, to receive ozanezumab (15 mg/kg) or placebo as intravenous infusions over 1 h every 2 weeks for 46 weeks, followed by assessments at week 48 and week 60. Patients and study personnel were masked to treatment assignment. The primary outcome was a joint-rank analysis of function (ALS Functional Rating Scale-Revised) and overall survival, analysed at 48 weeks in all patients who received at least one dose of study drug. This study is registered with ClinicalTrials.gov, number NCT01753076, and with GSK-ClinicalStudyRegister.com, NOG112264, and is completed.

Findings Between Dec 20, 2012, and Nov 1, 2013, we recruited 307 patients, of whom 303 were randomly assigned to receive placebo (n=151) or ozanezumab (n=152). The adjusted mean of the joint-rank score was –14·9 (SE 13·5) for the ozanezumab group and 15·0 (13·6) for the placebo group, with a least squares mean difference of –30·0 (95% CI –67·9 to 7·9; p=0·12). Overall, reported adverse events, serious adverse events, and adverse events leading to permanent discontinuation of study drug or withdrawal from study were similar between the treatment groups, except for dyspepsia (ten [7%] in the ozanezumab group *vs* four [3%] in the placebo group), depression (11 [7%] *vs* five [3%]), and diarrhoea (25 [16%] *vs* 12 [8%]). Respiratory failure was the most common serious adverse event (12 [8%] *vs* seven [5%]). At week 60, the number of deaths was higher in the ozanezumab group (20 [13%]) than in the placebo group (16 [11%]), mainly as a result of respiratory failure (ten [7%] *vs* five [3%]). Two deaths were considered related to the study drug (bladder transitional cell carcinoma in the ozanezumab group and cerebrovascular accident in the placebo group).

Interpretation Ozanezumab did not show 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 patients, ALS progressively involves muscles, leading to weakness and ultimately death, typically as a result of respiratory failure.[2] Most patients die within 5 years of symptom onset.[3] The pathophysiological mechanisms remain unconfirmed, but pathogenic changes are considered to involve interference with protein degradation and defects in RNA processing.[1] These changes lead to progressive cellular failure, disruption of axonal architecture and function, axonal retraction, and ultimately 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 stabilisation 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 drug, riluzole, has an effect, albeit small, on the survival of patients with ALS.[2] Data from randomised controlled trials suggest that riluzole extends survival by a median of 2–3 months, whereas results from uncontrolled registry studies suggest prolongation of survival by up to 21 months.[4] Although results from preclinical studies and many clinical trials were encouraging, no other treatments have shown any

Research in context

Evidence before this study

We searched PubMed up to Feb 3, 2016, with the term "(nogo-a OR RTN4 OR "neurite outgrowth inhibitor") AND (ALS OR motor neurone disease)" and no language restrictions. Evidence from mouse models of amyotrophic lateral sclerosis (ALS), together with molecular analysis of skeletal muscle from patients with ALS, suggests a role for neurite outgrowth inhibitor A (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 assessing the efficacy of ozanezumab or other drugs with this mechanism of action.

Added value of this study

This study was the first randomised, placebo-controlled trial designed to assess the safety and efficacy of an anti-Nogo-A monoclonal antibody for the treatment of patients with ALS. We did not find 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. These results 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

At present, only one approved drug, riluzole, has been shown to have a slight effect on the survival of patients with ALS. Thus, the identification of new options for ALS treatment remains a priority for clinical research.

effect on the disease course.[5] Apart from riluzole, multidisciplinary palliative care remains the main management approach for ALS.[2]

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] and 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 rapid 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 led 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 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 is a potential therapeutic target.

Ozanezumab (GSK1223249) is a humanised monoclonal antibody against Nogo-A that was well tolerated in a first-in-human study.[12] 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 roughly 2 weeks apart).[12] In this phase 2 trial, we assessed the effect of ozanezumab on the function and survival of patients with ALS.

Methods

Study design

This randomised, double-blind, placebo-controlled, phase 2 trial was done in 34 centres across 11 countries (Australia, Belgium, Canada, France, Germany, Italy, Japan, South Korea, the Netherlands, the UK, and the USA). The protocol was approved by the relevant ethics committee in each country. The study was done and monitored in accordance with Good Clinical Practice and the guiding principles of the Declaration of Helsinki 2008.

Participants

We recruited 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 criteria[13]) who had onset of muscle weakness no more than 30 months before the screening visit and slow vital capacity of at least 65% at screening (predicted for sex, age, ethnic origin, and height). Full study inclusion and exclusion criteria are listed in the appendix (p 1). Eligible patients were identified in the clinic at each of the study sites and 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 randomly assigned (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 randomisation schedule validated by GlaxoSmithKline. Anonymised patient numbers were provided to investigators via an interactive voice response system. Infusions were prepared by a non-masked pharmacist at the study site, and masking

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See Online for appendix

of infusion bags and drip chambers was achieved by covering with orange tape. Patients and all study personnel administering the interventions, assessing outcomes, and analysing data were masked to treatment assignment.

Procedures

We selected an ozanezumab dose regimen of 15 mg/kg once every 2 weeks for this study on the basis of analysis of drug biodistribution into muscle, co-localisation of Nogo-A with ozanezumab at the target site (ie, muscle cell membrane), plasma pharmacokinetic data, and the safety profile from clinical and non-clinical studies.[12,14,15] Patients received ozanezumab or placebo as intravenous infusions over 1 h. A total of 24 infusions were planned, starting at the baseline visit (week 0) and then every 2 weeks up to the last dose at week 46, followed by assessments at week 48 and week 60. Since this dosing regimen had not been tested in human beings previously, we used a two-part study design. In part A, a subgroup of 24 patients had intensive safety monitoring during the first four antibody infusions, with independent review of data before recruitment of the remaining patients for part B of the study (appendix).

Outcomes

The primary outcome was a joint-rank analysis of function (ALS Functional Rating Scale-Revised [ALSFRS-R]) and overall survival at 48 weeks. The ALSFRS-R questionnaire was administered at clinic visits; for participants who were unable to attend the clinic, it could also be administered by telephone, thus reducing risk of missing data and allowing data collection from patients who had withdrawn from study treatment but who did not withdraw consent.

Secondary efficacy outcomes were change from baseline in ALSFRS-R total score at week 48, monthly rate of decline in ALSFRS-R total score over 48 weeks, progression-free survival (with progression defined as at least a six-point decrease on ALSFRS-R) at week 48, overall survival (defined as time from randomisation until death or censoring) at week 48 and week 60, the proportion of responders to the Clinical Global Impression-Improvement Scale at week 48, and change from baseline in respiratory function (slow vital capacity) and muscle power (measured by hand-held dynamometry) at week 48. The secondary analysis of ALSFRS-R data was added at protocol amendment (May 29, 2013) to aid clinical interpretation of the results.[16] The protocol amendment was made during recruitment for part B of the study (after safety data from part A of the study had been reviewed but before data analysis), and was documented and approved by the relevant ethics committees.

Additional secondary outcomes were change from baseline to week 48 in health outcomes (based on the EuroQol-Short Form 5-level version [EQ-5D-5L]) and the Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40), plasma pharmacokinetics parameters of ozanezumab, and plasma concentrations of riluzole (to assess any pharmacokinetic interaction with ozanezumab).

Safety assessments were the monitoring of serious adverse events, adverse events, disease-related events, routine laboratory tests (clinical chemistry and haematology), vital signs, and electrocardiograms (ECGs). Adverse events and disease-related events were coded according to the Medical Dictionary for Regulatory Activities coding system. Values for clinical laboratory assessments 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 adverse event or a serious adverse event. Other safety measures included the 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 adverse events, neurological examination, and immunogenicity (incidence of anti-ozanezumab antibodies and relation with pharmacokinetics, safety, and efficacy).

Patients who stopped treatment, but who did not withdraw consent to continue in the study, were encouraged to continue to provide ALSFRS-R and safety data up to week 48 via telephone contact, and mortality was monitored up to week 60. They were also requested to return for a follow-up immunogenicity visit roughly 14 weeks after the last infusion.

Statistical analysis

We estimated that a sample size of 147 patients per group would provide roughly 86% power to detect a significant difference in the primary outcome between the two groups at 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 were based on the following assumptions: the mean weekly rate of decline in ALSFRS-R was 0·235 with placebo and 0·165 with ozanezumab; the weekly rate of decline in ALSFRS-R had a variance of 0·044 in both groups; the within-individual variance in change in ALSFRS-R from baseline was 4; overall survival followed an exponential distribution in both groups, the 48-week mortality with placebo was 10%, and the absolute reduction in mortality with ozanezumab was 5%; the dropout rate (excluding death) of the ALSFRS-R was 20% in both groups; and the correlation between ALSFRS-R and overall survival for each individual was zero. Refinements made to power calculations as part of the protocol amendment on May 29, 2013, indicated a slightly increased power (but still within 80–90%) and no change in sample size.

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 no difference in outcome existed, 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 on the basis of the sum of each individual score. The mean total score of patients receiving ozanezumab was compared with that of patients receiving placebo. This analysis differs from the combined analysis of function and survival, as used in the EMPOWER study[17] in ALS (published after the present study had commenced), in which the joint-rank score was ranked and then the mean rank was compared between groups.[17,18]

Efficacy and safety outcomes were analysed in the modified intention-to-treat population, which comprised all randomised patients who received at least one dose of study drug. The concentrations of ozanezumab and riluzole (pharmacokinetics) were assessed in all patients who received at least one dose of the drug and from whom at least one plasma sample was analysed for the respective drug.

We used on-treatment data (ie, data collected up to 21 days after the patient's last infusion) for the modified intention-to-treat population to analyse the primary outcome. We included retrieved follow-up data (ie, data collected more than 21 days after the patient's last infusion, typically by telephone) in additional, separate, prespecified analyses of joint-rank scores, ALSFRS-R, and survival. Data were adjusted for baseline ALSFRS-R total score, riluzole use, country, and treatment assignment. We used SAS, version 9.2, for statistical analyses.

An independent, unmasked data-monitoring committee reviewed safety data, including adverse events, laboratory results, ECGs, and other safety assessments at regular intervals (roughly every 3 months) throughout the study.

This study is registered with ClinicalTrials.gov, number NCT01753076, and with GSK-ClinicalStudyRegister.com, NOG112264.

Role of the funding source

The funder of the study was involved in study design, funding of the participating centres, data analysis, and writing and funding of the 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

We enrolled 307 patients, of whom 303 were randomised and included in the efficacy and safety analyses. Between Dec 20, 2012, and Jan 29, 2013, 24 patients entered into part A of the study, of whom 12 received ozanezumab and 12 received placebo. The independent data-monitoring committee did not identify any substantial safety concerns, and 279 patients entered into part B of the study between May 15, 2013, and Nov 1, 2013. Overall, 151 patients received placebo and 152 received ozanezumab (figure 1). 216 patients completed the follow-up visit 14 weeks after the last dose and provided data for at least 60 weeks after the baseline assessment. Demographic and baseline characteristics were well balanced between the two treatment groups (table 1).

At 48 weeks, the adjusted mean of the joint-rank score of function and survival was 15·0 (SE 13·6) in the placebo group and –14·9 (13·5) in the ozanezumab group, with a least squares means difference (ozanezumab minus placebo) of –30·0 (95% CI –67·9 to 7·9; p=0·12; table 2). For all secondary efficacy outcomes, the adjusted means were slightly higher, but non-significantly so, in the placebo group than in the ozanezumab group (table 2, appendix). The adjusted mean difference between the ozanezumab group and the placebo group in change from baseline in ALSFRS-R total score at week 48 was –1·3 (95% CI –3·1 to 0·4; p=0·14; figure 2A; table 2).

Survival outcomes were not significantly different between the treatment groups (figure 2B). At week 48, six (4%) patients in the placebo group and eight (5%) patients in the ozanezumab group had died. Inclusion of retrieved follow-up data showed that nine (6%) patients in the placebo group and 15 (10%) patients in the ozanezumab

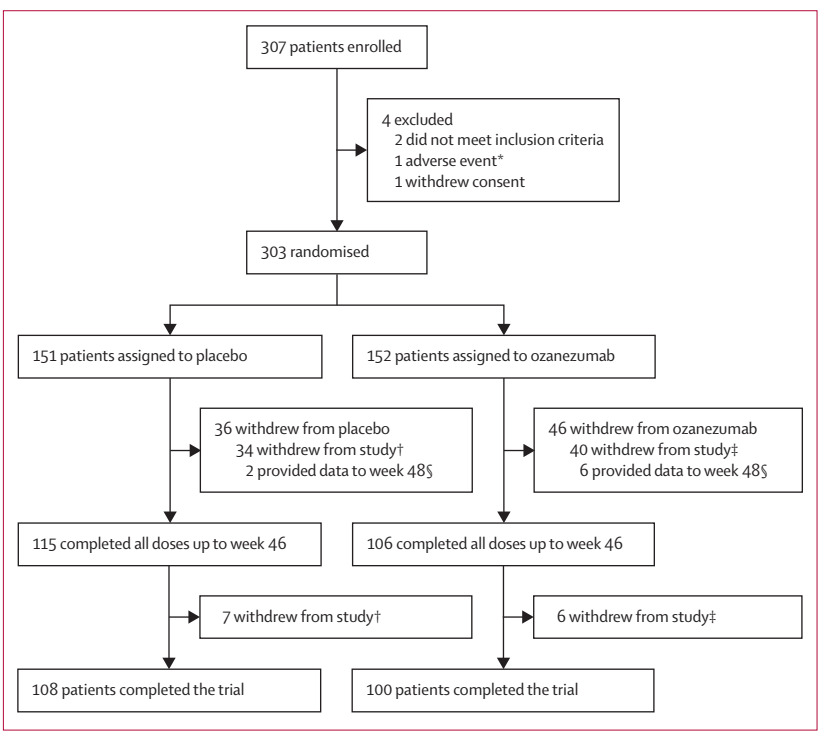

Figure 1: **Trial profile**
*The patient did not receive the study drug. †Reasons for withdrawal were adverse events (n=17), lack of efficacy (n=3), stopping criteria reached (n=1), lost to follow-up (n=2), physician decision (n=4), and withdrawal of consent (n=14). ‡Reasons for withdrawal were adverse events (n=18), lack of efficacy (n=1), lost to follow-up (n=1), physician decision (n=2), and withdrawal of consent (n=24). §These patients withdrew from study drug but continued to provide data for the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised and any relevant safety information by telephone up to week 48 and completed their follow-up visit.

	Placebo group (n=151)	Ozanezumab group (n=152)
Age, years		
Mean	55·5 (11·0)	55·7 (10·4)
18–64	115 (76%)	120 (79%)
65–74	34 (23%)	28 (18%)
75–81*	2 (1%)	4 (3%)
Sex		
Men	97 (64%)	103 (68%)
Women	54 (36%)	49 (32%)
Ethnic origin		
Not Hispanic or Latino	150 (99%)	149 (98%)
Hispanic or Latino	1 (1%)	3 (2%)
Height, cm	170·6 (9·2)	171·2 (10·3)
Weight, kg	72·8 (14·1)	75·1 (16·4)
Age at muscle weakness onset, years	54·4 (11·0)	54·6 (10·3)
Site of disease onset		
Upper limb or limbs	69 (46%)	63 (41%)
Lower limb or limbs	44 (29%)	46 (30%)
Both upper and lower limbs	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 (5·8)	8·8 (5·6)
Time since muscle weakness onset, months	17·9 (6·6)	18·5 (6·3)
Time since initial diagnosis, months	9·8 (7·2)	9·6 (6·7)
Age at initial diagnosis, years	55·1 (11·1)	55·3 (10·4)
Type of disease		
Sporadic	139 (92%)	143 (94%)
Familial	12 (8%)	9 (6%)
Certainty of diagnosis		
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		
One	26 (17%)	22 (14%)
Two	68 (45%)	62 (41%)
Three	38 (25%)	51 (34%)
Four	19 (13%)	17 (11%)
Percentage predicted slow vital capacity	95·7% (18·0)	93·3% (17·5)
Riluzole use	132 (87%)	131 (86%)
Riluzole plasma concentration, ng/mL	122·4 (100·9)	103·3 (99·5)
ALSFRS-R total score	38·4 (5·1)	37·7 (5·5)

Data are mean (SD) or n (%). ALSFRS-R=Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised. *Because exact dates of birth were not collected to protect anonymity, the oldest patient could have been aged 80 years or 81 years. †Brainstem, cervical, thoracic, or lumbosacral spinal cord.

Table 1: **Demographic and clinical baseline characteristics**

group had died at week 48, with a hazard ratio (HR) of 1·30 (95% CI 0·56–3·01; p=0·54). Progression-free survival at week 48 was 30·8% (23·0–38·6) in the placebo group and 28·5% (21·1–35·9) in the ozanezumab group (HR 1·07, 0·81–1·42; p=0·64). At week 60, 16 (11%) patients in the placebo group and 20 (13%) patients in the ozanezumab group had died (HR 1·03, 0·53–2·01; p=0·92; appendix). Since the proportionality assumptions were questionable for the Cox proportional hazard model because of the low number of events, we did a post-hoc analysis using the Kolmogorov-Smirnov test, the p values of which did not alter the interpretation of the results (appendix). No difference was seen between the placebo and ozanezumab groups for the health outcomes (change in ALSAQ–40 total score and EQ-5D-5L; appendix).

Plasma ozanezumab concentrations were consistent with those predicted before the study—ie, concentrations increased steadily with each dose and steady state was reached by week 12 (ie, dose 7). No evidence of change in ozanezumab elimination or clearance was seen (appendix). Plasma riluzole concentrations generally remained consistent with baseline values over the course of the study, with no evidence of change in elimination or clearance (appendix).

Overall, reported adverse events, serious adverse events, and adverse events leading to permanent discontinuation of study drug or withdrawal from study were similar between the treatment groups (table 3). Of 36 deaths reported, five (two in the ozanezumab group and three in the placebo group) occurred after follow-up contact, which was outside the timeframe specified for data collection of serious adverse events. No associated serious adverse events were reported for these five deaths, and all were judged by investigators to be due to ALS. The 31 remaining deaths occurred during the treatment or follow-up period (table 3). Two deaths were considered related to the study drug (bladder transitional cell carcinoma in the ozanezumab group and cerebrovascular accident in the placebo group). The higher number of deaths in the ozanezumab group than in the placebo group was attributable to a higher frequency of respiratory failure (ten [7%] *vs* five [3%]; appendix). 47 (31%) patients in the ozanezumab group had at least one serious adverse event, compared with 46 (30%) in the placebo group (table 3). Respiratory failure was the most common serious adverse event, reported in 12 (8%) patients in the ozanezumab group and seven (5%) in the placebo group. Three drug-related non-fatal serious adverse events (anaemia, appendicitis, and pulmonary embolism) were reported in the ozanezumab group, and two such events (unilateral blindness and thrombosis) were reported in the placebo group. The most common adverse events (reported by >10% of the overall population) were falls (125 [41%] patients), nasopharyngitis (67 [22%]), headache (55 [18%]), cough (37 [12%]), diarrhoea (37 [12%]), and constipation (36 [12%]; appendix). Some adverse events were roughly twice as frequent in the ozanezumab

group than in the placebo group—for example, dyspepsia (ten [7%] *vs* four [3%]), depression (11 [7%] *vs* five [3%]), and diarrhoea (25 [16%] *vs* 12 [8%]). No serious adverse events 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 after treatment with ozanezumab. The frequency of disease-related events was slightly higher in the ozanezumab group (123 [81%] patients) than in the placebo group (111 [74%] patients), mainly because of differences in the frequency of respiratory, thoracic, and mediastinal disorders (60 [39%] *vs* 44 [29%]). A slightly higher frequency of psychiatric disorders was reported in the ozanezumab group (23 [15%]) than in the placebo group (17 [11%]), mainly because of differences in the frequency of insomnia (six [4%] *vs* one [1%]). 20 (13%) patients in the ozanezumab group and 12 (8%) patients in the placebo group reported disease-related weight loss. More possible suicidality-related adverse events were recorded in the ozanezumab group (four [3%]) than in the placebo group (one [1%]).

15 (10%) patients in the ozanezumab group tested positive for anti-ozanezumab antibodies after the baseline assessment. One of these patients tested positive for neutralising anti-ozanezumab antibodies, but this patient did not show any evidence of adverse events related to immunogenicity (eg, hypersensitivity and rash) or any effect on efficacy or pharmacokinetics.

	Placebo group	Ozanezumab group	Ozanezumab *vs* placebo* (95% CI)	p value
Joint-rank score (primary outcome)†				
n	151	152	..	..
Adjusted mean	15·0 (13·6)	−14·9 (13·5)	−30·0 (−67·9 to 7·9)	0·12
Change from baseline in ALSFRS-R total score				
On-treatment data				
n	104	101	..	..
Adjusted mean	−9·1 (0·6)	−10·4 (0·6)	−1·3 (−3·1 to 0·4)	0·14
Including retrieved follow-up data				
n	120	111	..	..
Adjusted mean	−9·5 (0·7)	−10·8 (0·7)	−1·3 (−3·2 to 0·6)	0·17
Monthly rate of decline in ALSFRS-R total score (on-treatment data)				
n	149	150	..	..
Adjusted mean	−0·84 (0·06)	−0·96 (0·06)	−0·12 (−0·30 to 0·05)	0·17
Change in slow vital capacity, L				
n	96	98	..	..
Adjusted mean	−0·90 (0·08)	−1·03 (0·08)	−0·13 (−0·35 to 0·10)	0·27
Change in hand-held dynamometry (%)				
n‡	99	95	..	..
Adjusted mean	−34·7% (3·8)	−42·9% (3·8)	−8·2% (−18·7 to 2·3)	0·13

Data are adjusted mean (SE), unless specified otherwise. ALSFRS-R=Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised. *Least squares mean difference. †Data collected within 21 days of the patient's last infusion. ‡Patients with non-missing/non-zero measurements at baseline.

Table 2: **Primary and secondary efficacy endpoints at week 48**

Discussion

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

These findings are surprising because results from previous studies[8,9,19] had suggested a link between Nogo-A and ALS, illustrated by the upregulation of Nogo-A in the skeletal muscle of patients and its relation with disease severity. Although Nogo-A overexpression was also seen 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, neuromuscular junction destabilisation and neurite retraction have been suggested to precede degeneration of spinal motor neurons (ie, the so-called 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 a preclinical study,[15] ozanezumab resulted in a dose-dependent decrease or reversal of neurite outgrowth inhibition in a rat postnatal cerebellar granular neuron culture. Furthermore, the murine parent antibody of ozanezumab had a positive effect in the *SOD1*^{G93A} transgenic mouse model of ALS, in which it improved spinal motor neuron and motor unit survival, increased skeletal muscle force,[10] and 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 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,[12] ozanezumab was well tolerated, and although the study was not designed to assess efficacy, results for

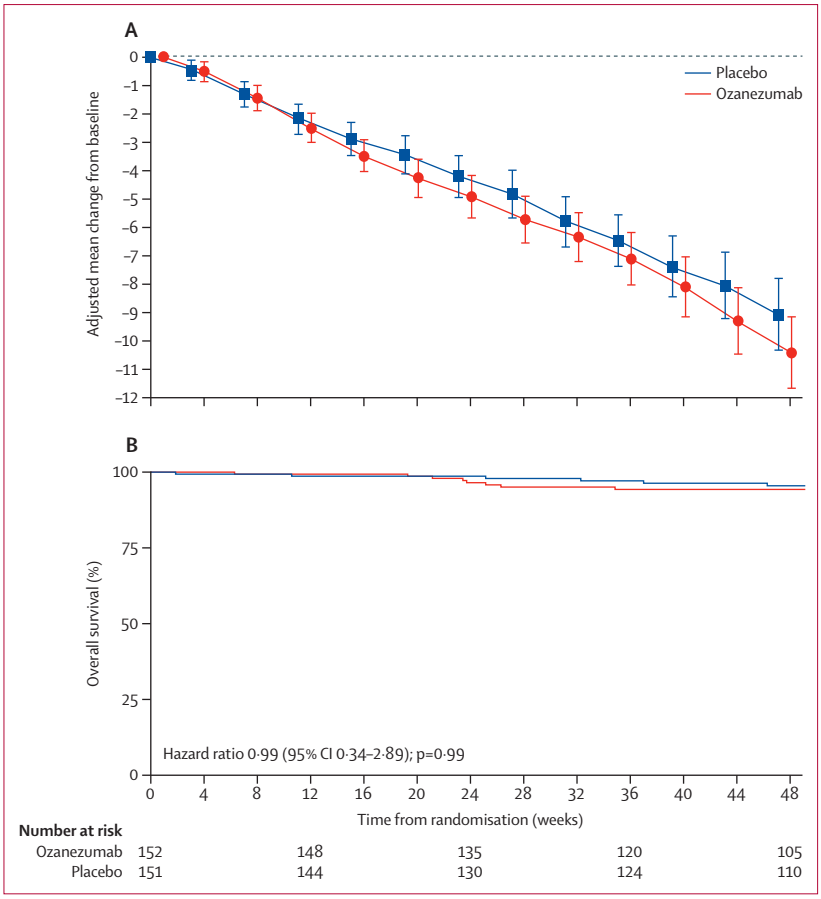

Figure 2: **(A) Adjusted mean change from baseline in ALSFRS-R total score and (B) overall survival**
Error bars represent 95% CI. ALSFRS-R=Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised.

	Placebo group (n=151)	Ozanezumab group (n=152)
All adverse events	139 (92%)	140 (92%)
Serious adverse events	46 (30%)	47 (31%)
Deaths		
Anytime after randomisation	16 (11%)*	20 (13%)*
During reporting period of serious adverse events	13 (9%)	18 (12%)
Drug-related serious adverse events	3 (2%)	3 (2%)
Adverse events leading to permanent discontinuation of study drug or withdrawal from study	18 (12%)	19 (13%)

*Three deaths in the placebo group and two deaths in the ozanezumab group occurred outside the data collection timeframe for serious adverse events specified in the protocol (ie, after follow-up contact); no serious adverse events were reported in the five patients who died.

Table 3: **Summary of adverse events**

functional endpoints (such as ALSFRS-R and slow vital capacity) and manual muscle testing were numerically in favour of ozanezumab at the highest dose of 15 mg/kg compared with placebo. Taken collectively, the results of these studies were deemed sufficient to initiate a phase 2 study.

The results of our study, although negative, are robust. The study population was representative of patients with mild to moderate ALS who had concomitant medical disorders that were expected for this population. Demographic and baseline characteristics were similar between the two treatment groups, and the functional decline observed in the placebo group was similar to that reported in other clinical studies of ALS.[16] A further strength is that the study had an independent data-monitoring committee in place for the periodic review of safety and efficacy data, and was done in accordance with a two-part design to ensure 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 dosing regimen used in this study was predicted to achieve at least 90% co-localisation of ozanezumab with Nogo-A, which was anticipated to achieve a relevant pharmacodynamics effect.[14] Plasma concentrations of ozanezumab during the study confirmed 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 no pharmacokinetic interaction existed 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 was 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 a method 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 this 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* mouse model of ALS.[10] Although this animal model of ALS is commonly used, no clinical translation has yet been shown.[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 are estimated to account for only around 2% of ALS cases, and SOD1-related ALS do not have the TDP-43 pathology associated with most forms of the disease.[1,29,30] Highly penetrant human *SOD1* mutations (eg, Ala4Val) do not induce the disease phenotype in the mouse model.[29] Finally, unlike in ALS clinical trials, treatment in *SOD1* mutant mice is often administered before symptom onset.[1,29] Although preclinical models such as the *SOD1* mouse can provide valuable information on the pharmacology of a new investigational drug, findings have not translated to the clinical setting so far.[26,27] One possible approach to address this issue 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. Evidence of the therapeutic target engagement (in preclinical models and then in early clinical studies) is also necessary before progressing to a clinical efficacy study. Clearly, advancing the 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, more deaths occurred in the ozanezumab group than in the placebo group (20 [13%] patients *vs* 16 [11%] patients at week 60), 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 might 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, since the effect in favour of placebo was not significant, another argument could be that targeting of Nogo-A had no effect. The differences in survival observed between groups could reflect natural variability in the rate of disease progression in patients with ALS (although disease onset, diagnosis characteristics, and phenotype were similar at baseline). Mortality in both groups was predominantly driven by respiratory failure, which is a common complication associated with disease progression and the main cause of death in patients with ALS.[30] Furthermore, Nogo-A overexpression previously observed in patients with ALS could be a downstream event in the disease process. This notion 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 our study.

Therefore, the mechanism of Nogo-A blockade 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.

Contributors

VM, JMS, PNL, KEM, WR, PR, DB, JB, MD, RM, and ALa were involved in study conception and design. VM, JMS, PNL, KEM, MCK, WR, AG, CD, SP, ALu, AC, SHK, and LHvdB were study investigators. VM, JMS, KEM, MCK, SP, AC, LHvdB, PR, DB, JB, MD, RM, JT, and ALa contributed to data analysis and data interpretation. All authors critically revised the manuscript.

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Declaration of interests

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