**Supplementary methods**

**Inclusion criteria**

1. Patients with diagnosis of familial or sporadic amyotrophic lateral sclerosis (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.18

2. Onset of muscle weakness no more than 30 months before screening visit.

3. Slow vital capacity (SVC) of at least 65% predicted for gender, age, ethnicity and height at Screening.

4. If on riluzole, the dose must have been stable for at least 28 days prior to Baseline visit.

5. Age 18–80 years inclusive.

6. Female patients may participate if they are of non-childbearing potential or if they are of childbearing potential and agree to use one of the protocol-approved methods for contraception during the study and 4 months after the last dose to avoid pregnancy. Women of childbearing potential must have a negative pregnancy test and be non-lactating. The list of protocol-approved contraceptive methods is as follows (note that this does not apply to females of childbearing potential with same-sex partners, when this is their preferred and usual lifestyle):

a. Abstinence from penile-vaginal intercourse, when this is the female’s preferred and usual lifestyle.

b. Oral contraceptive, either combined or progestogen alone.

c. Injectable progestogen.

d. Implants of etonogestrel or levonorgestrel.

e. Estrogenic vaginal ring.

f. Percutaneous contraceptive patches.

g. Intrauterine device or intrauterine system that meets the SOP effectiveness criteria as stated in the product label.

h. Male partner sterilisation prior to the female subject's entry into the study, and this male is the sole partner for that subject. The information on the male sterility can come from the site personnel’s: review of subject’s medical records; medical examination of the subject and/or semen analysis; or interview with the subject on his medical history.

i. Male condom combined with a vaginal spermicide\* (foam, gel, film, cream, or suppository) only for the following 3 situations when there is a very low risk for developmental toxicity:

i. Vaccines.

ii. Monoclonal antibodies when there is no target biology concern.

iii. Compounds that have a complete reproductive toxicology package and have not shown any signal for developmental toxicity.

j. Male condom combined with a female diaphragm, either with or without a vaginal spermicide\* (foam, gel, film, cream or suppository)

7. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2x upper limit of normal (ULN); alkaline phosphatase and bilirubin ≤1·5xULN.

8. QTc (both QTcB and QTcF) <450 milliseconds (msec) or <480 msec for patients with Bundle Branch Block at Screening and Baseline (average from triplicate electrocardiograms).

9. French patients: In France, a patient will be eligible for inclusion in this study only if either affiliated to or a beneficiary of a social security category.

\*Nonoxynol-9 is the critical component in most spermicides, and is regarded as an acceptable spermicidal agent. Concern has been raised that nonoxynol-9 damages the epithelial lining of the vagina, and exposure may facilitate transmission of viruses, particularly HIV. The World Health Organization conducted a technical consultation in October 2001 and concluded that the increased risk for such transmission was low to minimal.

**Exclusion criteria**

1. Patients with other neuromuscular disorders (including a history of polio), which in the opinion of the investigator could have contributed to the muscular atrophy or weakness caused by ALS or could have any other effect on the study efficacy or safety assessments that in the opinion of the investigator would impact participation in the study.

2. Patients with primary lateral sclerosis, monomelic ALS, ALS Parkinsonism dementia complex.

3. Patients requiring non-invasive or mechanical ventilation (non-invasive ventilation for sleep apnoea is allowed subject to discussion with Medical Monitor).

4. Patients on diaphragmatic pacing.

5. Presence of any of the following clinical conditions:

a. Drug abuse or alcoholism (according to Diagnostic and Statistical Manual of Mental Disorders –Fourth Edition criteria).

b. Uncontrolled hypertension despite optimal antihypertensive treatment; unstable cardiovascular, pulmonary, renal, endocrine or haematologic condition; current malignancy.

c. Active major infectious disease e.g. systemic infections with visceral involvement (pneumonia, pyelonephritis, endocarditis) and/or septicaemia.

d. Unstable psychiatric illness, such as psychosis or untreated major depression, within 90 days of the Screening visit.

6. Patients, who in the investigator's judgement, pose a significant suicide risk.

7. Current or chronic history of liver disease, known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones), positive Hepatitis B surface antigen or Hepatitis C antibody test.

8. Patients who have participated in a clinical trial involving receipt of a biopharmaceutical product (recombinant proteins, monoclonal antibodies, blood products, sera, allergens, and gene and cell therapy products) within 6 months prior to the first dosing day.

9. Patients who have received any type of vaccination within 2 weeks prior to study drug administration.

10. Chronic (>3 months) use of systemic immunosuppressants including systemic steroids.

11. Exposure to non-biological experimental agents (including investigational products and marketed medications used off-label) 1 month or 5 half-lives prior to Baseline visit (whichever is longer).

12. History of sensitivity to ozanezumab, or components thereof, or a history of other allergies that, in the opinion of the investigator, contraindicates participation in the study.

**Two-part study design**

The dosing regimen selected for the current study had not been tested previously in humans, and so a 2-part design was used. The first 24 patients randomised were included in Part A, and further recruitment was halted. In Part A, patients received intensive safety monitoring during the first four infusions of ozanezumab – patients were monitored at the study site for at least 24 hours after infusions 1 and 2, and for at least 6 hours after infusions 3 and 4. During this phase, sites were required to discuss safety for each patient with the Medical Monitor before proceeding to the next infusion. After all 24 subjects had received the first four infusions, unblinded safety data from these subjects were reviewed by an independent data monitoring committee (IDMC). After IDMC review, recruitment into Part B of the study commenced. Patients in Part B were monitored for at least 3 hours after each infusion up to infusion 13 (Week 24), after which the duration of monitoring could be adjusted. For Part B only, randomisation was stratified according to country and riluzole use. All subjects (whether recruited in Part A or B) received less intensive safety monitoring from this point. The IDMC also reviewed unblinded safety and selected efficacy data at regular intervals throughout the study.

**Statistical methods**

The joint rank scores were analysed using analysis of covariance (ANCOVA), adjusting for treatment, baseline ALS Functional Rating Scale-Revised (ALSFRS-R) score, riluzole use and country group. The ALSFRS-R total score, hand held dynamometry (HHD), slow vital capacity (SVC), Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40) score and EuroQol-Short Form 5-level version (EQ-5D) score were analysed using a mixed model repeated measures approach, adjusting for treatment, visit, baseline measure, riluzole use and country group, and including interaction terms for treatment by visit and baseline measure by visit. The ALSFRS-R score was also analysed using a linear mixed model adjusting for treatment, time, baseline ALSFRS-R, riluzole use, country group and interaction terms for treatment by time and baseline ALSFRS-R by time as fixed effects. Time and intercept were included in the model as random effects. The survival endpoints were analysed using a Cox proportional hazard model, adjusting for baseline ALSFRS-R score, riluzole use and country group. Clinical Global Impression-Improvement Scale (CGI-I) responders were analysed using a logistic regression analysis adjusting for Clinical Global Impression-Severity (CGI-S) at baseline, riluzole use and world region.

Due to small subject numbers, data from some countries were combined to create the covariate country groups used in the analysis. The countries that were combined were: Belgium and the Netherlands, Japan and South Korea, and the United States and Australia. The categories for world region were Europe, South-East Asia and the rest of the world.

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**Tables**

**Table S1: Statistical analysis of Clinical Global Impression–Improvement Scale (CGI-I) responders at Week 48 (ITT population)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **n** | | **Responders, n (%)** | |  |  |
|  | **Placebo**  **(N=151)** | **Ozanezumab**  **(N=152)** | **Placebo**  **(N=151)** | **Ozanezumab**  **(N=152)** | **Odds ratio (95% CI)** | **p-value** |
| CGI-I | 101 | 97 | 23 (23) | 18 (19) | 0·73  (0·36, 1·49) | 0·393 |

CGI-I, clinical global impression improvement scale; CI, confidence interval; ITT, intent-to-treat.

**Table S2: Statistical analysis of time-to-event endpoints (ITT population)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **n** | | **Kaplan–Meier survival estimate, % (95% CI)** | |  |  |
|  | **Placebo**  **(N=151)** | **Ozanezumab**  **(N=152)** | **Placebo**  **(N=151)** | **Ozanezumab**  **(N=152)** | **Hazard ratio (95% CI)** | **p-value for hazard ratio** |
| Progression-free survival at week 48a | 151 | 152 | 30·8  (23·0, 38·6) | 28·5  (21·1, 35·9) | 1·07  (0·81, 1·42) | 0·642 |
| Survival at week 60 | 151 | 152 | 87·4  (81·5, 93·3) | 85·4  (79·4, 91·3) | 1·03  (0·53, 2·01) | 0·923 |

aOn-treatment data. CI, confidence interval; ITT, intent-to-treat.

**Table S3: Kolmogorov-Smirnov analysis of time to event endpoints (ITT population, ad hoc analysis)**

|  |  |
| --- | --- |
| **Endpoint** | **p-value** |
| Survival at 48 weeks using on-treatment data | 0·9624 |
| Survival at 48 weeks including retrieved follow-up data | 0·9981 |
| Progression-free survival at 48 weeks using on-treatment data | 0·7725 |
| Respiratory support-free survival at 48 weeks using on-treatment data | 0·9127 |
| Survival at 60 weeks | 0·9723 |

ITT, intent-to-treat.

**Table S4: Health outcome measures, 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)** |
| Change in ALSAQ–40 total score | 102 | 96 | 19·2  (1·47) | 20·6 (1·49) | 1·4  (−2·8, 5·5) |
| Change in EQ-5D  (5 level) | 103 | 102 | −0·234  (0·0207) | −0·238 (0·0207) | −0·004  (−0·062, 0·053) |

ALSAQ–40, Amyotrophic lateral sclerosis assessment questionnaire-40; EQ-5D, EuroQoL-5 dimensions; SE, standard error; CI, confidence interval; ITT, intent-to-treat.

**Table S5:** **Riluzole concentrations over duration of ozanezumab dosing (PK Riluzole concentration population\*)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **Placebo group** | | **Ozanezumab group** | |
| **Analysis visit** | **Planned relative time** | **n** | **Mean (SD) concentration, ng/mL** | **n** | **Mean (SD) concentration, ng/mL** |
| Baseline | Pre-dose  IAD | 134  133 | 122·4 (100·9)  85·2 (63·5) | 132  132 | 103 (99·5)  69 (68·8) |
| Week 4 | Pre-dose  IAD | 128  130 | 142.3 (120·6)  90.2 (75·3) | 129  125 | 110 (102·3)  74·9 (65·4) |
| Week 8 | Pre-dose | 130 | 118·3 (106·2) | 129 | 106·5 (81·8) |
| Week 12 | Pre-dose  IAD | 128  128 | 108 (101·8)  80·7 (68·3) | 124  122 | 98·8 (82·3)  71·7 (55·5) |
| Week 24 | Pre-dose  IAD | 117  113 | 121 (98·6)  85·9 (57·9) | 110  109 | 104 (91·9)  74·7 (62·9) |
| Week 36 | Pre-dose  IAD | 109  107 | 110 (72·8)  88·9 (66·1) | 98  99 | 116 (99·8)  81 (74·0) |
| Week 44 | Pre-dose  IAD | 99  97 | 140 (101·5)  107 (78·7) | 81  82 | 118 (89·4)  81·1 (62·7) |
| Follow-up |  | 109 | 128·9 (90·0) | 94 | 123·7 (104·5) |

\*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.

IAD, immediately after dose; ITT, intent-to-treat; PK, pharmacokinetic; SD, standard deviation.

**Table S6. Summary of fatal serious adverse events (any time during on treatment or follow-up phase)**

|  |  |  |
| --- | --- | --- |
|  | **Number of patients (%)** | |
| **System organ class preferred term** | **Placebo (N=151)** | **Ozanezumab 15 mg/kg (N=152)** |
| **Any event** | 13 (9) | 18 (12) |
| Respiratory, thoracic and mediastinal disorders |  |  |
| Any event | 7 (5) | 14 (9) |
| Respiratory failure | 5 (3) | 10 (7) |
| Pneumonia aspiration | 1 (<1) | 1 (<1) |
| Acute respiratory failure | 0 | 1 (<1) |
| Asphyxia | 0 | 1 (<1) |
| Dyspnoea | 1 (<1) | 0 |
| Hypercapnia | 0 | 1 (<1) |
| Pulmonary embolism | 0 | 1 (<1) |
| Nervous system disorders |  |  |
| Any event | 3 (2) | 2 (1) |
| Amyotrophic lateral sclerosis | 2 (1) | 2 (1) |
| Cerebrovascular accident | 1 (<1) | 0 |
| Infections and infestations |  |  |
| Any event | 1 (<1) | 3 (2) |
| Pneumonia | 0 | 2 (1) |
| Sepsis | 1 (<1) | 1 (<1) |
| General disorders and administration site conditions |  |  |
| Any event | 2 (1) | 0 |
| Death\* | 1 (<1) | 0 |
| Euthanasia | 1 (<1) | 0 |
| Injury, poisoning and procedural complications |  |  |
| Any event | 0 | 1 (<1) |
| Fall | 0 | 1 (<1) |
| Neoplasms benign, malignant and unspecified (incl. cysts and polyps) |  |  |
| Any event | 0 | 1 (<1) |
| Bladder transitional cell carcinoma | 0 | 1 (<1) |

\*‘Death’ is used for this system organ class when the cause of death is not reported.