THE LANCET Neurology

Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Cudkowicz ME, Titus S, Kearney M, et al, for the Ceftriaxone Study Investigators. Safety and efficacy of ceftriaxone for amyotrophic lateral sclerosis: a multi-stage, randomised, double-blind, placebo-controlled trial. *Lancet Neurol* 2014; published online Oct 6. http://dx.doi.org/10.1016/S1474-4422(14)70222-4.

Webappendix A: Inclusion/Exclusion Criteria

Inclusion Criteria

1. Participants with familial or sporadic ALS diagnosed as possible, laboratory supported probable, probable or definite according to the World Federation of Neurology El Escorial criteria
2. Age 18 years or older
3. Capable of providing informed consent and complying with trial procedures
4. Vital capacity ≥60% predicted value for gender, height and age at screening
5. Women must not be able to become pregnant (eg, post menopausal, surgically sterile or using adequate birth control methods) for the duration of the study. Adequate contraception includes: abstinence, hormonal contraception (oral contraception, implanted contraception, injected contraception or other hormonal (eg, patch or contraceptive ring) contraception), intrauterine device in place for ≥3 months, barrier method in conjunction with spermicide or another adequate method (as determined by steering committee member review). Women of childbearing potential must have a negative pregnancy test at screening and be non-lactating.
6. First ALS symptoms occurred no more than 3 years prior to screening visit
7. Not taking riluzole or on a stable dosage for ≥14 days prior to the screening visit and ≥30 days prior to the baseline visit.
8. Subject has a competent caregiver who can and will be responsible for administration of study drug. If there is no caregiver, another qualified individual must be available to administer the study drug.
9. Geographic accessibility to the study site
10. Subjects medically able to undergo placement of central venous catheter as determined by the investigator (to include absence of systemic infection, a medical disorder which precludes catheter placement)

Exclusion Criteria

1. Dependence on mechanical ventilation (invasive or non-invasive, including Continuous Positive Airway Pressure or Bi-level Positive Airway Pressure for any part of the day or night prior to the screening visit. Dependence on mechanical ventilation is defined as being unable to lie flat (supine) without it, unable to sleep without it, or daytime use.
2. Exposure to ceftriaxone or any cephalosporin within 30 days prior to the screening visit
3. History of known sensitivity or intolerability to ceftriaxone or to any other cephalosporin
4. History of known sensitivity or intolerability to penicillin or any beta lactam (including mild rash)
5. Exposure to any other investigational agent within 30 days prior to screening visit
6. Known immune compromising illness or therapy
7. Active gastrointestinal disease within 30 days of the screening visit
8. History of antibiotic-induced colitis
9. Active biliary disease, including asymptomatic cholelithiasis (gallstones) or biliary sludge
10. Presence of any of the following clinical conditions
    a. Drug abuse or alcoholism
    b. Unstable cardiac, pulmonary, renal, hepatic, endocrine, hematologic, or active infectious disease, including current malignancy
    c. AIDS or AIDS-related complex
    d. Unstable psychiatric illness defined as psychosis or untreated major depression within 90 days of the Screening Visit
11. Laboratory values: screening alanine aminotransferase >3.0 times the upper limit of normal or, total bilirubin >1.5 times the upper limit of normal, absolute neutrophil count of ≤1000/µl, platelet concentration of <100,000/µl, hematocrit level of <33 for female or <35 for male, or coagulation tests >1.5 times upper limit of normal.
12. Women of childbearing potential not practicing adequate contraception
13. History of known sensitivity to bile acids or ursodiol

Allergy. Subjects with a remote or unclear history of sensitivity or allergy to ceftriaxone, other cephalosporins, penicillin or other beta lactams will require review and clearance by an Allergist or Infectious Disease specialist prior to entry. If this clearance is obtained, it will be documented and this will not be considered exclusionary for the study (exclusion # 3 and #4). Gastro-Esophogeal Reflux Disease is not considered active gastrointestinal disease, and is not exclusionary (exclusion # 7).

Riluzole. The use of riluzole will be permitted during the study. Subjects taking riluzole must be on a stable dosage for ≥14 days prior to the screening visit and ≥30 days prior to the baseline visit. Subjects are not allowed to start taking riluzole during the trial. About 60% of patients with ALS in the United States are currently taking riluzole. Allowing subjects the choice of taking riluzole reduces dropout rate and ensures that the investigators know which subjects are taking riluzole.

Webappendix B: Methods (additional information)

Stopping for efficacy

Efficacy stopping used the alpha spending rule approach, which provides flexibility in the selection of monitoring times. That is, a one-sided type I error of 0.025 (equivalent to a two-sided p=0.05 test) can be allocated during the course of the trial. Interim analyses were scheduled using the following alpha spending function: $0.025\ t^3$, where t (ie, information time) is the proportion of events (t) that occurred by the time of the analysis.

In the table below, efficacy stopping guidelines for the trial are provided based on assumptions about accrual and mortality rates. The two event rate estimates were calculated under the alternative and null hypothesis assuming a 1-year survival of 75%. It also was assumed that the accrual would take 5 months to ramp up to 25 participants per month. The p value to stop is one-sided:

Study month	Accrual	Number of events	p value to stop
3	NA	NA	NA
9	235	31-40	0·000064
15	385	61-75	0·000374
21	535	102-125	0·001686
27	600	153-187	0·005537
33	600	201-244	0·011807
39	600	243-293	0·019872

For example, by month 15, the study should have accrued 385 participants, and 61–75 events (deaths/permanent assisted ventilation) will have occurred. A log-rank test was performed to compare survival between the ceftriaxone and placebo groups. If this log-rank test produced to a one-sided p value <0·000374, the study could be stopped for efficacy.

Stopping for futility

Stopping for futility could occur if the trial did not reach the accrual targets, or if both Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised ***(***ALSFRS-R) and survival did not show sufficient trends toward efficacy. In addition, if the dropout rate was so high that the power to show a survival benefit was minimal, the primary endpoint of the trial was changed to compare 12-month changes in ALSFRS-R, and participants were given the option to discontinue study medication at 12 months.

Accrual targets. After allowing 5 months to reach an accrual rate of 25 participants per month, the Data Safety Monitoring Board (DSMB) could stop the study if the accrual futility boundary of ≥60% was not achieved.

Efficacy targets. The trial was considered futile if the co-primary endpoints, survival, and ALSFRS-R slopes failed to cross the futility boundary specified by the beta spending function .05*t. For the ALSFRS-R, it was defined as the ratio of the variance of the treatment time interaction at the final analysis over the variance at the interim analysis. Using linear regression of the variance of the efficacy parameter on different combinations of sample sizes and follow-up periods, t was assumed to be proportional to 75 times the number of participants plus ·25 times the number of subject-years of follow-up.

Treatment discontinuation rate targets. Although all analyses were based on the intention-to-treat (ITT) principle, discontinuations reduce the power of the study, as any effect of treatment is diminished by those who withdraw from that treatment group. Thus, the power calculation used an ITT hazard ratio (HR), which reflects the HR achieved by an effective treatment on all participants regardless if they were compliant and received treatment for the entire study period.

Under a Poisson event model, the primary effect of discontinuations was measured by the ratio, p: amount of time a participants is on treatment divided by the total time they was included in the study. The ITT HR was approximately $1/(p/R+(1-p))$, where R was the on-treatment HR (placebo/RX). The parameter p was estimated by the sum of the area under the time-to-discontinuation curves for each participant's potential follow-up, divided by the sum of the area under the time-to-death curve. At each DSMB meeting, p estimates were reported, and if the value was

significantly (p=0·05, one sided) less than 0·67, the board could recommend that the trial no longer have survival as a primary endpoint and be redesigned to focus on ALSFRS-R scores measured for 12 months.

If p=0·67, a participant was on treatment for 67% of the time they were in the study, and R=2 than 1/(p/R+(1-p))=1·5, meaning that treated participants would survive 1·5 times longer than untreated participants. Thus, an on-treatment HR of 2 gave an ITT HR of 1·5. Any HR larger than 2 was unlikely.

SECONDARY OUTCOME MEASURES

ALSFRS-R

The ALSFRS-R is an ordinal rating scale (range, 0–4) that is easy for patients to complete or for healthcare providers to administer. The instrument has been validated for assessing participant-perceived capability and independence in 12 functional activities relevant in ALS. The maximum possible score is 48; higher scores indicate better capabilities and more independence.

Vital capacity

Slow vital capacity was assessed three times at each visit, and values are expressed in litres (L). Expected normal value (based on age, gender, and height), and percentage predicted also were calculated. The maximum and mean value at each visit was used for data analysis. Date of birth and change in age during the study was factored into the analyses. The equations* are:

Expected normal value

Men

Limits: Age (18–85 years)
Height (147–203 cm)
Age <25: −6·8865 + 0·0739A + 0·0590Hc
Age ≥25: −8·7818 − 0·0298A + 0·0844Hc

Women

Limits: Age (18–88 years)
Height (142–183 cm)
Age <20: −4·4470 + 0·0699A + 0·0416Hc
Age ≥20, <70: −3·1947 − 0·0169A + 0·0444Hc
Age ≥70: −0·1889 − 0·0296A + 0·0313Hc

A = age in years: Date Performed − Date of Birth; Hc = height in centimeters.
*Revised formulae from NCTU (19 Sep 2011)

Percentage predicted value

VC % max = 100*maximum VC/expected normal VC
VC % mean = 100* mean VC/expected normal VC

Hand-held dynamometry: upper and lower extremity strength measurement
Bilateral muscle strength was measured using the Jaymar grip dynamometer. Secondarily, the maximum voluntary isometric contraction (MVIC), the mean of left and right grip strength (Z score). In patients with ALS, hand-held dynamometry (HHD) has been directly validated against MVIC. For upper and lower extremity muscles, correlations range from 0.84 to 0.92. It takes <30 min to complete a test of upper and lower extremities using HHD. Six proximal muscle groups–validated against MVIC–were examined bilaterally in upper (shoulder flexion, elbow flexion, elbow extension) and lower extremities (hip flexion knee flexion, knee extension). Wrist extension, first dorsal interosseous contraction, and ankle dorsiflexion, while not validated against MVIC, also were measured bilaterally. For each muscle group tested, the raw unit of measure was the maximum value of the trials, and tests that were not done were considered a zero score.

HHD outcomes were assessed using the percentage change from baseline. For participants without HHD testing at baseline, the first available measurement was used as the referent. Upper and lower extremity values were calculated as the sum of all tests for that extremity to create a mega score for upper extremity muscles and a mega score for lower extremity muscles. Scores for each muscle group were normalised to the participant's baseline value and expressed as percent of the baseline score.

ALS-Specific Quality of Life questionnaire

The ALS-Specific Quality of Life (ALSSQOL) questionnaire was developed and validated in individuals with ALS. It is not a health-related quality-of-life scale. The scale consists of 59 questions directed toward ALS symptom severity, mood and affect, intimacy, and social issues. Each item is scored on a scale ranging from 0 to10 (total score range, 0–590). However, because a score of 10 is maximally weighted toward negative values on some questions and positive values on others, some scales were reversed for consistency. Four of the questions were conditional (50, 53, 56, and 59), depending on a response to the previous question. They were not included in any quantitative analyses.

The ALSSQOL comprises six domains: Negative Emotion (12–14, 19–21, 23, 24, 28, 32, 35, 36, and 38); Interaction with People and the Environment (15, 17, 18, 22, 29, 30, 34, 37, 44, 45, and 49); Intimacy (48, 51, 52, 54, 55, 57, and 58); Religiosity (25, 33, 43, and 46); Physical Symptoms (1, 2, and 8–11); and Bulbar Function (3–6 and 26).

Caregiver Burden Inventory

The Caregiver Burden Inventory (CBI) has been validated to assess the quality of life of the caregiver as it relates to social, emotional, physical, time, and developmental aspects of the his or her relationship with the study participant with ALS. The CBI consists of 24 questions, and each item is rated on a scale from 0 to 4 (total score range, 0–96). Higher scores indicate more negative outcomes.

Webappendix C: Treatment-emergent adverse events

Table I. Treatment-emergent adverse events occurring in ≥5% of subjects in either treatment group, n (%)

CTCAE category ***CTCAE term***	**Ceftriaxone (n=340)**	**Placebo (n=173)**	**p value***
Gastrointestinal	245 (72)	97 (56)	0.0004
Diarrhea	*153 (45)*	*41 (24)*	*<0·0001*
Dermatology/skin	211 (62)	92 (53)	0·0578
Rash/desquamation	*92 (27)*	*29 (17)*	*0·0112*
Hepatobiliary/pancreas	211 (62)	19 (11)	<0·0001
Cholelithiasis	*181 (53)*	*5 (3)*	*<0·0001*
Biliary sludge	*100 (29)*	*12 (7)*	*<0.0001*
Cholecystitis	*15 (4)*	*0 (0)*	*0·0036*
Pain	200 (59)	88 (51)	0·0910
Abdominal (NOS)	*78 (23)*	*17 (10)*	*0·0003*
Infection	171 (50)	100 (58)	*0·1126*
ALT SGPT	*32 (9)*	*5 (3)*	*0·0063*
AST SGOT	*23 (7)*	*4 (2)*	*0·0360*
Line infections	*5 (1)*	*11 (6)*	*0·0052*
Infection with grade 3/4 neutropenia	*1 (0)*	*5 (3)*	*0·0182*
Neurology	162 (48)	83 (48)	1·0000
Pulmonary/upper respiratory	159 (47)	82 (47)	0·9256
Nasal/paranasal sinus reactions	*1 (0)*	*4 (2)*	*0·0464*
Musculoskeletal/soft tissue	140 (41)	61 (35)	0·2141
Constitutional symptoms	109 (32)	55 (32)	1·0000
Renal/genitourinary	80 (24)	35 (20)	0·4342
Metabolic/laboratory	70 (21)	27 (16)	0·1907
Hyponatremia	*0 (0)*	*3 (2)*	*0·0379*
Blood/bone marrow	61 (18)	15 (9)	0·0055
Neutrophils/granulocytes (ANC/AGC)	*31 (9)*	*2 (1)*	*0·0002*
Leukocytes (total WBC)	*21 (6)*	*2 (1)*	*0·0111*

Table I. Treatment-emergent adverse events occurring in ≥5% of subjects in either treatment group, n (%) (*cont'd*)

CTCAE Category ***CTCAE Term***	**Ceftriaxone (n=340)**	**Placebo (n=173)**	**p value***
Vascular	58 (17)	28 (16)	0·9006
Hot flashes	*1 (0)*	*4 (2)*	*0·0464*
Line thrombosis	*1 (0)*	*4 (2)*	*0·0464*
Thrombosis/thrombus/embolism	*30 (9)*	*6 (3)*	*0·0275*
Lymphatics	57 (17)	23 (13)	0·3677
Allergy/immunology	49 (14)	23 (13)	0·7890
Cardiac general	25 (7)	10 (17)	0·3944
Ocular/visual	17 (5)	3 (2)	0·0908

AGC=absolute granulocyte count; ALT=alanine aminotransferase; ANC=absolute neutrophil count; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; NOS=not otherwise specified; SGOT=serum glutamic oxaloacetic transaminase; SGPT=serum glutamic pyruvic transaminase; WBC=white blood cell.

*Fisher's exact test

Table II. Serious adverse events occurring in ≥5% of participants in each treatment group and all catheter-related serious adverse events, n (%)

CTCAE category	Ceftriaxone (n=340)	Placebo (n=173)	p value*
Pulmonary	88 (26)	38 (22)	0·3855
Hepatobiliary/pancreas	41 (12)	0 (0)	<0·0001
Infection	30 (9)	32 (18)	0·0024
Vascular	24 (7)	6 (3)	0·1142
Gastrointestinal	18 (5)	14 (8)	0·2473
Catheter-related event			
Vascular	11 (3)	2 (1)	0·2356
Infection	7 (2)	14 (8)	0·0018
Pulmonary	2 (1)	1 (1)	1·0000
Constitutional symptoms	1 (0)	2 (1)	0·2640
Hemorrhage	1 (0)	0 (0)	1·0000
Neurology	0 (0)	1 (1)	0·3372

CTCAE, Common Terminology Criteria for Adverse Events.

*Fisher's exact test.

Table III. Adverse event rate per month[*] in participants taking ceftriaxone alone (stages 1 and 2) and after add-on ursodiol (stage 3)

	Stages 1 and 2	stage 3	
	Ceftriaxone[†]	Ceftriaxone[†] + ursodiol	
CTCAE category	**(events/mo)**	**(events/mo)**	**p value**
Gastrointestinal	0·577	0·137	<0·0001
Pain	0·352	0·095	<0·0001
Hepatobiliary/pancreas	0·310	0·081	<0·0001
Constitutional symptoms	0·141	0·035	0·0006
Pulmonary/upper respiratory	0·127	0·059	0·0595
Neurology	0·084	0·075	0·8901
Blood/bone marrow	0·056	0·025	0·2139
Infection	0·056	0·073	0·8201

CTCAE=Common Terminology Criteria for Adverse Events.
[*]≥4 total events in either stages 1/2 or stage 3.
[†]4-g dosage.