Randomized double-blind placebo-controlled trial of acetyl-L-carnitine for ALS

Article · February 2013

DOI: 10.3109/21678421.2013.764568 · Source: PubMed

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Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2013; Early Online: 1–9

ORIGINAL ARTICLE

Randomized double-blind placebo-controlled trial of acetyl-L-carnitine for ALS

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Abstract

Our objective was to assess the effects of acetyl-L-carnitine (ALC) with riluzole on disability and mortality of amyotrophic lateral sclerosis (ALS). Definite/probable ALS patients, 40–70 years of age, duration 6–24 months, self-sufficient (i.e. able to swallow, cut food/handle utensils, and walk), and with forced vital capacity (FVC) $>80\%$ entered a pilot double-blind, placebo-controlled, parallel group trial and were followed for 48 weeks. ALC or placebo 3 g/day was added to riluzole 100 mg/day. Primary endpoint: number of patients no longer self-sufficient. Secondary endpoints: changes in ALSFRS-R, MRC, FVC and McGill Quality of Life (QoL) scores. Analysis was made in the intention-to-treat (ITT) and per-protocol (PP) population, completers and completers/compliers (i.e. taking $\geq 75\%$ of study drug). Forty-two patients received ALC and 40 placebo. In the ITT population, 34 (80.9%) patients receiving ALC and 39 (97.5%) receiving placebo became non-self-sufficient ($p = 0.0296$). In the PP analysis, percentages were 84.4 and 100.0% ($p = 0.0538$), respectively. Mean ALSFRS-R scores at 48 weeks were 33.6 (SD 10.4) and 27.6 (9.9) ($p = 0.0388$), respectively, and mean FVC scores 90.3 (32.6) and 58.6 (31.2) ($p = 0.0158$), respectively. Median survival was 45 months (ALC) and 22 months (placebo) ($p = 0.0176$). MRC, QoL and adverse events were similar. In conclusion, ALC may be effective, well-tolerated and safe in ALS. A pivotal phase III trial is needed.

Key words: *Acetyl-L-carnitine, amyotrophic lateral sclerosis, motor neuron disease, randomized trial*

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(Received 6 November 2012; accepted 3 January 2013)

ISSN 2167-8421 print/ISSN 2167-9223 online © 2013 Informa Healthcare
DOI: 10.3109/21678421.2013.764568

Introduction

Amyotrophic lateral sclerosis (ALS) is a rare, severe, progressive neurological disorder causing selective degeneration of the motor neurons. Several mechanisms have been proposed, including oxidative stress, neurofilament damage, mitochondrial abnormalities, glutamate-mediated excitotoxicity, and altered response to hypoxia (1). The only drug shown to improve survival in ALS is riluzole, which appears to block the presynaptic release of glutamate (2). However, the benefits consist of a three-month delay of death while disability and other outcome measures are virtually unaffected.

Acetyl-L-carnitine (ALC), the acetyl ester of L-carnitine, is a donor of acetyl groups that increases intracellular levels of carnitine and serves as a major transporter of fatty acids across the mitochondrial membranes (3). ALC counteracts motor neuron death induced by toxic agents or deprivation of trophic factors (4) and slows the disease progression in animal models with neurodegenerative disorders mimicking ALS (5). Its postulated mechanism of action is complementary to that of riluzole (6), which suggests the possibility of a combined action. An additive effect of ALC and riluzole was documented in the survival of transgenic mutant SOD1 mice when the combination was compared to ALC and riluzole singly and to vehicle recipients (Caterina Bendotti, personal communication).

These findings prompted a randomized trial in humans comparing patients with ALS receiving ALC and riluzole or riluzole monotherapy. The study aims were to assess: 1) the superiority of the combination over riluzole alone on functional disability in a clinically significant and measurable way; 2) the impact of the two treatments on mortality; 3) the tolerability and safety of the drugs at the doses used.

Methods

Study design and eligibility criteria

This was a not-for-profit, multicentre, randomized, placebo-controlled, parallel-arm (1:1), pilot phase II trial. Males and females aged 40 through 70 years with definite, probable (laboratory supported) or probable ALS according to the revised El Escorial diagnostic criteria (7) were eligible. Additional requirements were bulbar-onset or spinal-onset ALS with 6–24 months disease duration, satisfactory bulbar and spinal function (score 3 + on the ALS Functional Rating Scale- Revised (ALSFRS-R) (8) for swallowing, cutting food and handling utensils, and walking); satisfactory respiratory function (forced vital capacity, FVC, $>80\%$ of predicted); documented progression of symptoms in the last three months; ability to understand, comply with the study requirements, and give written informed consent. Exclusion criteria were familial ALS, antecedent polio infection, other motor neuron diseases (MND), involvement of other neurological systems, exposure to metals, other severe clinical conditions, poor compliance with previous treatments, experimental treatments, pregnancy or breast feeding, unwillingness or inability to take riluzole.

Treatment plan

All eligible patients were randomized to receive ALC or placebo in addition to riluzole tablets 50 mg b.i.d. Treatment allocation was centrally managed using a computer-generated, permuted block (with a block size of 4), 1:1 randomization scheme. A separate computer-generated randomization list was prepared and sent in sealed envelopes to each centre. Experimental treatment included ALC in powder 500 mg per packet, or an equivalent placebo, supplied by Sigma-Tau Industrie Farmaceutiche Riunite, Pomezia, Italy. The dose was two packets t.i.d. Active treatment and placebo were indistinguishable to sight and taste. Symptomatic and palliative treatments given during the study were permitted and recorded.

Study assessments

Patients' eligibility was tested, within 28 days before randomization visit (baseline). Eligible patients were then seen after 4, 12, 24, 36 and 48 weeks and data were collected using a structured web-based case-record form. At each visit, a general assessment was made, including vital signs, body mass index (BMI), comorbidity and medication record, with a neurological examination, including assessment of nerve function and quantitative and qualitative evaluation of the motor system. Functional disability was assessed using the ALSFRS-R scale. Muscle strength was measured with the Medical Research Council (MRC) scale (9) in the following muscles and muscle groups: deltoid, biceps brachii, triceps brachii, flexors and extensors carpi, finger flexors and extensors, opponent of the thumb, ileopsoas, quadriceps femori, leg flexors, tibialis anterior, triceps surae, extensor of the toe. Respiratory function was assessed using a spirometer to measure FVC before starting treatment (screening and baseline visit) and at 12, 24, 36 and 48 weeks.

Health-related quality of life (HRQoL), measured by the McGill Quality of Life questionnaire (10), was tested at baseline, 12 and 48 weeks. Biochemical and blood tests, chest X-rays and ECG were performed at baseline and whenever clinically indicated. Cognitive functions were not tested.

All adverse events (AEs) encountered and any serious events were to be recorded using the Coding Symbols for a Thesaurus of Adverse Reaction Terms (11). Severity was graded according to the modified WHO criteria for toxicity (http://www.regsource.com/_Adverse_Event_Reporting/_adverse_event_reporting.html) where applicable.

Compliance was tested by the local investigators, counting unused packages at each follow-up visit

and confirmed by the study monitor. As per study protocol, treating and evaluating physicians were blinded to the study treatment.

Statistical analysis

The primary endpoint was the proportion of patients no longer self-sufficient after 12 months, defined as individuals scoring 2 or lower on at least one of the ALSFRS-R items for swallowing, cutting food and handling utensils, or walking. Although this endpoint has not been formally validated, it is fairly similar to that used in other randomized trials conducted by us (12,13) and others (14). Secondary endpoints included total MRC and ALSFRS-R scores, FVC score, proportion of cases who died or needed tracheostomy, proportion of therapeutic failures (for ineffectiveness and/or AEs), total and subtotal (each domain) McGill scores.

Based on a previous study (13), non-self-sufficient patients after 12 months were expected to be approximately 60%. A total of 80 patients (40 in each arm) had to be randomized to have 80% power to detect a 30% absolute reduction (from 60% to 30%) in the primary endpoint in the group treated with ALC, with a 5% two-sided type 1 error.

Pre-planned statistical analyses were carried out on the following: 1) intention-to-treat (ITT) population, including all randomized patients receiving at least one dose of study medication (primary approach); 2) study completers, i.e. patients completing the 12-month follow-up (secondary analysis); 3) study completers and compliers, i.e. patients completing the 12-month follow-up and taking at least 75% of the assigned drug (secondary analysis). After withdrawal of all protocol violations, post hoc statistical analyses were carried out on the per-protocol population.

Demographic and clinical features were described using absolute and relative frequencies for discrete variables, and mean and standard deviation (SD) or median and range for continuous variables. The two treatment groups were compared using the χ^2 or Fisher's exact test for discrete variables and the Mann-Whitney test for continuous

Table I. Demographic and clinical characteristics of the sample.

	Treatment		
	Acetyl-L-carnitine (42) *n* (%)	Placebo (40) *n* (%)	*p*-value
Gender			
Female	18 (42.9)	14 (35.0)	0.4660
Male	24 (57.1)	26 (65.0)	
Age (years)			
<50	10 (23.8)	3 (7.5)	0.1159
50–59	10 (23.8)	11 (27.5)	
60–69	15 (35.7)	22 (55.0)	
70+	7 (16.7)	4 (10.0)	
Onset			
Bulbar	13 (31.0)	9 (22.5)	0.3879
Spinal	29 (69.0)	31 (77.5)	
Disease duration (months)*			
6–12	21 (51.2)	23 (57.5)	0.5705
13–24	20 (48.8)	17 (42.5)	
General examination			
Abnormal	9 (21.4)	11 (27.5)	0.5222
Normal	33 (78.6)	29 (72.5)	
Comorbidity			
0	23 (54.8)	17 (42.5)	0.2668
1+	19 (45.2)	23 (57.5)	
BMI			
<25	23 (56.1)	25 (62.5)	0.5577
≥ 25	18 (43.9)	15 (37.5)	
ns	1	–	

	Treatment		
	Acetyl-L-carnitine (42) median (min-max)	Placebo (40) median (min-max)	*p*-value
Age (years)	61 (38–74)	63 (39–73)	0.5421
BMI	23.9 (18.7–35.4)	24.0 (16.7–31.0)	0.3525
ALSFRS-R	43 (34–47)	42 (31–48)	0.3062
MRC	131 (86–150)	127 (68–150)	0.5782
FVC	99 (62–137)	94 (75–124)	0.1957
McGill	6.2 (4.9–9.0)	6.6 (4.7–8.4)	0.3040

*One patient had more than 24 months disease duration.

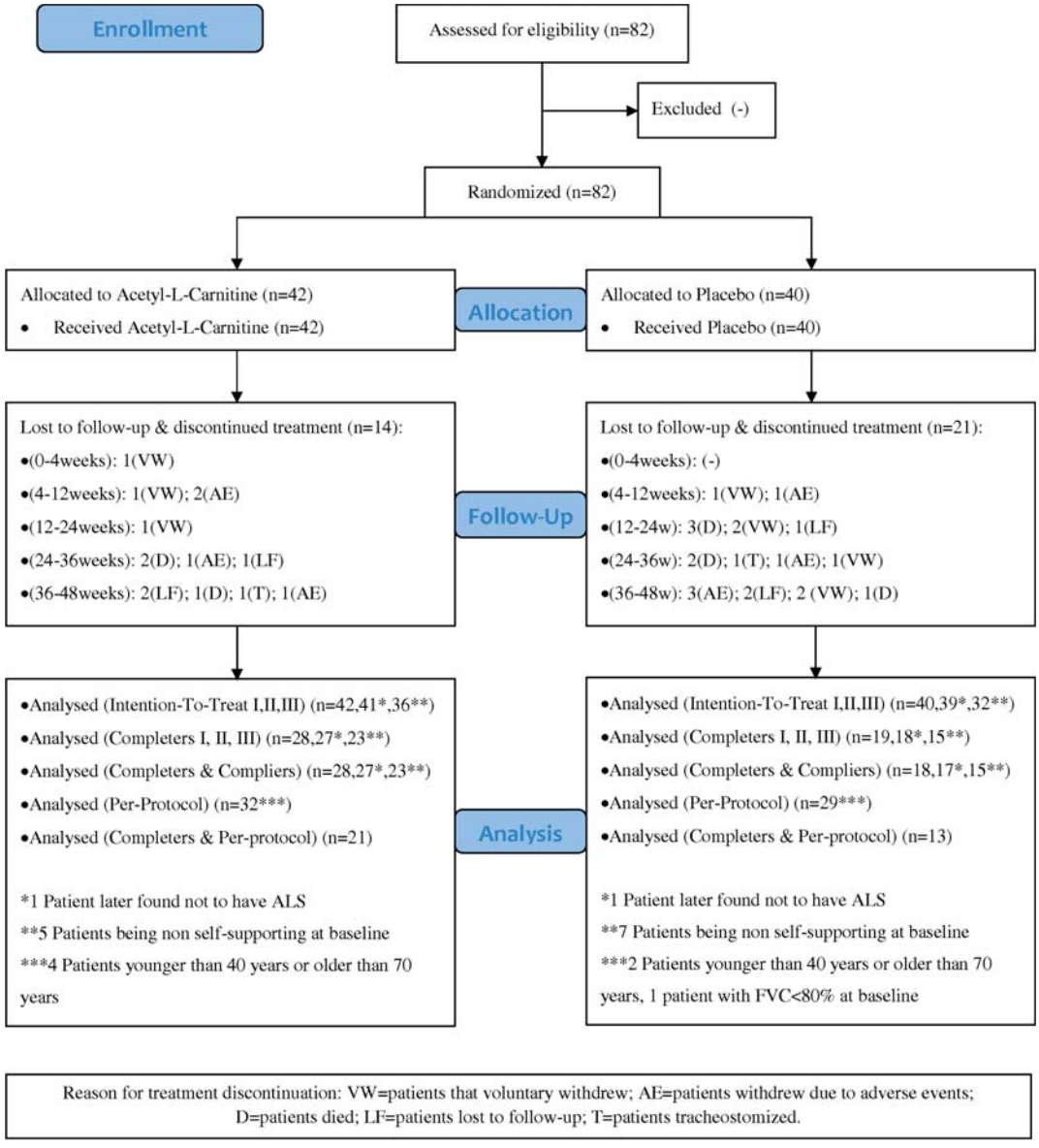

Figure 1. Study flow chart. The flow chart indicates the number of patients enrolled, the number withdrawn at each follow-up, and the number analysed in each dataset.

variables. A multivariate model was also used to test the primary hypothesis, adjusting for age, gender, onset, disease duration, comorbidity, and baseline ALSFRS-R score. This model was assessed using the SAS procedure PROC GENMOD and risks were computed as Relative Risks (RR) with 95% confidence intervals (95% CI). The same independent variables were used to perform a multivariable predictive logistic model in order to assess their prognostic value on self-sufficiency status at the end of the study in terms of Area Under the Curve (AUC) values.

Changes over time in the ALSFRS-R, MRC, FVC and McGill scores from baseline were assessed using the repeated measures analysis of variance (ANOVArm). The distribution of the values analysed according to the ANOVArm model was assessed with the Shapiro-Wilk's test. ANOVArm was assessed using a mixed effect model with the SAS procedure PROC MIXED using an approach already addressed by two of the authors (15). Correlations within patients were handled using the 'unstructured' correlation matrix. The results of ANOVArm are displayed as 'treatment', 'time' and 'treatment*time' effects. Overall survival and time to stopping treatment were calculated with the Kaplan-Meier method, and time to non-self-sufficient status with the actuarial method. Comparisons were assessed using the log-rank test and multivariable Cox's proportional hazard models. Risks were computed as hazard ratios (HR) with 95% CI. As post hoc analysis, the two treatment groups were ranked according to different outcomes and compared using the 'Joint-Rank' analysis (16,17) overall and by gender and ALS onset. To compare the progression rates of ALSFRS-R in the two groups from the onset of symptoms to the start of treatment, we used the formula proposed by Kimura et al. (18). Statistical significance was

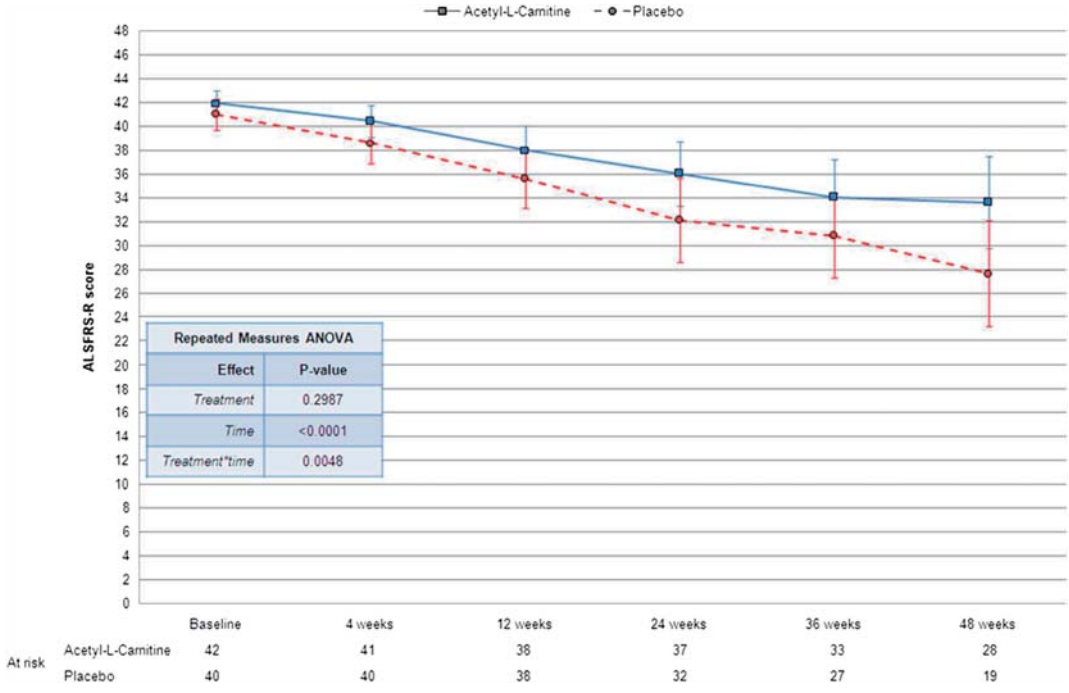

Figure 2. ALSFRS scores over time. Blue squares (acetyl-L-carnitine) and red dots (placebo) represent mean ALSFRS-R scores (with 95% confidence intervals) at baseline and each follow-up visit. The table reports the results of the mixed effect repeated measures model.

assessed with the signed rank test. To take into account the three analyses on the primary endpoint, the Holm-Bonferroni correction was applied to the type 1 error rate. The study statistician was blinded to the assigned treatment during the assessment of the primary endpoint.

The trial was approved by the ethics committees of each institution and coordinated by the Istituto Mario Negri, Milano, following the principles of Good Clinical Practice. A data monitoring committee (DMC) reviewed the confidential results of interim analyses.

Results

The trial was conducted in 16 experimental centres that enrolled and assigned participants to interventions. With only one exception (a patient with a serious adverse event), all investigators remained blind to treatment assignment throughout the study. Given the slow recruitment rate, after approval by the DMC, one interim analysis was performed in 46 patients (23 in each arm). The results on the primary endpoint did not show any difference ($p = 0.76$); however, a trend favouring active treatment was observed for the ALSFRS-R and for the MRC scores (data not shown) and led the investigators to complete the recruitment as established by the protocol.

Between 1 March 2007 and 9 February 2010, 82 patients were enrolled; 42 received ALC and 40 placebo. The sample comprised 50 males and 32 females, aged 38–74 years. Sixty patients had spinal-onset and 22 bulbar-onset ALS. Age, gender, site of onset, disease duration, general examination, comorbidity, BMI, mean ALSFRS-R, MRC, FVC and McGill scores were equally distributed in the two arms (Table I). The numbers of patients at each follow-up visit are depicted in Figure 1. Two patients (one in each arm) were later found not to have ALS. They had inclusion bodies myositis (placebo) and Kennedy syndrome (ALC). Other protocol violations included being non-self-sufficient at baseline (ALC, 5; placebo, 7), age younger than 40 years or older than 70 years (ALC, 4; placebo, 2), and FVC < 80% (placebo, 1). In one patient aged less than 40 years, disease duration exceeded 24 months. None received incorrect study treatment or doses or experimental medications.

In the ITT population ($n = 82$), 34 patients receiving ALC (80.9%) and 39 receiving placebo (97.5%) became non-self-sufficient ($p = 0.0296$). This gave a relative risk (RR) (95% CI) of 0.83 (0.71–0.97) and a number needed-to-treat (NNT) of 6 (95% CI 3–27). The multivariate model gave an RR (95% CI) of 0.86 (0.74–0.99) ($p = 0.0308$). When all protocol violations were removed from the analysis (per-protocol population, $n = 61$), the number of non-self-sufficient patients fell to 27 (84.4%) for ALC and 29 (100.0%) for placebo ($p = 0.0538$). The corresponding numbers among study completers ($n = 47$) were 20 (71.4%) and 18 (94.7%) ($p = 0.0640$). Table III gives details of the primary outcome in the study populations. Other details of the primary outcome in the other subpopulations are reported in e-Table 1 – which is only available in the online version of the journal. Please find this material with the following direct link to the article: http://informahealthcare.com/doi/abs/10.3109/21678421.2013.764568. The multivariable predictive model of self-sufficiency

reported ALC treatment (AUC = 0.72, *p*-value = 0.0272) and having a higher ALSFRS-R baseline score (AUC = 0.86, *p*-value = 0.0103) as the only significant predictors while age ($p = 0.8707$), gender ($p = 0.3415$), onset ($p = 0.1029$), disease duration ($p = 0.5667$), and comorbidity ($p = 0.2415$) were not significant.

The mean (standard deviation, SD) ALSFRS-R scores at 48 weeks were 33.6 (10.4) with ALC and 427.6 (9.9) with placebo ($p = 0.0388$) (see e-Table 2 – which is only available in the online version of the journal. Please find this material with the following direct link to the article: http://www. informahealthcare.com/(DOI:10.3109/21678421.2013.764568)), with a significant effect of time ($p < 0.0001$) and a significant interaction between treatment and time ($p = 0.0048$). There was more than a six-month difference in functional decline between the two groups (Figure 2). The mean (SD) monthly progression rate of ALSFRS-R from onset of symptoms to treatment start was similar in the two groups: respectively − 0.52 (0.44) and − 0.58 (0.37) for ALC and placebo ($p = 0.2867$). Differences arose after starting treatment, especially in the first six months, − 0.97 (1.09) and − 1.60 (1.39) ($p = 0.0247$), respectively.

The mean FVC at 48 weeks was higher with ALC than placebo (90.3 (32.6) vs. 58.6 (31.2); $p = 0.0158$) (see e-Table 2 – which is only available in the online version of the journal. Please find this material with the following direct link to the article: http://informahealthcare.com/doi/abs/ 10.3109/21678421.2013.764568), with no significant interaction between treatment and time ($p = 0.3833$). No differences were detected in the mean MRC score at 48 weeks (105.4 (36.9) and 97.3 (35.5) (e-Table 2 – which is only available in the online version of the journal. Please find this material with the following direct link to the article: http://informahealthcare.com/doi/abs/10.3109/21678421.2013.764568), but the interaction was significant between treatment and time ($p = 0.0316$). HRQoL at 48 weeks was similar, in general and for each individual domain (e-Table 3 – which is only available in the online version of the journal. Please find this material with the following direct link to the article: http://informahealthcare.com/doi/abs/ 10.3109/21678421.2013.764568).

The baseline distribution of ALSFRS-R, FVC, MRC and HRQoL was assessed using the Shapiro-Wilk's (SW) test. HRQoL had a normal distribution (SW = 0.983, $p = 0.3128$), while ALSFRS-R, FVC and MRC were skewed (SW = 0.961; 0.967; 0.932, $p = 0.038$; 0.0134; 0.0003, respectively). However, the quintiles analysis revealed distributed fairly normal data distribution and we decided to apply the planned ANOVArm on these data.

Survival analysis of the primary endpoint showed a trend towards greater efficacy of ALC over placebo ($p = 0.0780$), which disappeared in the per-protocol population ($p = 0.2298$). In the Cox's model, compared to placebo, patients receiving ALC had a 0.72 (0.45–1.16) hazard ratio (HR) of becoming non-self-sufficient ($p = 0.1804$). In the ITT population, the cumulative probability of death during the study was similar in the two treatment arms, both in the ITT ($p = 0.2054$) and in the per-protocol population ($p = 0.1547$). Results were similar in the per-protocol population (data not shown). Using the 'Joint Rank Analysis', significant between-treatment differences were found both in the ITT and in the per-protocol population (e-Table 4 – which is only available in the online version of the journal. Please find this material with the following direct link to the article: http://informahealthcare.com/doi/abs/10.3109/21678421.2013.764568). All the 'best five outcomes' received ALC.

Compliance was satisfactory in both treatment groups. The mean (SD) percentage of unused packages was 4.1 (7.9) in the ALC group and 7.7 (12.6) in the placebo group ($p = 0.2642$). Patients taking at least 80% of the study drug were 40 (ALC) and 34 (placebo). Twenty-seven patients receiving ALC and 21 receiving placebo reported one or more AEs. Seventeen AEs were mild (ALC, 9; placebo, 8), 10 moderate (ALC, 7; placebo, 3), three severe (ALC, 1; placebo, 2), and 17 serious (ALC, 9; placebo, 8). Table 3 lists the AEs by organ/system. Serious AEs included nine deaths (ALC, 3; placebo, 6). Only gastric pain, diarrhoea and gastric

Table II. Effects of treatment on loss of independence (primary endpoint).

	ALC *n* (%)	Placebo *n* (%)	*p*-value	RR (95% CI)	%RD (95% CI)	NNT (95% CI)
Primary analysis						
ITT I (82 pts)						
Non-self-sufficient	34 (80.9)	39 (97.5)	0.0296	0.83 (0.71–0.97)	− 16.6 (− 2.8, − 31.0)	6 (3–27)
Self-sufficient	8 (19.1)	1 (2.5)				
Secondary analysis						
PP (61 pts)						
Non-self-sufficient	27 (84.4)	29 (100.0)	0.0538	0.84 (0.73–0.98)	− 15.6 (− 1.0, − 31.8)	6 (4–33)
Self-sufficient	5 (15.6)	0 (−)				
Completers I (47 pts)						
Non-self-sufficient	20 (71.4)	18 (94.7)	0.0640	0.75 (0.58–0.98)	− 23.3 (+ 0.2, − 42.3)	4 (2–26)
Self-sufficient	8 (28.6)	1 (5.3)				

ITT I: intent to treat, randomized >= 1 dose; PP: fulfils all aspects per-protocol; Completers I: completed 12 months follow up

intolerance were considered drug-related. Biochemical and haematological assays, where available, were unrevealing.

Fourteen patients assigned to ALC (33.3%) discontinued treatment (death/tracheostomy, 4; withdrawal of consent, 4; AE, 3; lost to follow-up, 3). Twenty-one patients on placebo (52.5%) stopped treatment (death/tracheostomy, 7; withdrawal of consent, 6; AE, 5; lost to follow-up, 3) ($p = 0.0794$). Eight of the nine deaths were attributed to disease progression, and one (placebo) to traumatic brain injury following an accidental fall.

Subgroup analyses showed more evident efficacy of ALC in males; no difference was detected between spinal- and bulbar-onset ALS and between younger and older individuals (data not shown).

As of 30 June 2011, 17 (40.5%) patients treated with ALC and 26 (65.0) receiving placebo were reported dead. The Kaplan-Meier method found a significant difference in the median survival time (ALC, 45 months; placebo, 22 months; $p = 0.0176$). When adjusting survival for age, gender, general assessment at baseline, onset, disease duration and ALSFRS score at baseline in the Cox's model, we found an HR (95% CI) of 0.50 (0.27 − 0.93) ($p = 0.0282$).

Discussion

This study provides suggestions in support of the efficacy and safety of ALC for the treatment of ALS. After one year of treatment, more individuals were still self-sufficient with active treatment than placebo. A number of secondary analyses confirmed the superiority of active treatment. At the end of follow-up, the ALSFRS score and the FVC were higher among ALC than placebo recipients, with significant correlations with time and time-per-treatment interactions (only for ALSFRS-R score). However, as FVC varied widely within and between patients, we carried out a new repeated measures ANOVA transforming FVC% into its reciprocal (1/FVC%) to overcome this variability. The new estimates provided a significant interaction between treatment and time ($p = 0.0331$). Even the MRC score presented a significant correlation (and interaction) with time. The number of AEs, serious AEs, AEs leading to withdrawal and deaths did not differ between treatments, a finding in favour of ALC safety even when associated with riluzole. Furthermore, when combining efficacy and safety in the joint rank analysis, patients treated with ALC obtained the best results.

Table III. Adverse events and serious adverse events in patients receiving acetyl-L-carnitine (ALC) and placebo, by organ-system.

	AE			SAE		
	ALC	Placebo	Overall comparison	ALC	Placebo	Overall comparison
Death	–	–	–	3	6	3 vs. 6
Circulatory system						
Brugada syndrome	1	–	3 vs. 0	–	–	3 vs. 1
Deep vein thrombosis	1	–		–	–	
Myocardial infarction	–	–		–	1	
Pneumonia	–	–		1	–	
Respiratory insufficiency	–	–		2	–	
Water retention	1	–		–	–	
Digestive system						
Bowel irregularities and Constipation	–	4	10 vs. 12	–	–	1 vs. 1
Cholelithiasis		–	–		1	–
Diarrhoea	1	2		–	–	
Drooling	3	2		–	–	
Drug intolerance	–	1		–	–	
Epigastralgia	1	1		–	–	
Gastric/hepatic intolerance	2	–		–	–	
Gastroesophageal reflux	1	–		–	–	
Gastrorrhagia						
Haemorrhoids	1	–		–	–	
Hepatic insufficiency	–	1		–	–	
Intestinal disorder	1	–		–	–	
Meteorism	–	1		–	–	
Musculoskeletal system						
Fracture	1	1	2 vs. 1	1	–	2 vs. 0
Skull trauma	1	–		1	–	
Nervous system						
Retinal haemorrhage	1	–	1 vs. 0	–	–	–
Urinary system						
Urinary urgency	1	–	1 vs. 0	–	–	–
Vestibular system						
Dizziness	1	–	1 vs. 0	–	–	–

Our findings are in line with studies in animals and humans. In vitro ALC 10 mM had neurotrophic effects on cultured motor neurons. The drug also reduced motor neuron mortality induced by kainate exposure or serum/BDNF deprivation (5,19). In wobbler male mice treated with ALC 75 mg/kg/day for five weeks starting at age four weeks, the progression of symptoms was slower than in untreated controls (5).

The efficacy of treatment has also been tested in transgenic mutant SOD1 mice treated with ALC, riluzole, ALC and riluzole, or vehicle before the onset of symptoms (Caterina Bendotti, personal communication). In this unpublished study, animals receiving ALC or riluzole as monotherapy had better survival than animals treated with vehicle, and those on ALC-riluzole combination had significantly better survival. However, in a subsequent unpublished study this investigator found no differences in survival when the animals were treated after symptom onset. The use of a strain with more severe and short-lasting disease might explain the negative results.

Fatigue is a common symptom in patients with ALS (20,21). Compared to patients without, patients with fatigue are significantly more disabled (22). Treatment with ALC has been shown to improve fatigue in patients with chronic fatigue syndrome (23,24) or peripheral neuropathy (25–27).

L-carnitine improves fatigue and nutritional status in cancer patients (28). Long-term treatment with L-carnitine exerts a specific trophic action human < on type 1 muscle fibres (29). Altered nutritional status in patients with ALS is not uncommon (30) and malnutrition is an independent prognostic factor for survival during the course of the disease (31).

Study limitations

The first major limitation is the small sample size, but this was only a pilot trial whose results require confirmation through a large, pivotal, phase III trial. The second major limitation is the use of a non-validated primary endpoint (proportion of patients becoming non-self-sufficient); however, loss of independence during daily living activities, as measured by selected items of the ALSFRS-R scale, over a short period (12 months) is a strong indicator of treatment efficacy. Different measures (including ALSFRS-R, FVC and, in part, MRC scores) also support the putative effect of ALC. Thirdly, the proportion of patients becoming non-self-sufficient in the control group is higher than that expected from the power calculations; in our previous study we used the Norris disability scale (32), which was perhaps less sensitive than the ALSFRS-R scale in measuring functional deterioration. Our findings can be also explained by the study population, mostly represented by newly diagnosed patients. In this regard, some individuals presenting rapid worsening of symptoms at disease onset might have been included. Fourthly, there were protocol violations: two patients were found not to have ALS and in 12 others independence was not confirmed at randomization. However, a significant treatment effect was retained even after several sensitivity analyses. Fifthly, survival analysis did not confirm treatment efficacy during the 12-month follow-up. However, a significant difference in the death rates was found after extended follow-up. Sixth, with the present sample we could only test one dose, the highest ever used in clinical trials (33); we do not know the risk:benefit ratio of higher doses. Seventh, treatment effect was apparent only in males. This can be explained by the small numbers because there was a tendency towards greater efficacy even in females. Finally, the effects of ALC have been tested in self-sufficient patients and cannot be confirmed in those with more advanced disease.

In light of the above limitations, we are still uncertain whether the results of this study are sufficient to support a claim of efficacy for ALC. However, our data cannot be interpreted solely as chance findings. Given the paucity of effective treatments, this trial should not be discarded but considered the scientific background for a confirmatory trial.

Acknowledgements

The study was funded by Agenzia-Italiana-del-Farmaco (FARM5RLKR9). Sigma-Tau provided ALC and placebo free of charge. The authors are indebted to Susanna Franceschi and Stefania Moia for secretarial assistance. The study was also carried out on behalf of the 'Fondazione Aldo & Cele Daccò per la Ricerca Scientifica' and the European Union, grant agreement 259867. The manuscript was edited by J. D. Baggott.

Disclosure of interests: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. E. Beghi serves on the editorial advisory boards of Epilepsia, Amyotrophic Lateral Sclerosis, Clinical Neurology & Neurosurgery, and Neuroepidemiology; he has received funding for travel and speaker honoraria from UCB-Pharma, Sanofi-Aventis, GSK, EISAI; funding from GSK for educational presentations, and from AIFA, Sanofi-Aventis, Janssen-Cilag, EISAI, Lombardy Region, Istituto Superiore di Sanità and American ALS Association for the coordinating activity of RCT and observational study protocol. F. Giannini received funding from Kedrion SpA. V. La Bella received funding from the 'Mario Negri' Institute for pharmacological research, for patient recruitment and follow-up.

L. Mazzini received funding from the 'Mario Negri' Institute for pharmacological research and writing assistance. P. Messina has received funding from AIFA, Sanofi-Aventis, Janssen-Cilag, EISAI, Lombardy

Region, Istituto Superiore di Sanità and American ALS Association for the data analysis and data management of RCT and observational study protocol. E. Pupillo has received funding from AIFA, Italian Ministry of Health, Istituto Superiore di Sanità, American ALS Association for data management and data monitoring of RCT and observational study protocol.

V. Silani received funding from BIOGEN for ALS Clinical Trial. A. Chiò received funding from BIOGEN Idec. I. L. Simone has received funding from Sanofi-Aventis as personal honoraria for educational lectures.

The authors alone are responsible for the content and writing of the paper.

The study was funded by Agenzia-Italiana-del-Farmaco (FARM5RLKR9). Sigma-Tau provided ALC and placebo free of charge.

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Supplementary materials available online

e- Tables 1–3.

Supplementary material for Beghi E, et al. Randomized double-blind placebo-controlled trial of acetyl-L-carnitine for ALS, Amyotroph Lateral Scler. 2013; doi: 10.3109/21678421.2013.764568.

e- Tables 1–3

e-Table 1. Effects of treatment on loss of independence (primary endpoint) in other subpopulations.

			ALC	Placebo		RR (95% CI)	%RD (95% CI)	
			n (%)	*n* (%)	*p*-value			NNT (95% CI)
Primary analysis	ITT II (80 pts)	Non-self-sufficient	34 (82.9)	39 (100.0)	0.0357*	0.83 (0.72-0.95)	-17.1 (-4.7, -31.3)	6 (3-18)
		Self-sufficient	7 (17.1)	0 (-)				
	ITT III (68 pts)	Non-self-sufficient	29 (80.6)	32 (100.0)	0.0242*	0.81 (0.69-0.95)	-19.4 (-5.0, -35.0)	5 (3-15)
		Self-sufficient	7 (19.4)	0 (-)				
Secondary analysis	Completers II (45 pts)	Non-self-sufficient	20 (74.1)	18 (100.0)	0.0313	0.74 (0.59-0.93)	-25.9 (-4.2, -44.7)	4 (2-11)
		Self-sufficient	7 (25.9)	0 (-)				
	Completers III (38 pts)	Non-self-sufficient	16 (69.6)	15 (100.0)	0.0291	0.70 (0.53-0.91)	-30.4 (-5.2, -50.9)	3 (2-9)
		Self-sufficient	7 (30.4)	0 (-)				
	Completers & PP (34 pts)	Non-self-sufficient	16 (76.2)	13 (100.0)	0.1317	0.76 (0.60-0.97)	-23.8 (+2.5, -45.1)	4 (2-18)
		Self-sufficient	5 (23.8)	0 (-)				

* Adjusted *p* (Holm-Bonferroni Correction), original *p*-values (in order of appearance) were: 0.0119 and 0.0121.
ITT II: ITT population that actually has ALS; ITT III: same as ITT II and age 40• 70 years and FVC >=80%; Completers II: completed 12 months follow-up and actually have ALS; Completers III: same as Completers II and age 40• 70 years and FVC >=80%; Completers & PP: same as Completers III and fulfils all aspects per-protocol.

e-Table 2. Efficacy and tolerability of acetyl-L-carnitine (ALC) and placebo. Amyotrophic lateral sclerosis functional rating scale-revised (ALSFRS-R); forced vital capacity (FVC*); Medical Research Council (MRC) score.

	ITT population (n = 82)														
	ALSFRS-R					FVC					MRC				
	Acetyl-L-carnitine		Placebo		p-value	Acetyl-L-carnitine		Placebo		p-value	Acetyl-L-carnitine		Placebo		p-value
Visit	n	Mean (SD)	n	Mean (SD)		n	Mean (SD)	n	Mean (SD)		n	Mean (SD)	n	Mean (SD)	
Baseline	42	41.9 (3.4)	40	41.0 (4.2)	0.3062	40	99.7 (17.4)	39	93.9 (10.9)	0.1957	42	130.0 (16.2)	39	127.4 (17.9)	0.5782
4 weeks	41	40.4 (4.3)	40	38.6 (5.7)	0.1536	-	-	-	-	-	41	126.9 (18.8)	40	123.1 (21.0)	0.5563
12 weeks	38	38.0 (6.3)	38	35.6 (7.7)	0.1900	35	85.9 (26.6)	34	81.2 (19.5)	0.3679	37	118.9 (29.3)	38	116.0 (25.3)	0.3621
24 weeks	37	36.0 (8.5)	32	32.1 (10.2)	0.0731	33	80.7 (31.4)	26	73.2 (23.4)	0.2861	37	109.9 (33.3)	32	103.9 (32.9)	0.3451
36 weeks	33	34.0 (9.5)	27	30.8 (9.3)	0.1421	26	82.5 (32.8)	22	66.7 (22.3)	0.0617	33	111.4 (31.8)	27	100.4 (32.3)	0.1425
48 weeks	28	33.6 (10.4)	19	27.6 (9.9)	0.0388	19	90.3 (32.6)	14	58.6 (31.2)	0.0158	27	105.4 (36.9)	19	97.3 (35.5)	0.4260
	Completers population (n = 47)														
	ALSFRS-R					FVC					MRC				
	Acetyl-L-carnitine		Placebo			Acetyl-L-carnitine		Placebo			Acetyl-L-carnitine		Placebo		
Visit	n	Mean (SD)	n	Mean (SD)	p-value	n	Mean (SD)	n	Mean (SD)	p-value	n	Mean (SD)	n	Mean (SD)	p-value
Baseline	28	42.0 (3.6)	19	42.2 (4.0)	0.8879	26	103.3 (15.8)	18	95.2 (12.6)	0.1364	28	131.1 (16.8)	19	133.4 (15.2)	0.6300
4 weeks	28	41.4 (3.9)	19	40.2 (5.5)	0.4958	-	-	-	-	-	28	128.2 (19.1)	19	129.7 (17.5)	0.6899
12 weeks	28	39.5 (5.3)	19	38.1 (6.4)	0.4503	25	94.3 (23.6)	17	82.2 (25.9)	0.1287	28	123.5 (23.8)	19	123.8 (20.1)	0.7875
24 weeks	28	38.6 (6.7)	19	34.6 (8.3)	0.0776	25	89.3 (29.5)	15	75.0 (28.8)	0.1257	28	115.6 (29.5)	19	114.1 (25.4)	0.7061
36 weeks	28	35.5 (8.8)	19	32.1 (9.1)	0.1586	22	86.7 (32.9)	15	69.9 (24.6)	0.0889	28	111.2 (33.9)	19	107.9 (31.8)	0.6128
48 weeks	28	33.6 (10.4)	19	27.6 (9.9)	0.0388	19	90.3 (32.6)	14	58.6 (31.2)	0.0158	27	105.4 (36.9)	19	97.3 (35.5)	0.4260
	Repeated measures models														
	ALSFRS-R					FVC					MRC				
Effect	F-value$_{(df1,df2)}$			p-value		F-value$_{(df1,df2)}$			p-value		F-value$_{(df1,df2)}$			p-value	
of treatment	$1.09_{(1,81.7)}$			0.2987		$0.93_{(1,94.3)}$			0.3368		$0.12_{(1,82.1)}$			0.7257	
Time	$65.85_{(5,322)}$			<0.0001		$32.35_{(4,118)}$			<0.0001		$47.06_{(5,317)}$			<0.0001	
Treatment*Time	$3.45_{(5,322)}$			0.0048		$1.05_{(4,118)}$			0.3833		$2.48_{(5,320)}$			0.0316	

ITT: intention-to-treat; SD: standard deviation.
*In addition to missing data due to drop-outs, other missing responses were due to not applicable tests. These missing data were treated as 'completely at-random' in the repeated measures model.
All repeated measures models were mixed effect, both random (the individual) and fixed (treatment and time).
The pattern of drop-out was also accounted in all models using a dummy variable (Yes or No).

e-Table 3. Efficacy and tolerability of acetyl-L-carnitine (ALC) and placebo. McGill score.

Visit	Acetyl-L-carnitine (42)		Placebo (40)		*p*-value
	n	Mean (SD)	*n*	Mean (SD)	
Overall QOL over the past 2 days					
Baseline	42	6.4 (2.2)	40	6.2 (2.0)	0.6458
12 weeks	37	6.6 (1.6)	37	5.9 (2.3)	0.1746
48 weeks	26	6.2 (2.2)	19	6.2 (2.5)	0.8709
Physical symptoms					
Baseline	42	5.4 (2.8)	40	5.9 (2.2)	0.3581
12 weeks	37	5.0 (2.3)	38	5.5 (2.5)	0.3588
48 weeks	28	5.6 (2.6)	20	4.4 (2.4)	0.1410
Physical well-being					
Baseline	42	6.4 (1.9)	40	6.9 (2.1)	0.1118
12 weeks	37	6.1 (2.2)	38	6.0 (2.2)	0.8097
48 weeks	28	5.8 (2.6)	20	6.1 (2.6)	0.8913
Psychological symptoms					
Baseline	42	4.7 (2.4)	40	4.2 (2.2)	0.2992
12 weeks	37	4.8 (2.2)	38	4.3 (2.4)	0.3674
48 weeks	28	4.8 (2.5)	20	4.1 (2.7)	0.2911
Support system					
Baseline	42	8.0 (1.8)	40	8.2 (1.4)	0.8338
12 weeks	37	8.1 (1.7)	38	7.9 (1.9)	0.7371
48 weeks	28	7.4 (2.4)	20	7.7 (2.2)	0.5506
Existential well-being					
Baseline	42	7.4 (1.5)	40	7.5 (1.6)	0.9778
12 weeks	37	7.2 (1.2)	37	7.1 (1.6)	0.6123
48 weeks	26	6.8 (1.2)	19	6.9 (1.7)	0.5680
McGill (mean of the 5 items)					
Baseline	42	6.4 (1.0)	40	6.6 (0.9)	0.3040
12 weeks	37	6.2 (0.9)	37	6.2 (0.9)	0.9742
48 weeks	26	6.4 (0.8)	19	6.0 (1.0)	0.2384

	Repeated measures model						
	Overall QoL over the past 2 days	Physical symptoms	Physical well-being	Psychological symptoms	Support system	Existential well-being	Total McGill (mean of the 5 items)
Effects	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value
Treatment (a)	0.9594	0.2064	0.3994	0.2597	0.3120	0.8974	0.4169
Visit (b)	0.1681	0.3609	0.1925	0.4649	0.1744	0.0538	0.2565
Treatment* Visit (c)	0.9092	0.4827	0.4250	0.1863	0.8695	0.7447	0.0713

QoL: quality of life; SD: standard deviation.

e-Table 4. Efficacy and tolerability of acetyl-L-carnitine (ALC) and placebo. Joint-rank analysis.

Methods				
	Criteria		Ranking*	
1	If pt is 'dead' then rank according to (RAT) '< Time to death'	# of pts dead (9)	1 to 9	Worst
2	If criterion 1 = NO AND pt is 'tracheostomized' then RAT '< time to tracheostomy"'	# of pts tracheostomized (2)	10 to 11	
3	If criteria 1&2 = NO AND pt is 'withdrawal due to AEs' then RAT '< Time to withdrawal'	# of pts who withdrew due to AEs (8)	12 to 19	
4	If criteria 1&2&3 NO AND pt is 'voluntary withdrawal' or pt is 'lost to follow-up' then RAT > 'Delta (last-first visit) in ALSFRS-R score'	# of pts who voluntary withdrew or lost to follow-up (16)	20 to 35	
5	If criteria 1&2&3&4 = NO then RAT '> Delta (last-first visit) in ALSFRS-R score'	# of pts assessed ranked according to these criteria (47)	36 to 82	Best

Results						
	ALC Median (IQR)	Placebo Median (IQR)	*p*-value	Ranking ALC #Best 5/Worst 5	Ranking Placebo #Best 5/Worst 5	*p*-value
ITT	55 (25-70)	32 (17-50)	0.0087	5/1	0/4	0.0476
ITT – diagnostic errors	56 (32-70)	31 (16-50)	0.0033	5/0	0/5	0.0079
Per protocol	58 (33-71)	31 (16-47)	0.0040	5/1	0/4	0.0476

#Best 5: the five patients with the best outcomes; #Worst 5: the five patients with the worst outcomes.

IQR: interquartile range.

* In case of ties, rank was set on the basis of survival time to the primary endpoint.