

Full Length Research Paper

## Efficacy and effectiveness of drug treatments in amyotrophic lateral sclerosis: A systematic review with meta-analysis

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The results of published studies with various neuroprotectors seeking to preserve motor neuron function and improve survival in amyotrophic lateral sclerosis patients have poor evidence in humans, although there are several studies in animal models with positive results. A systematic review and meta-analysis of studies on drug treatment options and survival times in animal models and patients with amyotrophic lateral sclerosis from March, 2009 to March, 2015 was conducted. Four hundred eighty-nine (489) articles were found, and from these, we selected 30 preclinical '*in vivo*' studies, 18 randomized controlled trials, and four systematic reviews. A meta-analysis confirmed the effectiveness of various drugs in improving the life span in preclinical trials, in particular, Resveratrol, which had a mean difference of 10.8 days (95% CI: 9.57 to 12.02), whereas no drug showed efficacy in clinical trials. The positive results of preclinical studies should be interpreted with caution because there is a mismatch between those results and the negative results in clinical trials.

**Key words:** Amyotrophic lateral sclerosis (ALS), motor neuron disease, drug, treatment.

### INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease. It is more often sporadic and characterized by the progressive degeneration of both upper and lower motor neurons in the brain, brainstem and spinal cord (Gordon, 2013). Its incidence may vary

between 1.2 and 4.0 per 100,000 individuals per year (Logroscino et al., 2010; Marin et al., 2009). It is more predominant in males (3.0 per 100,000 individuals per year, 95% CI 2.8 to 3.3) than females (2.4 per 100,000 individuals per year, 95% CI 2.2 to 2.6). Its onset occurs

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between 58 and 63 years of age, and its incidence decreases considerably after the age of 80 (Chiò et al., 2013).

The few advances in knowledge about the mechanisms of development of ALS are primarily due to the understanding of familial forms, which correspond to 5 to 10% of cases (Ravits et al., 2013; Strong, 2010). The pathophysiology of the disease is still poorly understood, but it is believed that the disease's injury mechanisms involve both glial cells and neurons (Strong, 2010). The main known mechanisms are oxidative stress with damage to RNA species, mitochondrial dysfunction, impairment of axonal transport, glutamate excitotoxicity as a mechanism contributing to motor neuron injury, protein aggregation, endoplasmic reticulum stress, abnormal RNA processing, neuroinflammation, and excitability of peripheral axons (Mancuso, 2015).

Death occurs on average between 2 and 4 years after onset due to respiratory complications, but patients given multidisciplinary care and undergoing enteral nutrition and noninvasive ventilation have extended survival (Miller et al., 2009). A single drug, Riluzole, approved in 1996 by the Food and Drug Administration (FDA), slows the progression of the disease by approximately 2 to 4 months, but it does not prevent the disease's fatal outcome (Miller et al., 2009).

Studies on the use of various neuroprotectors seeking to preserve motor neuron function and reduce the toxic levels of glutamate have been giving any evidence of efficacy in humans, although their use has been fairly efficacious in experimental animal models (Orrell, 2010). Considering the need to identify new alternatives to treat ALS, the aim of this study was to investigate the efficacy and effectiveness of drug treatments in clinical and preclinical trials through a systematic review of the literature in the field.

## MATERIAL AND METHODS

### Strategies to search for and select studies

In May, 2015, we investigated primary preclinical *in vivo* studies, clinical trials and systematic reviews with subsequent meta-analyses published between March, 2009 and March, 2015 in the following electronic databases: Medline, Embase, Cochrane Library and Lilacs. The following Medical Subject Headings (MeSH) and Health Science Descriptors (HScDe) were used: 'Amyotrophic Lateral Sclerosis' OR 'Motor Neuron Disease' AND 'Treatment' AND 'drug' AND 'survival'. Two authors independently evaluated the titles and abstracts of all studies identified in the search in the aforementioned electronic databases based on the descriptors. The following inclusion criteria were adopted:

- i) In clinical studies, including prospective randomized trials and meta-analytical systematic reviews, patients diagnosed with a motor neuron disease by means of anamnesis and electromyography according to the El Escorial and Awaji criteria (Costa et al, 2012);
- ii) In preclinical studies, *in vivo* studies with assessment of survival compared to control group and studies of treatment after the onset

of weakness; and

- iii) Studies based on the use of any drug to increase survival time compared to placebo or other treatments used by the control group.

The exclusion criteria were studies in which participants presented with respiratory failure or spinal muscular atrophy; studies in which the treatment was administered only prior to disease; or narrative reviews, letters, editorials, case reports, duplicate publications or those without objective data to be evaluated. Articles published in all languages were included. The studies that met the inclusion criteria were obtained in full. References were also considered, and communication with the authors was established in cases of doubt. Disagreements were resolved by consensus, and when this was impossible, there was subsequent analysis by two additional reviewers.

### Data extraction

Data were obtained from each study using a review form with the following content: author, place where the work was conducted, year of publication, intervention, study design, number of participants, age, analysis by intention to treat, declaration of conflict of interest, evaluation by a research ethics committee, and animal species used if the study was preclinical. The following outcomes were assessed:

- i) Comparison between two drugs and/or placebo;
- ii) Analysis of mean survival and absolute days of survival. In preclinical studies, the authors converted the survival in days, when it was clearly reported as animals alive;
- iii) Mean duration of the disease until the start of intervention;
- iv) Alteration of the Revised ALS Functional Rating Scale - ALSFRS-R (The Amyotrophic Lateral Sclerosis Functional Rating Scale, 1996) between the start and end of the study;
- v) Incidence of reactions and adverse effects of proposed treatments.

### Assessing the quality of the studies

Quality was assessed by two independent authors, and in cases of disagreement, the situation was resolved by consensus among all authors. The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) model (Guyatt et al., 2008) was used for primary studies and/or the Assessment of Multiple Systematic Reviews (AMSTAR) criteria were used for systematic reviews (Kung et al., 2010). The following data were observed in the studies:

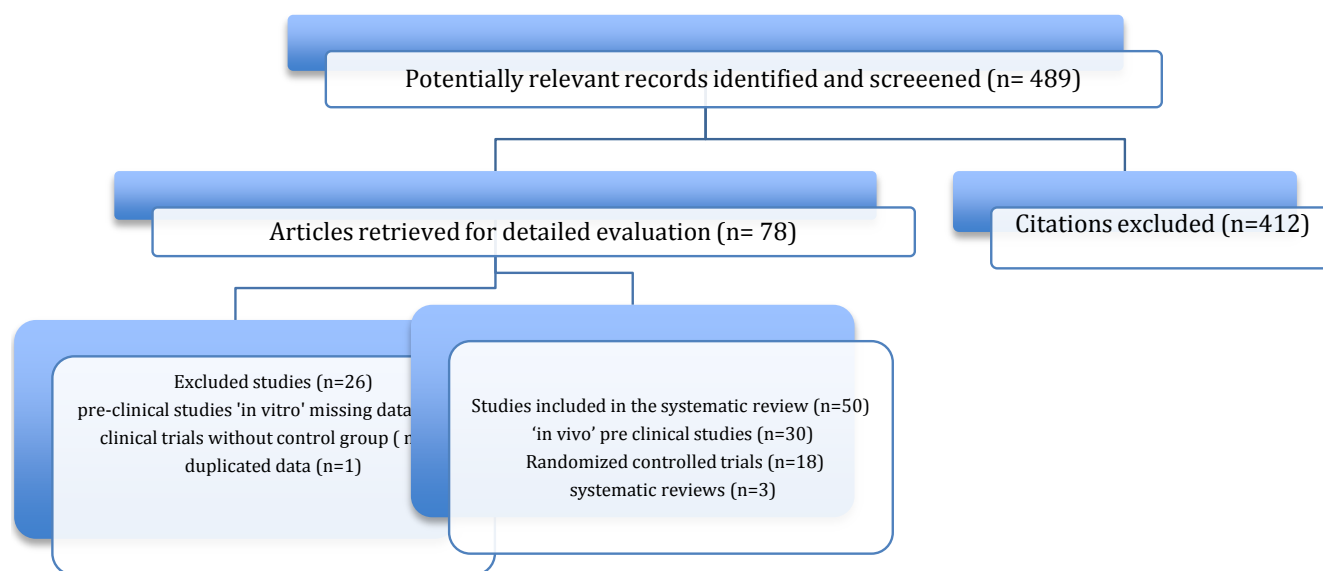
**Methods:** Research question, treatment sequence, allocation confidentiality, post-intervention follow-up, blinded outcome assessment, primary clinical outcome measures, location of study, protection against contamination, calculation of statistical power, sample representativeness, conflict of interest, and ethical aspects.

**Participants:** Inclusion criteria, exclusion criteria, age, gender, disease severity, and disease variants.

**Interventions:** Medications and doses or procedures, follow-up time, and method for monitoring disease progression.

**Outcomes assessed in the review:** Disease duration before intervention, survival time, and/or alteration of the ALSFRS-R.

The results of the primary outcomes were obtained based on the intention-to-treat principle: for each dichotomous outcome, the total number of participants in each group divided the number of events; for continuous outcomes, the following variables were calculated:



**Figure 1.** Systematic review flowchart.

mean, standard deviation and number of participants in each group. Data from work published more than once were obtained from the more thorough study. The reviewers rated each primary study according to the overall quality of evidence: A-high; B-moderate; C-low and D-very low, assigning scores of 1 to 5 according to the number of biases. The total AMSTAR score was used for systematic reviews. For analytical purposes, the studies were grouped as i) interventions in animal models and ii) clinical studies.

### Statistical analysis

Statistical analysis was performed in preclinical trials using RevMan software, version 5.3. All  $p$  values  $< 0.05$  were considered to be statistically significant. For dichotomous variables, such as patients who were alive at the end of the period analyzed, the absolute risk reduction method with a confidence interval of 95% (random effects model) was used.

For continuous variables, such as animal survival in days, the weighted mean difference (random effects model) was calculated based on the DerSimonian and Laird method, with a corresponding confidence interval of 95%. To evaluate heterogeneity among studies, a heterogeneity test was performed by calculating both the Q-test of heterogeneity and the  $I^2$  test of inconsistency. Heterogeneity was considered significant when  $p < 0.10$ . In addition, a sensitivity analysis was conducted using the funnel plot to quantify the presence of publication bias.

## RESULTS

Initially, 489 articles were obtained. Based on their abstracts, the following were selected: 23 prospective clinical trials, 48 preclinical trials, and 3 systematic reviews. After each original document was reviewed and data were obtained, 18 preclinical trials were excluded; of

these, 2 were '*in vitro*' (Calderó et al., 2010; Schuster et al., 2012), 6 did not include a quantitative evaluation of survival (Gu et al., 2010; Tovar-y-Romo et al., 2012; Cappello et al., 2012; Sunyach et al., 2012; Zhao et al., 2012, Yazhou et al., 2012), one had duplicate data (Jablonka et al., 2011), 7 only reported survival ratios and proportions (Seo et al., 2011; Ferrucci et al., 2010; Katsumata et al., 2012; Fidler et al., 2011; Gianforcaro et al., 2012, Gianforcaro et al., 2013; Schuster et al., 2010), and 2 involved interventions that occurred only prior to disease onset (Coughlan et al., 2015; Goursaud et al., 2015). The authors of 16 studies were contacted via email for completion of missing data, without any success. Five clinical trials were excluded. One contained duplicate data (Rudnicki et al., 2013), and 4 were not controlled (Chiò et al., 2011; Atassi et al., 2010; Fondell et al., 2012; Grassinger et al., 2014). Finally, 30 preclinical '*in vivo*' studies, 18 randomized and controlled clinical trials, and 3 systematic reviews of the Cochrane Collaboration were included. A flowchart illustrates the selection process adopted in the systematic review (Figure 1).

### Clinical studies

Table 1 lists the primary randomized clinical trials and systematic reviews of the Cochrane Collaboration, classifying them according to year, intervention, mean disease duration at baseline, follow-up time, number of participants, completion and quality. Only quality A and B primary studies were selected. Great heterogeneity

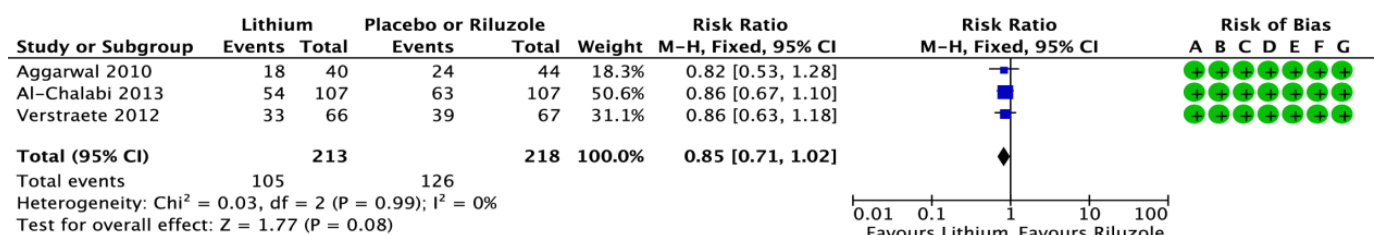
**Table 1.** Selected clinical trials including ALS patients with random allocation, according to intervention, mean time of disease, follow-up time, outcomes and quality (GRADE/AMSTAR) 2009 - 2015. E: experimental group; C: control group; TG: Therapeutic group; STG: sub therapeutic group; IV: intravenous.

Intervention (Ref)	N	Disease (Months)	Follow-up (Months)	Survival	ALSFRRS-R Slope	Grade
Talampanel 50 mg (Pascuzzi)	59	12	9	Not reported	E=(-7,1);C=(-10,2)(P=0,081)	1B
Ursodeoxycholic acid (min)	64	12.5	8	Not reported	E=1.04±0.28; C= 1.61±0.28 (P= 0.16 )	1B
Dexpropimexole 300 mg (Cudkowicz)	102	15.4	9	68% HR: 0,32 (95%CI 0.086 to 1.18) log rank p=0.07	31% Reduction:0,40 (95%: CI 0,38 to 1,18 )	1A
Dexpropimexole 300 mg (Cudkowicz)	-	-	-			-
COQ10 1.800 OU 2.700 mg (Kaufmann)	943	12	12	HR 1.03 (95%CI 0.75 to 1.43) p=0.84	E=13.34;C=-13.42( p=0.90)	1A
	185	27	18	Not reported	E=8.4± 7.3;C= 9.0±8.2(P=)	1A
BCAA/Threonin (Parton)	476/86	Not reported	6	BCAA HR 1.57 P=0.209 (95%CI 0.78 to 3.19); L-TR HR=2.76 P=0.151 (95%CI :0.71 to 9.27 )	Not reported	31*
	-	-	-			-
Ciliary neurotrophic factor –CTNF(Bongioanni)	1300	Not reported	6	RR 1.07(95% CI 0.81 to 1.41)	RR 1.07(95% CI 0.81 to 1.41)(P= 0.85)	31*
Creatine (Pastula)	386	Not reported	18	Dosing Escalation: 83%, 96% and 96%	Difference in slope 0.09(P= 0.76)	30*
Growth hormone- 2UI(Saccà)	40	15.6	18	RR: 1.03± 0.15	E=-45.2±6.1 C= 33.1±7.8(P=0.61)	1B
Acetyl-L-carnitine(Beghi)	82	Not reported	12	HR 0.72( 95%CI 0.45 to 1.16) P=0.1804	Monthly E=-0.97±1.09; C=1.60±1.39	1A
Ceftriaxone 2G 4G IV/DAY(Berry)	66	18	3	Not reported	E=36.8±6.0; C=35.2±5.7	1A
Lithium TG × STG (Chiò)	117	24	15	NR; did not differ P= 0.94	TG=1.26±1.43; STG=1.15±1.03(P= 0.60)	1B
Lithium + Riluzole (Aggarwal)	84	20.3	5.4	HR 1.13 (95%CI=0.61 to 2.07 )	0.15(95%CI -2.58 to 0.13) p=0.08	1A
Lithium + Riluzole (UKMND)	214	20.5	18	HR 1.35(95%CI 0.90 A 2.02)	9.50 (95% CI -10.31 to-8.70) slope 0.19(95% CI -1.28 to 0.90)	1A
Lithium × Placebo (Verstraete)	133	13	16	HR 1.03 (95%CI 0.66 A 1.63)	E= 40- 22; C= 40- 24 (P =0,74)	1A
G-CSF × Placebo (Duning)	39	22.4	12	Not reported	E=-4.66±3.37; C= -6.56±5.3 (P=0.289)	1B
G-CSF(Nefussy)	10	13.2	0.9	Not reported	E=35.3±9.4; C=34.4± 8.2	1B
Olesoxime (Lenglet)	512	17.5	18	E= 69.4%(95%CI 63.0 to 74.9);	HR 0.997 (95%CI 0.958 to 1.04)(p= 0.87)	1A
Pioglitazone (Dupuis)	219	18.9	15	HR:1.21 (95% CI: 0.71-2.07, p=0.48). C=67.5 %(95%CI 61.0 to 73.1)(P=0.71)	Not reported	1A
Lithium + Valproic acid (Boll)	49	46.5	17	HR 0.72± 0.6(12 months); 0.59± 0.07(16 months)(P= 0.016)	Better in experimental group (p<0.05)	2B
Ceftriaxone 4 G IV × Placebo (Cudkowicz)	340	18	72	No difference (P=0.5972)	HR 0.9 (95%CI 0.71 to 1.15) P= 0.4146	1A

among the studies was observed. Heterogeneity in relation to the method, time of patient follow-up

and intervention prevented us from performing a meta-analysis with respect to the proposed

outcomes with the exception of three studies using Lithium, in which it was possible to conduct



#### Risk of bias legend

- (A) Random sequence generation (selection bias)
- (B) Allocation concealment (selection bias)
- (C) Blinding of participants and personnel (performance bias)
- (D) Blinding of outcome assessment (detection bias)
- (E) Incomplete outcome data (attrition bias)
- (F) Selective reporting (reporting bias)
- (G) Other bias

**Figure 2.** Forest plot - use of lithium versus Placebo in ALS patients. Risk ratio analysis related to the endpoint in 15 to 16 months of follow-up. Period: 2009 to 2015.

a meta-analysis based on the number of survivors at the end of 15 to 16 months (Figure 2).

The mean disease duration at the time of randomization ranged from 12 to 72 months, with a weighted average of 16.2 months and a median of 15.6 months. The follow-up period after intervention ranged from 25 days to 18 months and was performed in a variable manner relative to the ALSFRS-R, survival, forced vital capacity (FVC) and occurrence of adverse events.

Lithium, dexamipexole and Granulocyte-colony stimulating factor (G-CSF) had greater consideration in the review because they were the most studied and a larger number of patients were enrolled in the trials. A phase II clinical trial using Dexamipexole (Cudkowicz et al., 2011) in 102 patients conducted in two phases with duration of nine months and dose escalation moderately slowed disease progression and increased survival time (HR: 0.32 95% CI; 0.086 to 1.18). With the dosage increase to 300 mg/day, in relation to the ALSFRS-R, a reduced functional decline of 31% was observed in the first stage compared to placebo, particularly in the subdomain of the scale relative to fine motor control, where the difference was greater (-1.4 SD 0.30 versus -0.60 SD 0.24), favoring larger studies with the drug. However, a phase III, multicenter, placebo-controlled clinical trial of 943 patients involving a 12-month follow-up was conducted and found no changes in survival time or ALSFRS-R scores (Cudkowicz et al., 2013).

The use of Lithium associated with Riluzole in ALS patients was evaluated in four randomized clinical trials (RCTs) (Aggarwal et al., 2010; Chiò et al., 2010; Verstraete et al., 2012; UKMND-LiCALS Study Group, 2013). In 2010, a multicenter study was conducted on the use of Riluzole and Lithium combined (Aggarwal et al., 2010) at a dose of 150 to 1050 mg per day in 84 patients

while maintaining serum concentration between 0.4 to 0.8 meq/L. The hazard ratio for final outcome (a drop of 6 points in the ALSFRS-R or death) was 1.13 (95% CI; 0.61 to 2.07). Patients were monitored for 5.4 months, and the study was interrupted because most patients in the experimental group presented with the final outcome. The mean difference in decline in the ALSFRS-R between the group that used lithium and the placebo group was 0.15 (95% CI; -0.43 to 0.73,  $p = 0.61$ ).

In that same year, in the USA and Canada (Chio et al., 2010), the drug was administered in two dosages-subtherapeutic (serum concentration of 0.2-0.4 mEq/L) and therapeutic (serum concentration of 0.4 to 0.8 mEq/L) to a group of 171 patients with a 15-month follow-up, but 85% had discontinued the drug by the end of this period due to adverse effects or lack of efficacy. No statistically significant difference was observed in the decline of the ALSFRS-R and FVC among the groups.

The results of two randomized trials using a similar method were published in the Netherlands in 2012 (Verstraete et al., 2012) and in Great Britain in 2013 (UKMND-LiCALS Study Group, 2013). Both studies evaluated Lithium and Riluzole versus placebo. The studies included 133 and 214 patients, respectively, with a 16- to 18-month follow-up. Hazard ratios (HR) of 1.13 (95% CI; 0.61 to 2.07) and 1.35 (95% CI; 0.90 to 2.02), respectively, were observed for survival, with no evidence of better performance of treated patients in relation to the ALSFRS-R or FVC. A meta-analysis was conducted to assess survival, including the number of patients who survived 15 to 16 months after treatment with Lithium at a variable dose with a serum level of 0.4 to 0.8 mEq/L, in the three studies that used Riluzole or a placebo as control (44, 46, 47). The study that used subtherapeutic Lithium concentrations as a control was excluded. Two hundred thirty-one events were observed in 431 patients

**Table 2.** Adverse events in clinical trials with ALS patients. 2009-2015. NR: Not Reported

Author (REF)	Drug	Adverse Events (AE)	N (EXPOSED)	N (AE)	N (DISCONTINUED)
Nefussy	G-CSF	Bone and muscle pain after the injections	19	2	0
Pascuzzi	TALAMPANEL	Dizziness, drowsiness, asthenia, depression,	40	24	2
Min	Oral solubilized ursodeoxycholic acid 500 mg	Abdominal pain, anorexia, dysphagia, dyspepsia, nausea, vomiting	40	25	5
Cudkowicz	Dexpramipexole 50 mg, 150 mg and 300 mg	Dizziness, headache, abdominal pain, anorexia, dysphagia, diarrhea, neutropenia	123 + 474	NR + 459	5 + 35
Kaufmann	COQ10 1.800 OU 2.700 MG	Respiratory and gastrointestinal events, fall, pain, nausea	110	63	0
Parton	Aminoacid - BCAA or threonin	Headache and gastrointestinal upset, gout	90	0.58 × control	1.35 × control
Bongioanni	Ciliary neurotrophic factor (cntf) -0,5 a 30 mcg/kg	Weight loss, anorexia, asthenia, cough	914	RR 1.55	NR
Pastula	Creatine - 5 a 10 g dia vo ou placebo	No significant	173	NR	NR
Saccà	Growth hormone	Increase of hepatic enzymes, joint swelling, hypertension, weakness, glucose INCR and INCR local reactions	20	15	NR
Beghi	Acetyl-L-carnitine 500 mg	Mai, pneumonia, urinary, dizziness, retinal haemorrhage, gastric intolerance	42	27	4
Berry	Ceftriaxone 2 g and 4 g iv	Pseudomembranous colitis, cholelithiasis, catheter related	21	5	4
Aggarwal	Lithium conc: 0.4-0.8 MEQ/L	Fatigue, sedation, raised tsh, anorexia, nausea, muscle weakness	40	20	3
Ukmond – alchalabi	Lithium 295 mg conc 0.4-0.8 mmol/l	Nausea, vomiting	107	61 HR=1.14	2
Verstraete	Lithium conc 0.4-0.8 mmol/L	Nausea, vomiting, polydipsia	66	47	16
Dupuis	Pioglitazone - 45 mg vo	Dysphagia, dyspnea, depression, oedema, weight loss,	109	35	NR
Duning	G-CSF	Leukocytosis, nervous system disorders, skin, musculoskeletal and connective tissue	5	11	0
Chiò	Lithium conc 0,4-0,8 mmol/l or 0,2-0,4 mmol/l	Cardiac, cystitis, deep vein thrombosis, edema, retinal, dehydration	171	38	117 (68.4%)

(Figure 2). The risk ratio obtained was 0.85 (95% CI; 0.71 to 1.02). The funnel graph showed that the studies presented similar findings.

A clinical trial conducted in Germany (Duning et al., 2011) used recombinant human Granulocyte-colony Stimulating Factor (G-CSF) to slow the progression of ALS symptoms in 10 patients at a dose of 10 mcg/kg/day versus placebo. Patients were monitored for 100 days and showed no differences in the percentage of decline in ALSFRS-R scores. The results of a previous pilot study conducted in Israel, in which half the suggested dose of the same drug was administered to 39 patients, suggested smaller declines in the ALSFRS-R variation (p: 0.289) and FVC (p: 0.854) in the experimental group (46). The studies did not assess survival. The

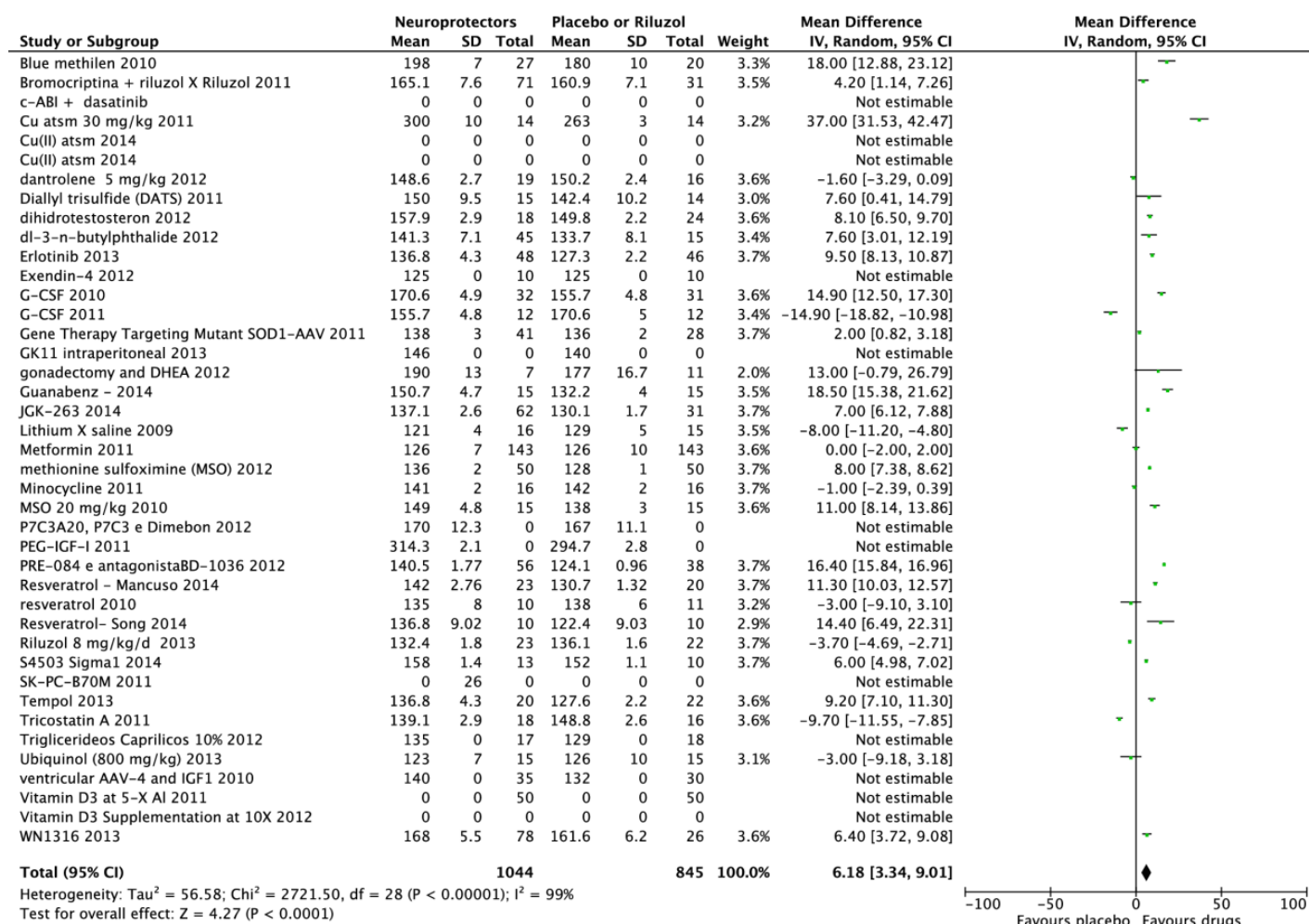
remaining interventions evaluated in single studies (Pascuzzi et al., 2010; Min et al., 2012; Kaufmann et al., 2009; Pastula, 2012; Sacca, 2012; Beghi et al., 2013; Berry et al., 2013; Dupuis et al., 2012; Lenglet et al., 2014; Boll et al., 2014; Cudkowicz et al., 2014) were not significantly successful in slowing disease progression or reducing ALSFRS-R variation compared to the control group.

Table 2 lists the adverse events (AEs) related to the use of drugs in clinical trials. It was observed that most of the studies reported weakness, gastrointestinal intolerance, and dizziness as adverse drug events, but they rarely reported severe events. Adverse events caused by Lithium are controversial because more adverse effects are known - 126 total, but with little

discontinuation except for a clinical trial that was interrupted due to an excessive number of adverse events (Verstraete et al., 2012). In summary, none of the drugs demonstrated unequivocal effectiveness in controlling the progression of the disease in humans.

### Preclinical studies

Preclinical 'in vivo' trials were conducted in transgenic mice expressing human mutated superoxide dismutase 1 (SOD1<sup>G93A</sup>) and showed experimental ALS treatments using neuroprotective therapies. The studies were considered homogeneous with respect to the method and the evaluation of outcomes in



**Figure 3.** Forest Plot – network meta-analysis including days of survival (life span) in pre-clinical studies with drugs. Period: 2009 to 2015.

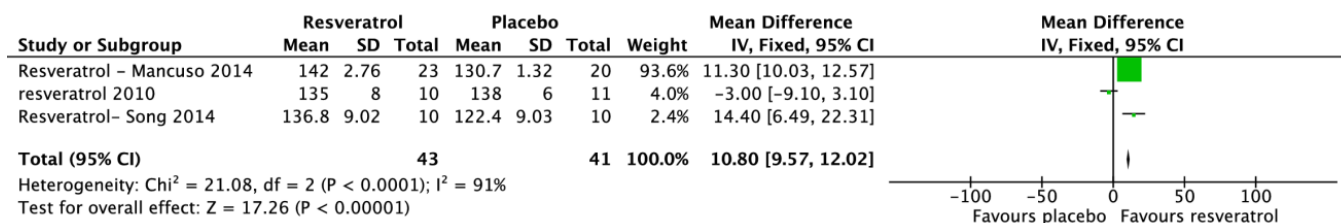
animals, making the comparison by meta-analytical methods possible. All included studies analyzed survival by the Kaplan-Meier method with the log-rank test, but only those studies that included disease prior to treatment and evaluated the survival of animals with a mean in days and standard deviation were included in our meta-analysis. The authors of all the studies that did not include such data were contacted via email.

Figure 3 describes the global effect of various drugs on the survival of animal models with ALS compared to placebo by means of network meta-analysis conducted from 2009 to 2015. It was observed that there are several effective medications. Studies on vascular endothelial growth factor (VEGF) (Tovar-y-Romo et al., 2012), Olesoxime (Sunyach et al., 2012), Exendin-4 (Yazhou et al., 2012), and SK-PC-B70M (Seo et al., 2011) were excluded from the meta-analysis for not including results about survival, although they did report improvements in motor performance and a neuroprotective effect of

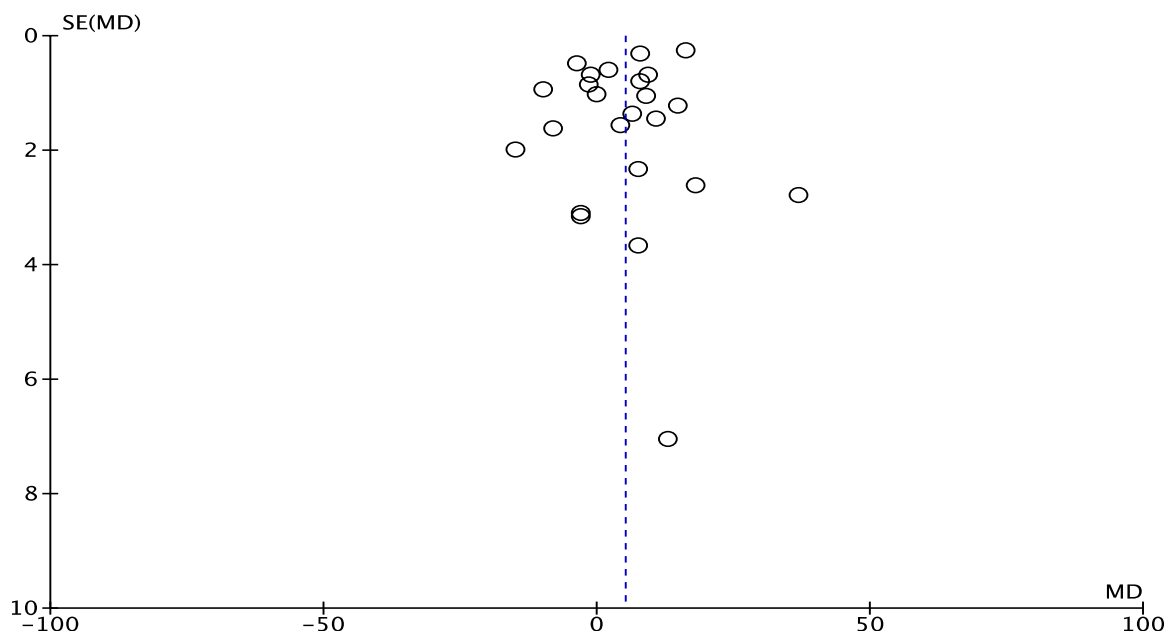
motor neurons in the spinal cord.

A study that investigated the effects of Dasatinib at a dose of 25 mg/kg/day (Le Pichon et al., 2013) reported improvement in animal survival associated with weight gain (log rank test,  $p < 0.01$ ). Studies on PEGylated insulin-like growth factor (Jablonka et al., 2011; Saenger et al., 2012) showed prolonged survival ( $p < 0.05$ ) associated with its use in the initial stage of the disease (Saenger et al., 2012), while a study of Gacyclidine (Gerber et al., 2013) showed a 4.3% increase in survival ( $p = 0.034$ ). It was also observed that dietary therapy with caprylic triglyceride (Zhao et al., 2012) and vitamin D3 (Gianforcaro et al., 2012; Gianforcaro et al., 2013) improved motor performance without influencing animal survival.

The most effective drugs in prolonging survival are Cull(atm) (Soon et al., 2011), dihydrotestosterone (Yoo et al., 2012), Granulocyte Colony-Stimulating Factor (G-CSF) (Henriques et al., 2011), Methionine Sulfoximine



**Figure 4.** Forest plot - meta-analysis including days of survival (life span) in pre-clinical studies with Resveratrol. Period: 2009 to 2015.



**Figure 5.** Funnel plot of preclinical studies in SOD1<sup>G93A</sup> mice. 2009-2015.

(MSO) (Ghoddoussi et al., 2010; Bame et al., 2012), PRE-084 (Mancuso et al., 2012), Tempol (Linares et al., 2013), WN1316 (Tanaka et al., 2014), n-butylphthalide (Feng et al., 2012), Guanabenz (Wang et al., 2014; Jiang et al., 2014) and Resveratrol (Markert et al., 2010; Song et al., 2014; Mancuso et al., 2014), with a total mean meta-analytical difference of 6.18 (95% CI 3.34 to 9.01) days of survival, favoring the group of drugs tested when compared to placebo. It was observed that Resveratrol showed major positive effect in three studies, and a meta-analysis (Figure 4) with this drug showed a mean difference of 10.8 (95% CI 9.57 to 12.02) days of survival, favoring the Resveratrol group. The authors analyzed the positive studies, as a group to demonstrate that more than one drug could potentially be effective in disease control. The funnel plot (Figure 5) showed a symmetrical distribution. The other drugs evaluated, including Riluzole

(Li et al., 2013) and Lithium (Pizzasegola et al., 2009), showed little or no impact on the survival of animal models.

**DISCUSSION**

Motor neuron disease is a group of progressive neurodegenerative disorders with different etiologies and clinical spectra that have a loss of lower and/or upper motor neurons in common (Mancuso, 2015). Although the heterogeneous and complex nature of ALS has been studied extensively, the absence of early detection biomarkers has not allowed the identification of patients at different stages or those developing the disease. With regard to treatments aimed at slowing the progression of ALS, an analysis of the results of preclinical trials in



animal models and clinical trials shows that there is great disparity between the findings of animal trials, which are often positive, and their replication in humans, which almost always yield negative results. In clinical studies, Riluzole (Miller et al., 1996) remains the single drug that has successfully slowed disease progression in a systematic review with a meta-analysis involving 1,477 patients; a 2- to 3-month increase in survival time was observed with a relative risk of 0.78 (95% CI 0.65 to 0.92) at 18 months.

According to some authors (Mancuso, 2015; Perrin, 2014), there are several possible explanations for the failure of translation from preclinical studies to effective human treatments. In preclinical studies, SOD1 animal models represented familial ALS more than sporadic ALS. In addition, pathophysiology of ALS spectra is poorly understood and it is possible that familial and sporadic ALS differ in some fundamental mechanisms that determine the effectiveness of treatments (Mancuso, 2015). On the other hand, some drugs used in animal models are used prior to symptom onset, which cannot be replicated in patients. It is also difficult to estimate the optimal target dose of an experimental drug in humans with the absence of the biomarkers.

The accessed preclinical trials used were relatively young, mutant SOD1<sup>G93A</sup> mice in homogeneous groups and a controlled environment, in which they showed a similar clinical condition. The drugs were used in the pre-symptomatic and early symptomatic stages, and many studies reported success in slowing the progression of symptoms and prolonging the survival of the animals, as shown in Figure 1. Most animal studies used the G93A SOD1 mice, but others used a low copy number G93A SOD1 strain or different familiar ALS mouse models with different onset and survival times. These studies have suggested that direct injury on the superoxide dismutase (SOD1) protein in neuronal tissues is crucial for the onset of motor neuron disease but not for the clarification of its progression, which is largely determined by microglia and astrocyte responses (Boillée et al., 2006).

The main mechanisms leading to neuronal death after onset of the disease include mitochondrial dysfunction, formation of free radicals and protein aggregates, glutamate excitotoxicity, axonal transport disruption, apoptosis, and inflammatory processes. There is evidence that protein aggregates can actively spread in the cerebral cortex and spinal cord via communication between cells, a process known as prion-like spread (Grad et al., 2015).

The role of autophagy in the injury mechanisms of the disease has also been discussed. Autophagy and the ubiquitin-proteasome (UP) system are two ways in which cells can degrade intracellular components. The UP system degrades short-lived proteins, whereas autophagy is responsible for the degradation of long-lasting proteins and damaged mitochondria, but when

present in excess, it can lead to self-digestion and cell death (Pasquali et al., 2009).

Despite the biases present in pre-clinical studies, negative results (95%) obtained in the randomized clinical trials are also influenced by several factors, among which are clinical heterogeneity, little knowledge about the disease prognosis, the highly variable course of the disease, the small number of participants, the inclusion of patients who had had the disease for a long time and not just incidental cases, withdrawal due to the adverse effects or lack of efficacy of drugs, and the use of different outcome measures (Traynor et al., 2006), as shown in Tables 1 and 2. The mean duration of the disease at the beginning of the studies ranged from 12 to 31.8 months, with a weighted average of 16.2 months and a median of 15.6 months. There was great variability among groups, which compromises the assessment of treatment effectiveness because it directly interferes with the scores of functional scales and survival time (Table 1).

Another factor that may be associated with inadequate group set up in clinical trials is the delay in establishing the diagnosis. A recent study conducted in the United States (Paganoni et al., 2014), in which logistic regression was used to assess 103 patients, showed that the total median time for diagnosis is 11.5 months. In this study, it was shown that 52% of patients had previously received another diagnosis; on average, evaluation by three doctors was necessary for a conclusive diagnosis. According to some authors (Paganoni et al., 2014; Gordon, 2011), dose escalation is important in phase II clinical trials before conducting phase III trials, in which efficacy and safety are determined for a greater number of patients. This did not occur in most of the studies selected and could explain why so many phase II trials are positive and phase III trials proved to be negative. Moreover, the ALSFRS-R, the only widely validated clinical scale and survival, are clinical outcomes that should be used to establish the efficacy of the tested compounds.

The effectiveness of various drugs in slowing the progression of motor neuron disease was tested in Cochrane systematic reviews, including Riluzole, Creatine, amino acids, and ciliary neurotrophic factor (CNTF), and only Riluzole yielded positive results. The systematic review of Riluzole included three clinical trials (Riluzole 876, placebo 406) and one of those included patients of advanced age (Mancuso, 2015). Daily treatment with 100 mg of Riluzole increased survival by two to three months in two studies ( $p = 0.039$ , hazard ratio (HR) 0.80, 95% CI 0.64 to 0.99), but in patients presenting with advanced disease, the result was not significant ( $p = 0.056$ , HR 0.84, 95% CI 0.70 to 1.01). Since then, various drugs have been used in clinical trials in an attempt to slow disease progression without success. Lithium was used in four clinical trials for

this purpose, motivated by positive results in multiple cell culture and animal assays (Fornai et al., 2008), but to date, the same results have not been observed in clinical trials (Aggarwal et al., 2010; Chiò et al., 2010; Verstraete et al., 2012; UKMND-LiCALS Study Group, 2013).

The most significant multicenter clinical trial was conducted in the UK and used Lithium carbonate to treat ALS - LiCALS (UKMND-LiCALS Study Group, 2013) in 214 patients over 18 months. Although the number of adverse events observed was not significant (hazard ratio for serious adverse events 1.14, 95% CI 0.79 to 1.65), the drug was not beneficial (Mantel-Cox log-rank  $\chi^2$  on 1 df = 1.64,  $p = 0.20$ ). Three published articles that were included in the review reported negative results in terms of disease progression and many adverse events (Table 2) with the use of Lithium. However, these studies used a non-traditional method, included few patients and did not exclude the possibility of a minor drug effect on survival and disease progression (Aggarwal et al, 2010; Chiò et al, 2010; Verstraete et al, 2012). A meta-analysis of these studies that considered the dichotomous variable survivors in the experimental and control groups at 15 to 16 months yielded a hazard ratio of 0.85 (95% CI: 0.71 to 1.02), without statistical significance.

In preclinical trials, a significant improvement in survival with the use of recombinant human granulocyte-colony stimulating factor (G-CSF) was observed in a selected study (Henriques et al., 2011). The efficacy and safety of the drug in humans with ALS was evaluated based on two articles that had a total of 49 patients and a maximum follow-up time of 12 months (Duning et al., 2011; Nefussy et al., 2010). Subcutaneous injections of G-CSF or saline solution (5 to 10 mg/kg/day of G-CSF for 4 days every three months during 1 year or during 10 days, from day 20th to 25th) were administered. Survival was not assessed, and the primary outcome was disease progression. The ALSFRS-R score was evaluated, and it was observed that the drug was well tolerated, with no significant evidence of efficacy.

In addition, some researchers have found that hypermetabolism is present in ALS patients and that there is a correlation between dyslipidemia, good nutrition status and a better prognosis (Schmitt et al., 2014). However, studies with caprylic triglyceride in animal models (Zhao et al., 2012) and hypercaloric enteral nutrition in patients with ALS with a short-term follow up that assessed safety and tolerability found that survival was not modified (Dorst et al., 2014).

To standardize the results of clinical trials and make them more reliable, approximating the results obtained in animal models, some authors (Gordon, 2011; Beghi et al., 2011) have suggested using samples of a representative number of patients, having a shorter diagnosis time; avoiding prevalent cases; stratifying patients into groups with a homogeneous clinical condition; and having as an endpoint validated functional

scales such as the ALSFRS-R, death or survival, or mechanical ventilation use. It is also important to conduct studies of different populations to compare patients with different genetic susceptibility and exposure to various environmental risk factors. However, the implementation of these strategies involves wider samples and higher costs.

Guidelines have been introduced (Ludolph et al., 2010) that should reduce the number of false positives in preclinical studies and therefore prevent unnecessary clinical trials. These recommendations include rigorously assessing animals' physical and biochemical traits in terms of human disease; characterizing when disease symptoms and death occur and being alert to unexpected variations; and creating a mathematical model to aid experimental design, including how many mice must be included in a study. Perrin (2014) also suggested excluding irrelevant animals; balancing for gender; avoiding putting siblings into the same treatment group; and tracking genes that induce non-inherited disease.

## Conclusion

Amyotrophic lateral sclerosis (ALS) is a rare, heterogeneous disease that is still poorly understood in its pathophysiology and is difficult to manage from a clinical point of view. Great efficacy of preclinical studies was observed, whereas the clinical ones showed no effectiveness in improving survival. Thus, the positive results of preclinical studies should be interpreted with caution. Translatability of the preclinical findings to clinical studies requires accurate standardization of preclinical research. On the other hand, a better control of the bias of clinical trials is needed to allow a greater potential of generalizing the findings. Additionally, interventions should be tested in patients who have been more recently—diagnosed, and samples should be stratified into more homogeneous groups. The most promising drugs observed in preclinical studies were Resveratrol, Cull(atm), Dihydrotestosterone, Erlotinib, Granulocyte Colony-Stimulating Factor (G-CSF), Methionine Sulfoximine (MSO), PRE-084, Tempol, WN1316, and n-butylphthalide. The new experimental drugs that demonstrate success in slowing the progression of the disease could be used alone compared to placebos but also in combination.

## Conflicts of interest

The authors declare no conflicts of interest and have followed the Sources of Funding Statement.

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