Safety and efficacy of edaravone in well-defined Iranian patients with amyotrophic lateral sclerosis: A parallel-group single-blind trial

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Abstract

Background: This parallel-group single-blind trial evaluates the safety and efficacy of Edaravone, as a free radical scavenger, in a highly selective subgroup of Iranian patients with amyotrophic lateral sclerosis (ALS).

Methods: The study was registered in ClinicalTrials.gov (registration number: NCT03272802) and Iranian Registry of Clinical Trials (registration number: IRCT20190324043105N). Patients were included into the study, who were diagnosed as probable or definite ALS (according to revised El Escorial criteria), mildly to moderately affected by the disease [according to Amyotrophic Lateral Sclerosis Health State Scale (ALS/HSS)], scored ≥ 2 points on all items of the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R), and had forced vital capacity (FVC) of at least 80%. 20 patients (10 cases, 10 controls) were observed for 12 cycles (each cycle lasted four weeks). Cases received Edaravone for the first 14 days in the first cycle and for the first 10 days in the next cycles. In addition, all patients received Riluzole. The 40-item Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40), ALSFRS-R, and Manual Muscle Testing (MMT) scores were measured every 3 cycles to evaluate the physical and functional status of the patients. Besides, injection reactions, adverse events (AEs), and serious adverse events (SAEs) were measured during the study.

Results: ALSAQ-40, ALSFRS-R, and MMT scores were not significantly different between cases and controls in 5 different time points. During the study, no injection reactions were observed. AEs and SAEs were not significantly different between cases and controls.

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Conclusion: Our data did not demonstrate efficacy of Edaravone in ALS treatment, but showed its safety for use in patients with ALS. Further studies are necessary to investigate Edaravone efficacy in patients with ALS before prescribing this new drug outside Japan.

Introduction

Amyotrophic lateral sclerosis (ALS) is known as a fatal neurodegenerative disease. The disease is characterized by progressive muscle weakness with evidence of upper and lower motor neuron involvement. Usually, patients will die within 2-3 years after diagnosis. Familial form of the disease causes about 5%-10% of all cases. Few genes were identified as the cause of the familial form.[1] Sporadic form is more complex. The gene-time-environment model was proposed for explaining the sporadic form. This model clarifies that environmental factors and senescence react with susceptible genetic bases to form the disease phenotype.[2] Complex nature and unknown pathogenesis of ALS are 2 important reasons for the failure of therapeutic interventions. Except for Riluzole, more than 50 other drugs were introduced, but all were ineffective in human-based studies. Oxidative stress is one of the proposed theories that can cause neuronal injuries. Experiments showed an increased level of oxidative stress markers like 3-nitrotyrosine (3NT) in biopsies taken from the mouse model for ALS and also the familial form of this disease.[3] Edaravone is known as a potent free radical scavenger. It has proven neuroprotective effects in other neurologic diseases like stroke. 2 phase II clinical trials approved Edaravone effectiveness in patients with ALS. Edaravone administration reduced levels of tissue oxidative damage markers like plasma palmitoleic and oleic acids and cerebrospinal fluid (CSF) 3NT and increased plasma uric acid level, a potent free radical scavenger. In both studies, Edaravone-treated patients preserved their functional status better than Edaravone-untreated patients [measured by Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R)].[4,5] In this study, we aimed to assess the safety and efficacy of Edaravone in a well-defined group of patients with ALS in the Iranian population.

Materials and Methods

Standard protocol approvals: This parallel-group single-blind clinical trial was conducted in Al-Zahra University Hospital (the major referral hospital of Isfahan University of Medical Sciences, Isfahan, Iran) from March 2017 to March 2019. Before enrolling the patients into the study, all of them provided written informed consent. The study was conducted in compliance with Good Clinical Practice (GCP) and Consolidated Standards of Reporting Trials (CONSORT) statement. Protocol of this study was approved by the Ethical Committee of Isfahan University of Medical Sciences (ethical code: IR.mui.rec.1396.3.569). The study was registered in ClinicalTrials.gov (registration number: NCT03272802) and Iranian Registry of Clinical Trials (registration number: IRCT20190324043105N). This study was supported by Isfahan University of Medical Sciences.

Study design and participants: Inclusion criteria for our study were: age between 18 and 75 years, probable or definite ALS diagnosis (according to revised El Escorial criteria),[6] mild or moderate status according to Amyotrophic Lateral Sclerosis Health State Scale (ALS/HSS),[7] score of ≥ 2 points on all items of the ALSFRS-R (preserved function of the patient),[8] and forced vital capacity (FVC) of at least 80% (patients without an obstructive pattern in spirometry). From 48 patients with ALS who referred to our center from March 2017 to March 2018, 23 met our inclusion criteria. CONSORT flow diagram is shown in figure 1. These patients were invited to the first visit. At the first visit, patients were informed about our study. Besides, demographic data, disease type (sporadic, familial), disease onset (limb, bulbar), parental consanguinity, family history of ALS, and drug history were documented. 12 patients with ALS were selected as the case group. 11 age and sex-matched patients with ALS were selected as the control group. Patients in the case group were injected 60 mg intravenous (IV) Edaravone (brand name: Radicut/Radicava from Mitsubishi Tanabe Pharma Corporation, Japan) daily, for 12 cycles. Each cycle included four weeks. In the first cycle, the drug was administered for the first 14 days and in the next cycles, for the first 10 days of the cycle.[9] Before and after injection, vital signs (temperature, respiratory rate, pulse rate, blood pressure) of all patients were measured. If any vital sign disturbance or drug reaction occurred during or after injection, it would be recorded. Riluzole also was prescribed for all of the patients (100 mg daily, in 2 divided doses).[10]
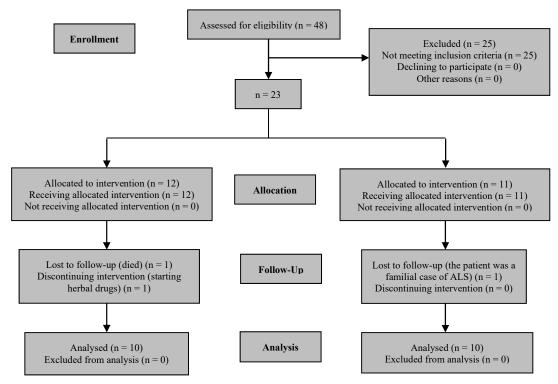
Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram

During the study, patients would be excluded if any side effects was observed, the patient started any other drug or herb for ALS treatment, or the patient had other muscular or neurologic disease that could interfere with the evaluation of ALS progression. Before starting the study and every 3 cycles, patients were invited for follow-up visits. In these visits, physical and functional status of patients was evaluated by 40-item Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40),[11,12] ALSFRS-R,[8] and Manual Muscle Testing (MMT).[13,14] In addition, during these visits, adverse events (AEs) and serious adverse events (SAEs) were documented (according to the GCP guideline).

Scores and tools

El Escorial criteria: This is a well-known criterion for ALS diagnosis. This criterion is designed to standardize diagnostic steps in ALS and make sure that the diagnosis is correct. This criterion classifies patients into 3 levels: possible, probable, and definite.[6]

ALS/HSS: ALS/HSS classifies patients with ALS into 4 categories based on their functional impairments in 3 body regions (i.e., bulbar, arm, and leg). Mild disease is defined when the patient is functionally independent and has a mild defect in one region. The moderate disease is defined

when one region is moderate to severely involved, or there is mild involvement in all regions. Severe form is when 2 or 3 regions are moderate to severely involved. In the terminal form, the patient is severely dependent.[7]

ALSAQ-40: This questionnaire assesses the quality of life (QOL) in patients with neuromuscular disease, especially ALS.[11] Shamshiri et al. translated and validated this questionnaire in the Persian language.[12]

ALSFRS-R: It is a valid patient-centered scale consisting of 12 items. ALSFRS-R evaluates impairments in 4 clinical domains in patients with ALS: bulbar function (items 1-3), fine motor (items 4-6), gross motor (items 7-9), and respiratory function (items 10-12). This tool is a good predictor of prognosis in patients with ALS.[8]

MMT: Muscle strength measurements using clinical scales like the six-point Medical Research Council (MRC) Scale are conventional in ALS clinical trials.[13] Based on methodology of Iran-ALS clinical registry, six upper extremity, six lower extremity, and neck flexor and extensor muscle groups were selected for bilateral evaluation.[14]

Data analysis was carried out using SPSS software (version 23, IBM Corporation, Armonk, NY, USA). To fulfil blindness, the analyzer was not

aware of the intervention groups. Quantitative variables were presented as mean ± standard deviation (SD) and qualitative variables were presented as frequencies. P-values less than 0.05 were regarded as statistically significant. Kolmogorov-Smirnov Z-test was used to check the normality of data. Independent samples t-test was conducted to compare quantitative variables (age, weight, Riluzole consumption), and chi-square test was used to compare qualitative variables (sex, city, consanguinity, disease onset, ALS/HSS) between case and control groups. Independent samples t-test was used to compare ALSAQ-40, ALSFRS-R, and MMT scores between case and control groups in 5 time points (before starting the study and after 3, 6, 9, and 12 cycles). Repeated measures analysis of variance (ANOVA) was used to compare ALSAQ-40, ALSFRS-R, and MMT scores within mentioned time points in case and control groups.

Results

Subject background: From 23 patients, 3 were excluded during the study. A 67-year-old woman from the case group discontinued prescribed drugs and started herbal medicine after 6 months. A 74-year-old woman from the control group died 4 months after the first visit because of stroke. A 47-year-old woman from the control group was a familial case. Unlike other subjects, she showed very slow disease progression. From 20 patients, 17 (85%) were men, 17 (85%) showed limb-onset (others bulbar onset), and 15 (75%) had no parental consanguinity. All the patients had no family history of ALS (sporadic form). Mean age was 57.20 ± 13.02 years and mean weight was 70.60 ± 11.73 kg. 15 patients (75%) showed a mild form of ALS (according to ALS/HSS). On average, Riluzole was consumed 8.45 ± 6.41 months before the first visit.

Efficacy: Mean ALSAQ-40 score was 90.60 ± 20.74 at the first visit and 101.20 ± 21.08, 116.50 ± 26.12, 127.75 ± 26.72, and 140.45 ± 27.66 after 3, 6, 9, and 12 cycles, respectively. Mean ALSFRS-R score was 41.00 ± 4.34 at the first visit and 35.60 ± 6.30, 30.10 ± 7.76, 26.80 ± 8.56, and 21.90 ± 9.17 after 3, 6, 9, and 12 cycles, respectively. Mean MMT score was 114.45 ± 13.86 at the first visit and 102.55 ± 16.56, 87.45 ± 20.82, 76.05 ± 25.14, and 65.80 ± 24.47 after 3, 6, 9, and 12 cycles, respectively. In bivariate analysis, demographic variables (age, sex, weight, and city) and other measured variables (Riluzole consumption, disease onset, disease type, parental consanguinity, and ALS/HSS) were not significantly different between cases and controls (relevant data and P-values are shown in table 1). In independent samples t-test results, primary outcome variables (ALSAQ-40, ALSFRS-R, MMT) were not significantly different between cases and controls in 5 time points (before starting the study and after 3, 6, 9, and 12 cycles). Primary outcome variables (ALSAQ-40, ALSFRS-R, MMT) were significantly different within cases in mentioned 5 time points and also in controls (no difference between two groups in repeated measures ANOVA results). Relevant data and P-values are summarized in table 2.

Table 1. Bivariate analysis, between case and control groups

Variable	Case (n = 10)	Control (n = 10)	P
Sex (male/female)	9/1	8/2	> 0.99
Age (year) (mean ± SD)	55.20 ± 13.50	59.20 ± 12.92	0.50
Weight (kg) (mean ± SD)	72.10 ± 12.44	69.10 ± 11.44	0.58
City[†]			0.37
Esfahan	5	4	
Najafabad	0	2	
Lenjan	1	0	
Shahreza	2	1	
Lordegan	1	0	
Shahrekord	0	2	
Yazd	1	1	
Riluzole consumption[‡] (month) (mean ± SD)	8.80 ± 7.31	8.10 ± 5.76	0.81
Disease onset (limb/bulb)	9/1	8/2	> 0.99
Disease type (sporadic/familial)	10/0	10/0	-
Parental consanguinity (yes/no)	3/7	2/8	> 0.99
ALS/HSS (mild/moderate)	2/8	3/7	> 0.99

[†]This variable shows patient's place of residence
[‡]This variable shows the duration of Riluzole consumption before the first visit
ALS/HSS: Amyotrophic Lateral Sclerosis/Health State Scale; SD: Standard deviation

Table 2. Changes in 3 primary outcome measures in case and control groups

Variable	Case	Control	P
MMT score (mean ± SD)			
Base	115.80 ± 16.13	113.10 ± 11.88	0.67
After 3 cycles	102.20 ± 20.35	102.90 ± 12.83	0.92
After 6 cycles	86.20 ± 25.48	88.70 ± 16.20	0.79
After 9 cycles	75.10 ± 28.51	77.00 ± 22.79	0.87
After 12 cycles	62.70 ± 24.63	68.90 ± 25.22	0.58
P	< 0.01	< 0.01	
ALSFRS-R score (mean ± SD)			
Base	40.20 ± 5.02	41.80 ± 3.61	0.42
After 3 cycles	36.60 ± 6.13	34.60 ± 6.63	0.49
After 6 cycles	31.80 ± 7.84	28.40 ± 7.70	0.34
After 9 cycles	28.70 ± 8.95	24.90 ± 8.15	0.33
After 12 cycles	24.20 ± 10.07	19.60 ± 8.01	0.27
P	< 0.01	< 0.01	
ALSAQ score (mean ± SD)			
Base	88.60 ± 20.31	92.60 ± 22.06	0.67
After 3 cycles	97.80 ± 20.95	104.60 ± 21.76	0.48
After 6 cycles	113.10 ± 28.50	119.90 ± 24.55	0.57
After 9 cycles	122.90 ± 27.48	132.60 ± 26.44	0.43
After 12 cycles	131.60 ± 26.67	149.30 ± 27.02	0.15
P	< 0.01	< 0.01	

MMT: Manual Muscle Testing; ALSFRS-R: Revised Amyotrophic Lateral Sclerosis Functional Rating Scale; ALSAQ: Amyotrophic Lateral Sclerosis Assessment Questionnaire; SD: Standard deviation

Safety: There was no injection reaction in case and control groups. A patient from the case group reported increased blood pressure a few hours after injection every time when she arrived home (not measured by our staff). All AEs and SAEs are listed in table 3. There was no significant difference in the incidence of AEs and SAEs between cases and controls. Gait disturbance, dysphagia, and muscular weakness occurred almost always due to the underlying disease. One patient from the case group underwent mechanical ventilation due to respiratory failure (the only SAE of this study). In this patient, respiratory failure was diagnosed as a complication of the underlying disease.

Discussion

Unlike previous phase 2 clinical trials,[4,5] the results of the phase 3 studies were ambivalent. MCI186-16 study, the first phase III clinical trial, in 2014 did not approve the efficacy of Edaravone for treatment of ALS. In the first cycle, patients received 60 mg Edaravone (n = 102) or placebo (n = 104) daily for 14 days and were off for the next 14 days. In following 5 cycles, they received treatment for the first 10 days of the cycle.[9] An extension of MCI186-16 study (MCI186-17 study) for another 6 cycles also did not approve efficacy of Edaravone for treatment of ALS.

In this study, patients who received Edaravone in MCI186-16 study, received Edaravone (the E-E group, n = 48) or placebo (the E-P group, n = 45) after randomization; and all of the patients who received placebo were switched to Edaravone (the P-E group, n = 90). Analyses were performed in all patients with ALS and in efficacy-expected subpopulation (EESP). EESP was defined as FVC ≥ 80% and a score ≥ 2 for all items of ALSFRS-R at baseline.[15]

Table 3. Adverse events (AEs) and serious adverse events (SAEs)

	AEs		P[†]	SAEs		P[†]
	Case (n = 10)	Control (n = 10)		Case (n = 10)	Control (n = 10)	
Gait disturbance	3	5	0.65			
Dysphagia	4	2	0.62			
Muscular weakness	7	8	> 0.99	1	0	> 0.99
Constipation	3	0	0.21			
Myalgia	2	5	0.35			
Anemia	1	0	> 0.99			
Hyperglycemia	0	2	0.47			
Total	20	22		1	0	

[†]All P-values were calculated by chi-square test

AE: Adverse event; SAE: Serious adverse event

When analysis of MCI186-17 study was performed on definite/probable EESP 2 years (dpEESP2y) subpopulation (probable or definite ALS diagnosed according to revised El Escorial criteria, disease duration of ≤ 2 years in addition to EESP criteria), a greater difference in ALSFRS-R score changes between E-E and E-P groups was observed; but the results were not statistically significant.[16] Besides, MCI186-18 study did not show efficacy of Edaravone for treatment of ALS. In this study, definite or probable patients with ALS (according to revised El Escorial criteria) with a Japan ALS severity classification of grade 3 (requiring assistance for eating, excretion, or ambulation), FVC ≥ 60%, disease duration less than 3 years, and 1 to 4 points decrease in ALSFRS-R score in 12-week pre-observation period were treated with Edaravone (n = 13) or placebo (n = 12) for 6 cycles (cycles the same as MCI186-16 and MCI186-17 studies).[17] In another phase III clinical trial (MCI186-19 study) published in 2017, post-hoc analysis of MCI186-16 study demonstrated Edaravone effectiveness in treatment of a selected subgroup of patients with ALS, who had independent living status (grade 1 or 2 according to the Japan ALS severity classification) in addition to dpEESP2y criteria.[18] In another study, post-hoc analysis of MCI186-19 study approved statistical analysis of the MCI186-19 study. In this study, further analysis showed Edaravone effectiveness in all 4 clinical domains of ALSFRS-R.[19] Post-hoc analysis of MCI186-19 study after 6 cycles of open-label extension period showed a statistically significant decrease in ALSFRS-R score in P-E group compared to E-E group, suggesting better preserved function when Edaravone was started early and administered continuously.[20]

There are a few methodological defects in these clinical trials. The first defect is that all of these studies were conducted in Japan; as a reason, the results are not conclusive for other populations. Another issue is that Mitsubishi Tanabe Pharma Corporation supported all of these studies. This issue can lead to bias in data analysis. Another important point is that only a small subgroup of patients with ALS benefited from this drug (dpEESP2y subgroup). Based on findings from phase 3 studies, we decided to conduct a clinical trial in a small group of Iranian patients with ALS to see the effect of Edaravone on the Iranian population. In our study, we selected patients with ALS according to dpEESP2y criteria to maximize the efficacy of Edaravone. Drug injection protocol was the same as previous phase 3 studies. We followed our patients for 12 cycles (48 weeks). According to the previous phase 3 studies, it was an acceptable following period. After all these efforts, our study did not show the efficacy of Edaravone in patients with ALS. Clinical and statistical evaluations did not demonstrate any physical or functional improvement in the cases compared to the controls. These finding may be due to less effectiveness of Edaravone in Iranian subjects or may be due to the modest effect of this drug in ALS treatment. During past 2-3 decades, more than 50 other drugs were introduced based on preclinical studies, but all were ineffective in clinical trials.[3] Even Riluzole showed a mild effect on patient survival (9% increase in 1-year survival) and also the mechanism of action for this drug was poorly described. This issue reflects the inability of scientists and clinicians to find a better treatment option for this fatal disease.[21] As the secondary endpoint, our study showed the safety of Edaravone in patients with ALS. This finding was consistent with the results of previous phase 3 studies.[9,15,17,18]

Conclusion

Our trial did not prove the efficacy of Edaravone to delay the worsening of ALS, but showed its safety. Ethnical differences could explain contradictory results of different studies. According to the authors' opinion, further trials are necessary before prescribing Edaravone outside Japan. As a final important point, taking stronger steps towards identifying molecular pathogenesis of ALS must be the priority of scientists. By explaining molecular pathways more exactly, maybe we could find better treatment options in the future.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

References

1. Wijesekera LC, Leigh PN. Amyotrophic lateral sclerosis. Orphanet J Rare Dis 2009; 4: 3.

2. Al-Chalabi A, Hardiman O. The epidemiology of ALS: A conspiracy of genes, environment and time. Nat Rev Neurol 2013; 9(11): 617-28.

3. Noto Y, Shibuya K, Vucic S, Kiernan MC. Novel therapies in development that

inhibit motor neuron hyperexcitability in amyotrophic lateral sclerosis. Expert Rev Neurother 2016; 16(10): 1147-54.

4. Yoshino H, Kimura A. Investigation of the therapeutic effects of edaravone, a free radical scavenger, on amyotrophic lateral sclerosis (Phase II study). Amyotroph Lateral Scler 2006; 7(4): 241-5.

5. Nagase M, Yamamoto Y, Miyazaki Y, Yoshino H. Increased oxidative stress in patients with amyotrophic lateral sclerosis and the effect of edaravone administration. Redox Rep 2016; 21(3): 104-12.

6. Agosta F, Al-Chalabi A, Filippi M, Hardiman O, Kaji R, Meininger V, et al. The El Escorial criteria: Strengths and weaknesses. Amyotroph Lateral Scler Frontotemporal Degener 2015; 16(1-2): 1-7.

7. Kiebert GM, Green C, Murphy C, Mitchell JD, O'Brien M, Burrell A, et al. Patients' health-related quality of life and utilities associated with different stages of amyotrophic lateral sclerosis. J Neurol Sci 2001; 191(1-2): 87-93.

8. Kaufmann P, Levy G, Thompson JL, Delbene ML, Battista V, Gordon PH, et al. The ALSFRSr predicts survival time in an ALS clinic population. Neurology 2005; 64(1): 38-43.

9. Abe K, Itoyama Y, Sobue G, Tsuji S, Aoki M, Doyu M, et al. Confirmatory double-blind, parallel-group, placebo-controlled study of efficacy and safety of edaravone (MCI-186) in amyotrophic lateral sclerosis patients. Amyotroph Lateral Scler Frontotemporal Degener 2014;

15(7-8): 610-7.

10. Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Cochrane Database Syst Rev 2012; (3): CD001447.

11. Jenkinson C, Harris R, Fitzpatrick R. The Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40): Evidence for a method of imputing missing data. Amyotroph Lateral Scler 2007; 8(2): 90-5.

12. Shamshiri H, Eshraghian MR, Ameli N, Nafissi S. Validation of the Persian version of the 40-item amyotrophic lateral sclerosis assessment questionnaire. Iran J Neurol 2013; 12(3): 102-5.

13. Paganoni S, Cudkowicz M, Berry JD. Outcome measures in amyotrophic lateral sclerosis clinical trials. Clin Investig (Lond) 2014; 4(7): 605-18.

14. Shamshiri H, Fatehi F, Davoudi F, Mir E, Pourmirza B, Abolfazli R, et al. Amyotrophic lateral sclerosis progression: Iran-ALS clinical registry, a multicentre study. Amyotroph Lateral Scler Frontotemporal Degener 2015; 16(7-8): 506-11.

15. Exploratory double-blind, parallel-group, placebo-controlled extension study of edaravone (MCI-186) in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2017; 18(sup1): 20-31.

16. Takahashi F, Takei K, Tsuda K, Palumbo J. Post-hoc analysis of MCI186-17, the extension study to MCI186-16, the

confirmatory double-blind, parallel-group, placebo-controlled study of edaravone in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2017; 18(sup1): 32-9.

17. Exploratory double-blind, parallel-group, placebo-controlled study of edaravone (MCI-186) in amyotrophic lateral sclerosis (Japan ALS severity classification: Grade 3, requiring assistance for eating, excretion or ambulation). Amyotroph Lateral Scler Frontotemporal Degener 2017; 18(sup1): 40-8.

18. Writing Group; Edaravone (MCI-186) ALS 19 Study Group. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: A randomised, double-blind, placebo-controlled trial. Lancet Neurol 2017; 16(7): 505-12.

19. Takei K, Takahashi F, Liu S, Tsuda K, Palumbo J. Post-hoc analysis of randomised, placebo-controlled, double-blind study (MCI186-19) of edaravone (MCI-186) in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2017; 18(sup1): 49-54.

20. Takei K, Tsuda K, Takahashi F, Palumbo J. Post-hoc analysis of open-label extension period of study MCI186-19 in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2017; 18(sup1): 64-70.

21. Dharmadasa T, Kiernan MC. Riluzole, disease stage and survival in ALS. Lancet Neurol 2018; 17(5): 385-6.