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The Italian multicenter experience with Edaravone in Amyotrophic Lateral Sclerosis.

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Abstract

Objectives: The aim of the study is to analyze the ALS disease progression and respiratory function of Italian patients treated with Edaravone (EVN), as well as the adherence to, and the effects of, the therapy.

Methods: We performed an observational study of patients treated with EVN from May 2017 to May 2019, in 39 Italian ALS Centers. Taking into account ALS patients with at least 12 months of EVN treatment, we compared the decline of ALSFRS-R and FVC with a group of matched historical controls from the Pooled Resource Open-Access ALS Clinical Trials (PROACT) database, using both descriptive and survival analysis approaches.

Results: A total of 331 ALS Italian patients treated with EVN and 290 matched historical controls were recruited in this study. No significant differences on disease progression or respiratory function were found comparing the two cohorts in both descriptive and survival analyses. The EVN treatment was overall well tolerated.

Conclusions: The study showed that EVN treatment was well tolerated. No significant differences were reported in ALS patients treated and not treated with EVN, in terms of both disease progression and respiratory function. These findings prove that further studies are required to better clarify whether EVN could be considered an effective treatment for ALS disease.

Keywords: Amyotrophic Lateral Sclerosis, Edaravone, multicenter study, observational study

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Consent for publication: All authors have given final approval of the version of the manuscript to be submitted. Each author takes public responsibility for appropriate portions of the content and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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1. Introduction

An urgent unmet need persists for effective therapies significantly improving disease progression, survival and/or quality of life in Amyotrophic lateral sclerosis (ALS).

Edaravone (EVN) phase II study showed that in the group treated with 60 mg/day there was a significant lower decline of ALSFRS score in the 6 months of treatment. A phase III clinical trial, suggested no significant effects in ALS, although there was a trend towards slower disease progression in patients taking the drug [1]. A post-hoc analysis was performed in a subgroup of patients with the following findings: % forced vital capacity (FVC) of $\geq 80\%$, ≥ 2 points for all item scores in the ALS Functional Rating Scale-Revised (ALSFRS-R) score before treatment, a diagnosis of 'definite' or 'probable' ALS according to the El Escorial revised diagnostic criteria (EEC) and onset of disease within 2 years. The results showed a significant reduction in the ALSFRS-R score decline in patients treated with EVN [2]. Subsequently, a double-blind trial (MCI-186-J19) showed a slowing of disease progression over the 24-week treatment period in patients of this specific subgroup [3]. Based on these results, the drug was approved for ALS in Japan and in the United States. Unfortunately, the only available data about the effects of the EVN on disease progression are limited to one small clinical trial [2] and the scientific community raised some questions about its real effect [4].

The aim of this study was to evaluate data in a “real world” context about the safety and the adherence to the EVN treatment of patients that was treated in different Italian Regions. Moreover, we evaluated the effect of EVN on disease progression, respiratory function and survival comparing these patients with those extracted from the Pooled Resource Open-Access ALS Clinical Trials (PRO-ACT) database [5].

2. Patients and methods

All the patients included in the study fulfilled the MCI-186-J19 criteria (Table 1) such as a diagnosis of a clinically "probable" or "definite" ALS according to revised El Escorial Criteria, an age equal to or greater than, 18 years old, a FVC% equal or more than 80% predicted normal value for gender, height, and age in seated position at the screening visit, a score ≥ 2 in all items of ALSFRS-R at the screening visit, a disease duration from symptoms onset no longer than 24 months, and a decrease in the ALSFRS-R score of 1-4 during a 12-week observation period between the screening and the baseline. Otherwise, the exclusion criteria for the EVN treatment included concomitant significant neurological or neurodegenerative disease, concomitant significant disease in other systems or organs, creatine clearance lower than 50mL/min, women who are pregnant or breastfeeding, and patients who do not understand or provide an informed consent.

Study design

We collected data of 331 ALS patients treated with EVN (EDARAV-ALS cohort). The study was approved by the local ethical committees of participating centers. All subjects signed an approved Informed Consent Form after receiving detailed written and verbal information about the reason, the nature, the required procedures, the intended duration and the possible risks and benefits and any discomfort associated with the study. The informed consent procedure was in according to the guidelines provided in the Declaration of Helsinki and the ICH E6 Guideline for Good Clinical Practice [6].

To compare the disease progression of patients treated and not treated with EVN, a matched PRO-ACT cohort with the same inclusion and exclusion criteria of the EDARAV-ALS cohort (n=290) was extrapolated [5]. This is a useful tool for investigators interested in ALS clinical research and is a useful publicly available repository of longitudinal ALS data (<https://nctu.partners.org/ProACT/>).

The disease progression was analyzed by both descriptive and survival analysis.

Objectives

The objectives of this observational study could be summarized as follows:

- i. to assess whether the disease progression, quarterly measured through ALSFRS-R, is different between the EDARAV-ALS and PRO-ACT cohorts [5];
- ii. to assess whether the decline of the FVC% score, quarterly measured, is different between the EDARAV-ALS and PRO-ACT cohorts;
- iii. to assess whether EVN improves the survival time until the D-50 (defined as the time point when ALSFRS-R drops to 24 [7]), or the time to reach the 60% of the FVC, comparing the two cohorts of EDARAV-ALS and PRO-ACT;
- iv. to assess the EVN safety and tolerability in a long-term period (48 weeks), taking into account the following variables: adverse events (AEs) occurrence and arising changes in physical examination due to treatment, vital signs (blood pressure, pulse rate and body temperature), body weight, and clinical laboratory tests (biochemistry, hematology).

The following clinical data were collected: age at the start of treatment; sex; date of symptoms onset; type of onset (Bulbar or Spinal); El Escorial revised diagnostic criteria at the start of treatment; date of treatment start; number of treatment cycles; ALSFRS-R at treatment beginning and at each follow-up visit; disease progression rate (ΔFS)¹, considered as categorical variable stratified using Kimura et al. (2006) criteria; FVC% at the start of treatment and at each follow-up visit; date of interruption of the treatment; reason for the interruption of the treatment; date of death.

Statistical analysis

ALSFRS-R and FVC measures were quarterly extracted during 1-year follow-up, achieving a total of 5 time points. Changes in ALSFRS-R total score and FVC % were calculated as the difference between the baseline value and each time point's value.

¹ $\Delta FS = \frac{48 - \text{ALSFRS-R at start of treatment}}{\text{start date of treatment} - \text{date of onset (month)}}$

Disease progression and survival analysis were performed in accordance with the intention-to-treat principle, considering patients with at least one follow-up visit in addition to the baseline visit (n=197 for the EDARAV-ALS group and n=290 for the PRO-ACT cohort).

For each variable, Shapiro Wilk test and Levene test were used to evaluate the normality of the distribution and the homogeneity of the variance, respectively. Data were summarized using median and interquartile range for continuous variables. Wilcoxon rank sum test and chi square test were used to assess the differences in demographic and clinical features between EDARAV-ALS and PRO-ACT cohorts at baseline. Wilcoxon rank sum test was used to compare the difference in changes in both ALSFRS_r total score and FVC% between the two groups.

Moreover, comparing the EDARAV-ALS and PRO-ACT cohorts, Chi-square test and Wilcoxon rank sum test were used to analyse the proportion of patients who reached the D-50 (for the ALSFRS_r total score), the 60% FVC, and the time between the first visit and the achievement of D-50 or 60% FVC.

Finally, a survival analysis was performed, with a follow-up from the first ALS symptoms to D-50 and/or 60% FVC. Patients who died or who did not reach the milestones by the end of the 1-year follow-up period were considered as censored at the end of the follow-up. Survival curves between the two groups were estimated by the Kaplan-Meier analysis and were compared using the log rank test. Multivariable Cox proportional hazards regression model was used to analyze the survival effect of the EVN treatment, adjusted for age, sex, site of onset, diagnostic delay and Δ FS. Effect size was expressed in hazard ratio (with 95% confidence interval). These two outcomes, derived from ALSFRS-R and FVC, were chosen because of the floor effect of the ALS disease at the end of its trajectory, and because of the lack of information about date of tracheostomy in the PRO-ACT database.

All tests were two-tailed, and a p-value $<.05$ was considered as statistically significant.

Numbers and percentages of side effects related to the treatment were reported, considering also the patients who reported only the baseline visit (n=331).

The analyses were performed by two biostatisticians of two different ALS Centers, using common criteria and independent scripts, wrote by using SAS version 9.3 (SAS Institute, Inc, Cary, NC) and STATA version 13.1 (StataCorp, College Station, Texas, USA).

Protocol approvals

The study was approved by the ethical committee of each centres. Patients signed a written informed consent.

Data availability statement

Data will be available upon request by interested researchers.

3. Results

A total of 331 ALS patients treated with EVN between May 2017 to May 2019 in 39 ALS Italian Centres were collected for this study; a descriptive analysis of treated patients is reported in the Table 2.

In the survival analysis, we excluded 134 patients due to incomplete data at follow-up, therefore only 197 ALS patients were considered.

Baseline clinical features of EDARAV-ALS and PRO-ACT cohorts are reported in Table 2. No statistical significant differences emerged from the analysis, except for the age at the beginning of the treatment/first evaluation – which was significantly lower in the PRO-ACT group (60 years [54.0 – 68.0] for EDARAV-ALS cohort vs 55 years [47.5 – 63.0] for the PRO-ACT cohort; $p < 0.01$) – and for the Δ FS for which we found a significantly higher proportion of fast progressors' patients in the PRO-ACT group.

To clearly articulate findings, the results section will be structured following the objectives of the study (see above).

3.1. Comparison between patients treated vs. not treated with EVN, using ALSFRS-R and FVC scores (objectives 1 and 2)

There were no significant differences between EDARAV-ALS and PRO-ACT cohorts in any of the five time points, except for the difference between baseline and T3, and between baseline and T6 for both the ALSFRS_r and the FVC%, in which the scores were significantly higher in the PRO-ACT (Figure 1).

No significant differences emerged considering the proportion of patients that reached the D-50 and of patients that reached the 60% FVC, between the two cohorts. During the 1-year follow-up period, the D-50 was reached by 46 patients (23.3 %) in the EDARAV-ALS cohort and by 62 patients (21.4 %) in the PRO-ACT cohort ($p=0.61$). Similar results were obtained considering the 60% FVC as event, reached by 51 patients (25.9 %) in the EDARAV-ALS group and by 68 patients (23.45 %) in the PRO-ACT group ($p=0.54$). For both events, the differences in proportions between the two cohorts were represented in Figure 2.

Moreover, no significant differences were reported as far as the interval between time of the first evaluation and time of reaching D-50 (6 months [6.0 – 9.0] vs 7 months [5.0 – 9.0], $p=0.98$), or 60% FVC (6 months [3.0 – 6.0] vs 6 months [3.0 – 9.0], $p=0.76$).

3.2. Survival analysis for EVN treated vs. not treated patients, from ALS onset to D-50 and/or time to reach 60% FVC (objective 3).

No significant differences were found regarding survival among the two groups, considering both the end points: D-50 (log-rank $p=0.94$) and 60% FVC (log-rank $p=0.77$) (Figure 3). These results were confirmed also in the multivariable Cox proportional hazard model (HR=1.40; 95% CI=0.81 – 2.41, $p=0.23$ considering D-50 as endpoint, and HR=1.42; 95% CI=0.88 – 2.29, $p=0.15$ considering 60% FVC as endpoint).

Table 4 summarizes the aforementioned results, in accordance with the three objective sessions.

3.3. EVN safety and tolerability in a long-term period (objective 4).

Side effects were collected for all the patients who received at least one dose of the treatment (n=331). A qualitative analysis revealed that EVN was generally well tolerated. The adverse events were similar to those reported in the EVN literature. In particular, we recorded six cases of nephrotic syndrome and four cases of deep venous thrombosis. Minor adverse events included mild dizziness (four cases) and burning sensation at the injection site (five cases). No remarkable events were observed after interruption of treatment.

Twenty-two patients voluntarily suspended the treatment after approximately six months due to lack of effects and to the burdensome treatment in terms of duration and route of administration.

Discussion

In this observational study, EVN resulted to be overall well tolerated, without significant adverse events, in keeping with previous studies performed in Japan, Europe and South Korea [1, 2, 3, 8, 9]. Although in Italy the treatment with EVN was allowed in ALS patients with the specific clinical findings of the MCI-186-J19 trial, our multi-centre analysis did not show a positive effects of EVN on ALS progression [1, 2,3] found in the previous studies: a trend towards slower disease progression in the patients taking the drug in the phase III clinical trial [1], a significant reduction in the ALSFRS-R score decline in patients treated with EVN compared to those treated with placebo in a post-hoc analysis performed in a subgroup of patients [2], a statistically significant slowing of disease progression over the 24-week treatment period in patients of this specific subgroup taking EVN in a double blind trial [3]. Also the limited improvement in a more advanced stage of ALS shown in a Korean observational study [9], which considered 22 patients treated with EVN, was not observed in our study.

Our results confirm the findings of an Italian retrospective study, conducted by Fortuna et al. [8], performed right after July 2017, when Edaravone was formally admitted as compassionate therapy for ALS by the Italian Medicines Agency (AIFA). The study recruited 31 consecutive patients with ALS treated with EVN in the Motor Neuron Disease Clinic of the University of Padova (Italy), and showed no significant effects on disease progression.

The higher number of patients recruited in our study (n=331), compared to the previous studies on this topic, represents an important strength. This was possible thanks to a huge effort on data collection and an excellent coordination among several ALS Academic and non-Academic centres located all over Italy.

Among the main limitations of this study, some important considerations need to be addressed. Since there was not a parallel control group, a cohort of controls was extrapolated by PRO-ACT database, including only patients with the same inclusion and exclusion criteria of the EDARAV-ALS cohort. This represents the main limitation of the study, even considering only patients monitored by the revised ALSFRS-R measures. To minimize this possible selection bias, the analysis was performed using two different approaches, each one with pros and cons. The descriptive analysis proposed for the first and second objectives, represents a clear and efficient way of comparing the ALS disease progression between EDARAV-ALS and PRO-ACT groups. Nevertheless, this approach could be affected by a selection bias due to the exclusion of ALS patients for whom ALS measures were collected out of the quarter time windows.

For this reason, we performed also a survival analysis up to the D-50 for both EDARAV-ALS and PRO-ACT groups. The strength of this approach is to consider all ALSFRS-R collected for both groups, avoiding the possible selection bias of the quarterly-time windows analysis. Nevertheless, this approach could be still biased because of the different time intervals among the ALSFRS-R measures between the two groups (20 days for PRO-ACT and 35 days for EDARAV-ALS group). Anyhow, it is reasonable to assume that the different time-intervals gradients between the two groups of subjects were not so relevant in the light of negative findings resulted from the analysis.

The two approaches did not show significant differences among the two groups in terms of disease progression, with findings very similar to each other, proving that we need further studies, in a placebo-controlled setting, to well clarify whether EVN could be considered an efficacious treatment for ALS disease.

This study showed data in a “real world” context about the safety, the adherence to the therapy and its effect on the disease progression and respiratory function in patients treated with EVN, administered intravenously, in Italy. Compared to all previous researches on EVN effectiveness, this study involved the largest number of patients (331).

The treatment was overall well tolerated, but no significant differences in disease progression and respiratory functions was observed between the two cohorts of ALS patients treated and not treated with EVN.

Additional large studies with longer follow up should be performed to further assess the effectiveness of this treatment among “real world” ALS patients focusing as well on determination of its long-term effects.

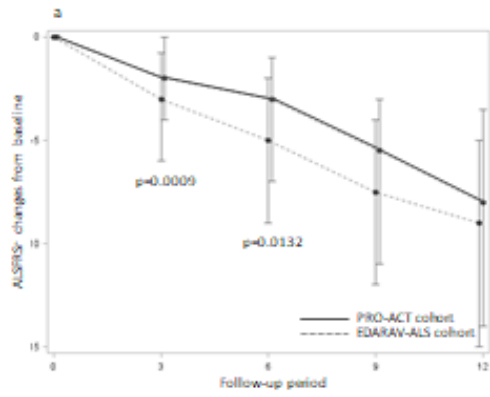
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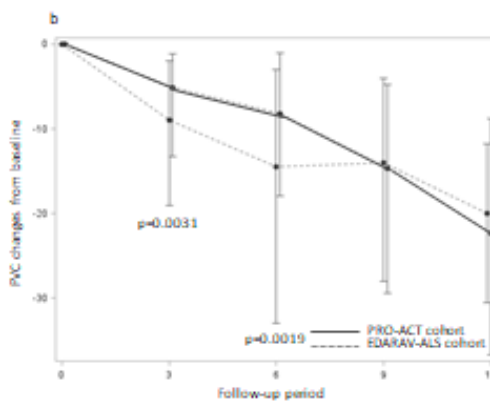
Figure Legends

Figure 1. Differences between EDARAV-ALS and PRO-ACT cohorts in any of the five time

points .



EDARAV-ALS cohort	N=197	N=184	N=128	N=74	N=53
PRO-ACT cohort	N=290	N=222	N=215	N=60	N=39



EDARAV-ALS cohort	N=197	N=173	N=122	N=67	N=44
PRO-ACT cohort	N=290	N=214	N=199	N=51	N=39

Figure 2. Differences in proportions between the EDARAV-ALS and the PRO-ACT cohorts.

Figure 2: differences in proportions between the EDARAV-ALS and the PRO-ACT cohorts

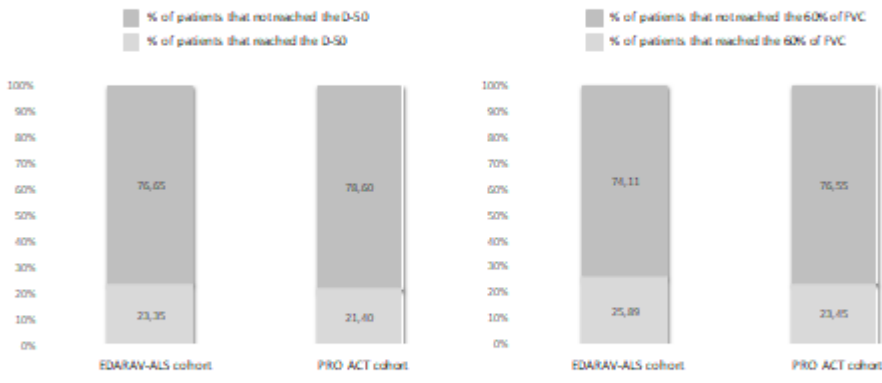


Figure 3. Differences of survival among the two groups, considering both the end points: D-50 (log-rank $p=0.94$) and 60% of FVC (log-rank $p=0.77$).

Figure 3: differences of survival among the two groups, considering both the end points: D-50 (log-rank $p=0.94$) and 60% of FVC (log-rank $p=0.77$)

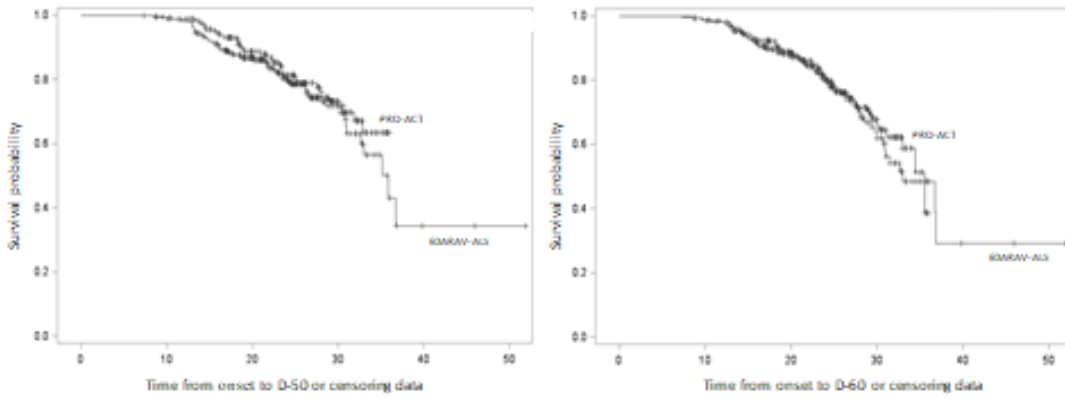


Table-1- MCI-186-J19 Inclusion and Exclusion criteria [3]

Inclusion Criteria:

- Patients whose conditions are defined as "definite ALS" or "probable ALS" diagnostic criteria El Escorial and revised Airlie House.
- Patients who can eat a meal, excrete, or move with oneself alone, and do not need assistance in everyday life.
- Patients of less than 2 years after the onset of ALS.
- Patients whose progress of the condition during 12 weeks before administration meet other requirements.
- FVC>80%

Exclusion Criteria:

- Patients with such complications as Parkinson's disease, schizophrenia, dementia, renal failure, or other severe complication, and patients who have the anamnesis of hypersensitivity to edaravone.
 - Pregnant, lactating, and probably pregnant patients, and patients who want to become pregnant, and patients who cannot agree to contraception.
 - Patients who have participated in other trials within 12 weeks before consent, or who are participating in other clinical trials at present.
 - In addition to the above exclusion criteria, patients judged to be inadequate to participate in this study by their physician.
 - FVC<80%
-

Table 2 – Demographic and clinical characteristics of ALS patients who received at least one dose of the treatment.

		EDARAV-ALS
		(n=331)
Sex, n (%)		
	Male	203 (61.3)
	Female	128 (38.7)
Age at baseline, median [IQR]		60.1 [52.9 – 68.6]
Site of onset, n (%)		
	Bulbar	66 (22.2)
	Spinal	231 (77.8)
	<i>Missing</i>	34
Diagnostic Delay, median [IQR]		9.2 [6.1 – 13.2]
	<i>Missing</i>	130
ALSFRS _r total score at baseline, median [IQR]		40.0 [36.0 – 43.0]
Δ FS, n (%)		
	Δ FS < 0.50	171 (60.6)
	$0.50 \leq \Delta$ FS < 1.00	77 (27.3)
	Δ FS \geq 1.00	34 (12.1)
	<i>Missing</i>	48
FVC % at baseline, median [IQR]		95.0 [84.0 – 105.0]

Table 3 – Demographic and clinical characteristics of EDARAV-ALS and PROACT cohorts, with complete follow-up data.

	EDARAV-ALS (n=197)	PROACT (n=290)	<i>p-value</i>
Sex, n (%)			0.07
Male	111 (56.3)	187 (64.5)	
Female	86 (43.7)	103 (35.5)	
Age at baseline, median [IQR]	60.0 [54.0 – 68.0]	55 [47.5 – 63.0]	<0.01
Site of onset, n (%)			0.07
Bulbar	42 (23.7)	48 (16.5)	
Spinal	135 (76.3)	242 (83.5)	
Missing	20	0	
Diagnostic Delay, median [IQR]	8.2 [6.0 – 12.2]	8.0 [5.1 – 11.9]	0.11
Missing	75	0	
ALSFRS _r total score at baseline, median [IQR]	41.0 [38.0 – 43.0]	41.0 [38.0 – 43.0]	0.76
Δ FS, n (%)			0.02
Δ FS < 0.50	101 (58.1)	132 (45.5)	
$0.50 \leq \Delta$ FS < 1.00	54 (31.0)	108 (37.2)	
Δ FS \geq 1.00	19 (10.9)	50 (17.2)	
Missing	23	0	
FVC % at baseline, median [IQR]	96.0 [89.0 – 108.0]	96.0 [89.0 – 108.0]	0.07

Abbreviations: ALSFRS_r, Amyotrophic Lateral Sclerosis Functional Rating Scale revised; Δ FS, Disease Progression Rate; FVC, Forced Vital Capacity.
In bold, significant *p*-values.

Table 4 – Comparison between the two groups in accordance with the preselected outcomes

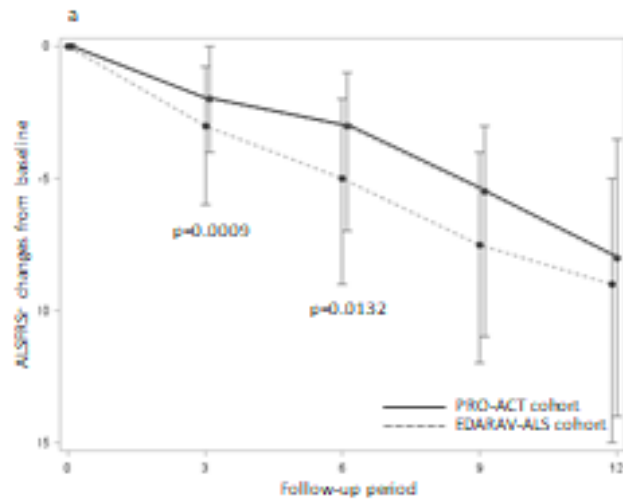
	EDARAV-ALS	PRO-ACT	<i>p-value</i>
Objective (i)			
ALSFRS _r changes from baseline to T3	3.0 [0.5 – 6.0]	2.0 [0.0 – 4.0]	<.01 ¥
ALSFRS _r changes from baseline to T6	5.0 [2.0 – 9.0]	3.0 [1.0 – 7.0]	0.01 ¥
ALSFRS _r changes from baseline to T9	7.5 [4.0 – 12.0]	5.5 [3.0 – 11.0]	0.29¥
ALSFRS _r changes from baseline to T12	9.0 [5.0 – 15.0]	8.0 [3.0 – 14.0]	0.56¥
N° of patients that reached the D-50	46 (23.3 %)†	62 (21.4 %)†	0.61¶
Time from first evaluation to D-50, months	6.0 [6.0 – 9.0]	7.0 [5.0 – 9.0]	0.98¥
Objective (ii)			
FVC changes from baseline to T3	9.0 [2.0 – 19.0]	5.1 [1.7 – 13.3]	<.01 ¥
FVC changes from baseline to T6	14.5 [3.0 – 33.0]	8.2 [1.0 – 18.0]	<.01 ¥
FVC changes from baseline to T9	14.0 [3.0 – 31.0]	14.7 [4.3 – 31.3]	0.92¥
FVC changes from baseline to T12	20.0 [11.5 – 31.0]	22.3 [8.6 – 40.1]	0.85¥
N° of patients that reached 60% FVC	51 (25.9 %)†	68 (23.45 %)†	0.54¶
Time from first evaluation to 60% FVC, months	6.0 [3.0 – 6.0]	6.0 [3.0 – 9.0]	0.76¥
Objective (iii)			
Survival from onset to D-50	0.99 [0.66 – 1.47]*		0.94‡
Survival from onset to 60% FVC	1.06 [0.73 – 1.54]*		0.77‡

Data are median [IQR] unless otherwise stated.

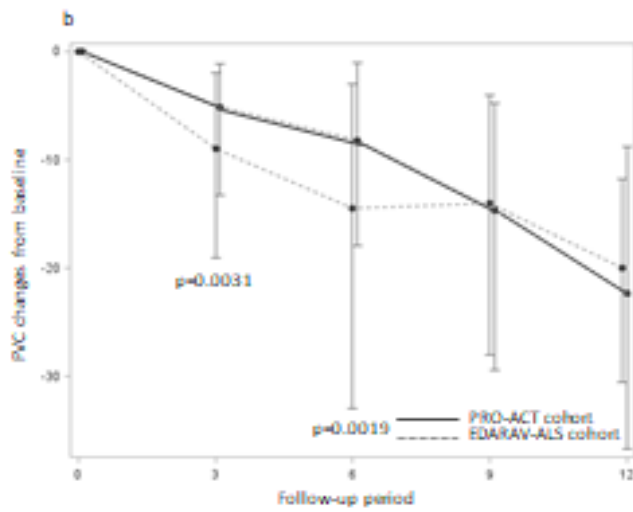
Abbreviations: ALSFRS_r, Amyotrophic Lateral Sclerosis Functional Rating Scale revised; FVC, Forced Vital Capacity.

*HR [95% CI]. †n(%). ¥ Wilcoxon rank sum test. ¶ Chi square test. ‡ Log-rank test.

In bold, significant *p*-values.



EDARAV-ALS cohort	N=197	N=184	N=128	N=74	N=53
PRO-ACT cohort	N=290	N=222	N=215	N=60	N=39



EDARAV-ALS cohort	N=197	N=173	N=122	N=67	N=44
PRO-ACT cohort	N=290	N=214	N=199	N=51	N=39

