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# Journal of Neurology

## Riluzole and other prognostic factors in ALS: a population-based registry study in Italy --Manuscript Draft--

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<b>Abstract:</b>	<p><b>Objective:</b> In this prospective population-based registry study on ALS survival, we investigated the role of riluzole treatment, together with other clinical factors, on the prognosis in incident ALS cases in Emilia Romagna Region (ERR), Italy.</p> <p><b>Methods:</b> A registry for ALS has been collecting all incident cases in ERR since 2009. Detailed clinical data from all patients diagnosed with ALS between 1.1.2009 and 31.12.2014 have been analyzed for this study, with last follow up date set at 31.12.2015.</p> <p><b>Results:</b> During the 6 years of the study, there were 681 incident cases with a median tracheostomy-free survival of 40 months (95%CI 36-44) from onset and of 26 months (95%CI 24-30) from diagnosis; 573 patients (84.14%) were treated with riluzole, 207 (30.39%) patients underwent gastrostomy, 246 (36.12%) non invasive ventilation, and 103 (15.15%) invasive ventilation. Patients who took treatment for <math>\geq 75\%</math> of disease duration from diagnosis had a median survival of 29 months compared to 18 months in patients with <math>&lt; 75\%</math> treatment duration. In multivariable analysis, factors independently influencing survival were age at onset (HR 1.04, 95%CI 1.02-1.05, <math>p &lt; 0.001</math>), dementia (HR 1.56, 95%CI 1.05-2.32, <math>p = 0.027</math>), degree of diagnostic certainty (HR 0.88, 95%CI 0.78-0.98, <math>p = 0.021</math>), gastrostomy (HR 1.46, 95%CI 1.14-1.88, <math>p = 0.003</math>), NIV (HR 1.43, 95%CI 1.12-1.82, <math>p = 0.004</math>), and weight loss at diagnosis (HR 1.05, 95%CI 1.03-1.07, <math>p &lt; 0.001</math>), diagnostic delay (HR 0.98, 95%CI 0.97-0.99, <math>p = 0.004</math>), and % treatment duration (HR 0.98, 95%CI 0.98-0.99, <math>p &lt; 0.001</math>).</p> <p><b>Conclusions:</b> Independently from other prognostic factors, patients who received riluzole for a longer period of time survived longer, but further population based studies are needed to verify if long-term use of riluzole prolongs survival.</p>	
<b>Response to Reviewers:</b>	<p>Response to reviewer comments on "Riluzole and other prognostic factors in ALS: a population-based registry study in Italy"</p> <p>We wish to thank the reviewers and editor for their comprehensive review of our manuscript. We have done our best to address each concern. We are grateful to Journal of Neurology for the opportunity to submit the revised manuscript.</p> <p>Best regards, Jessica Mandrioli (on behalf of all coauthors)</p> <p>Reviewer: 1</p> <p>Comments for the Author:</p> <p>Reviewer #1: COMMENTS FOR AUTHOR:</p> <p>Reviewer #1:</p> <p>1) Survival is defined as "diagnosis to death" (page 6, line 40) but time-varying covariate is used for "time from onset to riluzole exposure" (page 7, line 10-14 and page 8, line 34-36). The time-varying covariate should be in accordance with the survival period definition. Thus, if survival is used "from diagnosis to death" then the time-varying covariate should also be used "from diagnosis" to riluzole exposure (and not from onset).</p> <p>We agree and we made the suggested changes (page 7, lines 10-12 and page 8, lines 34-36) Results were corrected accordingly (page 8 lines 38-45, 54-61; page 9 lines 4-6, 16-26, table 4)</p> <p>2) Figure 1a: Crossing of curves at about 24 months with the riluzole group associated with a rather detrimental effect after 84 months. This is an interesting finding also described in other studies. The authors may wish to discuss the discrepancy between</p>	

	<p>the lack of an overall riluzole effect (figure 1a) and the remarkably beneficial effect in the other analysis, i.e. % treatment duration with HR = 0.18.</p> <p>We discuss this finding as suggested (discussion page 10 lines 12-24)</p> <p>3) Figure 1c: Should be PDC &gt;90</p> <p>We changed it accordingly.</p> <p>4) "% treatment duration (HR 0.18...)" (on page 4, line 32): This seems to be the main result but is not mentioned in the result section (it is only listed in table 4 on the last page). Moreover, such a HR is extremely low and would mean that each % increase in treatment duration would reduce ALS mortality to only 18%. The authors may wish to double-check their calculations and analysis.</p> <p>We apologize for the mistake, due to the fact that we calculated PDC as the ratio between Riluzole treatment duration/disease duration and not as % (as a consequence the HR of 0.18 refers to an increase of PDC of 1 unit, that is not possible). Considering PDC as %, after correcting for immortal time bias, we obtained a HR of 0.98, i.e. a decrease of the risk of death/tracheostomy of 2% for each % increase in treatment duration.</p> <p>We added the main result in the results section (page 8, lines 38-45).</p> <p>We corrected the mistake also on page 8 lines 58-60, on page 9 lines 4-6 and 16-19 and in table 2 and table 4.</p>
<p><b>Author Comments:</b></p>	<p>Modena, 23rd January 2018</p> <p>Dear Sir,</p> <p>we would like to submit our revised manuscript entitled "Riluzole and other prognostic factors in ALS: a population-based registry study in Emilia Romagna, Italy" for consideration by Journal of Neurology, as an original communication.</p> <p>We performed a prospective population-based registry study on ALS survival, aiming at studying the role of Riluzole treatment, together with other clinical factors, on the prognosis in incident ALS cases in Emilia Romagna Region (ERR), Italy. A registry for ALS has been collecting all incident cases in ERR since 2009. Detailed clinical data from all patients diagnosed with ALS between 1.1.2009 and 31.12.2014 have been analyzed for this study, with last follow up date set at 31.12.2015. During the 6 years of the study, there were 681 incident cases with a median tracheostomy-free survival of 40 months (95%C.I. 36-44) from onset and of 26 months (95%C.I. 24-30) from diagnosis; 573 patients (84.14%) were treated with riluzole, 207 (30.39%) patients underwent gastrostomy, 246 (36.12%) non invasive ventilation, and 103 (15.15%) invasive ventilation. Patients who took treatment for <math>\geq 75\%</math> of disease duration had a median survival of 29 months compared to 18 months in patients with <math>&lt; 75\%</math> treatment duration. In multivariable analysis, factors independently influencing survival were age at onset (HR 1.04, 95%CI 1.02-1.05, <math>p &lt; 0.001</math>), dementia (HR 1.56, 95%CI 1.05-2.32, <math>p = 0.027</math>), degree of diagnostic certainty (HR 0.88, 95%CI 0.78-0.98, <math>p = 0.021</math>), gastrostomy (HR 1.46, 95%CI 1.14-1.88, <math>p = 0.003</math>), NIV (HR 1.43, 95%CI 1.12-1.82, <math>p = 0.004</math>), and weight loss at diagnosis (HR 1.05, 95%CI 1.03-1.07, <math>p &lt; 0.001</math>), diagnostic delay (HR 0.98, 95%CI 0.97-0.99, <math>p = 0.004</math>), and % treatment duration (HR 0.98, 95%CI 0.98-0.99, <math>p &lt; 0.001</math>).</p> <p>In summary, in our study, independently from other prognostic factors, patients who received riluzole for a longer period of time survived longer, but further population based studies are needed to verify if long-term use of riluzole prolongs survival.</p> <p>We hope to have accomplished with all the reviewers requests.</p> <p>We hope that you will find our work to be of interest to Journal of Neurology readership and to the scientific community.</p> <p>The authors declare that they do not have any conflict of interest. All the authors have read the final draft of the manuscript and declare that there isn't any other author not included in the list. The manuscript has not been previously published and it is not under simultaneous consideration by other journals.</p>

Yours sincerely,

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The authors declare no conflicts of interest

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## ABSTRACT

**Objective:** In this prospective population-based registry study on ALS survival, we investigated the role of riluzole treatment, together with other clinical factors, on the prognosis in incident ALS cases in Emilia Romagna Region (ERR), Italy.

**Methods:** A registry for ALS has been collecting all incident cases in ERR since 2009. Detailed clinical data from all patients diagnosed with ALS between 1.1.2009 and 31.12.2014 have been analyzed for this study, with last follow up date set at 31.12.2015.

**Results:** During the 6 years of the study, there were 681 incident cases with a median tracheostomy-free survival of 40 months (95% C.I. 36-44) from onset and of 26 months (95% C.I. 24-30) from diagnosis; 573 patients (84.14%) were treated with riluzole, 207 (30.39%) patients underwent gastrostomy, 246 (36.12%) non invasive ventilation, and 103 (15.15%) invasive ventilation. Patients who took treatment for  $\geq 75\%$  of disease duration from diagnosis had a median survival of 29 months compared to 18 months in patients with  $< 75\%$  treatment duration. **In multivariable analysis,**

**factors independently influencing survival were age at onset (HR 1.04, 95% CI 1.02-1.05,  $p < 0.001$ ), dementia (HR 1.56, 95% CI 1.05-2.32,  $p = 0.027$ ), degree of diagnostic certainty (HR 0.88, 95% CI 0.78-0.98,  $p = 0.021$ ), gastrostomy (HR 1.46, 95% CI 1.14-1.88,  $p = 0.003$ ), NIV (HR 1.43, 95% CI 1.12-1.82,  $p = 0.004$ ), and weight loss at diagnosis (HR 1.05, 95% CI 1.03-1.07,  $p < 0.001$ ), diagnostic delay (HR 0.98, 95% CI 0.97-0.99,  $p = 0.004$ ), and % treatment duration (HR 0.98, 95% CI 0.98-0.99,  $p < 0.001$ ).**

**Conclusions:** Independently from other prognostic factors, patients who received riluzole for a longer period of time survived longer, but further population based studies are needed to verify if long-term use of riluzole prolongs survival.

**Key words:** amyotrophic lateral sclerosis, survival, prognostic factors, therapeutic intervention, Riluzole.

## TEXT

### INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease characterized by progressive motor deficits over the course of weeks to months leading to severe disability and death. The disease is highly heterogeneous in clinical presentation, age at onset, involvement of other than motor systems (cognitive impairment in up to 50%, variable sensory, autonomic, extrapyramidal signs), family history and genetic background, disease course and survival [1, 2]. Although survival of ALS patients from symptom onset is reported to be 3-5 years, published studies report a wide range of outcomes influenced by age and site of onset, severity and rate of disease progression, diagnostic delay, cognitive impairment, nutritional and respiratory status, functional/disability scores, Revised El Escorial diagnostic Criteria (EEC-R), multidisciplinary approach, and therapeutic interventions [3–5].

Despite significant efforts and a large number of well-designed clinical trials, published studies on ALS treatments gave negative results, probably because of ALS phenotypic, genetic and pathophysiological heterogeneity [6]. So far, riluzole is the only drug that has been shown to increase survival in ALS patients [7], although with a poorly understood mechanism of action[8] and a way of drug delivery still investigational [9, 10].

Riluzole has been tested in four randomized clinical trials (RCTs) involving 1477 patients, showing a significant effect on survival which was quantified as a gain of 3 months in 50% of patients or a 9% gain in the probability of surviving one year [11].

Conversely, subsequent cohort studies have suggested that treatment with riluzole may be associated with a median survival increase of 6 months to as many as 12 months, with diverse impact on different subpopulations of ALS patients [5, 12–15]. Two recent studies (one retrospective [16] and one prospective [17]) considered the cumulative defined daily dose to measure treatment duration, and obtained contrasting results.

In this prospective population-based registry study on ALS survival, our aim was to study the role of riluzole treatment and its duration on prognosis in incident ALS cases in Emilia Romagna Region (ERR), Italy, between 2009 and 2014.

### METHODS

#### Patient data collection

The study was performed in Emilia Romagna Region (ERR), northern Italy, considering patients diagnosed with ALS from 1/1/2009 to 31/12/2014. The region of Emilia-Romagna covers an area of 22,453 km<sup>2</sup>, ranking 6th in Italy, and during the evaluated time period, the local population increased from 4,395,569 (2009) to 4,450,508 (2014).

A prospective registry (Emilia Romagna Registry for ALS–ERRALS) has been active in our region since 2009 [18], collecting all incident ALS cases among residents of ERR, diagnosed according to EEC-R [19]. Caring physicians

collected a detailed phenotypic profile of each ALS patient, including the following parameters: age at onset and

diagnosis, gender, type of onset, site and time of onset, affected regions, upper and lower motor neuron signs, EEC-R

classification, clinical phenotype [1], presence of dementia (diagnosed by neuropsychological testing) and/or

extrapyramidal signs, family history, and drugs (including riluzole).

These data have been included at diagnosis into an electronic database available through a dedicated web-site,

accessible only to the investigators of ERR Neurological Departments. In each department, one or more investigators

were identified as study referents, and had to upload data on new ALS cases as soon as possible after diagnosis. Clinical

follow up has been performed in the 17 neurological departments of ERR until death, collecting and uploading

information on ALS course, including forced vital capacity (FVC), ALS Functional Rating Scale – Revised (ALSFRS-

R) [20, 21], riluzole intake and discontinuation, gastrostomy [22], non-invasive (NIV) or invasive (IV) ventilation

support, and cause, place and time of death. A regular supervision on the data has been performed by the coordinating

center, checking for accuracy and completeness.

Conforming to the EFNS Guidelines [23], patients underwent a regular multidisciplinary follow-up at least every 3-4

months. When patients were no longer able to reach the centers, they were monitored at their home or in a nursing home

by the Integrated Home Care of the Italian National Health System (INHS)[24].

For this study we focused on clinical variables and riluzole intake, considering date of riluzole administration and

discontinuation (if applicable) with respect to disease duration.

The study was approved by the ethical committees of the coordinating center and of ERR provinces.

## Statistical Methods

Continuous variables are reported as mean and standard deviation (SD) from the mean. Discrete variables are reported

as number and proportion of subjects with the characteristic of interest. Descriptive statistics were performed using

Student's t-test and chi-square test where appropriate. Survival was calculated from onset and from diagnosis to

death/tracheostomy or the censoring date (last day of follow-up, 31/12/2015) using the Kaplan-Meier method. The

curves were compared with the Log-Rank test. Multivariable analysis was performed with Cox's proportional hazards

model, to evaluate the association between demographic and clinical factors, riluzole use/treatment duration and

survival.

Riluzole use was defined as a categorical variable (0="no drug", 1="drug user" if treatment was taken at a dosage of

100 mg for at least 1 month), but we also classified individuals in different groups based on the duration of riluzole

treatment (in days from treatment initiation to treatment end or death/tracheostomy) with respect to ALS duration (in

days from diagnosis to death/tracheostomy) (proportion of days covered -PDC)[16]. PDC definition was adopted

because an evaluation of survival times exclusively in dependence of the days of riluzole treatment would have biased

the results since patients with longer therapy durations will have lived necessarily longer [16]. Mean PDC was 75%, median PDC was 90%; we considered these values as cut off values for the individuation of three treatment groups. We also calculated the delay from onset of symptoms to start of riluzole (months). Regarding the analysis of riluzole's effect on survival we accounted for a possible bias due to a delay in the start of riluzole treatment after diagnosis by adjusting for the immortal time bias, which refers, in observational studies, to the time during which the outcome could not have occurred [25]. To correct for this bias we used a time-varying covariate **for time from diagnosis to Riluzole exposure** in order to avoid misclassification of exposed patients' survival time before the first prescription as the exposed follow-up time [25].

We measured ALSFRS-R at diagnosis and ALSFRS-R monthly decline, subtracting ALSFRS-R total score at diagnosis from the score of 48 presumed at onset and dividing the results for the number of months of diagnostic delay [26].

For all Cox proportional hazard regression analyses, we considered age at onset, phenotypes, type of onset, EEC-R classification, presence of dementia and/or extrapyramidal signs, comorbidities using the Charlson Comorbidity Index (CCI) [27], family history of ALS, monthly ALSFRS-R decline at diagnosis and riluzole use, to assess the independent contribution of the variables of interest to death or tracheostomy.

A p value <0.05 was considered significant. All calculations were performed with STATA statistical package, V.12 (2013).

## RESULTS

### Clinical characteristics of ALS patients

During the 6 years of study, there were 681, 371 men and 310 women (men:women =1.20), incident cases in ERR. Table 1 shows the clinical features of ALS patients. Median survival time was 40 months (95% C.I. 36-44) from onset and 26 (95% C.I. 24-30) from diagnosis. The overall 1-year, 2-year, 3-year and 5-year survival rates from onset were 89.6%, 68.8%, 53.8% and 36.6% respectively. The overall 1-year, 2-year, 3-year and 5-year survival rates from diagnosis were 73.3%, 52.8%, 39.9% and 32.7% respectively. Five hundred and seventy-three patients (84.14%) were treated with riluzole, 207 (30.39%) underwent gastrostomy, 246 (36.12%) NIV, and 103 (15.15%) IV.

### Univariate analysis of factors associated with ALS survival

According to univariate analysis, factors related to tracheostomy-free survival from diagnosis were (table 2): age at onset, diagnostic delay, site of onset, phenotype, presence of dementia, degree of diagnostic certainty according to EEC-R, weight change between healthy status and diagnosis, ALSFRS-R score at diagnosis, and ALSFRS-R monthly decline, NIV and gastrostomy, comorbidities (CCI). The same factors influenced survival from disease onset (table 2). Overall riluzole treatment did not influence ALS survival (figure 1A). In contrast, the duration of treatment in relation to disease duration, expressed as percent of days of riluzole use (until discontinuation, tracheostomy or death)(PDC),

influenced survival (Table 2). Standard daily dose of riluzole was 50 mg twice a day. Median PDC was 90%. Mean PDC was 75%. Patients that took riluzole for a number of days corresponding to at least 75% of disease duration from diagnosis ( $\geq 75\%$ PDC) had a median survival of 29 months as compared to 18 months in patients with a shorter percentage of riluzole treatment (figure 1B). Patients that took riluzole for a number of days corresponding to at least 90% of disease duration from diagnosis ( $\geq 90\%$ PDC) had a median survival of 46 months as compared to 15 months in patients with a shorter percentage of riluzole treatment (figure 1C). The delay from disease onset to start of treatment significantly influenced survival (table 2) as the earlier the patients took riluzole, the worse the prognosis. Half of treated patients took riluzole treatment after 317 days (~10 months) from onset.

Patients who took riluzole for  $\geq 90\%$ PDC were younger, had a shorter diagnostic delay, a lower CCI and ALSFRS-R decline, had more frequently a spinal onset, were less frequently affected by dementia and underwent more often NIV and IV (table 3). There was a mean difference of 2 months in the delay from diagnosis to treatment initiation between the two groups.

#### **Multivariable analysis of factors associated with ALS survival**

We then did a multivariable analysis including PDC, the delay from onset to start of treatment, and all variables possibly influencing survival available at time of diagnosis. In the initial Cox multivariable model, we included the following variables (retention criterion of  $<0.10$ ): diagnostic delay, age at onset, site of onset, phenotypes, presence of dementia, EEC-R classification, PDC, delay from onset to start of treatment, ALSFRS-R score at diagnosis, ALSFRS-R monthly decline at diagnosis, weight loss at diagnosis, comorbidities (CCI), gastrostomy and NIV. We also used a time-varying covariate for time from diagnosis to riluzole exposure.

After dropping non-significant terms (stepwise backward), the final model included age at onset, diagnostic delay, dementia, EEC-R classification, gastrostomy, NIV, weight loss at diagnosis, PDC (table 4). As for PDC (%), after correcting for immortal time bias, we obtained a HR of 0.98, i.e. a decrease of the risk of death/tracheostomy of 2% for each % increase in treatment duration.

#### **Subgroups analysis of survival**

Hypothesizing that early treatment discontinuation was most frequent in more severely affected patients, we excluded from our population those patients who died before 6 months from diagnosis (79 cases). In the remaining 602 patients the results were unchanged and factors independently influencing survival (multivariable analysis) were age at onset (HR 1.03, 95% C.I. 1.02-1.04,  $p<0.001$ ), diagnostic delay (HR 0.97, 95% C.I. 0.96-0.99,  $p=0.001$ ), EEC-R classification (HR 0.86, 95% C.I. 0.76-0.98,  $p=0.020$ ), NIV (HR 1.71, 95% C.I. 1.31-2.24,  $p<0.001$ ), gastrostomy (HR 1.61, 95% C.I. 1.23-2.11,  $p=0.001$ ), weight loss at diagnosis (HR 1.04, 95% C.I. 1.01-1.06,  $p=0.002$ ), and PDC (HR 0.98, 95% C.I. 0.98-0.99,  $p=0.001$ ). As compared to the remaining study population, the 79 patients dying early were older (mean age

at onset  $73.78 \pm 7.89$  years vs  $65.53 \pm 11.10$  years), with bulbar onset (55.70% vs. 44.30%), more frequently demented (78.48% vs. 21.52%), and with a greater monthly decline of the ALSFRS-R ( $2.15 \pm 2.87$  points/months vs.  $1.00 \pm 1.36$ ).

The factors influencing survival in these patients were PDC (HR 0.95, 95% C.I. 0.94-0.97,  $p=0.001$ ), and CCI (HR 1.71, 95% C.I. 1.08-2.69,  $p=0.021$ ).

The same results were obtained removing from the initial population patients who survived less than 4 months (46 patients) and less than 5 months (64 patients)(data not shown).

We investigated if treatment with riluzole and its duration were ineffective in the patients with the lowest ALSFRS-R score at diagnosis i.e. the lowest quartile according to this variable (ALSFRS-R global score  $<35$ ). In this group, multivariable analysis showed that factors predicting survival were PDC (HR 0.98, 95% C.I. 0.97-0.99,  $p<0.001$ ), weight loss at diagnosis (HR 1.07, 95% C.I. 1.03-1.11,  $p<0.001$ ), age at onset I (HR 1.05, 95% C.I. 1.02-1.08,  $p=0.002$ ), and diagnostic delay (HR 0.97, 95% C.I. 0.94-0.99,  $p=0.009$ ). When we limited the analysis to 25% of patients having the highest ALSFRS-R monthly decline ( $>1.3$  points/month), factors predicting survival were age at onset (HR 1.04, 95% C.I. 1.01-1.06,  $p=0.007$ ), dementia (HR 3.47, 95% C.I. 1.63-7.35,  $p=0.015$ ), weight loss at diagnosis (HR 1.06, 95% C.I. 1.01-1.10,  $p=0.009$ ), and gastrostomy (HR 2.47, 95% C.I. 1.35-4.50,  $p=0.003$ ).

## DISCUSSION

Riluzole is the only drug approved for the treatment of ALS by the European Medical Agency in Europe. After  $>20$  years from its discovery [28], a number of concerns about its therapeutic effects persist: the relatively unknown mechanism of action, the modest prolongation of survival (on average a few months), with concerns about cost effectiveness, the lack of benefit on some secondary measures of efficacy, and some controversies related to the drug efficacy in population-based studies and clinical practice [11]. Otherwise ALS has no cure, currently, and riluzole has a satisfactory safety profile, leading to recommend the drug to slow disease progression for patients with ALS [29].

Because ALS treatment (including all therapeutic interventions and drugs) is completely covered by INHS and considering that it has a relatively low cost, the percentage of ERR residents with ALS using riluzole is very high. In our study, 84% of patients received riluzole treatment for at least one month, a fraction that is higher than that reported in other studies carried on in Europe [30], USA [31] and Asia [17] probably due to the different socio-economic context. Given the widespread use of this drug in our country it is therefore important to determine whether and to what extent it is effective in the real-world ALS population, to improve patient counseling and the design of clinical trials.

In previous non-RCTs studies, the effect of riluzole on ALS survival was controversial, with some studies showing no gain of survival [32], and others showing an effect ranging from 6 to 12 months [3, 5, 12], a few studies documenting an effect for the first 6 months of treatment only, with a 15% reduction in mortality at 6 months [16]. Some investigators suggested that riluzole could only slow down motor neuron degeneration and it is more effective in early-

stage patients [15, 33]; others suggest a possible beneficial effect only after long-term use of the drug [17]. This different effectiveness may be attributed to the different target populations (registry populations or referral cohorts). Moreover, in most studies riluzole use was analyzed as a categorical factor (use versus non use)[3, 5, 12, 15, 34–37], whereas only two recent studies considered the cumulative defined daily dose [16, 17] coming to opposite conclusions: a limited effect for the first 6 months in Austrian ALS patients [16], a possible effect due to long-term use in a Chinese cohort [17].

In our study, analysing survival in dependence of riluzole treatment showed a beneficial effect of the drug for the initial months of therapy only, because at 24 months after diagnosis the survival curves of riluzole-treated and untreated patients crossed with untreated patients showing an apparently better survival thereafter (figure 1A), as already reported [12, 16]. Although patients who took riluzole did not survive significantly more than patients who did not, prognosis of patients who took riluzole for more time (in relation to disease duration) was better. Our apparently inconsistent results, in agreement with a recent study [17], may reflect the hypothesis that drug efficacy can be confirmed only after long-term treatment. Moreover, patients enrolled in riluzole RCTs were perhaps more motivated to carry on the study treatment until the study end (hoping in a new therapy for ALS) than current real-world patients who may discontinue the drug because they saw themselves worsening or were aware of its limited effectiveness [37]. Nevertheless, since the duration of treatment with riluzole would be longer in patients living longer, this might be a potential confounder of the dose-dependent effect of riluzole on survival, and the lack of difference between patients treated with riluzole and patients who were untreated may be due to the fact that more severely affected individuals do not take it or discontinue its intake because of a rapidly worsening condition. For this reason, we also adjusted our analysis for immortal time bias, but results were similar, suggesting that this potential bias did not substantially affect our findings.

We also eliminated from our sample patients died within 6 months from diagnosis, but the results were unchanged. To test the hypothesis of a lack of efficacy of riluzole treatment in more severely affected patients at diagnosis, we also evaluated patients with a low ALSFRS-R score at diagnosis: in that group, the extent of treatment duration independently influenced survival.

Conversely, our analyses in patients who had a high ALSFRS-R monthly decline showed that in this small sample PDC did not influenced survival: in this rapidly progressive population riluzole, whenever given, has no effect on survival.

As efficacy of riluzole seemed related to the duration of its intake, we hypothesized that the earlier it is given to patients the longer the patients will survive, as already suggested [38]. Therefore we studied the relationship between delay from disease onset and start of riluzole, and survival. We found that the earlier the patients took riluzole, the worse the prognosis, probably because of the well known negative prognostic role of a short diagnostic delay in ALS [3].



On the contrary, we identified the characteristics of patients who took riluzole for a longer period of time: these patients were younger, more frequently with spinal onset, without cognitive impairment, and with a lower CCI. Younger age and spinal onset, and absence of dementia, are well known factors related to a better survival [3]. These patients may be more compliant and more prone to comply with medical counseling and pharmacological treatment: we found that patients treated for a longer time with riluzole underwent more frequently NIV and IV, suggesting a general attitude to accept all therapeutic interventions which may influence survival [3]. These findings are similar to what reported by others [17].

The study has strengths and limitations. The major strength is its population-based design. In addition, we are fairly confident that case ascertainment in the study area was almost complete, as shown by the incidence rate of the disease [18].

A limitation of this study is the observational design, compared to the experimental context of RCTs. However, observational studies particularly if population-based, have the advantage of a longer follow-up than the RCTs, and also include participants who approximate real world population [39]. This also means that, since age at onset and disease severity are more heterogeneous in a population-based registry study than in RCTs [28, 40, 41], older and more severely affected patients were included in our study in comparison with RCTs. A recent Cochrane review [11] showed that analyzing pooled riluzole RCTs data, the effects of riluzole on survival were significant when the homogeneous group of participants in the first two trials were considered [28, 40], but when all three trials were analyzed [41], there was a high heterogeneity due to the addition of more seriously affected and older patients, and the combined treatment effect fell just short of significance [11].

Another possible limitation of the study is the fact that the vast majority of ALS patients received Riluzole [23]. In addition, we did not collect data on advance directives of patients in relation to nutritional and respiratory supports which can influence survival and we do not have data about reasons for riluzole treatment discontinuations or related side effects. Furthermore, we had genetic status only in a limited number of cases and this information would have impact survival especially in relation to C9orf72 hexanucleotide repeat expansion [42].

Finally, as discussed before, a specific and important weakness of our study is that, since the duration of treatment would be greater in patients living longer, this may be a potential confounder of the dose-dependent effect of riluzole on survival.

In summary, in our study patients who received riluzole for a proportionally longer period of time survived longer, thus suggesting that long-term use of riluzole may prolong survival. Younger and cognitively normal ALS patients and patients with a spinal onset, a lower CCI, and lower decline of ALSFRS-R were more likely to use riluzole for a longer time and survived longer. However, further studies are needed to verify if long-term use of riluzole prolongs survival.

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## TABLES AND FIGURES LEGENDS

**Table 1: Patients characteristics (681 patients)**

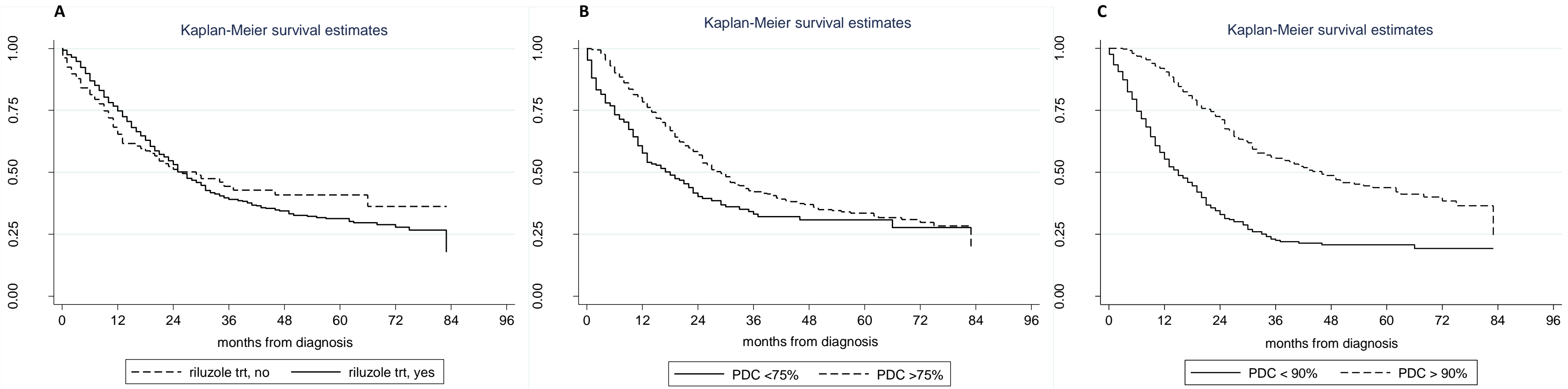
**Table 2: Clinical factors and survival (from onset/diagnosis to death or tracheostomy) in incident patients of Emilia Romagna, Italy (univariate analysis) (681 patients)**

**Table 3: Clinical characteristics of patients with respect to % of riluzole treatment duration in relation to survival ( $\geq 90\%$  versus 1-90% of time of disease duration, versus no treatment) (681 patients)**

**Table 4: Independent prognostic factors (multivariable Cox analysis)**

**Figure 1: A) Tracheostomy-free survival from diagnosis of incident ALS cases in Emilia Romagna Region based on riluzole treatment; B) Tracheostomy-free survival from diagnosis of incident ALS cases in Emilia Romagna Region based on percentage of time of riluzole treatment in relation to disease duration ( $\geq 75\%$  versus  $< 75\%$  of time of disease duration); C) Tracheostomy-free survival from diagnosis of incident ALS cases in Emilia Romagna Region based on percentage of time of riluzole treatment in relation to disease duration ( $\geq 90\%$  versus  $< 90\%$  of time of disease duration).**

Figure 1



**Table 1: Patients' characteristics (N = 681)**

<i>Explanatory variables</i>	<b>Total</b> <b>N=681</b> <b>n (%)</b> <b>m [SD]<sup>a</sup></b>	<b>Men N=371</b> <b>n (%)</b> <b>m [SD]</b>	<b>Women N=310</b> <b>n (%)</b> <b>m [SD]</b>	<b>p-value</b>
<b>ALS Onset</b> (Bulbar)	238 (34.95)	107 (28.84)	131 (42.26)	<b>&lt;0.001</b>
<b>Age at onset</b>	66.50 [±11.09]	65.87 [±10.66]	67.26 [±11.57]	0.102
<b>Diagnostic delay</b>	13.16 [±13.09]	13.13 [±13.85]	13.20 [±12.14]	0.947
<b>Phenotype</b>				
Bulbar	211 (30.98)	92 (24.80)	119 (38.39)	<b>&lt;0.001</b>
Classic	286 (42.00)	177 (47.71)	109 (35.16)	
Flail arm and leg	119 (17.47)	68 (18.33)	51 (16.45)	
UMNp <sup>c</sup>	38 (5.58)	16 (4.31)	22 (7.10)	
Respiratory	21 (3.96)	15 (4.04)	6 (1.94)	
Unknown	6 (0.88)	3 (0.81)	3 (0.97)	
<b>Revised El Escorial criteria</b>				
Definite	189 (27.75)	94 (25.34)	95 (30.65)	0.250
Clinically probable	216 (31.72)	122 (32.88)	94 (30.32)	
Probable lab-supported	92 (13.51)	48 (12.94)	44 (14.19)	
Possible	136 (19.97)	82 (22.10)	54 (17.42)	
<b>Dementia</b>	64 (9.39)	31 (8.36)	33 (10.65)	0.308
<b>BMI<sup>d</sup> at diagnosis (Kg/m<sup>2</sup>)</b>	24.39 [±3.98]	24.94 [±3.72]	23.73 [±4.19]	<b>&lt;0.001</b>
<b>Riluzole</b> (Yes)	573 (84.14)	319 (85.98)	254 (81.94)	0.150
<b>Treatment duration</b> (days)	869.24 [±645.00]	867.84 [±639.67]	870.99 [±652.84]	0.954
<b>Delay from onset to riluzole intake</b> (days)	421.13 [±372.12]	408.82 [±367.45]	436.48 [±378.02]	0.378
<b>Delay from diagnosis to riluzole intake</b> (days)	41.53 [±97.98]	39.74 [±80.31]	43.76 [±116.46]	0.626
<b>Gastrostomy</b> (Yes)	207 (30.39)	96 (25.88)	111 (35.81)	<b>0.005</b>

<b>Non invasive ventilation</b> (Yes)	246 (36.12)	147 (39.62)	99 (31.94)	0.038
<b>Invasive ventilation</b> (Yes)	103 (15.15)	65 (17.52)	38 (12.26)	0.059
<b>ALSFRS-R<sup>e</sup> at diagnosis</b>	38.58 [ $\pm$ 7.95]	39.28 [ $\pm$ 7.26]	37.75 [ $\pm$ 8.63]	0.013
<b>ALSFRS-R monthly decline (points/month) measured at diagnosis</b>	1.14 [ $\pm$ 1.66]	1.08 [ $\pm$ 1.41]	1.21 [ $\pm$ 1.92]	0.300
<b>Charlson Comorbidity Index</b>	2.73 [ $\pm$ 1.56]	2.69 [ $\pm$ 1.50]	2.77 [ $\pm$ 1.62]	0.221

SD<sup>a</sup>: Standard Deviation; NA<sup>b</sup>: Not Appropriate; UMN-P<sup>c</sup>: Upper Motor Neuron predominant; BMI<sup>d</sup>: Body Mass

Index; ALSFRS-R<sup>e</sup>: ALS Functional Rating Scale – Revised.



**Table 2: Demographic and clinical factors and survival (from onset/diagnosis to death or tracheostomy) in incident patients of Emilia Romagna, Italy (univariate analysis) (N = 681)**

Variable	Categories	Survival from onset			Survival from diagnosis		
		HR <sup>c</sup>	95%CI <sup>d</sup>	p-value	HR	95%CI	p-value
Sex	Woman	1	(reference)	0.239	1	(reference)	0.308
	Man	1.12	0.93-1.37		1.11	0.91-1.35	
Onset	Spinal	1	(reference)	<0.001	1	(reference)	<0.001
	Bulbar	1.77	1.45-2.16		1.69	1.39-2.06	
Diagnostic delay	Months	0.41	0.33-0.50	<0.001	0.98	0.97-0.99	<0.001
Age at onset	Years	1.04	1.03-1.05	<0.001	1.04	1.03-1.05	<0.001
Phenotype	Bulbar	1	(reference)	<0.001	1	(reference)	0.015
	Classic	0.63	0.50-0.78		0.80	0.64-1.00	
	Flail arm/leg	0.47	0.35-0.64		0.70	0.52-0.94	
	Upper MN-predominant	0.32	0.19-0.56		0.52	0.31-0.86	
	Respiratory	1.29	0.77-2.16		1.15	0.65-2.09	
Dementia	No	1	(reference)	<0.001	1	(reference)	<0.001
	Yes	2.02	1.50-2.73		2.26	1.67-3.07	
Familial ALS	No	1	(reference)	0.711	1	(reference)	0.679
	Yes	1.09	0.68-1.75		1.10	0.69-1.771	
EEC-R <sup>a</sup>	Definite	1	(reference)	<0.001	1	(reference)	0.025
	Probable	0.71	0.56-0.91		0.89	0.70-1.13	
	Probable lab. supported	0.43	0.31-0.61		0.68	0.49-0.95	
	Possible	0.62	0.47-0.82		0.68	0.51-0.92	
ALSFRS-R <sup>b</sup> at diagnosis	Points	0.99	0.97-1.00	0.032	0.98	0.97-0.99	<0.001
ALSFRS-R monthly decline	Points	1.32	1.27-1.38	<0.001	1.24	1.10-1.39	<0.001
ALSFRS-R monthly decline	<1 point/month	1	(reference)	<0.001	1	(reference)	0.014
	≥1 point/month	3.75	2.97-4.74		1.33	1.06-1.65	

<b>BMI at diagnosis</b>	Kg/m <sup>2</sup>	0.98	0.95-1.01	0.118	0.97	0.95-1.00	0.086
<b>Weight variation between healthy state and diagnosis</b>	kg	1.04	1.03-1.05	<b>&lt;0.001</b>	1.05	1.04-1.07	<b>&lt;0.001</b>
<b>Riluzole treatment (No/Yes)</b>	No	1	(reference)	0.39	1	(reference)	0.703
	Yes	1.12	0.86-1.48		1.05	0.80-1.39	
<b>Riluzole treatment duration/disease duration (onset to death/tracheostomy)</b>	<60%	1	(reference)	<b>&lt;0.001</b>	NA	NA	NA
	≥60%	0.57	0.47-0.69		NA	NA	NA
<b>Riluzole treatment duration/disease duration (onset to death/tracheostomy)</b>	%	0.49	0.37-0.66	<b>&lt;0.001</b>	NA	NA	NA
<b>Riluzole treatment duration/disease duration (diagnosis to death/tracheostomy)</b>	<75%	NA	NA	NA	1	(reference)	<b>0.001</b>
	≥75%	NA	NA	NA	0.69	0.55-0.86	
<b>Riluzole treatment duration/disease duration (diagnosis to death/tracheostomy)</b>	%	NA	NA	NA	0.99	0.99-1.00	<b>0.010</b>
<b>Delay from onset to riluzole intake</b>	months	0.96	0.95-0.97	<b>&lt;0.001</b>	0.98	0.97-0.99	<b>&lt;0.001</b>
<b>Delay from onset to riluzole intake</b>	<10 months	1	(reference)	<b>&lt;0.001</b>	1	(reference)	<b>&lt;0.001</b>
	≥10 months	0.43	0.35-0.53		0.64	0.52-0.79	
<b>Non invasive ventilation</b>	No	1	(reference)	<b>&lt;0.001</b>	1	(reference)	<b>&lt;0.001</b>
	Yes	1.58	1.30-1.92		1.59	1.318-1.94	
<b>Gastrostomy</b>	No	1	(reference)	<b>&lt;0.001</b>	1	(reference)	<b>&lt;0.001</b>
	Yes	1.86	1.53-2.27		1.80	1.47-2.19	
<b>Charlson Comorbidity Index</b>	Points	1.21	1.15-1.29	<b>&lt;0.001</b>	1.24	1.17-1.31	<b>&lt;0.001</b>

<sup>a</sup>EEC-R: El Escorial Criteria- Revised; <sup>b</sup>ALSFRS-R: ALS Functional Rating Scale –Revised; <sup>c</sup>HR= Hazard Ratio; <sup>d</sup>CI=

Confidence Interval; Significant results in bold

**Table 3: Clinical characteristics of patients with respect to % of riluzole treatment duration in relation to survival from diagnosis (>90% versus 1-90% of time of disease duration, versus no treatment) (N = 681)**

<i>Explanatory variables</i>	<b>No riluzole treatment</b> <b>N=106</b> <b>n (%) m [SD]<sup>d</sup></b>	<b>Riluzole treatment ≤90%</b> <b>N=228</b> <b>n (%) m [SD]</b>	<b>Riluzole treatment &gt;90%</b> <b>N=347</b> <b>n (%) m [SD]</b>	<b>p-value</b>
<b>ALS Onset</b> (Bulbar)	44 (41.51)	88 (38.59)	106 (30.55)	<b>0.043</b>
<b>Sex</b> (Man)	51 (48.11)	125 (54.82)	195 (56.19)	0.340
<b>Age at onset</b>	69.85 [±11.04]	68.33 [±9.99]	64.27 [±11.35]	<b>&lt;0.001</b>
<b>Diagnostic delay</b>	15.77 [±17.98]	12.34 [±13.30]	12.81 [±10.85]	0.067
<b>Phenotype</b>				
Bulbar	42 (39.62)	73 (32.02)	97 (27.95)	0.187
Classic	41 (38.68)	100 (43.86)	150 (43.23)	
Flail arm and leg	14 (13.21)	32 (14.03)	73 (21.04)	
UMNp <sup>a</sup>	6 (5.66)	13 (5.70)	19 (5.47)	
Respiratory	3 (2.83)	10 (4.38)	8 (2.30)	
<b>Revised El Escorial Criteria</b>				
Definite	27 (28.72)	71 (33.65)	91 (27.74)	0.562
Clinically probable	37 (39.36)	71 (33.65)	108 (32.93)	
Probable lab-supported	12 (12.76)	26 (12.32)	54 (16.46)	
Possible	18 (19.15)	43 (20.38)	75 (22.86)	
<b>Dementia</b>	13 (12.26)	32 (14.03)	19 (5.47)	<b>0.001</b>
<b>BMI<sup>b</sup> at diagnosis</b> (Kg/m <sup>2</sup> )	24.00 [±4.71]	24.16 [±4.04]	24.64 [±3.75]	0.290
<b>Gastrostomy</b> (Yes)	24 (22.64)	78 (34.21)	105 (30.26)	0.101
<b>Non invasive ventilation</b> (Yes)	28 (26.41)	77 (33.77)	141 (40.63)	<b>0.019</b>
<b>Invasive ventilation</b> (Yes)	8 (7.55)	49 (21.58)	46 (13.26)	<b>0.001</b>
<b>ALSFRS-R<sup>c</sup> monthly decline</b> (points/month)	0.83 [±0.69]	0.86 [±0.85]	0.69 [±0.67]	<b>0.016</b>
<b>Days from diagnosis to riluzole intake</b>	NA	78.98 [±148.67]	17.62 [±16.04]	<b>&lt;0.001</b>
<b>Days from onset to riluzole intake</b>	NA	442.37 [±438.73]	401.91 [±326.05]	<b>&lt;0.001</b>
<b>Absolute duration of Riluzole treatment</b>	NA	361.38 [±368.55]	1027.25 [±	<b>&lt;0.001</b>

(days)			611.16]	
<b>Charlson Comorbidity Index</b>				<b>0.027</b>
<b>&lt;3</b>	41 (38.68)	88 (38.60)	173 (49.86)	
<b>3-5</b>	59 (55.66)	127 (55.70)	165 (47.55)	
<b>6-10</b>	6 (5.66)	13 (5.70)	9 (2.59)	

<sup>a</sup>UMN-p= Upper Motor Neuron- predominant, <sup>b</sup>BMI= Body Mass Index, <sup>c</sup>ALSFRS-R: ALS Functional Rating Scale –

Revised; <sup>d</sup>SD= Standard Deviation; Significant results in bold

**Table 4: Independent prognostic factors (multivariable Cox analysis)**

Variable	Survival from diagnosis to death or tracheostomy		
	Hazard Ratio	95%CI	p-value
Age at onset (years)	1.04	1.02-1.05	<0.001
Dementia (yes/no)	1.56	1.05-2.32	0.027
Revised El Escorial Criteria classification	0.88	0.78-0.98	0.021
Weight variation between healthy state and diagnosis (kg)	1.05	1.03-1.07	<0.001
Gastrostomy (Yes/No)	1.46	1.14-1.88	0.003
Non invasive ventilation (Yes/No)	1.43	1.12-1.82	0.004
Time from onset to diagnosis (months)	0.98	0.97-0.99	0.004
Riluzole treatment duration/disease duration (diagnosis to death/tracheostomy) (%)	0.98	0.98-0.99	<0.001

<sup>a</sup>ALSFRS-R: ALS Functional Rating Scale - Revised. Significant results in bold