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ORIGINAL ARTICLE

In-depth analysis of data from the RAS-ALS study reveals new insights in rasagiline treatment for amyotrophic lateral sclerosis

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Abstract

Background and purpose: In 2016, we concluded a randomized controlled trial testing 1 mg rasagiline per day add-on to standard therapy in 252 amyotrophic lateral sclerosis (ALS) patients. This article aims at better characterizing ALS patients who could possibly benefit from rasagiline by reporting new subgroup analysis and genetic data.

Methods: We performed further exploratory in-depth analyses of the study population and investigated the relevance of single nucleotide polymorphisms (SNPs) related to the dopaminergic system.

Results: Placebo-treated patients with very slow disease progression (loss of Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised [ALSFRS-R] per month before randomization of ≤0.328 points) showed a per se survival probability after 24 months of 0.85 (95% confidence interval = 0.65–0.94). The large group of intermediate to fast progressing ALS patients showed a prolonged survival in the rasagiline group compared to placebo after 6 and 12 months ($p = 0.02$, $p = 0.04$), and a reduced decline of ALSFRS-R after 18 months ($p = 0.049$). SNP genotypes in the *MAOB* gene and *DRD2* gene did not show clear associations with rasagiline treatment effects.

Conclusions: These results underline the need to consider individual disease progression at baseline in future ALS studies. Very slow disease progressors compromise the statistical power of studies with treatment durations of 12–18 months using clinical endpoints. Analysis of *MAOB* and *DRD2* SNPs revealed no clear relationship to any outcome parameter. More insights are expected from future studies elucidating whether patients with $DRD2_{CC}$ genotype (Rs2283265) show a pronounced benefit from treatment with

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rasagiline, pointing to the opportunities precision medicine could open up for ALS patients in the future.

KEYWORDS
amyotrophic lateral sclerosis, randomized controlled trial, rasagiline, subgroup analysis

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, leading to progressive paralysis of most voluntarily innervated muscles, and to death caused by respiratory failure after a mean disease duration of 3 years [1]. The causes of ALS are largely unknown; it has been shown that pTDP-43, a pathological, misfolded protein, continuously spreads over the brain and spinal cord [2, 3]. Despite decades of intensive research, no effective therapy for ALS has been found. To date, riluzole, a glutamate antagonist, is the only approved drug for ALS in Europe; it marginally prolongs survival in the pivotal studies by about 3 months [4].

Rasagiline is a monoamine oxidase B (MAOB) inhibitor that is known to beneficially modify the course of Parkinson disease [5]. By inhibiting MAOB, rasagiline reduces dopamine and serotonin catabolism, thereby increasing the availability of dopamine and serotonin for neurotransmission. In ALS, a prominent pathological involvement of dopaminergic [6, 7] and serotonergic neurons [8, 9] has been repeatedly described.

Moreover, it has been shown for MAOB inhibitors like for example rasagiline and selegiline that both reveal antiapoptotic and antioxidative activities [10–13], induce growth factor secretion, and reduce pro-inflammatory cytokines like IL-1ß, IL-6, and TNF-alpha [14–17]. Both apoptosis and oxidative stress are known to contribute to ALS pathogenesis [18]. Therefore, rasagiline was identified as a promising therapeutic candidate for ALS and tested in the G93A mouse model for familial ALS, in which a dose-dependent therapeutic effect on motor function and prolonged survival by 20% was observed [19]. In 2016, we concluded a randomized controlled trial (RCT) with 1 mg rasagiline versus placebo as an add-on to standard therapy with riluzole in 252 ALS patients. Although the primary endpoint (survival at the end of the trial) was negative, exploratory analyses revealed a statistically significant benefit on survival after 6 months in all study patients (full analysis set [FAS]). Subgroup analysis of normal to fast progressing patients additionally revealed a decreased death rate after 6 months ($p = 0.007$) and 12 months ($p = 0.02$), as well as a reduced decline of Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R) scores after 6 ($p = 0.01$), 12 ($p = 0.01$), and 18 months ($p = 0.005$) [20]. However, it remained unclear why the drug was apparently only effective in this subset of patients.

In this context, recent publications reported single nucleotide polymorphisms (SNPs) in the *MAOB* gene and the D2 autoreceptor coding gene (*DRD2*) influencing treatment effects with rasagiline in Parkinson disease [21–23]. Hence, the role and importance of these SNPs for the treatment of ALS with rasagiline has become of great interest as well.

This article aims to better understand the treatment effect in a subset of patients whose course of disease can potentially be modified beneficially by rasagiline by performing additional subgroup analyses and SNP analysis of the 2016 concluded RCT.

METHODS

Study design of the original RAS-ALS trial

The RAS-ALS study [20] was a randomized, double-blind, parallel group, placebo-controlled trial of rasagiline as an add-on therapy to riluzole in patients with ALS. It was conducted at 15 sites of the clinical and scientific German Network for Motor Neuron Diseases (MND-NET) and was executed in accordance with the Declaration of Helsinki, International Conference on Harmonization Guideline for Good Clinical Practice, European Union Clinical Trials Directive, and applicable local regulations. The Competent Ethics Committee of Ulm University, Germany, in consultation with the involved local ethics committees, approved the study protocol (approval number 378/12). Additional genetic analysis with biomaterial derived from this trial was approved by the ethics committee of Ulm University, Germany (approval number 472/17).

The study population was defined as follows: patients with possible, probable (clinically or laboratory supported), or definite ALS, according to the revised version of the El Escorial World Federation of Neurology criteria [24]; patients aged at least 18 years; onset of progressive weakness within 36 months before the study; disease duration of >6 months and <3 years (with disease onset defined as date of first muscle weakness); and best sitting slow vital capacity of at least 50%. All included patients had been treated with 100 mg riluzole per day for at least 3 months before inclusion. Please refer to Ludolph et al. [20] for more detailed information on the inclusion and exclusion criteria of the study.

Enrolled patients underwent a screening phase, which lasted up to 4 weeks, and an 18-month treatment phase. Clinical and physical examinations (outcome measures), blood sampling, and drug compliance were recorded at on-site visits (2, 6, 12, and 18 months after baseline visit) and/or via telephone (1, 3, 9, and 15 months after baseline). Study participants were asked to provide a voluntary blood sample for genetic analysis of the dopaminergic system. This sample was collected either at screening or at baseline visit. Informed consent in line with abovementioned ethics committee approvals was obtained. Long-term survival status of all study participants was collected at the end of the study (i.e., the last patient's last visit plus the 14-day follow-up for adverse events).

DNA human blood extraction

Hemolysis was performed using isotonic ammonium chloride buffer in EDTA, and leukocytes collected after centrifugation. The leukocyte pellet was treated with 10 mg/mL proteinase K in buffer with the presence of 20% sodium dodecyl sulfate and 250 mM EDTA, and DNA was precipitated with saturated sodium chloride solution (6 M), washed with 70% ethanol, and dissolved in TE buffer before SNP genotyping.

MAOB genotyping

Intron 13 polymorphism in the *MAOB* gene (Rs1799836) was genotyped using polymerase chain reaction (PCR)-based restriction fragment length polymorphism as previously described [25]. Briefly, PCR was performed with MasterMix Taq DNApolymerase (VWR International, Ref. 733–1320) and the following primers: Maob-For: 5′-GGAACCTCTTATACCACAGG-3′ and 5′-GACTGCCA GATTTCATCCTC-3′ in the following program: 4 min 94°C (40 s 94°C, 30 s 54°C, 50 s 72°C) × 34, 10 min 72°C. PCR products were then digested with Tsp45I enzyme and loaded on a 2% agarose (Euromedex, Ref. D5-E) gel electrophoresis with Low Molecular Weight DNA Ladder (New England Biolabs, Ref. N3233L) and stained with ethidium bromide using standard procedures. The G allele yields a single 232-bp band, and the A allele yields two bands of 146 and 86 bp.

SNPs: *DRD2*

DRD2 SNPs were genotyped using allele-specific quantitative PCR assays previously published [26] and following primers Rs2283265 (wild: GGA-AAC-AGG-CTC-ATA-GAA-GGT-ATG-C; SNP: CCG-GCG-CGG-CCG-CCG-GAA-ACA-GGC-TCA-AAG-GTA-CGA; forward: TTT-TGC-TGA-GTG-ACC-TTA-GGC-AA) and the PCR program 30 s 95°C; (10 s 95°C, 1 min 60°C) × 39; 5 s 90°C with Sso Advanced Universal SYBR Green Supermix (Bio-Rad 1725274).

Statistical analysis

Descriptive analyses are based on mean and SD or median and interquartile range as appropriate for continuous data and absolute/relative frequencies for categorical data. We used the two-sample *t*-test or the Mann–Whitney *U*-test as appropriate for group comparisons of continuous data. For group comparisons of categorical data, the chi-squared test or Fisher exact test was used as appropriate. Kaplan–Meier plots and the log-rank test were used for group comparisons of survival time. Additionally, Cox proportional hazard regression models were fitted to calculate the hazard ratio (HR).

To define the slope of ALSFRS-R at baseline, we collected the date of first paresis and calculated the progression rate according to the following formula: (48 − score at randomization) / (date of randomization − date of first symptom). Missing data were not replaced. For dropouts, survival time was treated as censored at the time of dropout.

We did all statistical tests two-sided at a significance level of 5%. An adjustment for multiple testing was not done. Therefore, all results were interpreted as hypothesis-generating and not as proof of efficacy. Statistical analyses were done using SAS, version 9.4, and GraphPad Prism, version 7.04.

RESULTS

In 2016, we concluded the RAS-ALS trial testing 1 mg rasagiline versus placebo as an add-on to standard therapy with riluzole in 252 ALS patients. Although the primary endpoint was negative, exploratory analyses revealed a statistically significant benefit on survival after 6 months in all study patients (FAS) [20]. We also found a potential disease-modifying effect of rasagiline in the subgroup of normal to fast progressing patients ($n = 122$), as the decline of ALSFRS-R was significantly reduced compared to placebo. This protective effect was substantiated by a prolonged survival after 6 and 12 months in the rasagiline group [20].

Clinical outcome measures in slow progressing study participants

To approach the question of which ALS patients responded to the treatment with rasagiline, we now defined new subgroups using the lower quartile of progression rate at study baseline. This cutoff divided the slopes at a loss of 0.328 points on the ALSFRS-R per month between onset of first symptom and baseline. Patients with a progression rate ≤ 0.328 were defined as very slow progressors, and patients with a progression rate > 0.328 were defined as intermediate to fast progressors. Baseline characteristics for very slow progressors and intermediate to fast progressors were similar in both treatment groups (rasagiline vs. placebo; Table 1); rasagiline and placebo patients had similar age, body mass index, and functional status as measured by the ALSFRS-R and slow vital capacity.

Survival analysis in patients with very slow disease progression revealed no difference after 6 ($p = 0.36$), 12 ($p = 0.47$), and 18 months ($p = 0.81$) between rasagiline and placebo. The survival probability after 24 months in patients treated with rasagiline was 0.86 (95% confidence interval [CI] = 0.61–0.95), and for patients in the placebo group 0.85 (95% CI = 0.65–0.94). Only three patients in the placebo group and two patients in the rasagiline group died within the treatment period of 18 months. These results indicate that a survival benefit of rasagiline in the subgroup of very slow progressing patients was unlikely to be detected within the given treatment period of 18 months.

Survival analysis in patients with intermediate to fast disease progression showed prolonged survival in the rasagiline group

TABLE 1 Patient characteristics at baseline according to progression rate in the original RAS-ALS trial (very slow progressors vs. intermediate to fast progressors).

	Progression rate ≤ 0.328 points of ALSFRS-R per month upfront randomization [25th percentile cutoff]				Progression rate > 0.328 points of ALSFRS-R per month upfront randomization			
Characteristic	Placebo, $n=31$	Rasagiline, $n=25$	Total, $n=56$	p	Placebo, $n=82$	Rasagiline, $n=88$	Total, $n=170$	p
Age, years[a]	57.6 ± 11.1	60.7 ± 10.3	59.0 ± 10.8	0.29[b]	61.0 ± 9.9	59.7 ± 11.6	60.3 ± 10.8	0.44[b]
Sex								
Female	9 (29.0%)	8 (32.0%)	17 (30.4%)	0.81[c]	27 (32.9%)	40 (45.5%)	67 (39.4%)	0.09[c]
Male	22 (71.0%)	17 (68.0%)	39 (69.6%)		55 (67.1%)	48 (54.5%)	103 (60.6%)	
BMI, kg/m^2[a]	25.3 ± 3.3	25.7 ± 3.4	25.4 ± 3.3	0.65[b]	25.8 ± 3.7	25.4 ± 3.8	25.6 ± 3.8	0.41[b]
Onset								
Bulbar	6 (19.4%)	8 (32.0%)	14 (25.0%)	0.28[c]	17 (20.7%)	20 (22.7%)	37 (21.8%)	0.75[c]
Spinal	25 (80.6%)	17 (68.0%)	42 (75.0%)		65 (79.3%)	68 (77.3%)	133 (78.2%)	
Duration of disease, months[a,d]	24.6 ± 9.4	26.7 ± 17.5	25.6 ± 13.5	0.59[b]	15.4 ± 8.6	16.7 ± 8.5	16.1 ± 8.5	0.31[b]
Certainty of diagnosis								
Definite	5 (16.1%)	6 (24.0%)	11 (19.6%)	0.04[c]	15 (18.3%)	16 (18.2%)	31 (18.2%)	1.00[c]
Probable	20 (64.5%)	7 (28.0%)	27 (48.2%)		43 (52.4%)	46 (52.3%)	89 (52.4%)	
Laboratory-supported probable	5 (16.1%)	9 (36.0%)	14 (25.0%)		18 (22.0%)	19 (21.6%)	37 (21.8%)	
Possible	1 (3.2%)	3 (12.0%)	4 (7.1%)		6 (7.3%)	7 (8.0%)	13 (7.7%)	
ALSFRS-R [sum score][a]	42.4 ± 3.1	42.5 ± 2.9	42.4 ± 3.0	0.94[b]	36.5 ± 4.9	36.8 ± 5.5	36.7 ± 5.2	0.69[b]
SVC, %[a]	91.1 ± 18.2	86.9 ± 17.6	89.3 ± 17.9	0.49[b]	83.9 ± 16.5	82.1 ± 18.9	83.0 ± 17.7	0.49[b]
SEIQoL [sum score][a]	68.6 ± 22.3	73.5 ± 13.9	70.8 ± 19.0	0.50[b]	67.3 ± 19.8	65.1 ± 21.2	66.2 ± 20.5	0.50[b]

Note: Data are mean ± SD or *n* (%).

Abbreviations: ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; BMI, body mass index; SEIQoL, Schedule for Evaluation of Individual Quality of Life; SVC, slow vital capacity.

[a]Mean ± SD.

[b]Two-sample *t*-test.

[c]Chi-squared test.

[d]Placebo: $n=113$, rasagiline: $n=114$.

compared to placebo after 6 months (HR = 0.29, 95% CI = 0.09–0.90, $p=0.02$) and 12 months (HR = 0.52, 95% CI = 0.27–0.99, $p=0.04$; Figure 1). The survival probability after 6 months was 0.85 (95% CI = 0.75–0.91) in the placebo group and 0.95 (95% CI = 0.88–0.98) in the rasagiline group. The survival probability after 12 months was 0.69 (95% CI = 0.58–0.78) in the placebo group and 0.82 (95% CI = 0.72–0.89) in the rasagiline group. However, after 18 months, survival between rasagiline and placebo was not significantly different (HR = 0.72, 95% CI = 0.45–1.17, $p=0.19$); the survival probability was 0.55 (95% CI = 0.43–0.65) in the placebo group and 0.63 (95% CI = 0.52–0.72) in the rasagiline group.

We also found a positive effect of rasagiline on disease progression in this group of intermediate to fast progressing patients after 18 months treatment, indicating a slower decline of ALSFRS-R in the verum group compared to placebo ($p=0.049$; Figure 1). However, after 6 and 12 months, the difference between rasagiline and placebo was not significant (6 months: $p=0.17$; 12 months: $p=0.10$). There was no difference between the rasagiline and placebo groups for other secondary outcome measures.

SNPs in the *MAOB* gene

$MAOB_A$ alleles of Rs1799836 have been found to be associated with a highly increased splicing frequency compared to $MAOB_G$, leading to an increased MAOB protein biosynthesis [22, 27]. The increased levels of MAOB result in an increased deamination and hence reduced availability of dopamine. Because the *MAOB* gene is located on the X chromosome, we analyzed SNP data and clinical outcome parameters separately for male and female study

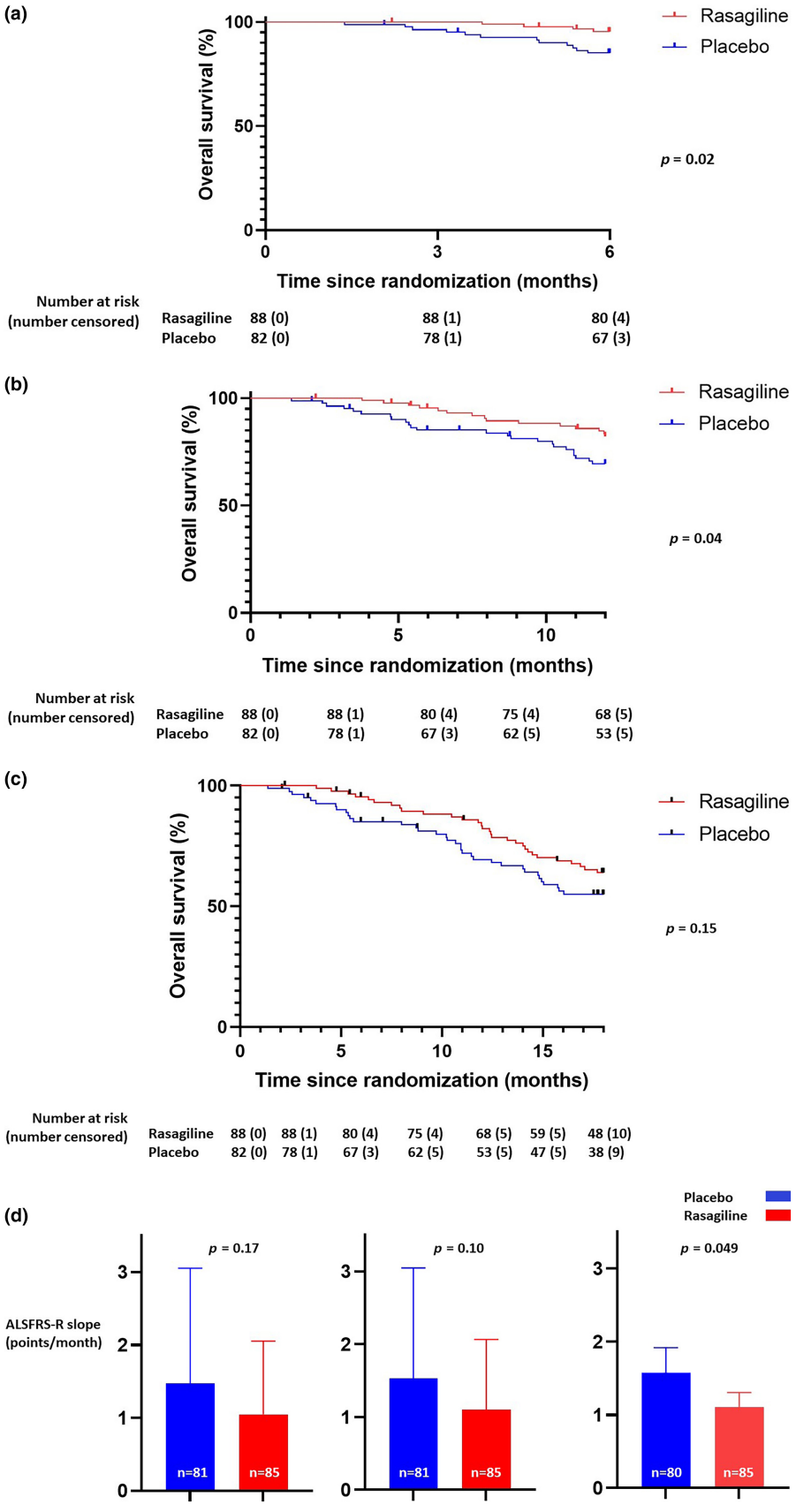

FIGURE 1 Treatment effects in intermediate to fast progressing amyotrophic lateral sclerosis patients (prebaseline progression rate of >0.328 points of Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised [ALSFRS-R] lost per month). (a–c) Kaplan–Meier survival curves for overall survival are shown. *p* = unadjusted log-rank *p*-value. (d) Slope of ALSFRS-R score at 6, 12, and 18 months.

participants. We investigated treatment effects (verum vs. placebo) for each genotype as well as the effect of genotypes in each treatment group.

Survival analysis did not reveal differences between rasagiline and placebo for *MAOB* genotype subgroups A ($p=0.96$), G ($p=0.98$), A/G ($p=0.73$), and G/G ($p=0.30$). In female patients with $MAOB_{AA}$ genotype, a survival difference was observed ($p=0.03$). However, the result is based on a very small sample size (rasagiline: $n=9$, placebo: $n=11$) and thus prone for bias. Further survival analysis did not show a superiority of any genotype in the whole study population ($p=0.21$) as well as in each treatment group (rasagiline: $p=0.47$, placebo: $p=0.13$).

Disease progression during 18 months of treatment (verum versus placebo) did also not differ in each SNP genotype (A: $p=0.30$, G: $p=0.98$, A/A: $p=0.11$, A/G: $p=0.67$, G/G: $p=0.84$). However, the informative value of these results is limited, because SNP subgroups were quite small (ranging from $n=34$ to $n=9$). Other outcome parameters were not analyzed in the context of *MAOB* SNP genotypes.

Subgroup analysis of all male study participants showed no significant difference for patients with $MAOB_A$ ($n=62$) compared to $MAOB_G$ ($n=60$, $p=0.07$). The survival probability after 18 months was 0.76 (95% CI=0.63–0.85) for patients with $MAOB_A$ and 0.59 (95% CI=0.45–0.70) for patients with $MAOB_G$. The HR was 0.55 (95% CI=0.29–1.06; Figure 2). Disease progression rates (ALSFRS-R slope) at 6, 12, and 18 months were similar for both genotypes (Table 2). Baseline characteristics per genotype are displayed in Table 2.

SNPs in the *DRD2* gene

A SNP on the dopamine D2 receptor gene (*DRD2*) has been found to be relevant in the context of rasagiline response in Parkinson disease. The homozygous $DRD2_{CC}$ genotype (Rs2283265) leads to an increase of presynaptic D2 autoreceptors, an increased sensitivity to dopamine, and, as a consequence, to a better response to rasagiline treatment [23]. Baseline characteristics (verum vs. placebo) for patients with this genotype are displayed in Table 3.

The survival probability in patients with C/C alleles after 6 months was 0.85 (95% CI=0.72–0.93) in the placebo group and 0.93 (95% CI=0.80–0.98) in the rasagiline group (HR=0.46, 95% CI=0.12–1.78, $p=0.26$), after 12 months 0.71 (95% CI=0.56–0.82) in the placebo group and 0.81 (95% CI=0.66–0.90) in the rasagiline group (HR=0.60, 95% CI=0.25–1.43, $p=0.25$), and after 18 months 0.55 (95% CI=0.40–0.68) in the placebo group and 0.67 (95% CI=0.50–0.79) in the rasagiline group (HR=0.66, 95% CI=0.34–1.30, $p=0.23$).

Disease progression rates (ALSFRS-R slope) at 6, 12, and 18 months were similar in both treatment groups (Table 3).

Analyzing long-term survival at the end of the study (i.e., after the last patient completed the 18-month study treatment) revealed no significant difference between study participants with C/C alleles receiving rasagiline compared to placebo (HR=0.59, 95% CI=0.32–1.08, $p=0.08$; Figure 3) or patients with C/A alleles (HR=1.85, 95% CI=0.49–7.02, $p=0.36$).

DISCUSSION

In 2016, we concluded a randomized controlled clinical trial testing 1 mg rasagiline per day add-on to standard therapy (100 mg riluzole) in patients with ALS [20]. In this article, we report new genetic data and subgroup analyses aiming at clarifying the question of whether patients with specific clinical and genetic characteristics may have beneficially responded to the treatment.

Survival analysis of placebo-treated very slow progressors revealed a survival probability after 24 months of 0.85 (95% CI=0.65–0.94). As this RCT was designed to compare the frequency of survival events between rasagiline and placebo within a treatment period of 18 months, we can assume that the intervention period might have been too short to detect treatment effects for very slow progressing patients. Thus, studies with treatment durations of 6–18 months, as commonly applied in ALS trials today, will likely not enable proving or disproving efficacy for rasagiline or any other treatment approach with moderate treatment effects in this subgroup of patients and carry the risk that the whole study might be underpowered. Hence,

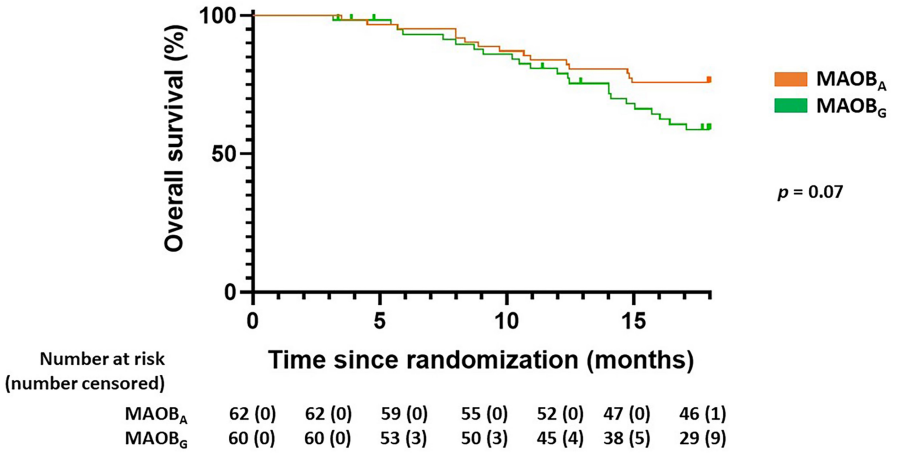

FIGURE 2 Survival analysis for male study participants with $MAOB_A$ or $MAOB_G$ genotype in Rs1799836. Kaplan–Meier survival curves for overall survival are shown. p=unadjusted log-rank p-value.

TABLE 2 Patient characteristics per *MAOB* genotype of male study participants at baseline.

	Male study participants with $MAOB_A$ or $MAOB_G$ genotype in Rs1799836			
Characteristic	**A allele, $n=62$**	**G allele, $n=60$**	**Total, $n=122$**	***p***
Age, years[a]	60.1 ± 10.9	58.1 ± 10.8	59.1 ± 10.9	0.30[b]
BMI, kg/m^2[a]	26.8 ± 3.1	25.5 ± 3.1	26.2 ± 3.2	0.03[b]
Onset				
Bulbar	13 (21.0%)	13 (21.7%)	26 (21.3%)	0.92[c]
Spinal	49 (79.0%)	47 (78.3%)	96 (78.7%)	
Duration of disease, months[a,d]	19.3 ± 11.1	18.0 ± 9.4	18.7 ± 10.3	0.51[b]
Certainty of diagnosis				
Definite	17 (27.4%)	7 (11.7%)	24 (19.7%)	0.07[c]
Probable	28 (45.2%)	33 (55.0%)	61 (50.0%)	
Laboratory-supported probable	14 (22.6%)	12 (20.0%)	26 (21.3%)	
Possible	3 (4.8%)	8 (13.3%)	11 (9.0%)	
SVC, %[a]	83.4 ± 16.1	80.9 ± 15.9	82.1 ± 16.0	0.39[b]
ALSFRS-R [sum score] at baseline[a]	39.0 ± 5.3	38.8 ± 5.2	38.9 ± 5.2	0.87[b]
ALSFRS-R slope at baseline[a]	0.63 ± 0.53	0.61 ± 0.42	0.62 ± 0.48	0.84[b]
ALSFRS-R slope at month 6[a]	0.91 ± 0.94	1.29 ± 1.61	1.10 ± 1.33	0.11[b]
ALSFRS-R slope at month 12[a]	0.96 ± 0.87	1.25 ± 1.56	1.11 ± 1.27	0.19[b]
ALSFRS-R slope at month 18[a]	0.98 ± 0.90	1.31 ± 1.55	1.15 ± 1.28	0.16[b]

Note: Data are mean ± SD or *n* (%).

Abbreviations: ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; BMI, body mass index; SVC, slow vital capacity.

[a]Mean ± SD.

[b]Two-sample *t*-test.

[c]Chi-squared test.

[d]A allele: $n=58$, G allele: $n=56$.

future ALS studies should take into consideration individual disease progression at baseline to align the study population and study design.

Considering this finding, when excluding 25% of patients with the slowest disease progression at baseline in this trial, the remaining 75% of patients with intermediate to fast disease progression showed beneficial effects with regard to survival after 6 and 12 months, as well as motor function (ALSFRS-R) after 18 months. Interestingly, these beneficial effects are detected in a more heterogenic subset of ALS patients than studied in the Centaur trial [28] leading to a marketing authorization by the US Food and Drug Administration.

However, our results did not show a survival benefit after 18 months, which may be caused by reduced statistical power due to the decreased sample size in this subgroup ($n=170$ at baseline, $n=147$ at month 6, $n=121$ at month 12, $n=86$ at month 18). Likewise, group comparisons of disease progression (ALSFRS-R) under study treatment missed statistical significance by a rather narrow margin after 6 and 12 months. This might indicate that sufficient changes of ALSFRS-R to demonstrate a treatment effect were only reached at the end of the study intervention. As our data refer to explorative post hoc analysis without adjustment for multiple testing, we consider them as hypothesis-generating, laying the foundation for future confirmatory studies.

Another part of the post hoc analyses focused on the potential relevance of SNPs related to the dopaminergic system. Subgroup analysis revealed a trend toward better long-term survival for $DRD2_{CC}$ patients (Rs2283265) treated with rasagiline compared to placebo, whereas $DRD2_{CA}$ patients did not show any positive effects. MAOB inhibition by rasagiline significantly reduces dopamine catabolism. Hence, availability of dopamine is steadily increased but relies on a sufficient number of presynaptic receptors to be effective. The presence of A alleles on the *DRD2* gene results in an imbalance of D2 receptor types in the so-called "indirect pathway" of the basal ganglia motor circuit [23]. This has been shown for Parkinson disease and could potentially be relevant for the treatment of ALS patients as well. In this context, results from our *DRD2* SNP analysis might suggest that ALS patients with an increased number of presynaptic D2 autoreceptors associated with $DRD2_{CC}$ might have better prerequisites to benefit from increased dopamine levels caused by a pharmacological treatment with rasagiline.

TABLE 3 Patient characteristics at baseline for study participants with $DRD2_{CC}$ genotype in Rs2283265.

Characteristic	Study participants with $DRD2_{CC}$ genotype in Rs2283265			
	Placebo, $n=48$	Rasagiline, $n=44$	Total, $n=92$	p
Age, years[a]	61.4 ± 8.6	62.2 ± 11.8	61.8 ± 10.2	0.68[b]
Sex				
Female	17 (35.4%)	17 (38.6%)	34 (37.0%)	0.75[c]
Male	31 (64.6%)	27 (61.4%)	58 (63.0%)	
BMI, kg/m^2[a]	25.1 ± 3.5	25.0 ± 3.8	25.1 ± 3.7	0.82[b]
Onset				
Bulbar	12 (25.0%)	14 (31.8%)	26 (28.3%)	0.47[c]
Spinal	36 (75.0%)	30 (68.2%)	66 (71.7%)	
Duration of disease, months[a,d]	18.0 ± 10.2	18.0 ± 7.7	18.0 ± 9.1	0.97[b]
Certainty of diagnosis				
Definite	8 (16.7%)	7 (15.9%)	15 (16.3%)	0.51[e]
Probable	26 (54.2%)	20 (45.5%)	46 (50.0%)	
Laboratory-supported probable	13 (27.1%)	13 (29.6%)	26 (28.3%)	
Possible	1 (2.1%)	4 (9.1%)	5 (5.4%)	
SVC, %[a]	85.1 ± 16.3	80.5 ± 19.8	82.9 ± 18.1	0.22[b]
ALSFRS-R [sum score] at baseline[a]	38.5 ± 5.4	38.3 ± 5.7	38.4 ± 5.5	0.87[b]
ALSFRS-R slope at baseline[a]	0.69 ± 0.55	0.62 ± 0.46	0.66 ± 0.51	0.51[b]
ALSFRS-R slope at month 6[a]	1.35 ± 1.68	1.43 ± 1.19	1.39 ± 1.46	0.37[b]
ALSFRS-R slope at month 12[a]	1.41 ± 1.59	1.32 ± 1.16	1.37 ± 1.39	0.14[b]
ALSFRS-R slope at month 18[a]	1.47 ± 1.60	1.35 ± 1.14	1.42 ± 1.39	0.10[b]

Note: Data are mean ± SD or *n* (%).

Abbreviations: ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; BMI, body mass index; SVC, slow vital capacity.

[a]Mean ± SD.

[b]Two sample *t*-test.

[c]Chi-squared test.

[d]Placebo: $n=44$, rasagiline: $n=38$.

[e]Fisher exact test.

The analysis of SNPs in the *MAOB* gene did not provide a clear picture. Survival analysis between both treatment groups in male as well as in female patients did not show significant differences for the analyzed *MAOB* SNPs. Interestingly, survival analysis in all male patients revealed a trend towards longer survival for the $MAOB_A$ genotype ($p=0.07$), indicating that the Rs1799836 SNP might constitute a potential prognostic factor in ALS, which has to be further evaluated in future trials. At first glance, it appears surprising that increased MAOB activity might be associated with prolonged survival, because $MAOB_A$ is associated with an increased emergence of toxins and free radicals resulting from MAOB oxidation of dopamine and other substrates, as amines are catalyzed by MAOB to aldehyde, ammoniac, and hydrogen peroxide [14]. However, the exact role of MAOB in ALS is still unclear; thus, the results should be regarded as hypothesis-generating.

A confirmation of both SNP analyses in a subsequent larger RCT might potentially have significant impact on ALS treatment in the future. Precision medicine in general and especially in a heterogenic disorder like ALS is an important topic of current research [29, 30]. Results from *MAOB* SNPs, if confirmed and well understood in a future trial, might be used along with other established prognostic factors of ALS to better assess prognosis. Results from *DRD2* SNPs might help to perform a personalized risk–benefit assessment of whether to start treatment with rasagiline, provided that the efficacy of rasagiline in ALS can be proven by a follow-up trial.

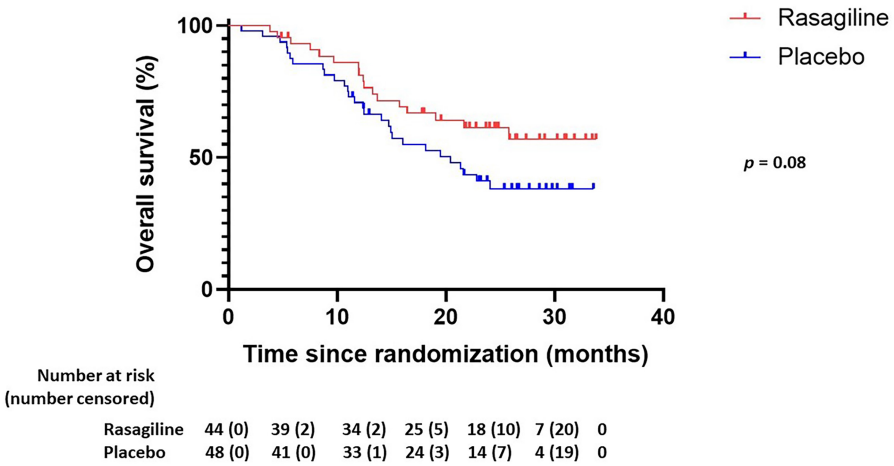

FIGURE 3 Long-term survival of study participants with $DRD2_{CC}$ genotype in Rs2283265. Kaplan–Meier survival curves for overall survival are shown. *p* = unadjusted log-rank *p*-value.

In summary, these post hoc results indicate that rasagiline, despite missing the primary endpoint in the original study, still constitutes a promising candidate for the treatment of ALS, as demonstrated by a significant effect on survival and disease progression after excluding patients with very slow disease progression. Further insights regarding the exact underlying mechanisms of action need to come from a future RCT.

AUTHOR CONTRIBUTIONS

Joachim Schuster: Conceptualization; methodology; writing – original draft; writing – review and editing; formal analysis. **Jens Dreyhaupt:** Conceptualization; methodology; writing – original draft; writing – review and editing; formal analysis. **Karla Mönkemöller:** Writing – review and editing; formal analysis. **Luc Dupuis:** Conceptualization; methodology; formal analysis; writing – review and editing. **Stéphane Dieterlé:** Formal analysis; writing – review and editing. **Jochen H. Weishaupt:** Investigation; writing – review and editing. **Jan Kassubek:** Investigation; writing – review and editing. **Susanne Petri:** Investigation; writing – review and editing. **Thomas Meyer:** Investigation; writing – review and editing. **Julian Grosskreutz:** Investigation; writing – review and editing. **Berthold Schrank:** Investigation; writing – review and editing. **Matthias Boentert:** Investigation; writing – review and editing. **Alexander Emmer:** Investigation; writing – review and editing. **Andreas Hermann:** Investigation; writing – review and editing. **Daniel Zeller:** Investigation; writing – review and editing. **Johannes Prudlo:** Investigation; writing – review and editing. **Andrea S. Winkler:** Investigation; writing – review and editing. **Torsten Grehl:** Investigation; writing – review and editing. **Michael T. Heneka:** Investigation; writing – review and editing. **Siw Johannesen:** Investigation; writing – review and editing. **Bettina Göricke:** Investigation; writing – review and editing. **Simon Witzel:** Formal analysis; writing – review and editing. **Johannes Dorst:** Conceptualization; methodology; formal analysis; writing – review and editing; investigation. **Albert C. Ludolph:** Conceptualization; methodology; investigation; writing – review and editing; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The data that support the findings will be shared with researchers upon reasonable request.

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