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RESEARCH ARTICLE

Efficacy and tolerability of EH301 for amyotrophic lateral sclerosis: a randomized, double-blind, placebo-controlled human pilot study

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Abstract

Background: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease, characterized by progressive loss of spinal and cortical motor neurons, leading to muscular atrophy, respiratory failure, and ultimately death. There is no known cure, and the clinical benefit of the two drugs approved to treat ALS remains unclear. Novel disease-modifying therapeutics that are able to modulate the disease course are desperately needed. Our objective was to evaluate the efficacy and tolerability of Elysium Health's candidate drug EH301 in people with ALS (PALS). *Methods*: This was a single-center, prospective, double-blind, randomized, placebo-controlled pilot study. Thirty-two PALS were recruited thanks to the collaboration of the Spanish Foundation for ALS Research (FUNDELA). Study participants were randomized to receive either EH301 or placebo and underwent evaluation for 4 months. Differences between EH301 and placebo-treated participants were evaluated based on standard clinical endpoints, including the revised ALS functional rating scale (ALSFRS-R), forced vital capacity (FVC), and the Medical Research Council (MRC) grading scale. *Results*: Compared to placebo, participants treated with EH301 demonstrated significant improvements in the ALSFRS-R score, pulmonary function, muscular strength, and in skeletal muscle/fat weight ratio. EH301 was shown to significantly slow the progression of ALS relative to placebo, and even showed improvements in several key outcome measures compared with baseline. *Conclusions*: This study provides evidence in support of the disease-modifying effects of EH301 for the treatment of ALS.

Keywords: Amyotrophic lateral sclerosis, 1-(beta-D-Ribofuranosyl)nicotinamide chloride, 3,5-Dimethoxy-4′-hydroxy-trans-stilbene, randomized control study, human

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Introduction

It has been nearly 150 years since Charcot and Joffroy first described the symptoms of amyotrophic lateral sclerosis (ALS) (1); yet, we are still no closer to a curative treatment for this debilitating disease. In 1995, riluzole became the first approved treatment for ALS. However, it is only mildly efficacious and extends survival by 2–3 months (2). More recently, edaravone received FDA approval for the treatment of ALS, based on the results from a Phase III showing a difference in the 24-week change in ALSFRS-R score in the edaravone-treated group relative to placebo (−5.01 compared to −7.50, respectively) (3). However, edaravone is only beneficial to a subset of people with early-stage ALS (3). It is also costly (~$140,000 annually in the US) and requires daily intravenous infusions over 2 weeks, followed by a 2-week drug-free period (4,5). These factors may make edaravone treatment unattainable for some people with ALS (PALS). Effective, disease-modifying treatments which serve the wider ALS community are desperately needed.

There is substantial interest in the NAD^+ dependent class III histone deacylases, or sirtuins, as therapeutic targets in ALS (6). Sirtuins regulate a myriad of cellular processes that are implicated in ALS, such as the regulation of energy metabolism, mitochondria function, inflammation, and DNA repair. Altered sirtuin 1 (SIRT1) levels have been observed both in mouse models of ALS (7) and postmortem tissues from ALS patients (8); therefore, techniques to increase sirtuin activity represent viable therapeutic approaches in the treatment of ALS.

In this study, we employed EH301—a combination of 1-(β-D-Ribofuranosyl) nicotinamide chloride and 3,5-Dimethoxy-4′-hydroxy-trans-stilbene—in a placebo-controlled, double-blinded, randomized pilot study of ALS patients. The two active compounds are predicted to work synergistically to increase NAD^+ levels and support sirtuin activity: 1-(β-D-Ribofuranosyl) nicotinamide chloride has been shown to increase circulating levels of NAD^+ in published human clinical trials (9), whereas 3,5-dimethoxy-4′-hydroxy-trans-stilbene has been shown to directly activate SIRT1 (10,11). The objective of this study was to assess the safety, tolerability, and efficacy of EH301 in the treatment of ALS, based on standard clinical criteria.

Materials and methods

Study design and eligibility

This study was a single-center, randomized, double-blinded, placebo-controlled parallel-group pilot trial of EH301-treated versus placebo-treated patients with ALS. This trial is listed on ClinicalTrials.gov (NCT03489200).

Eligible participants were 18 years of age or older and diagnosed with probable or definite (sporadic or familial) ALS by El Escorial criteria (12), with an onset of symptomatology for more than 6 months. The study recruited both female and male participants; females were not lactating, had a negative pregnancy test and agreed to use an effective method of birth control throughout the duration of the study. All patients received riluzole treatment according to standard dosage. The exclusion criteria can be found in the Supplementary Methodology.

This study was approved by the University of Valencia Institutional Review Board on Human Studies (ref. H1479983999044) and participants provided written informed consent. All the procedures followed the declaration of Helsinki.

Randomization and blinding

Patients were recruited thanks to the Spanish Foundation for ALS Research (FUNDELA). We randomly allocated participants in a 1:1 ratio to receive 1200 mg EH301 or placebo (capsules containing brown sugar; matched in size and color). Study product was provided by Elysium Health. Half of the study drug was administered in the morning and the other half in the afternoon. Randomization was stratified by age, duration of symptoms and baseline ALSFRS-R score. The study was double-blinded, including patients and the health staff, except for the directors (J.E.R., J.M.E.). Both directors were responsible for screening, enrollment, and randomization of the study participants. Neither director collected nor analyzed data prior to un-blinding. All evaluators who collected outcome data were blinded to the treatment status of the study participants. The corresponding author was fully responsible for the strict compliance with these conditions during the months the trial lasted to avoid the possibility of bias.

Diet

All patients were recommended a Mediterranean style diet (approx. 2300 kcal/day; 55% carbohydrates +30% fat +15% proteins) (see e.g. (13)). Carbohydrates in the diet corresponded to slow-release carbohydrate foods. Vitamins and oligoelements in the diet of both groups were adjusted following recommended dietary allowance by the EFSA (European Food Safety Authority).

Follow-up, outcomes, and data collection

Following baseline evaluation, study participants were evaluated following 2 and 4 months of treatment. The primary outcome measurement was the ALSFRS-R score (14). Secondary outcome

measurements included pulmonary function measured using FVC, muscular strength using a modified 11-step MRC grading scale (see Supplementary methodology), BMI, fat and skeletal weight according to standard procedures (15), and electrical activity of skeletal muscles, measured using surface electromyogram (EMG). FVC was measured using a Datospir touch spirometer (Sibelmed, Barcelona, Spain) (16), and expressed as the % of the standard value that corresponds to human adults depending on sex, weight and age parameters (17). Muscle groups evaluated using surface electromyogram (EMG) were identical to those evaluated using the MRC grading scale; these include the R and L biceps, triceps, quadriceps and tibial muscles. A surface EMG measuring device (BTS FreeEMG 300, BTS S.p.A., Milan, Italy) was used to evaluate EMG signals per muscle, which were subsequently analyzed by the root mean square (RMS) method which reflects the physiological activity in the motor unit during contraction. The RMS represents the square root of the average power of the EMG signal for a given period of time (18).

Throughout the entire duration of the study, the patients had permanent communication with the medical team. Data were collected at clinical facilities dependent on the Catholic University San Vicente Mártir (Valencia, Spain). After the study concluded at 4 months, it was un-blinded and significant differences were confirmed. All study participants had the option to continue taking EH301 on an open-label extension.

Statistical analysis

Within-group (2- and 4-month changes relative to baseline) and between-group (2- and 4-month measurements in EH301 relative to placebo) differences were identified using a *t*-test. Data are presented as mean values ± standard deviation for the number of different experiments.

Results

Participant demographics

Thirty-two PALS fulfilling the inclusion criteria were recruited between October 2016 and January 2017 and randomly distributed in two groups of 17 (placebo) and 15 (EH301) patients. Participants in both groups were similar in age, duration of symptoms, BMI, and anthropometric measurements (fat weight and skeletal muscle weight), as well as ALSFRS-R score and FVC (Table 1). Although every effort was made to randomize the patients fairly into the EH301 and placebo groups, the small size of this pilot study, coupled with the inherent heterogeneity of the disease, meant that some small differences in the baseline measurements were found. We observed a significant difference in baseline MRC grading scale index and surface EMG measurements taken within the right tibial muscles; in both instances, the mean baseline value was lower in the EH301 group. Participants randomized into the EH301 group also had a shorter duration of symptoms (Δ = 3.4 months) and a slightly lower ALSFRS-R score (Δ = 2.7) at baseline; however, these differences were not significant. These differences must be taken into account when interpreting the data, as these outcome measures could be influenced by faster-progressing patients. Nevertheless, although it is a possibility, it cannot be predicted if a specific patient is going to progress faster than another.

The study began in February 2017, once participant eligibility was confirmed. A total of 12 patients withdrew from the study (7 placebo, 5 EH301); the reasons are described in Figure 1. The study concluded in June 2017, following 4 months of observation.

Primary outcome measurement

We observed a significant improvement ($p < 0.01$) in the ALSFRS-R in participants treated with EH301 relative to placebo following 4 months of treatment (Table 1). At this time-point, participants within the placebo group had deteriorated significantly relative to their baseline measurements ($p < 0.05$), whereas the EH301 group showed no significant decline. When comparing the change from baseline between EH301 and placebo, a significant improvement ($p < 0.01$) was observed in the EH301 group at the 2- and 4-month evaluation (Figure 2(A-i)). This corresponded to a 3.4- and 2.5-point improvement in the EH301 group at 2 and 4 months respectively, compared to a 3.0- and 5.5-point decline in the placebo group.

Secondary outcome measurements

We observed a significant slowing or reversal associated with treatment with EH301 in other outcome measurements. At 2 months, participants in the EH301 group showed a significant improvement ($p < 0.01$) in the MRC grading scale index relative to placebo (Table 1) despite the baseline MRC grading scale index being lower at baseline in the EH301 group. This corresponded to a 9.6 (±3.2) improvement in the EH301 group, compared to a 10.0 (±2.9) decline in the placebo group (Figure 2(A-iii)). This observation was upheld at 4 months, resulting in a difference of 23.2 between EH301- and placebo-treated participants ($p < 0.01$) (Figure 2(A-iii)).

We observed a significant improvement in measured forced vital capacity (FVC) at 2 months

Table 1. Baseline characteristics and change in outcome measures following 2 months (2M) and 4 months (4M) of treatment with either placebo or EH301.

		Placebo			EH301		
		BL $n = 14$	2M $n = 14$	4M $n = 10$	BL $n = 13$	2M $n = 13$	4M $n = 10$
Baseline characteristics							
Age (years)		55.6 (10.5)			56.9 (9.1)		
Sex (Male/Female)		8/6			9/4		
Duration of symptoms (months)		24.0 (9.6)			20.6 (12.5) $n = 12$; 1 case =64		
Onset: Spinal/bulbar		10/4			10/3		
Riluzole use		14/14			13/13		
Primary outcome measurement							
ALSFRS-R		41.5 (5.2)	38.5 (6.0)	35.2 (3.1)*	38.8 (4.9)	42.4 (4.7)	41.3 (2.8) ++
Secondary outcome measurement							
FVC (%)		92.3 (12.6)	88.5 (9.7)	77.8 (10.0)**	95.3 (7.4)	101.4 (4.5)*++	97.2 (7.4) ++
MRC scale index		45.5 (3.3)	35.6 (4.4)**	34.2 (6.6)**	40.6 (2.9) —	50.1 (2.7)**++	57.4 (14.6)**++
BMI (kg/m^2)		24.6 (2.1)	23.8 (1.9)	22.3 (2.4)*	24.7 (3.7)	23.5 (2.3)	24.0 (2.0)
Fat weight (kg)		12.5 (3.8)	13.6 (4.5)	13.9 (4.0)	14.7 (3.6)	13.3 (3.7)	13.0 (4.2)
Skeletal muscle weight (kg)		26.3 (2.2)	25.8 (2.7)	25.0 (2.9)	24.4 (4.0)	24.8 (2.2)	24.9 (2.0)
Surface EMG (μV)							
Biceps	R	233 (87)	201 (56)	184 (46)	285 (103)	292 (87) ++	237 (77)
	L	245 (91)	246 (101)	190 (38)	264 (85)	288 (109)	276 (84) ++
Triceps	R	184 (53)	134 (71)*	126 (51)**	170 (66)	195 (74) +	212 (55) ++
	L	211 (77)	177 (65)	155 (45)	234 (59)	250 (71) +	327 (74)*++
Quadriceps	R	151 (46)	136 (44)	141 (35)	133 (36)	147 (75)	152 (50)
	L	177 (65)	146 (68)	133 (47)	138 (55)	148 (69)	148 (39)
Tibial	R	167 (49)	135 (53)	125 (42)	101 (57) —	97 (50)	164 (44)**
	L	139 (50)	116 (55)	94 (33)	122 (57)	123 (68)	182 (56)**++

Data are presented as the mean (SD) and are representative of patients that completed the follow-up assessments.

ALSFRS-R: amyotrophic lateral sclerosis functional rating scale—revised; BMI: body mass index; EMG: electromyography; FVC: forced vital capacity; MRC: Medical Research Council Grading Scale.

Statistical analyses were performed using a *t*-test. −−$p < 0.01$: comparison of baseline characteristics between participants randomized to EH301 versus baseline; *$p < 0.05$, and **$p < 0.01$: comparison of 2M or 4M measurements versus baseline (within-group differences); +$p < 0.05$ and ++$p < 0.01$: comparison of EH301 versus placebo-treated ALS patients (between-group differences).

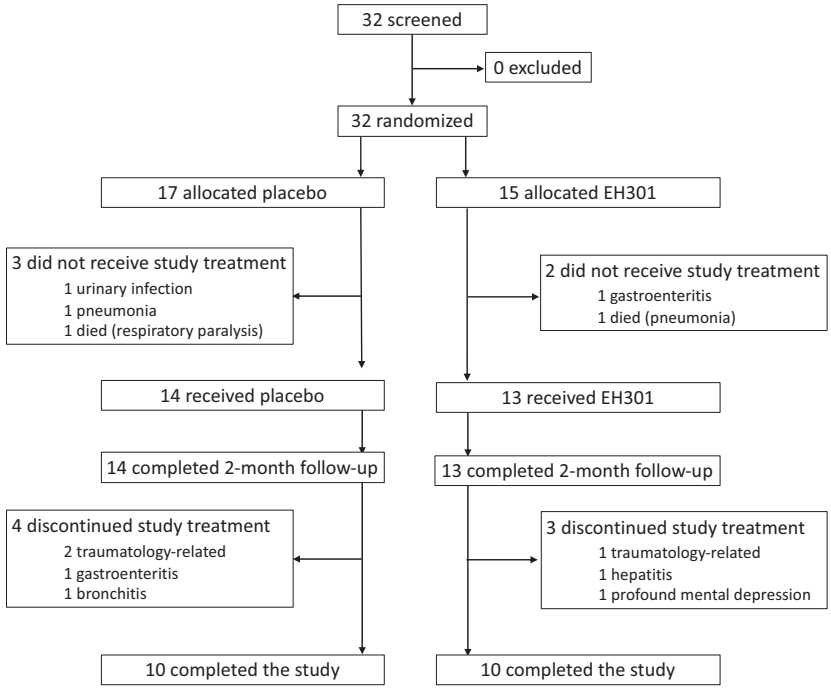

Figure 1. Study flow diagram.

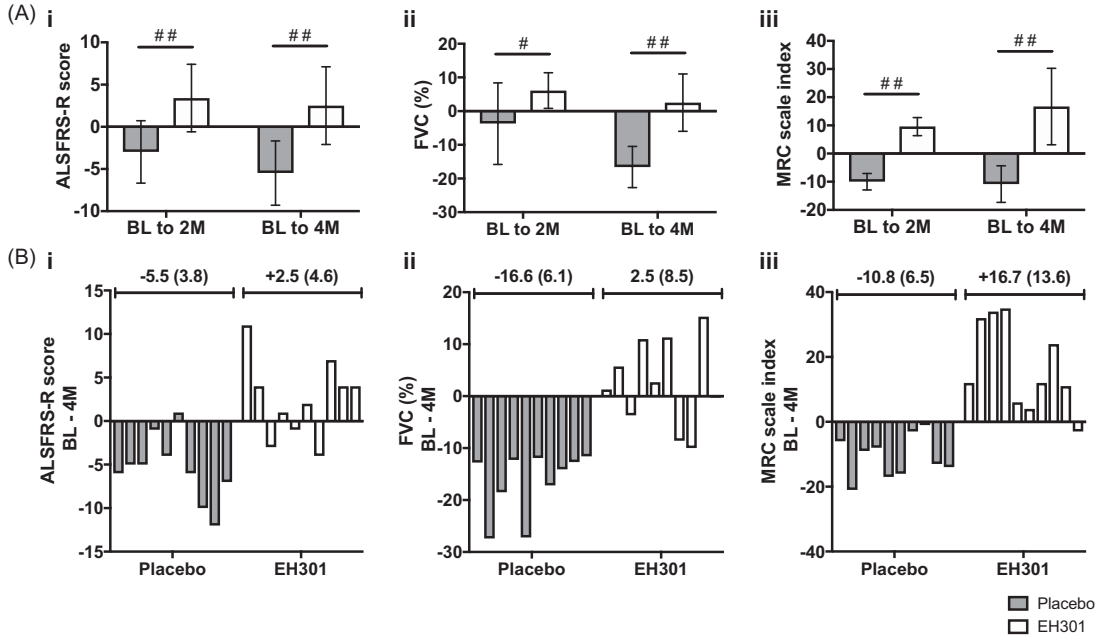

Figure 2. Main clinical endpoints. (A) 2-month (2M) and 4-month (4M) mean change from baseline (BL) in outcome measures in ALS participants treated with either placebo or EH301. Data are representative of patients that completed the follow-up assessments. Error bars represent the SD. ALSFRS-R = Revised Amyotrophic Lateral Sclerosis Functional Rating Scale; MRC = Medical Research Council grading scale; FVC = forced vital capacity. Statistical analyses were performed using a *t*-test. #$p < 0.05$, and ##$p < 0.01$: comparison of the 2M or 4M change from baseline in EH301 vs. placebo. (B) Summary of individual study participant's 4-month change from baseline. All participants enrolled in the trial exhibited a positive response in at least one of the outcome measurements.

within the EH301 group relative to baseline ($p < 0.05$) and placebo ($p < 0.01$) (Table 1). At this time-point, we detected a 6.1% (±5.3) increase in FVC in the EH301 group relative to baseline, compared to a 3.8% (±12.1) decline in placebo (Table 1). At 4 months, these alterations manifested as a 19.4% difference between EH301 and placebo (Figure 2(A-ii)).

Evaluation of muscle groups using surface EMG revealed a significant increase in electrical activity within the right (R) and left (L) tibial muscles in the EH301 group at 4 months, relative to baseline ($p < 0.01$ for both R and L) (Table 1). We also observed greater electrical activity in the L biceps, R and L triceps, and L tibial muscles (all $p < 0.01$) in the EH301 group at 4 months, relative to the placebo group (Table 1). When we further explored the 2- and 4-month change from baseline across all the muscle groups, we observed an increase in the 2-month change in electrical activity in the EH301 group in 5 of the 8 muscle groups under investigation (Figure 3). The 4-month evaluation revealed a significant difference in the change from baseline between the EH301 and placebo groups within the R and L triceps and R and L tibial (all $p < 0.01$, respectively) (Figure 3).

Individual response data comparing 4-month data to baseline for three main clinical endpoints (MRC grading scale, FVC, and ALSFRS-R) are presented in Figure 2(b). Progression of ALS was observed in everyone within the placebo group across all clinical endpoints (with the exception of one participant's ALSFRS-R score, which improved by 1 point). Interestingly, the entire EH301 group showed an improvement in at least one clinical endpoint. Specifically, 7 of 10 participants in the EH301 group showed improvement in ALSFRS-R scores (Figure 2(b-i)). Similarly, 6 out of 10 participants in the EH301 group showed improvement in FVC (Figure 2(B-ii)), whereas 9 out of 10 participants showed improvements in muscle strength as measured by the MRC grading scale (Figure 2(B-iii)). Only 1 participant in the EH301 group failed to show improvement in at least two of the clinical endpoints; this individual still showed significant improvement in the MRC grading scale (Figure 2(B-iii)).

Finally, we noted that treatment with EH301 induced a significant decrease in fat and a significant increase in skeletal muscle weights, effects opposite to the placebo group at both 2- and 4-months (Table 1 and Supplementary Figure S1). These anthropometric observations support the findings from the main clinical outcomes, indicating that the PALS within the EH301 group were gaining muscular strength.

After this study concluded in June 2017, all participants were given the option to continue treatment on an open-label extension study. All study participants elected to continue taking EH301. In February 2018, we completed a 1-year evaluation of participants initially randomized to EH301; the outcome measurements are presented in the supplementary material (Supplementary Table S1). Relative to baseline, we did not observe any

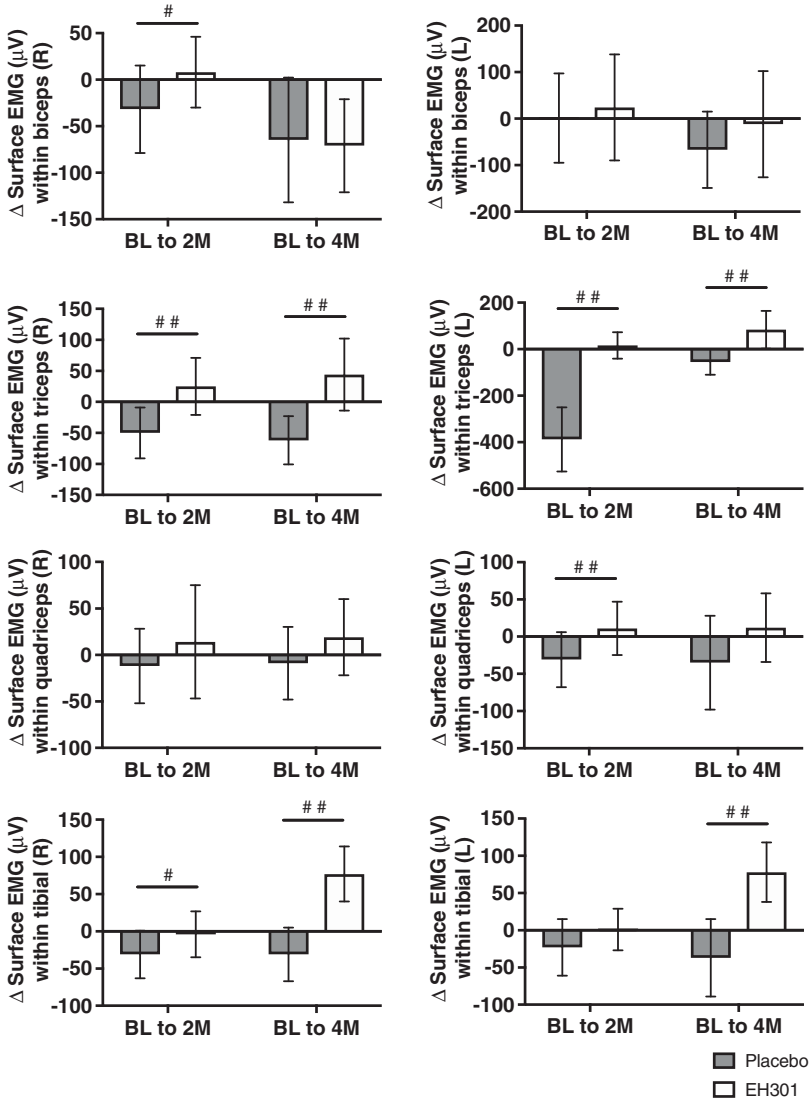

Figure 3. Changes in electromyography (EMG) graphed according to muscle (biceps, triceps, quadriceps, tibial). 2-month (2M) and 4-month (4M) mean change from baseline (BL) in outcome measures in ALS participants treated with either placebo or EH301. Data are representative of patients that completed the follow-up assessments. Error bars represent the SD. Statistical analyses were performed using a *t*-test. #$p < 0.05$, and ##$p < 0.01$: comparison of the 2M or 4M change from baseline in EH301 vs. placebo.

significant deterioration in the ALSFRS-R score or muscle function, measured using the MRC grading scale. In addition, 6 of the 8 muscle groups investigated using EMG did not show deterioration. We did, however, detect an 11.5% reduction in FVC, suggesting some decline in pulmonary function between baseline and 1 year. However, this reduction in FVC at 1-year is still less than the reduction in FVC observed in the placebo group at 4 months (11.5% mean reduction vs. 16.7%).

Safety and tolerability

Adverse events (AEs; including mild headache, moderate dyspepsia, and moderate diarrhea) were reported by four participants in the placebo group and five participants of the EH301 group that completed the study. All AEs were short in duration with full recovery of the participant, and none could be attributed to the investigational product. No other serious AEs were reported during this study (with the exception of those that caused patient withdrawal; see Figure 1). None of the reasons for patient withdrawal could be directly attributed to the treatment (EH301 or placebo).

This exploratory trial has been subject to a mock FDA Biometrics Monitoring audit by PAREXEL International, Inc. using the international standards for human clinical trials as a framework. In this audit, PAREXEL concluded that this study was conducted ethically, morally, and in a controlled manner, and the outputs lend credence to the efficacy and safety of EH301 in the treatment of ALS.

Discussion

The results presented here demonstrate that EH301 is safe. Our preliminary results show its

positive effect against ALS progression and provide support for a larger study to formally test treatment efficacy. All participants treated with EH301 demonstrated improvement in at least one outcome measure, with the majority of participants showing improvement in at least two clinical measures.

It is important to note that all study participants were taking riluzole. This is not uncommon in clinical trials of ALS; recently published clinical trials have reported riluzole use in the range of 73% (18) to 91% (3). In this study, with 100% reported riluzole use in both groups, all observed clinical benefits should be attributed to EH301.

None of the participants were taking edaravone, which has previously shown to lessen the 6-month decline in the ALSFRS-R score by 33% after 24 weeks of treatment (3). In contrast, EH301 demonstrated significant improvements in pulmonary function and muscle strength while showing no significant decline in the ALSFRS-R score over the 4-month controlled trial.

The 1-year evaluation of EH301 provides further support despite the absence of a placebo group. We observed no further decline in the ALSFRS-R score or the MRC grading scale index relative to baseline (Supplementary Table S1). In contrast, a prospective study of progression in PALS reported a significant decline in both of these outcome measures following 12 months of observation (19). Indeed, the ALSFRS-R score is known to reliably decline by approximately 1 point/month (0.92 ± 0.08 (SEM) according to the NEALS database (20)); meanwhile, our findings of no significant difference relative to baseline imply that we may have minimized further deterioration of functionality.

We observed a significant decline in pulmonary function in the 1-year evaluation relative to baseline; however, the 11.5% decline in FVC was smaller than what we observed in the placebo group at 4 months (Supplementary Table S1 compared to Figure 2(A-ii)), in addition to estimates of predicted decline, which anticipate a decrease in FVC of ~25% over 1 year (21). We also noted that one study participant within the EH301group exhibited a 49% decline in FVC at the 1-year evaluation relative to baseline (individual data not shown), which is reflected in the 1-year mean FVC measurement. Nevertheless, the small sample size of our study is insufficient to achieve definitive results, and further investigation is necessary.

Both components of EH301 activate sirtuins; NAD^+ dependent deacylases that play important roles in mitochondrial function (22). Recent studies have shown that mitochondrial deficiencies may play unexpected roles in diseases not thought to be related to these organelles. For example, genetic defects in DNA repair lead to cancer syndromes and also trigger mitochondrial defects via NAD^+ depletion, due to activation of the NAD^+ consuming enzymes, poly [ADP-ribose] polymerase 1 (PARP1) and PARP2 (23). This, in turn, inactivates SIRT1, resulting in a break-down in mitophagy, yielding defective mitochondria that produce excessive levels of reactive oxygen species (ROS) at the expense of ATP (24). In addition, the inappropriate activation of sterile alpha and TIR motif-constraining 1 (SARM1) may cause a depletion of NAD^+ and axon destruction in motor neurons (25). Hypothetically NAD^+ and ATP deficiencies, if occurring in ALS motor neurons, could lead to mitochondrial decline and excessive production of ROS.

In the ALS patients, we suggest that the combination treatment of EH301 may exert beneficial effects on mitochondria, including increased ATP production due to 1-(β-D-Ribofuranosyl) nicotinamide chloride and decreased ROS due to the SIRT1-activation activity of 3,5-dimethoxy-4′-hydroxy-trans-stilbene (11). Moreover, 1-(β-D-Ribofuranosyl) nicotinamide chloride-induced activation of SIRT 1 and SIRT3 could lead to deacetylation and activation of mitochondrial superoxide dismutase (26), and deacetylation and upregulation of PGC-1α (a master regulator of mitochondrial function and known neuroprotective factor) (27), thus, contributing to the prevention of ROS-mediated damage in the motor neurons.

3,5-Dimethoxy-4′-hydroxy-trans-stilbene can cross the BBB (28) and has been shown to increase nuclear Nrf2 (29,30); a redox-sensitive transcription factor involved in transcriptional regulation of many antioxidant genes, including γ-glutamate-cysteine ligase, the rate-limiting step in glutathione (GSH) synthesis (31). It is noteworthy that maintenance of the mitochondrial GSH pool is essential to protect neurons from oxidative and nitrosative stress (32).

This pilot study indicates that EH301 may offer disease-modifying benefits for the treatment of ALS. The findings from this pilot trial must be further validated in a larger Phase II trial, to explore the efficacy of EH301 in a larger patient population.

Declaration of interest

The authors report no disclosures relevant to the manuscript with the exception of L. Guarente, H. E. Holmes and R.W. Dellinger who report salary support and stock options from Elysium Health, Inc.

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