

The effects of dexamipexole (KNS-760704) in individuals with amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is characterized by upper and lower motor neuron dysfunction and loss, rapidly progressive muscle weakness, wasting and death^{1–3}. Many factors, including mitochondrial dysfunction, may contribute to ALS pathogenesis^{4–9}. Riluzole, which has shown only modest benefits in a measure of survival time without demonstrated effects on muscle strength or function, is the only approved treatment for ALS^{10,11}. We tested the putative mitochondrial modulator dexamipexole (KNS-760704; (6*R*)-4,5,6,7-tetrahydro-*N*6-propyl-2,6-benzothiazole-diamine)^{12–14} in subjects with ALS in a two-part, double-blind safety and tolerability study, with a preliminary assessment of its effects on functional decline and mortality. In part 1, the effects of dexamipexole (50, 150 or 300 mg d⁻¹) versus placebo were assessed over 12 weeks. In part 2, after a 4-week, single-blind placebo washout, continuing subjects were re-randomized to dexamipexole at 50 mg d⁻¹ or 300 mg d⁻¹ as double-blind active treatment for 24 weeks. Dexamipexole was safe and well tolerated. Trends showing a dose-dependent attenuation of the slope of decline of the ALS Functional Rating Scale-Revised (ALSF_{RS}-R) in part 1 and a statistically significant ($P = 0.046$) difference between groups in a joint rank test of change from baseline in ALSF_{RS}-R and mortality in part 2 strongly support further testing of dexamipexole in ALS.

ALS clinical investigators have employed various approaches to increase the efficiency of phase 2 studies, including futility designs and dose-selection studies^{15–21}. Our phase 2 study used a unique study design and tested a drug, dexamipexole, that had not been previously tested in a double-blind, placebo-controlled clinical trial in subjects with ALS. A previous open-label study of dexamipexole in subjects with ALS suggested a dose-dependent attenuation of functional decline¹³, and we now report the results of a two-part, multicenter, double-blind study to evaluate the safety and tolerability of dexamipexole, as well as the preliminary effects on measures of clinical function and

mortality for up to 9 months of dexamipexole treatment in subjects with ALS. The clinical trial design is presented in **Figure 1**.

In part 1, 102 subjects with ALS were randomized at 20 US sites to receive placebo ($n = 27$), dexamipexole at 50 mg d⁻¹ ($n = 23$; 25 mg orally every 12 h), dexamipexole at 150 mg d⁻¹ ($n = 26$; 75 mg orally every 12 h) or dexamipexole at 300 mg d⁻¹ ($n = 26$; 150 mg orally every 12 h) (**Fig. 1**). All 102 randomized subjects had at least one post-baseline clinical status evaluation (**Supplementary Table 1**) and were included in the intent-to-treat population; four subjects discontinued treatment, but none died during part 1. An additional subject withdrew consent prior to entering part 2. In part 2 (**Fig. 1**), 97 subjects entered a single-blind 4-week placebo washout period, during which five subjects discontinued (including three deaths due to disease progression). The remaining 92 subjects were re-randomized to one of two double-blind groups (48 subjects received 50 mg d⁻¹ (as 25 mg orally every 12 h) and 44 subjects received 300 mg d⁻¹ (as 150 mg orally every 12 h)); 90 of the 92 randomized subjects had at least one post-baseline clinical status evaluation (**Supplementary Table 2**). A total of 71 subjects completed 24 weeks of double-blind active treatment, including 34 subjects (71%) in the 50-mg group and 37 subjects (84%) in the 300-mg group. Discontinuations (21) occurred in part 2 because of death (nine subjects; seven in the 50-mg group and two in the 300-mg group), withdrawal of consent (seven subjects; five in the 50-mg group and two in the 300-mg group), adverse events (dizziness and neutropenia) (two subjects; both in the 300-mg group), and other, including loss to follow-up (three subjects; two in the 50-mg group and one in the 300-mg group). Three subjects (two in the 50-mg group and one in the 300-mg group), who discontinued for reasons other than death, died before what would have been their active treatment week 24 visit. We included these deaths in analyses of mortality. Treatment groups were generally well balanced with regard to baseline characteristics, including >50% of subjects on concomitant riluzole in all groups (**Table 1**). There was a nonsignificant ($P = 0.07$) difference in the number of subjects with bulbar-onset ALS randomized in part 2 to the 300-mg group (11 of 44; 25%) compared with the 50 mg group (5 of 48; 10%).

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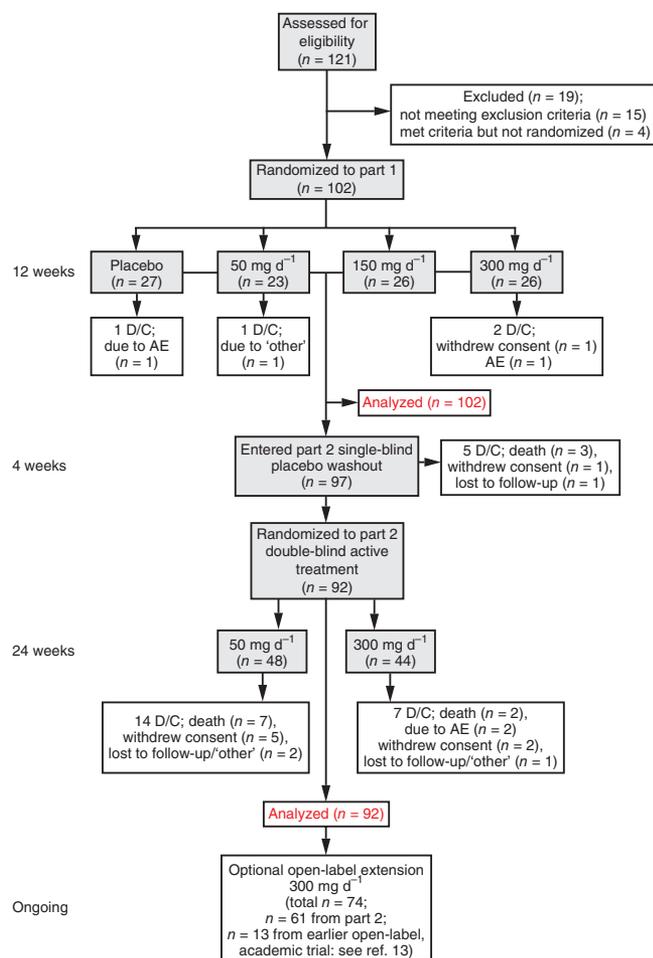


Figure 1 Schematic of the design of parts 1 and 2 of the study. The number of subjects with ALS randomized to each dose group, events leading to changes in subject number in each group and the final numbers of subjects contributing to analyses are indicated for the part 1 12-week active treatment period, the part 2 4-week placebo washout period, and the part 2 24-week active treatment period. The ongoing safety extension study, in which subjects with ALS continuing from part 2 and subjects with ALS enrolled from a previous open-label study of dexamipexole are dosed with 300 mg d⁻¹, is indicated at the bottom of the schematic. D/C, discontinued.

Pharmacokinetic analyses in part 1 (**Supplementary Results and Supplementary Table 3**) showed that plasma pharmacokinetics were linear across dosages, and steady state was achieved before study day 10, the earliest pharmacokinetic sample day, consistent with the observed elimination half-life of 6.7 to 8.2 h (**Fig. 2a**). There were no dosage-related trends in either oral clearance or volume of distribution, and no evidence of drug accumulation after steady state was reached. Plasma concentrations were achieved that should have produced pharmacodynamic central nervous system concentrations (**Fig. 2a**).

We observed no differences between dose groups in the incidence of vital sign, electrocardiogram or laboratory abnormalities that met prespecified clinical significance criteria. Safety result details are available in the **Supplementary Results and Supplementary Tables 4 and 5**). There were no deaths or treatment-related serious adverse events (SAEs) during part 1 of the study. All but four subjects completed the 12 weeks of treatment: two subjects withdrew consent and two subjects discontinued due to adverse events (see **Supplementary Table 5**). During the placebo washout period, 5 of 97 subjects (5%)

had SAEs (six events in five subjects: disease progression in two subjects, dyspnea in two subjects and both urethral obstruction and urinary retention in one subject), none of which were considered to be treatment related. Of these five subjects, three (one placebo subject and two 50-mg subjects from part 1) died as a result of disease progression. Twelve deaths occurred during the double-blind active treatment period in part 2: nine deaths occurred in subjects receiving 50 mg d⁻¹ and three occurred in subjects receiving 300 mg d⁻¹. There were 17 subjects with SAEs in part 2 (**Supplementary Tables 4 and 6**), 11 of 48 (23%) in the group receiving 50 mg d⁻¹ and 6 of 44 (14%) in the group receiving 300 mg d⁻¹. Five subjects (two in part 1 and three in part 2) receiving 300 mg d⁻¹ experienced reversible Common Terminology Criteria for adverse events grade 2 asymptomatic (four subjects) or grade 4 febrile (one subject) neutropenia; there was no recurrence in three of four subjects who resumed active treatment (**Supplementary Table 7**). We considered dexamipexole to be safe and well tolerated in both parts of the study.

Mean ALSFRS-R total scores at baseline in part 1 were similar in the four treatment groups (**Table 1**). The treatment group slope estimates of ALSFRS-R scores (in units per month) from the linear mixed-effects model were -1.28 (placebo, 95% confidence interval (CI): -1.82 to -0.74), -1.89 (50 mg, 95% CI: -2.48 to -1.29), -1.17 (150 mg, 95% CI: -1.71 to -0.62) and -0.88 (300 mg, 95% CI: -1.44 to -0.31). The difference in mean slope between 300 mg d⁻¹ and placebo was 0.40 (95% CI: -0.38 to 1.18), a reduction of 31% in part 1 of the study. The primary analysis of overall treatment effect by the linear mixed-effects model applied to the slope²² was not significant ($P = 0.11$). Study endpoint was defined as the last observation of the subject before dropout or at study completion, the last observation carried forward. The treatment group mean \pm s.e.m. change from baseline to study endpoint in ALSFRS-R scores was -3.6 ± 0.8 (placebo), -5.0 ± 1.1 (50 mg), -3.3 ± 0.6 (150 mg) and -2.2 ± 0.6 (300 mg). The slope of the ALSFRS-R and the mean change from baseline were influenced in the 50-mg group by two very rapidly declining subjects (see **Fig. 2b**). The median change from baseline to endpoint was less affected by outliers and showed a strong trend of reduced decline with increasing dose; the median values were -4.0 (placebo), -3.0 (50 mg), -2.5 (150 mg) and -2.0 (300 mg). Median decline in ALSFRS-R score in the 300-mg group was attenuated by 50% relative to the placebo group in part 1 of the study. Riluzole use showed no interactions with dexamipexole use in any of the treatment groups.

When a six-point or greater drop in ALSFRS-R total score from baseline to 12 weeks in part 1 was used to define subjects that failed to respond to drug treatment²³, a significant dose-dependent effect was observed. The number of failures totaled nine subjects (33%) in the placebo group, eight subjects (35%) in the 50-mg group, four subjects (15%) in the 150-mg group and two subjects (8%) in the 300-mg group (logistic regression analysis, $P = 0.01$; **Fig. 2b**).

The ALSFRS-R is divided equally into four subdomains measuring fine-motor, gross-motor, bulbar and respiratory functions^{24,25}, which decline at varying rates, from highest to lowest in the order listed²⁵. Consistent with previous results, the fine-motor subdomain score in subjects receiving placebo in part 1 declined at a higher rate than the gross-motor, bulbar or respiratory subdomains (mean \pm s.e.m., percentage change from baseline in ALSFRS-R subdomain score; -1.4 ± 0.30 , 38%; -0.9 ± 0.36 , 24%; -0.8 ± 0.25 , 22%; -0.6 ± 0.22 , 16%, respectively). The greatest difference between subjects receiving placebo and those receiving 300 mg d⁻¹ dexamipexole in mean change from baseline was in the fine motor subdomain (-1.4 ± 0.30 versus -0.6 ± 0.24 , **Fig. 2c**).

At baseline in part 1, upright vital capacity values (calculated as the percentage of the predicted pulmonary capacity for healthy individuals,

Table 1 Summary of demographic and baseline characteristics for randomized groups in parts 1 and 2 of the study

Characteristic	Part 1				Part 2 ^a	
	PBO (<i>n</i> = 27)	50 mg (<i>n</i> = 23)	150 mg (<i>n</i> = 26)	300 mg (<i>n</i> = 26)	50 mg (<i>n</i> = 48)	300 mg (<i>n</i> = 44)
Mean age in years (s.d.)	55.8 (9.07)	58.1 (10.20)	56.0 (11.03)	58.2 (10.96)	56.7 (10.26)	57.1 (10.96)
Males (%)	51.9	60.9	69.2	73.1	66.7	63.6
Mean BMI in kg (m ²) ⁻¹ (s.d.)	25.66 (3.93)	26.86 (6.37)	25.78 (4.49)	26.81 (4.19)	26.71 (5.32)	25.55 (4.47)
Mean symptom duration at baseline in months (s.d.)	15.5 (5.57)	12.5 (4.65)	15.1 (5.65)	12.9 (6.36)	17.8 (5.97)	18.6 (5.94)
Mean time from diagnosis to baseline in months (s.d.)	7.1 (5.69)	6.1 (4.35)	6.6 (5.72)	6.0 (5.90)	9.94 (5.21)	11.15 (5.99)
Bulbar onset (%)	25.9%	13.0%	7.7%	23.1%	10.4%	25.0%
Number of subjects with familial ALS	1 (4%)	3 (14%)	1 (4%)	2 (8%)	3 (6%)	3 (7%)
Mean ALSFRS-R at baseline (s.d.)	37.3 (5.14)	37.2 (5.61)	39.1 (4.44)	38.5 (5.97)	34.0 (7.69)	33.8 (8.29)
Mean vital capacity at baseline (s.d.)	90.4 (14.39)	89.0 (11.22)	88.4 (14.94)	91.7 (16.01)	76.7 (18.85)	81.7 (21.19)
Concomitant riluzole (%)	59.3	52.2	61.6	69.2	52.1	65.9

^aBaseline for part 2 was obtained at the beginning of the 24-week active treatment phase (part 2, week 4, which corresponded with the end of the placebo washout period), for vital capacity at baseline *n* = 45 for 50 mg d⁻¹, *n* = 42 for 300 mg d⁻¹. PBO, placebo.

matched for age and gender, in a test of upright slow expiratory effort) were similar in the four treatment groups (Table 1). Based on a linear mixed-effects model, the slope of upright vital capacity did not differ significantly across treatment groups (*P* = 0.54). However, the number of treatment failures, defined as a reduction in vital capacity of 20% or greater from baseline to week 12, was significantly different and totaled eight subjects (30%) in the placebo group, three subjects (13%) in the 50-mg group, three subjects (12%) in the 150-mg group and one subject (4%) in the 300-mg group (logistic regression on dosage; *P* = 0.03; Fig. 2d).

Subjects were re-randomized into the active treatment component of part 2 as described previously; the results of re-randomization are presented in Table 2. The treatment group slope estimates of ALSFRS-R

scores from the linear mixed-effects model at week 24 of active treatment were -1.28 for the 50-mg group (95% CI: -1.56 to -1.01) and -1.02 for the 300-mg group (95% CI: -1.29 to -0.75), treatment group difference of 0.26 (95% CI: -0.12 to 0.64). This was an attenuation of 20.5% in the rate of decline in ALSFRS-R scores for the 300-mg group relative to the 50-mg group over 24 weeks of active treatment (*P* = 0.177; Fig. 2e). There also was a 68% reduction in the hazard of mortality for the 300-mg group relative to the 50-mg group (log-rank test, *P* = 0.07; hazard ratio 0.32, 95% CI: 0.086 to 1.18, Kaplan-Meier estimates, Fig. 2f).

Deaths and informative discontinuations can affect the slope estimates and analyses of mean changes in ALSFRS-R scores. We therefore conducted prespecified sensitivity analyses to assess the effects

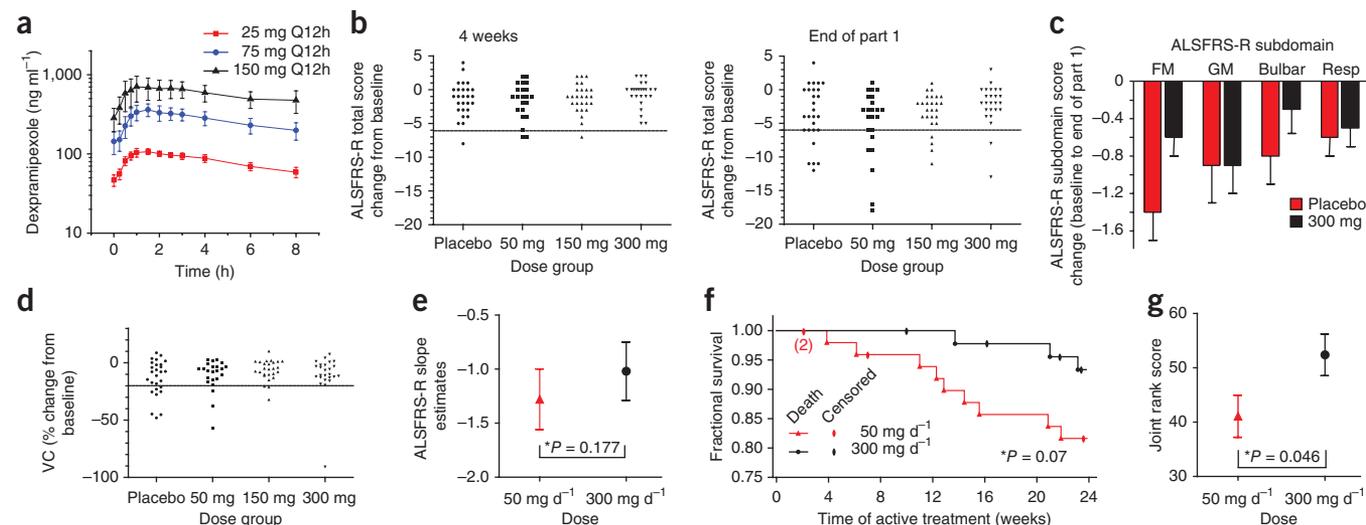


Figure 2 The effects of dexampipexole in parts 1 and 2 of the study. (a) Pharmacokinetic analyses of plasma drug concentrations (mean \pm s.e.m.) following oral administration of 25 mg (50 mg d⁻¹), 75 mg (150 mg d⁻¹) and 150 mg (300 mg d⁻¹) doses of dexampipexole twice daily (Q12h) for at least 10 d in a subgroup of patients in part 1 of the study. (b) Scatterplots of individual changes from baseline to week 4 (left) and to endpoint (right) for ALSFRS-R total scores by treatment group in part 1 of the study, with the dashed line indicating the criterion used for analysis of treatment failure (decrease in ALSFRS-R total score of ≥ 6 points; *P* = 0.01 for ALSFRS-R total score; logistic regression analysis). (c) Bar chart showing change from baseline, (mean \pm s.e.m.) to endpoint in the fine-motor (FM), gross-motor (GM), bulbar and respiratory subdomains of the ALSFRS-R for the placebo and 300-mg groups in part 1 of the study. (d) Scatterplots of individual changes from baseline to endpoint for percentage vital capacity (VC) by treatment group in part 1 of the study, with the dashed line indicating the criterion used for analysis of treatment failure (decrease in vital capacity of $\geq 20\%$). (*P* = 0.03; logistic regression analysis). (e) Plot of the ALSFRS-R slope estimates, in units per month, from the linear mixed-effects model ($\pm 95\%$ CI), for the two groups (50 mg d⁻¹ and 300 mg d⁻¹) studied in part 2. (f) Kaplan-Meier estimates of the probability of survival by treatment group in the active treatment phase of part 2 of the study (*P* = 0.07; log-rank test). This analysis includes four subjects from each group that were censored because of discontinuation. (g) Mean (\pm s.e.m.) rank scores from the joint-rank analysis of survival and change from baseline in ALSFRS-R by treatment group in part 2 of the study (*P* = 0.046; Gehan-Wilcoxon test).

Table 2 Results of re-randomization of subjects to part 2 double-blind active treatment groups: number and percentage of subjects by part 1 treatment group assignment

Part 1 treatment assignment	Part 2 treatment assignment		Part 1 treatment assignments for subjects re-randomized to DBAT in part 2
	50 mg d ⁻¹	300 mg d ⁻¹	
Placebo	10 (21%)	14 (32%)	24 (26%)
50 mg d ⁻¹	14 (29%)	6 (14%)	20 (22%)
150 mg d ⁻¹	12 (25%)	13 (30%)	25 (27%)
300 mg d ⁻¹	12 (25%)	11 (25%)	23 (25%)
Total	48 (100%)	44 (100%)	92 (100%)

DBAT, double-blind active treatment.

of the large imbalance in deaths and discontinuations between the treatment groups in part 2. We conducted a joint rank analysis (see Online Methods and **Supplementary Methods**) to account for mortality in the analysis of the functional data; subject outcomes were first ranked on the basis of mortality (earliest deaths ranked worst, or lowest), then on the basis of change from baseline in ALSFRS-R total score for surviving subjects, with the greatest decrease in a survivor ranked just above the highest death ranking. The mean rank score for the 300-mg group (52.39 ± 3.84) was significantly higher than the mean score for the 50-mg group (41.10 ± 3.88 ; Gehan-Wilcoxon test; $P = 0.046$; **Fig. 2g**). In an analysis of covariance (ANCOVA) on the rank scores to adjust for baseline variables (ALSFRS-R score, time from symptom onset, site of disease onset and riluzole use), the significance level was $P = 0.01$. Imputation of an ALSFRS-R score of 0 for the next visit that would have occurred (had they survived) for those subjects who died during the study, within the linear mixed-effects model²⁶, resulted in treatment group \pm s.e.m. slope estimates of -2.07 ± 0.26 for the 50-mg group versus -1.19 ± 0.26 for the 300-mg group, a reduction in decline of 43% ($P = 0.02$). There was no apparent effect of dexamipexole on the slope of McGill Single-Item Scale (SIS) scores in either part 1 or part 2 ($P = 0.49$ and $P = 0.59$, respectively).

An ANCOVA run on the joint rank scores from part 2 included fixed effects for the part 1 and part 2 treatment group. Part 1 treatment group was not a significant predictor of part 2 joint rank score, $P = 0.51$. The ANCOVA model was expanded to include a part 1 treatment group by part 2 treatment group interaction; the interaction was not significant ($P = 0.89$).

The linear mixed-effects model mean estimates of decline in vital capacity slope from baseline through week 24 of part 2 active treatment were -2.45 ± 0.53 and -3.07 ± 0.51 for the 50-mg and 300-mg groups, respectively ($P = 0.40$). When we imputed zero values as described above for subjects who died, we observed a 20.3% attenuation of decline in the 300-mg group compared with the 50-mg group ($P = 0.39$).

In summary, our study data indicate that twice daily oral dexamipexole at 50–300 mg d⁻¹, in 102 subjects with ALS, was safe and well tolerated. Infrequent, reversible neutropenia was the only possible drug-related safety event of note observed during either part 1 or part 2 of the study, and it will be monitored in future clinical trials. Subjects choosing to do so after part 2 ($n = 61$), and additional subjects enrolled from a previous open label study ($n = 13$)¹³, receive the highest dose (300 mg d⁻¹) of dexamipexole in a long-term open-label safety study that is currently ongoing. Subjects in this long-term study have been exposed to active high-dose drug for up to 26 months, and 22 subjects are currently still receiving drug. The adverse event profile continues to support the conclusion that the drug is safe and well tolerated.

Furthermore, we observed preliminary evidence of drug activity in recognized measures of function and disease progression. In part 1, we observed a dose-dependent trend in the reduction of the rate of decline in the ALSFRS-R total score. Relative to the placebo group, the 300-mg slope of decline was reduced by 31%, and the median rate of decline from baseline to week 12 was reduced by 50%, representing clinically significant treatment effects²⁴. Whereas the reduction produced by 150 mg d⁻¹ dexamipexole was intermediate between that achieved by 300 mg d⁻¹ and placebo, it is currently unclear whether there was any effect of 50 mg d⁻¹ dexamipexole. Failure analyses of ALSFRS-R and vital capacity decline in part 1 both showed significant dosage-dependent effects.

In ALS, there is wide variation in the rate of decline in the ALSFRS-R (see **Supplementary Figs. 1 and 2**), but, in general, declines in neuromuscular function and mortality are correlated^{27–30}, and deaths and informative discontinuations can skew group estimates of the decline of ALSFRS-R scores. Usually subjects with the most rapidly declining ALSFRS-R scores have the shortest survival³⁰. Group mean ALSFRS-R scores generally improve after dropouts, as the dropout scores no longer contribute to subsequent group means. If a reduction in deaths or discontinuations results from the action of an effective drug, true differences in ALSFRS-R scores between groups experiencing the most deaths and discontinuations and dose-effective drug groups can be underestimated, and analysis techniques have been developed to attempt to fairly and systematically compensate for this. The decline in the 300-mg mean ALSFRS-R slope in part 2, relative to the slope of decline for the 50-mg group, was reduced by ~20%, which is a potentially clinically significant level of effect²⁴. This is in contrast to the group mean changes in ALSFRS-R total score relative to baseline. The 50-mg and 300-mg group means had differentiated early in the 24-week active treatment period, but the difference in raw means had largely disappeared by active treatment week 24 because increased deaths and discontinuations in the 50-mg group inflated the raw mean scores at subsequent visits. Imputing a score of 0 for the first missing visit after death adjusts for the artificial improvement in the group mean ALSFRS-R total score following deaths and discontinuations. When we performed this imputation analysis, the result was a significant difference between the two dose groups. In the joint rank analysis of functional decline and mortality, we detected a significant difference ($P = 0.046$) between the groups, favoring the 300-mg group. The joint ranking appropriately accounts for the missing functional data from deaths and discontinuations and makes no assumptions about the linearity of change in ALSFRS-R.

Our unique study design allowed the same cohort of subjects to participate in what are essentially two separate studies of the drug. There was no evidence that carry-over effects of part 1 treatment explained the effects observed in part 2. Furthermore, including only subjects ≤ 24 months from the onset of symptoms resulted in a greater-than-typical rate of decline in the ALSFRS-R total score, which may have increased the dynamic range to detect a drug effect. To our knowledge, no other drug has shown a clinically significant effect on the decline of the ALSFRS-R in a properly controlled clinical trial, and no other study has shown effects on both function and mortality. Although a primary analysis of the overall treatment effect by the linear mixed-effects model applied to the slope was not statistically significant, treatment effects on function in parts 1 and 2 were similar, favoring 300-mg dexamipexole over either placebo or low-dose drug.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturemedicine/>.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

M.C., R.M., H.M., J.S., D.H.M. and D.S. served as expert clinical and statistical advisors to the sponsor, Knopp Biosciences, and collaborated with M.E.B., E.W.I., J.L.M., D.A., M.S., C.A., J.M. and V.K.G. with respect to the design of the study. E.W.I., J.L.M., C.A., and J.M. were principally responsible for the clinical operations and execution of the study. M.E.B. was principally responsible for medical monitoring and patient safety assessments for the study. D.A. was principally responsible for the statistical analysis of the study with input from D.H.M. and D.S. who served in an advisory capacity. M.S. and E.W.I. were principally responsible for ensuring regulatory compliance with US federal regulations governing sponsored clinical research for the duration of the study. V.K.G., E.W.I., M.E.B., D.A., J.L.M. and M.C. collaborated to write the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/naturemedicine/>.

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- Rowland, L.P. & Shneider, N.A. Amyotrophic lateral sclerosis. *N. Engl. J. Med.* **344**, 1688–1700 (2001).
- Wijesekera, L.C. & Leigh, P.N. Amyotrophic lateral sclerosis. *Orphanet J. Rare Dis.* **4**, 3 (2009).
- Vucic, S. & Kiernan, M.C. Pathophysiology of neurodegeneration in familial amyotrophic lateral sclerosis. *Curr. Mol. Med.* **9**, 255–272 (2009).
- Hervias, I., Beal, M.F. & Manfredi, G. Mitochondrial dysfunction and amyotrophic lateral sclerosis. *Muscle Nerve* **33**, 598–608 (2006).
- Manfredi, G. & Xu, Z. Mitochondrial dysfunction and its role in motor neuron degeneration in ALS. *Mitochondrion* **5**, 77–87 (2005).
- Baron, M., Kudin, A.P. & Kunz, W.S. Mitochondrial dysfunction in neurodegenerative disorders. *Biochem. Soc. Trans.* **35**, 1228–1231 (2007).
- Corti, S. *et al.* Amyotrophic lateral sclerosis linked to a novel SOD1 mutation with muscle mitochondrial dysfunction. *J. Neurol. Sci.* **276**, 170–174 (2009).
- Kawamata, H. & Manfredi, G. Mitochondrial dysfunction and intracellular calcium dysregulation in ALS. *Mech. Ageing Dev.* **131**, 517–526 (2010).
- Shi, P., Wei, Y., Zhang, J., Gal, J. & Zhu, H. Mitochondrial dysfunction is a converging point of multiple pathological pathways in amyotrophic lateral sclerosis. *J. Alzheimers Dis.* **20** (suppl. 2), S311–S324 (2010).
- Messori, A., Trippoli, S., Becagli, P. & Zaccara, G. Cost effectiveness of riluzole in amyotrophic lateral sclerosis. Italian Cooperative Group for the Study of Meta-Analysis and the Osservatorio SIFO sui Farmaci. *Pharmacoeconomics* **16**, 153–163 (1999).
- Stewart, A. *et al.* The clinical effectiveness and cost-effectiveness of riluzole for motor neurone disease: a rapid and systematic review. *Health Technol. Assess.* **5**, 1–97 (2001).
- Gribkoff, V.K. & Bozik, M.E. KNS-760704 [(6R)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine dihydrochloride monohydrate] for the treatment of amyotrophic lateral sclerosis. *CNS Neurosci. Ther.* **14**, 215–226 (2008).
- Wang, H. *et al.* R+ pramipexole as a mitochondrially focused neuroprotectant: initial early phase studies in ALS. *Amyotroph. Lateral Scler.* **9**, 50–58 (2008).
- Bozik, M.E., Mather, J.M., Kramer, W.H., Gribkoff, V.K. & Ingersoll, E.W. Safety, tolerability and pharmacokinetics of KNS-760704 (dextramipexole) in healthy adult subjects. *J. Clin. Pharmacol.* **51**, 1177–1185 (2010).
- Cudkovicz, M.E. *et al.* Toward more efficient clinical trials for amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* **11**, 259–265 (2010).
- Cheung, Y.K., Gordon, P.H. & Levin, B. Selecting promising ALS therapies in clinical trials. *Neurology* **67**, 1748–1751 (2006).
- Groeneveld, G.J., Graf, M., van, d.T.I., van den Berg, L.H. & Ludolph, A.C. Alternative trial design in amyotrophic lateral sclerosis saves time and patients. *Amyotroph. Lateral Scler.* **8**, 266–269 (2007).
- Gordon, P.H. *et al.* Outcome measures for early phase clinical trials. *Amyotroph. Lateral Scler.* **8**, 270–273 (2007).
- Schoenfeld, D.A. & Cudkovicz, M. Design of phase II ALS clinical trials. *Amyotroph. Lateral Scler.* **9**, 16–23 (2008).
- Shefner, J.M. Designing clinical trials in amyotrophic lateral sclerosis. *Phys. Med. Rehabil. Clin. N. Am.* **19**, 495–508 (2008).
- Bedlack, R.S., Pastula, D.M., Welsh, E., Pulley, D. & Cudkovicz, M.E. Scrutinizing enrollment in ALS clinical trials: room for improvement? *Amyotroph. Lateral Scler.* **9**, 257–265 (2008).
- Laird, N.M. & Ware, J.H. Random-effects models for longitudinal data. *Biometrics* **38**, 963–974 (1982).
- Aggarwal, S.P. *et al.* Safety and efficacy of lithium in combination with riluzole for treatment of amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol.* **9**, 481–488 (2010).
- Castrillo-Viguera, C., Grasso, D.L., Simpson, E., Shefner, J. & Cudkovicz, M.E. Clinical significance in the change of decline in ALSFRS-R. *Amyotroph. Lateral Scler.* **11**, 178–180 (2010).
- Cedarbaum, J.M. *et al.* The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (phase III). *J. Neurol. Sci.* **169**, 13–21 (1999).
- Miller, R. *et al.* Phase II/III randomized trial of TCH346 in patients with ALS. *Neurology* **69**, 776–784 (2007).
- Gordon, P.H. & Cheung, Y.K. Progression rate of ALSFRS-R at time of diagnosis predicts survival time in ALS. *Neurology* **67**, 1314–1315 (2006).
- Kollewe, K. *et al.* ALSFRS-R score and its ratio: a useful predictor for ALS-progression. *J. Neurol. Sci.* **275**, 69–73 (2008).
- Liu, X.X., Fan, D.S., Zhang, J., Zhang, S. & Zheng, J.Y. *Zhonghua Yi Xue Za Zhi* [Revised amyotrophic lateral sclerosis functional rating scale at time of diagnosis predicts survival time in amyotrophic lateral sclerosis] **89**, 2472–2475 (2009).
- Kaufmann, P. *et al.* The ALSFRS-R predicts survival time in an ALS clinic population. *Neurology* **64**, 38–43 (2005).

ONLINE METHODS

Study design and sample size. See **Supplementary Methods** for enrollment criteria. An original feature of the protocol was the division of the study into two double-blind components (**Fig. 1**). Part 1 was a randomized, placebo controlled, double-blind study of dexamipexole (50, 150, and 300 mg d⁻¹) versus placebo for 12 weeks; we administered drug every 12 h as divided oral doses. In part 2, after a 4-week, single-blind placebo-washout period, we re-randomized subjects to 50 or 300 mg d⁻¹ dexamipexole for 24 weeks. Safety was the primary objective, and it was assessed by measurement of vital signs, physical examinations, 12-lead electrocardiograms, adverse events and clinical laboratory evaluations. We evaluated the clinical effects of dexamipexole as secondary objectives by administration of the ALSFRS-R^{31,32}, vital capacity³³, the McGill SIS^{34,35} and estimates of survival. (See **Supplementary Tables 1 and 2**) for the study visit schedules. Study sample size was calculated using safety considerations, including the number of subjects needed to determine the common short-term side effects and risks associated with the drug. Part 1 was designed to enroll 20 subjects per arm (and was slightly overenrolled); with 20 patients per dose group, if an adverse event had a true frequency of 8% or greater, there was a ≥80% probability of observing at least one occurrence of that adverse event. The sample size for part 2 was determined by the number of subjects who completed part 1 and continued to part 2. The study was not powered for efficacy and had only 13% power to detect a 30% decrease in the slope of ALSFRS-R across treatment groups at 20 subjects per arm. In this light, the effects of dexamipexole on function and mortality described in this manuscript should be seen as preliminary indications of drug activity rather than definitive indications of drug efficacy.

Human subjects. This study was conducted in full accordance with US and international standards of Good Clinical Practice (GCP) guidelines and as per Title 21 Code of Federal Regulations Parts 50, 56 and 312, International Conference of Harmonisation guidelines, applicable government regulations, the ethical principles stated in the Declaration of Helsinki, local ethical and legal requirements, and institutional research policies and procedures. All subjects provided written informed consent, and institutional review board (IRB) approval was obtained for each participating center. The IRBs are as follows: Human Subjects Committee, University of Kansas Medical Center; University of Virginia IRB-HSR; Partners Human Research Committee; Vanderbilt University Medical Center Institutional Review Board; Office for Protection of Research Subjects, Los Angeles, California; Western Institutional Review Board, Olympia, Washington; University of Texas Health Science Center Institutional Review Board; California Pacific Medical Center Institutional Review Board; Colorado Multiple Institutional Review Board, University of Colorado at Denver and Health Sciences Center; Combined Institutional Review Board, Saint Elizabeth Community Health Center; Washington University School of Medicine Human Research Protection Office; The Johns Hopkins Medicine Institutional Review Board; The University of Arkansas for Medical Sciences Institutional Review Board; University of Miami Human Subjects Research Office; Institutional Review Board for the Protection of Human Subjects, State University of New York Upstate Medical University; Oregon Health and Sciences University Research Integrity Office-IRB; University of Utah Institutional Review Board; Institutional Review Board, Human Subjects Protection Office, Penn State Milton S. Hershey Medical Center.

Statistical analyses. All statistical tests were two tailed. Unless otherwise noted, mean values are accompanied by s.e.m.

For analysis of part 1 data, in prespecified analyses, we summarized the clinical effects on the ALSFRS-R, vital capacity and McGill SIS scores by treatment group, with slope estimates and 95% CI (in number of points per month) derived from the linear mixed-effects slopes model, which has been used in numerous recent ALS clinical trials^{36–38}. In this model, the interaction term between the time variable and the treatment group indicator variable represents the difference between the placebo slope and the treatment group slope; the estimated coefficient of the time variable is the placebo slope (see details in **Supplementary Methods** and **Supplementary Table 8**).

We conducted the following exploratory analyses for ALSFRS-R and vital capacity data at study endpoint last observation carried forward (see **Supplementary Methods**): (i) scatter plots of the data to illustrate trends and the effect of outliers and (ii) analysis of the rate of failures in ALSFRS-R and vital capacity by logistic regression on dosage (see **Supplementary Methods**).

For analysis of part 2 data, we plotted survival time using Kaplan-Meier estimates of the probability of survival as a prespecified analysis. We used the log-rank test to compare the treatment groups with respect to time to death and the Cox life-table proportional hazards regression model to provide estimates of the hazard ratio and 95% CI. Reduction in hazard for mortality was defined as 1 minus the hazard ratio. In other prespecified analyses, we summarized clinical effects on the ALSFRS-R, vital capacity and McGill SIS scores by treatment group, with the rate-of-change estimates (expressed as a slope in units per month) derived from the linear mixed-effects slopes model as described above for part 1 (see **Supplementary Table 9**).

We conducted a prespecified sensitivity analysis using a generalized Gehan-Wilcoxon rank test based on a ranking of subjects' outcome based jointly on change from baseline in ALSFRS-R and time to survival (see **Supplementary Methods**)³⁹. As an exploratory analysis, we ran an ANCOVA on the joint ranked scores to adjust for the following covariates: duration of ALS symptoms at baseline, baseline ALSFRS-R score, site of disease onset and concomitant riluzole use (see **Supplementary Methods**).

In a second exploratory sensitivity analysis, we imputed a score of zero as the ALSFRS-R total score for the first post-death visit for subjects who died during the double-blind active treatment period, and the treatment groups were compared using the linear mixed-effects slopes model (see **Supplementary Methods**).

Additional methods. Detailed methodology is described in the **Supplementary Methods**.

- Gordon, P.H., Miller, R.G. & Moore, D.H. ALSFRS-R. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* **5** (suppl. 1), 90–93 (2004).
- Kaufmann, P. *et al.* Excellent inter-rater, intra-rater and telephone-administered reliability of the ALSFRS-R in a multicenter clinical trial. *Amyotroph. Lateral Scler.* **8**, 42–46 (2007).
- Aggarwal, S.P. *et al.* Safety and efficacy of lithium in combination with riluzole for treatment of amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol.* **9**, 481–488 (2010).
- Cohen, S.R. *et al.* Validity of the McGill Quality of Life Questionnaire in the palliative care setting: a multi-centre Canadian study demonstrating the importance of the existential domain. *Palliat. Med.* **11**, 3–20 (1997).
- Simmons, Z., Bremer, B.A., Robbins, R.A., Walsh, S.M. & Fischer, S. Quality of life in ALS depends on factors other than strength and physical function. *Neurology* **55**, 388–392 (2000).
- Gordon, P.H. *et al.* Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. *Lancet Neurol.* **6**, 1045–1053 (2007).
- Miller, R. *et al.* Phase II/III randomized trial of TCH346 in patients with ALS. *Neurology* **69**, 776–784 (2007).
- Cudkovicz, M.E. *et al.* Trial of celecoxib in amyotrophic lateral sclerosis. *Ann. Neurol.* **60**, 22–31 (2006).
- Finkelstein, D.M. & Schoenfeld, D.A. Combining mortality and longitudinal measures in clinical trials. *Stat. Med.* **18**, 1341–1354 (1999).