1 **JAMA Neurology Original Investigation**

2

3 **Title**

4 Efficacy and Safety of Ultra-High Dose Methylcobalamin in Early-Stage Amyotrophic Lateral Sclerosis:

5 A Randomized Clinical Trial

6

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80   **Key Points**

81   **Question:** Does twice-weekly intramuscular injection of ultra-high dose methylcobalamin 50 mg retard

82   clinical progression in early-stage amyotrophic lateral sclerosis?

83   **Findings:** In this randomized phase 3 clinical trial that included 130 participants who were enrolled within 1

84   year from symptom onset and presented 1- or 2-point decrease in the Revised Amyotrophic Lateral Sclerosis

85   Functional Rating Scale total score during 12-week observation, the changes in the score were -2.66 with

86   methylcobalamin vs -4.63 with placebo during the 16-week treatment, which significantly differed.

87   **Meaning:** Ultra-high dose methylcobalamin can slow functional decline in early-stage amyotrophic lateral

88   sclerosis with moderate progression rate.

89

90 **Abstract**

91 **Importance:**

92 Post hoc analysis in a phase 2/3 trial indicated ultra-high dose methylcobalamin slowed decline of the Revised

93 Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) total score at week 16 as well as at week

94 182, without increase of adverse events, in patients with amyotrophic lateral sclerosis (ALS) who were

95 enrolled within 1 year from onset.

96 **Objective:**

97 To validate the efficacy and safety of ultra-high dose methylcobalamin for patients with ALS enrolled within 1

98 year of onset.

99 **Design:**

100 A multicenter, placebo-controlled, double-blind, randomized phase 3 trial with 12-week observation and 16-

101 week randomized period, conducted from October 2017 to September 2019.

102 **Setting:**

103 Twenty-five neurology centers in Japan.

104 **Participants:**

105 Patients with ALS diagnosed within 1 year of onset by the updated Awaji criteria were initially enrolled. Of

106 those, patients fulfilling the following criteria after 12-week observation were eligible for randomization: 1- or

107 2-point decrease in ALSFRS-R total score, a percent forced vital capacity over 60%, no history of noninvasive

108 respiratory support and tracheostomy, and being ambulant. The target number was 64 in both

109　methylcobalamin and placebo groups. Of 203 patients enrolled in the observation, 130 patients (age, 61.0 ±

110　11.7 years; female, 56) met the criteria and were randomly assigned through an electronic web-response

111　system to methylcobalamin or placebo (65 for each). Of these, 129 patients were eligible for the full analysis

112　set, and 126 completed the double-blind stage.

113　**Interventions:**

114　Intramuscular injection of methylcobalamin 50 mg or placebo twice weekly for 16 weeks.

115　**Main outcomes and measures:**

116　The primary endpoint was change in ALSFRS-R total score from baseline to week 16 in the full analysis set.

117　**Results:**

118　The least-squares mean difference in ALSFRS-R total score at week 16 of the randomized period was 1.97

119　points greater with methylcobalamin than placebo (−2.66 versus −4.63; 95% CI, 0.44–3.50; $P = 0.012$). The

120　incidence of adverse events was similar between the two groups.

121　**Conclusions and relevance:**

122　Ultra-high dose methylcobalamin was efficacious in slowing functional decline and safe in the 16-week

123　treatment period in ALS patients in the early stage and with moderate progression rate.

124　**Trial registration:**

125　UMIN-CTR Identifier: UMIN000029588 (umin.ac.jp/ctr); ClinicalTrials.gov Identifier: NCT03548311

126　(clinicaltrials.gov)

127

Introduction

Amyotrophic lateral sclerosis (ALS) is an intractable disease affecting the upper and lower motor neurons and resulting in progressive systemic muscle weakness and atrophy. The duration from symptom onset to the use of invasive respiratory support or death is 20–48 months.[1] Although riluzole[2,3] and edaravone[4] have been approved by the U.S. Food and Drug Administration to modify the disease progression of ALS, the effectiveness of these drugs is restricted, warranting the development of further treatments.

In vivo studies have shown that ultra-high dose methylcobalamin injections inhibited the progression of motor symptoms and neuropathological changes in a wobbler mouse model of ALS.[5] A clinical pilot study demonstrated that intramuscular administration of ultra-high dose methylcobalamin increased the amplitude of compound muscle action potentials in patients with ALS.[6] A phase 2/3 clinical trial including 373 Japanese patients with ALS within 3 years of clinical onset diagnosed by the El Escorial/Revised Airlie House Criteria (the Airlie House criteria) found that ultra-high dose methylcobalamin 25 mg or 50 mg was safe and tolerable, although it did not show significant efficacy in the overall cohort.[7] Nonetheless, post hoc analyses of patients who were enrolled within 1 year from symptom onset and showed 1- or 2-point decrease in ALSFRS-R total score during 12-week observation before treatment, most likely classified as the moderate progressors in a Japanese ALS cohort,[8] revealed dose-dependent efficacy of ultra-high dose methylcobalamin. Intramuscular injection of methylcobalamin 50 mg twice weekly prolonged the intervals to primary events (full ventilation support or death) by over 600 days compared to the placebo. Additionally, the Revised Amyotrophic Lateral

147 Sclerosis Functional Rating Scale (ALSFRS-R) total score significantly differed between the two groups by

148 2.6 points at 16 weeks and by 5.3 points at 182 weeks, in favor of methylcobalamin. These results suggest that

149 this treatment is beneficial for ALS patients in the early stage and with moderate progression rate. Since ALS

150 is a disease with heterogeneity, patient stratification especially by disease stages and progression rates is

151 important when assessing the efficacy of a compound in clinical trials.[9] Therefore, we conducted a phase 3

152 clinical trial to confirm the efficacy of ultra-high dose methylcobalamin (50 mg intramuscularly twice a week)

153 to slow the progression of clinical symptoms for ALS patients in the early stage and moderate progressions

154 (the Japan Early-stage Trial of ultra-high dose methylcobalamin for ALS, JETALS).

155

156 **Methods**

157 **Study design and participants**

158 This randomized, double-blind, placebo-controlled, investigator-initiated trial was conducted at 25 neurology

159 centers in Japan. The trial design and protocol have been published previously.[10] The trial protocol is available

160 in Supplement 1. This trial comprised three stages: the observation period (12 weeks), the treatment period (16

161 weeks), and the open label extended period (until March 2024); the latter two included randomized

162 participants.

163

164 Ambulatory patients aged 20 years or older who were diagnosed as sporadic or familial ALS with definite,

165 probable, or probable laboratory-supported categories using the updated Awaji criteria[11] (Supplement 2) and

166   within 1 year of symptom onset were enrolled for the observation period (primary enrollment). Patients who

167   remained ambulatory and presented a 1- or 2-point decrease in the ALSFRS-R total score during the 12-week

168   observation period were entered into the 16-week treatment period (secondary enrollment). Patients were

169   excluded before randomization if they met any of the following conditions: no change or a decrease of 3 or

170   more points in ALSFRS-R total score during the observation period; a percent forced vital capacity (%FVC)

171   of 60% or less; or a history of noninvasive respiratory support or tracheostomy. Concomitant stable use of

172   riluzole was allowed during the double-blind period. Use of edaravone was prohibited from 4 weeks prior to

173   enrollment in the observation period and throughout the double-blind period (Supplement 2).

174

175   This trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice

176   guidelines. The trial protocol was approved by the institutional review board of each site before trial initiation.

177   Amendments to the trial protocol were made when needed and were approved by each institutional review

178   board. The major revisions were the addition of investigational sites, changes of investigators, and the addition

179   of prohibited concomitant drugs and therapies. All patients provided written informed consent. The

180   researchers assume responsibility for the accuracy and completeness of the data and analyses, as well as for

181   the fidelity of the trial and this report to the protocol. An overview of the trial design is provided in

182   Supplement 2 (eFigure 1).

183

184 **Randomization, masking, and procedures**

185 At the end of the observation period, the patients were randomly assigned in a 1:1 ratio to receive the

186 investigational drug (either methylcobalamin 50 mg or placebo) with an electronic web-response

187 randomization system on the basis of a complete randomization scheme prepared by the independent

188 randomization expert (Supplement 2). Efficacy was evaluated by blinded neurologists and safety was assessed

189 by unblinded neurologists; both groups of neurologists were prohibited from sharing information that may

190 lead to patient identification. The investigational drug contained lyophilized masses and powders with or

191 without methylcobalamin 50 mg. The drug was stored in a light-shielded vial and the vial was packaged and

192 guaranteed to be indistinguishable from its appearance. Each vial of investigational drug was dissolved in 2.2

193 ml of saline and administered 2.0 ml into the muscles of two of the following points: thigh, buttock, and

194 deltoid (4.0 ml total). Trained doctors or nurses not involved in the efficacy evaluations injected the

195 investigational drug so patients or care givers could not observe it throughout the administration process. The

196 patients were informed that administration of the investigational drug might cause reddening of the urine, but

197 that there should be no health problems; it was not informed whether methylcobalamin or placebo would

198 cause this coloration. To avoid the bias, the evaluators at each institution were requested not to question the

199 patient or care givers about the color of the urine. Eligible patients for secondary enrollment were

200 administered the investigational drug intramuscularly twice weekly during the 16-week treatment period.

201 Efficacy and safety outcomes were evaluated at weeks 0 and 12 during the observation period and at weeks 4,

202 8, and 16 during the treatment period. Patients who wished to receive methylcobalamin after week 16 of

203 treatment were entered into the open label extended period and were allowed to continue treatment until

204 March 2024.

205

206 **Outcomes**

207 The primary endpoint was the change in the ALSFRS-R total score from the allocation day (baseline) to week

208 16 of the treatment period. We set the treatment period to 16 weeks since a significant effect was detected at as

209 early as 16 weeks in the post hoc analysis of the previous trial (more details in Supplement 2). This also

210 minimized patient exposure to the placebo. The secondary endpoints were time from the allocation day to the

211 onset of an event (24-h use of noninvasive respiratory support, use of invasive respiratory support, or death),

212 or a change in %FVC, plasma homocysteine concentration, manual muscle test total score, left and right grip

213 strength, Norris scale total score, and ALS assessment questionnaire (ALSAQ-40) total score. The safety

214 endpoints were adverse events, laboratory test results, electrocardiogram results, and vital sign measurements.

215

216 **Sample size**

217 Based on post hoc analysis of the previous trial,[7] the required number of patients for a type I error probability

218 to ≤2.5% in one-sided tests and a statistical power of ≥80% was a minimum of 60 patients per group.

219 Considering that there would be dropout during the trial, the target number of patients was 64 patients per

220 group (Supplement 2).

221

Statistical analysis

223 The primary efficacy analysis set was the full analysis set (FAS). The FAS included eligible patients who

224 received the investigational drug at least once. The safety analysis set (SAS) included eligible patients who

225 received the investigational drug at least once, excluding those who had no assessable safety data. Analysis of

226 the primary endpoint was performed with a linear mixed effect model for repeated measures with an

227 unstructured covariance structure of the error variance to estimate the change in the ALSFRS-R total score

228 from baseline. Response variables were changes in ALSFRS-R total score at 4, 8, and 16 weeks. Missing

229 values were not imputed. In the mixed model repeated measure model, we estimated the least-squares mean

230 difference between methylcobalamin and placebo in the change from baseline to week 16. Fixed effects

231 included the treatment group, time points, minimization factors, and interactions between treatment groups

232 and time points. The significance level was set at a one-tailed $P < 0.025$. Sensitivity analysis was also

233 performed for the Per Protocol Set, excluding patients with deviation from the protocol. We also compared the

234 slope between groups using the ALSFRS-R total score at the preinitiation, 4-, 8-, and 16-week time points as

235 response variables, fitting the primary regression equation to the time points and response variables, and

236 analyzed the data with a mixed model with intercept and slope as varying effects. An independent data and

237 safety monitoring board periodically reviewed the efficacy and safety data. The data were obtained using

238 electronic data capture. Additional information on the statistical analyses is provided in the Statistical Analysis

239 Plan in the attachment. Statistical analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC,

240 USA). The trial protocol was reported to the Pharmaceuticals and Medical Devices Agency (PMDA, No. 29-

241   3331).

242

243   **Results**

244   **Study participants**

245   Between October 17, 2017 and September 30, 2019, we entered 203 patients to the observation period, 130 of

246   whom were enrolled for the treatment period and were randomly assigned to the methylcobalamin group (n =

247   65) or the placebo group (n = 65) (Figure 1). A total of 129 patients were included the FAS and SAS; one

248   patient in the placebo group was excluded from the FAS and SAS as the patient was initially diagnosed with

249   probable ALS using the updated Awaji criteria and the Airlie House criteria but was later re-diagnosed with

250   cervical spinal canal stenosis based on clinical course and examination after randomization. Otherwise, one

251   patient in the placebo group and two patients in the methylcobalamin group discontinued due to withdrawal of

252   consent, and 126 patients (63 patients in each group; 97%) completed the trial. The baseline demographic and

253   disease characteristics were similar between the groups, without significant differences (Table 1).

254

255   **Efficacy outcomes**

256   The least-squares mean changes in the ALSFRS-R total score at week 16 were -2.66 ± 0.61 in the

257   methylcobalamin group and -4.63 ± 0.60 in the placebo group, and the difference was 1.97 in favor of

258   methylcobalamin (95% CI, 0.44–3.50; $P = 0.012$; Table 2). There were no deaths, 24-h use of noninvasive

259   respiratory support, or use of invasive respiratory support during the 16-week treatment period (Table 2). In

260   the sensitivity analysis, the slope of the ALSFRS-R total score through the treatment period was significantly

261   smaller in the methylcobalamin group (P = 0.018; Figure 2). Additional analyses related to changes in the

262   ALSFRS-R score are shown in Supplement 2 (eTables 1–4). The least-squares mean changes in the plasma

263   homocysteine concentration at week 16 were significantly lower in the methylcobalamin group (least-squares

264   mean difference, −1.71; 95% CI, −1.14−2.29; P < 0.001; eFigure 2 in Supplement 2). The least-squares mean

265   changes in %FVC, Norris scale total score, and manual muscle test total score did not show significant

266   differences between the methylcobalamin and placebo groups.

267

268   **Safety outcomes**

269   Adverse events were reported in 62% of patients in the methylcobalamin group and in 66% of patients in the

270   placebo group (Table 3). Three patients experienced serious adverse events that were not causally related to

271   the investigational drugs: cerebral infarction in the methylcobalamin group and hemorrhoid surgery and

272   stenosis of the tracheostoma after laryngotracheal separation in the placebo group. Regarding the tracheal

273   stenosis, the patient underwent a laryngotracheal separation for dysphagia due to ALS progression at week 5

274   of the treatment period and developed the stenosis at week 13 of the treatment period. No other patients

275   underwent a tracheostomy during the treatment period. There were no adverse events leading to

276   discontinuation. Events reported by at least 5% in either group are shown in Table 3. Details of adverse events

277   are shown in Supplement 2 (eTables 5–7). No notable differences in changes of laboratory measurements,

278   electrocardiogram parameters, and vital signs were observed between the two groups (eTable 8 in Supplement

279   2).

280

Discussion

282 This trial demonstrated ultra-high dose methylcobalamin resulted in a 43% reduction in clinical deterioration

283 as evaluated with the ALSFRS-R total score throughout the 16-week treatment period in the patients with

284 early-stage ALS. The reduction ratio was virtually equivalent to that observed in the post hoc analysis in the

285 previous phase 2/3 trial (45%).[7] Our results indicate disease-modifying, reproducible, and clinically

286 meaningful[12] effects of ultra-high dose methylcobalamin for ALS patients in the early stage and with moderate

287 progression rate. In the ALSFRS-R sub-scores, decrease in the fine-motor, gross-motor, and total limb (the

288 sum of both) functions were significantly smaller with methylcobalamin; changes in bulbar and respiratory

289 functions did not differ probably because they would be more affected in later stages (eTables 1–4 in

290 Supplement 2). Our results also confirmed that ultra-high dose methylcobalamin was safe during the 16-week

291 treatment.

292

293 A total of 116 patients (90%) concomitantly used riluzole. In these participants, the least-squares mean

294 difference in ALSFRS-R score was 2.11 in favor of methylcobalamin (95% CI, 0.46–3.76; $P = 0.013$),

295 implying the combination of riluzole and methylcobalamin has a greater therapeutic effect than riluzole alone

296 (eTable 4 in Supplement 2). Correspondingly, a mutant human SOD1(G93A)-mediated in vitro ALS model

297 showed combination therapy with methylcobalamin and riluzole enhanced the survival of motor neurons

298 compared with monotherapy of either drug alone.[13]

299

300   Methylcobalamin acts as a coenzyme of methionine synthase, which is required for the formation of

301   methionine from homocysteine in the methylation cycle. The methylation cycle in central nervous tissue

302   seems to have an indispensable role in the elimination of homocysteine.[14] Multiple lines of evidence suggest

303   homocysteine is neurotoxic,[15] which provides a promising therapeutic target in neurological disorders such as

304   stroke[16] and dementia.[17] In particular, homocysteine induces excitotoxicity, oxidative stress, mitochondrial

305   dysfunction, activation of inflammation, and motor neuron death.[18,19] In fact, plasma homocysteine levels are

306   reported to be elevated in patients with ALS.[20] The current trial showed plasma homocysteine levels indeed

307   highly significantly decreased with methylcobalamin. On the other hand, changes in plasma homocysteine

308   levels were not correlated with those in ALSFRS-R scores in the treatment period (eFigure 3 in Supplement

309   2). It should be noted, however, homocysteine levels are affected by several factors,[21] such as diet, smoking,

310   methylenetetrahydrofolate reductase genetic polymorphisms, and baseline B-vitamin status, which were not

311   adjusted in our trial. Meanwhile, methylcobalamin may also exert a therapeutic effect independent of lowering

312   homocysteine. Cobalamin exhibits antioxidant and anti-inflammatory effects in homocysteine-independent

313   systems.[22,23] Moreover, methylcobalamin protects against glutamate neurotoxicity.[24] We also note the gut

314   microbiome has been indicated to play a disease modifying role in SOD1 model mice[25] and to be conceivably

315   changed in ALS patients.[26] It would thus be interesting to speculate that methylcobalamin, which may

316   modulate gut microbiome, could exert its effect via microbiome in patients with ALS. Collectively,

317   methylcobalamin potentially antagonizes many adverse cellular processes likely involved in ALS. The anti-

318   ALS effect might be related to the attenuation of multiple processes rather than any single process.

319

320   Our trials showed methylcobalamin should be in ultra-high dose and 50 rather than 25 mg.[6] It is suggested

321   methylcobalamin at high concentration paradoxically upregulates gene transcription and thereby protein

322   synthesis in vitro.[27] In vivo models of rat peripheral neuropathy also demonstrate methylcobalamin at high,

323   but not low, concentration promotes nerve regeneration.[28,29] These results seem to reinforce the necessity of

324   ultra-high dose for ALS treatment. We decided the dose of 50 mg because the post-hoc analysis of the

325   previous trial with placebo and methylcobalamin 25 mg and 50 mg groups showed methylcobalamin

326   prolonged survival and inhibited ALSFRS-R decline in a dose-dependent manner. The effect of

327   methylcobalamin on ALS may correlate with increasing dose in the nervous system. As the nervous system

328   can retain an extremely small portion of the total dose, it would need much higher dose than other tissues.

329

330   Our results indicate the benefit of post hoc analyses of clinical trials. Based on the post hoc analysis, we

331   adopted the 16-week treatment period. While it was shorter than the usual 24-week period, it could reduce the

332   site visits, likely reduce the dropouts during the intervention, allow early enrollment in the open label period,

333   and save the cost. Furthermore, since the post hoc analysis indicated early diagnosis and enrollment would be

334   critical, we used the updated Awaji criteria to efficiently enroll patients with early-stage ALS. In parallel, we

335   also evaluated the categories in the Airlie House criteria; 12 of the 203 patients enrolled in the observation

336   period satisfied the updated Awaji criteria but not the Airlie House criteria (eTable 6 in Supplement 2),

337   suggesting the updated Awaji criteria played a critical role in successful patient enrollment.

338

339   **Limitations**

340   This trial was optimized to replicate the results of the post-hoc analysis in the previous trial,[7] and the trial

341   design has several limitations. First, since the trial was designed to enroll patients in the early stage and with

342   moderate progressions,[8] efficacy of ultra-high dose methylcobalamin remains unvalidated in patients with

343   other profiles; the previous trial enrolling 373 ALS patients within 3 years from symptom onset failed to show

344   the efficacy.[7] Second, the inclusion criteria for patients in the early stage posed a risk of inclusion of ALS

345   mimics;[30] we actually detected, via careful monitoring, one case with cervical spinal canal stenosis. Third, the

346   treatment duration of 16 weeks was different from the duration of 24 weeks adopted in most other clinical

347   trials for ALS. Fourth, the sample size was relatively small for a phase 3 trial, although it was twice as large as

348   that in the post-hoc analysis. Fifth, because the patients were in early stages and without rapid progression, no

349   24-hour use of noninvasive respiratory support, use of invasive respiratory support, or death was observed

350   during the 16-week treatment period, and other secondary endpoints did not attain significance; meanwhile,

351   the least squares mean changes in %FVC, Norris scale total score, and manual muscle test total score showed

352   a tendency toward smaller decline with methylcobalamin (eFigure 2 in Supplement 2). Lastly, biomarkers

353   such as neurofilament light chain[31], phosphorylated neurofilament heavy chain[32], urinary p75[33], motor unit

354   number index[34], homocysteine in the cerebrospinal fluid[35], and genetic factors[36] were not evaluated.

355

356   **Conclusions**

357   This phase 3 clinical trial enrolling patients with early-stage ALS and moderate progression rate validated that

358   ultra-high dose methylcobalamin significantly slowed clinical progression as assessed with the ALSFRS-R

359   total score in the 16-week treatment period. The safety of ultra-high dose methylcobalamin for ALS patients

360   were also reproduced.

361

Author Contributions

Ryosuke Oki and Yuishin Izumi had full access to all the data in the study and take responsibility for the

integrity of the data and the accuracy of the data analysis. Ryosuke Oki and Yuishin Izumi contributed equally

as co-first authors.

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Conflict of Interest Disclosures

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414   **Role of Funder/Sponsor**

419

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422   and the trial teams involved in JETALS.

423

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524

525 **Figure Legends**

526 **Figure 1. Screening, randomization, and follow up**

527 One patient in the placebo group was excluded from the full analysis set and safety analysis set as the patient

528 was initially diagnosed with probable amyotrophic lateral sclerosis but was later re-diagnosed with cervical

529 spinal canal stenosis. One patient in the placebo group and two in the methylcobalamin group discontinued by

530 consent withdrawal, and 126 patients (97%) ultimately completed the 16-week trial.

531

532 **Figure 2. Primary efficacy outcomes**

533 The mean and slope of the Revised ALS Functional Rating Scale (ALSFRS-R) total score in the full analysis

534 set are shown. Data are shown as means ± SE. Error bars indicate 95% CIs. Grid lines indicate the ALSFRS-R

535 total score during the treatment periods. Dot lines indicate the slopes of the ALSFRS-R total score from baseline

536 to week 16.

Tables

Table 1. Baseline demographic and clinical characteristics (full analysis set)[a]

	Placebo (n = 64)	Methylcobalamin (n = 65)	Total (n = 129)	P value
Male sex, no. (%)	40 (63)	34 (52)	74 (57)	0.242[e]
Age, years	60.8 ± 12.1	61.2 ± 11.4	61.0 ± 11.7	0.852[e]
Period from ALS onset at the enrollment of the observation period, months	8.5 ± 2.3	8.2 ± 2.4	8.3 ± 2.3	0.412[f]
ALSFRS-R total score at baseline	42.3 ± 2.7	42.4 ± 2.6	42.4 ± 2.6	0.851[f]
%FVC at baseline, %	90.6 ± 16.9	93.4 ± 16.9	92.0 ± 16.9	0.333[f]
Body mass index, kg/m^2	22.6 ± 3.9	21.8 ± 2.8	22.2 ± 3.4	0.185[f]
Vitamin B12 level at the enrollment of the observation period, pg/ml	571.8 ± 719.9	585.9 ± 373.0	578.9 ± 570.2	0.921[f]
Disease type, no. (%)				
Upper extremity	32 (50)	33 (51)	65 (50)	1.000[e]
Lower extremity	13 (20)	13 (20)	26 (20)	
Bulbar	19 (30)	19 (29)	38 (30)	
ALS type, no. (%)				
Familial ALS	0 (0)	1 (2)	1 (1)	1.000[e]
Sporadic ALS	64 (100)	64 (98)	128 (99)	
Concomitant use of riluzole, no. (%)	58 (91)	58 (89)	119 (92)	1.000[e]
History of edaravone use, no. (%)	6 (9)	4 (6)	10 (8)	0.530[e]
ALS diagnosis of updated Awaji criteria, no. (%)[b]				

	Placebo	Methylcobalamin	Total	P value
	(n = 64)	(n = 65)	(n = 129)	
Definite	16 (25)	23 (35)	39 (30)	0.385 [e]
Probable	32 (50)	30 (46)	62 (48)	
Probable laboratory-supported	16 (25)	12 (19)	28 (22)	
ALS severity at baseline, no. (%)[c]				
Grade 1	21 (33)	21 (32)	42 (33)	0.954 [g]
Grade 2	43 (67)	44 (68)	87 (67)	
Change in ALSFRS-R total score in the observation period, no. (%)[d]				
−2	28 (44)	31 (48)	59 (46)	0.656 [g]
−1	36 (56)	34 (52)	70 (54)	

539   [a] Plus–minus values indicate mean ± SD. No significant differences in baseline demographic

540   and disease characteristics were observed between the groups.

541   [b] The updated Awaji criteria, adopted as the ALS diagnostic criteria in this trial, comprised the

542   categories of definite, probable, probable laboratory-supported, and possible. ALS patients who

543   met the criteria of definite, probable, or probable laboratory-supported categories were eligible

544   for enrollment.[14]

545   [c] ALS severity: The severity of ALS symptoms was graded according to the Japan ALS severity

546   classification of grades 1–5, with grade 5 being the most severe.

547   [d] Change in the Revised ALS Functional Rating Scale (ALSFRS-R) total score during the 12-

548   week observation period before randomization. ALSFRS-R ranges from 0 to 48, with a lower

549   score indicating more severe symptoms.

550   [e.] Fisher's exact test.

551   [f.] Unpaired t-test.

552   [g.] Wilcoxon two-sample test.

553

554   **Table 2. Primary and secondary efficacy outcomes (full analysis set)[a]**

End Point[b]	Placebo	Methylcobalamin	Difference	P value
	(n = 64)	(n = 65)	(95% CI)	
Change in ALSFRS-R total score from baseline to week 4, 8, and 16				
Change value from baseline to week 4	−1.19 ± 0.35	−0.20 ± 0.36	0.99 (0.34–1.65)	0.003
Change value from baseline to week 8	−2.33 ± 0.43	−1.34 ± 0.44	0.99 (0.04–1.95)	0.042
Change value from baseline to week 16 (primary endpoint)	−4.63 ± 0.60	−2.66 ± 0.61	1.97 (0.44–3.50)	0.012
Secondary end points (change from baseline to week 16)				
Events (24-hour use of noninvasive respiratory support, use of invasive respiratory support, or death)[c]	0	0		
Change in plasma homocysteine concentration	0.00 ± 0.28	−1.71 ± 0.29	−1.71 (−2.23−−1.20)	<0.001
Change in %FVC	−9.4 ± 1.8	−7.4 ± 1.8	2.0 (−1.9–5.8)	0.313
Change in manual muscle test total score	−3.7 ± 0.7	−2.9 ± 0.7	0.8 (−0.6–2.3)	0.266
Change in Norris scale total score	−9.9 ± 1.5	−7.0 ± 1.6	2.9 (−0.5–6.3)	0.095
Change in grip strength (Right)	−2.5 ± 0.7	−2.7 ± 0.7	−0.2 (−1.6–1.3)	0.834
Change in grip strength (Left)	−2.5 ± 0.6	−2.1 ± 0.7	0.4 (−0.9–1.7)	0.538
Change in ALSAQ-40 total score	18.2 ± 3.5	15.4 ± 3.7	−2.8 (−10.0–4.5)	0.455

555   [a] Plus–minus values are least-squares means ± SE.

556   [b] Primary end point and all secondary end points are the change from baseline to week 16.

557   [c] No predefined events (24-h use of noninvasive respiratory support, use of invasive respiratory

558   support, or death) occurred throughout the 16-week treatment period.

559 **Table 3. Adverse events (safety analysis set)[a]**

	Placebo (n = 64)	Methylcobalamin (n = 65)
	No. of patients (%)	
Adverse events	42 (66)	40 (62)
Adverse drug reactions	1 (2)	5 (8)
Severe adverse events	1 (2)	1 (2)
Severe adverse drug reactions	0	0
Adverse events leading to discontinuation	0	0
Adverse drug reactions leading to discontinuation	0	0
Serious adverse event	2 (3)	1 (2)
Serious adverse drug reactions	0	0
Adverse events reported by ≥5% of patients in either group[b]		
Constipation	4 (6)	3 (5)
Nasopharyngitis	7 (11)	4 (6)
Contusion	7 (11)	5 (8)
Fall	2 (3)	4 (6)
Back pain	4 (6)	3 (5)
Insomnia	4 (6)	1 (2)

560   [a] Adverse events that emerged during the observation and treatment periods are shown.

561   [b] Events are shown according to the preferred term in the Japanese translation of the MedDRA,

562 version 22.1.

Figure 1. Screening, Randomization, and Follow up

Figure 2. Primary Efficacy Outcomes (Full Analysis Set)