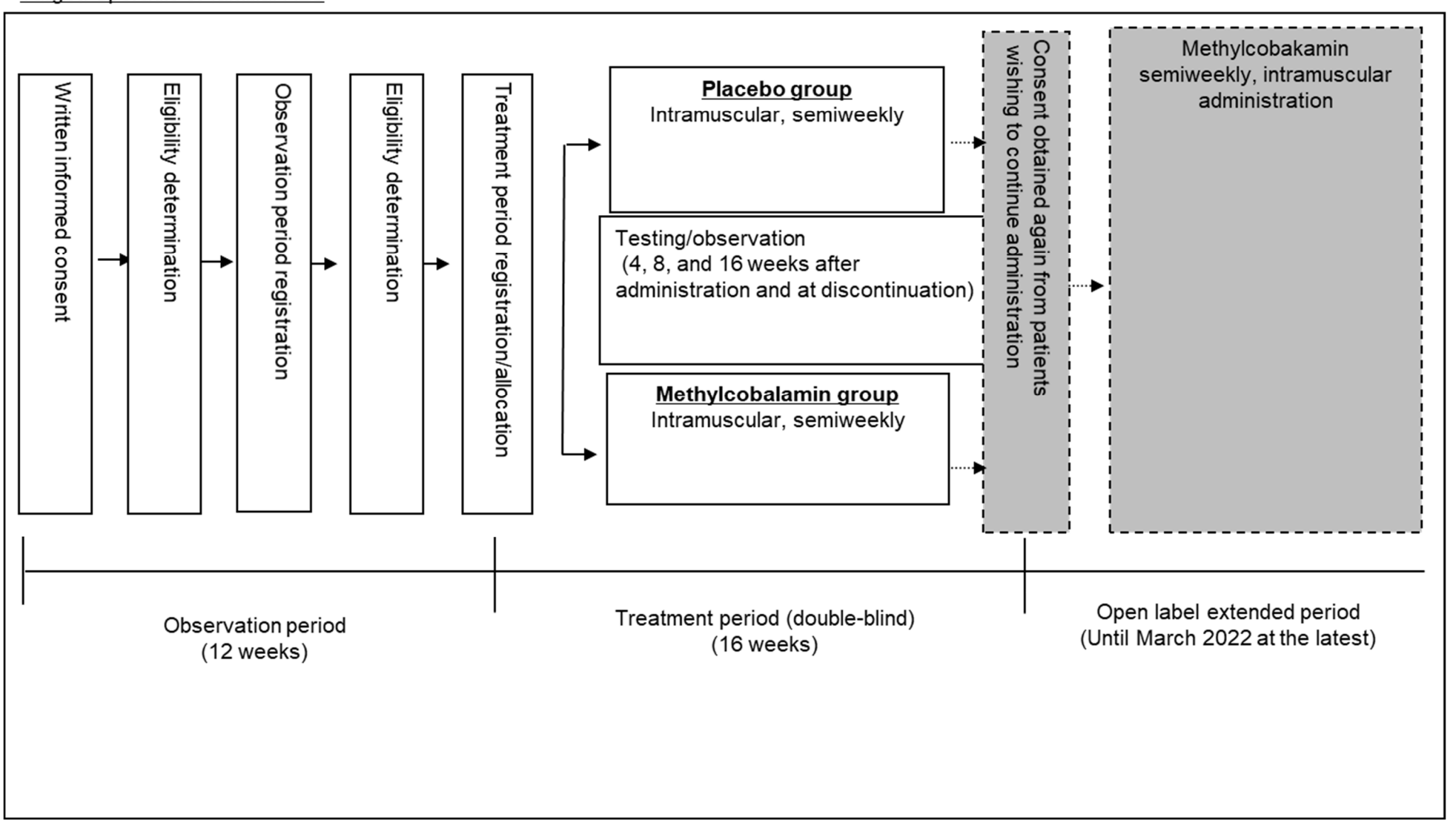
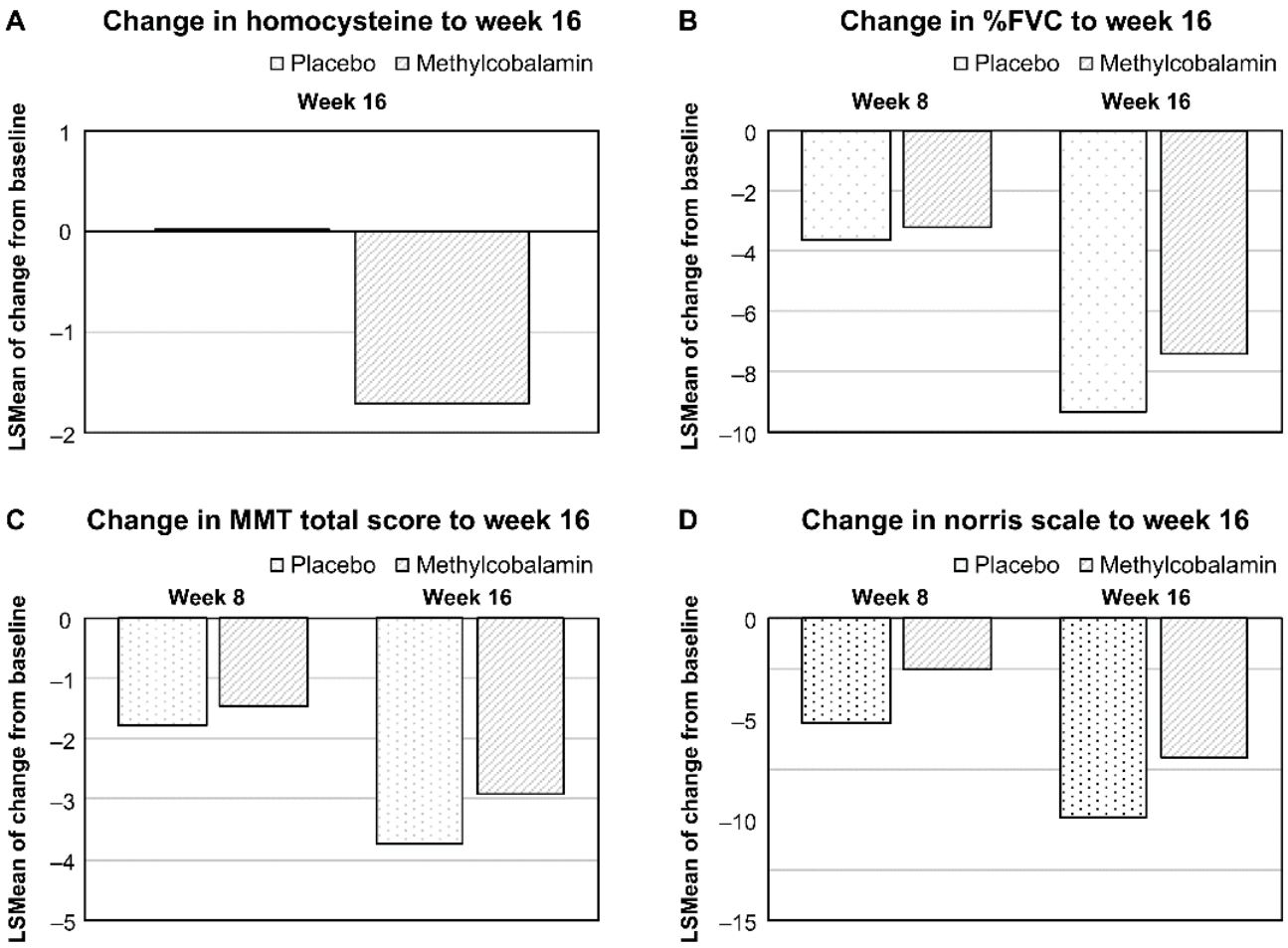
Supplementary File

eFigure 1. Trial Design

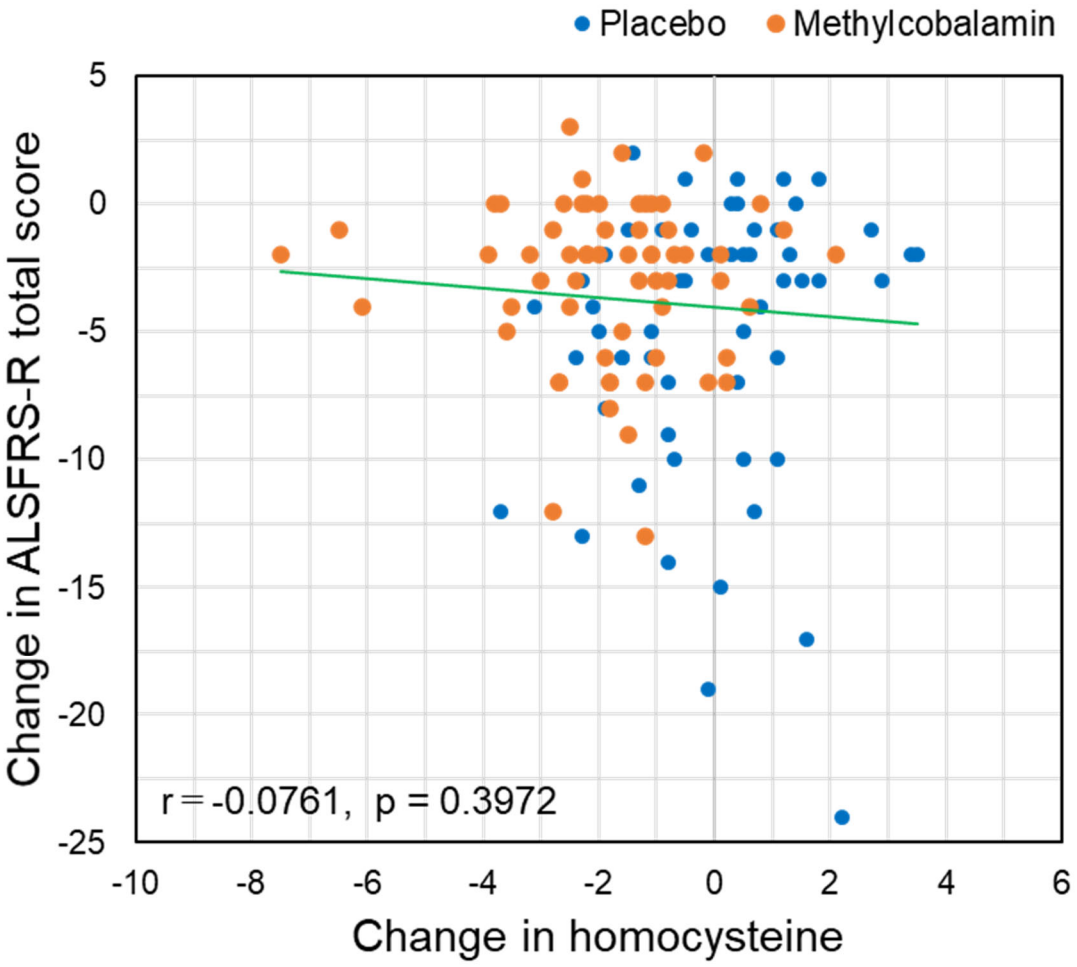


eFigure 2. Secondary Efficacy Outcomes (Full Analysis Set)



Panels A to D show the secondary efficacy outcomes. Data are shown as least-squares means. Panel A shows the change in the plasma homocysteine concentration from baseline to week 16. Panel B shows the change in % forced vital capacity (%FVC) from baseline to weeks 8 and 16. Panel C shows the variation in manual muscle test (MMT) total score from baseline to weeks 8 and 16. Panel D shows the variation in Norris scale total score from baseline to weeks 8 and 16.

eFigure 3. Association between Changes in ALSFRS-R and Homocysteine



eFigure 3 show the association between changes in the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) and the plasma homocysteine concentration from baseline to week 16. Red and blue circles indicate the methylcobalamin and placebo groups, respectively. There were no associations between changes in ALSFRS-R total score and homocysteine.

eTable 1. Change in ALSFRS-R Total Score in the FAS (Sensitivity Analysis)

Change in ALSFRS-R Total Score	Placebo	Methylcobalamin	Difference (95% CI)	*P* value
	(n = 64)	(n = 65)		
Change value from baseline to week 4	−1.41 ± 0.23	−0.43 ± 0.23	0.98 (0.33–1.63)	0.004
	(n = 64)	(n = 64)		
Change value from baseline to week 8	−2.55 ± 0.34	−1.57 ± 0.34	0.98 (0.03–1.93)	0.044
	(n = 63)	(n = 63)		
Change value from baseline to week 16	−4.84 ± 0.55	−2.89 ± 0.55	1.95 (0.42–3.48)	0.013

eTable 2. The Slope of ALSFRS-R Total Score in the FAS (Sensitivity Analysis)

			Estimated coefficient			Difference between coefficient		
	Statistics	**Group**	**Estimated**	**SE**	**P value**	**Estimated**	**SE**	***P* value**
Regression to the quadratic curve	Zero intercept	Placebo	42.2822	0.3433	<0.0001			
		Methylcobalamin	42.4914	0.3405	<0.0001			
	Primary coefficient	Placebo	−0.0473	0.0077	<0.0001	−0.0213	0.0109	0.052
		Methylcobalamin	−0.0260	0.0077	0.0008			
	Secondary coefficient	Placebo	0.0000	0.0001	0.6581	0.0000	0.0001	0.611
		Methylcobalamin	−0.0000	0.0001	0.7820			
Regression to the linear equation	Zero intercept	Placebo	42.2487	0.3349	<0.0001			
		Methylcobalamin	42.5119	0.3323	<0.0001			
	Primary coefficient	Placebo	−0.0447	0.0051	<0.0001	−0.0171	0.0072	0.018
		Methylcobalamin	−0.0276	0.0051	<0.0001			
	Slope (/week)	Placebo	−0.3129	0.0357	<0.001	−0.1197	0.0504	0.018
		Methylcobalamin	−0.1932	0.0357	<0.001			

eTable 3. Summary Statistics of ALSFRS-R Total Score and Sub-score in the FAS

						Change from baseline	
ALSFRS-R	**Group**	**Visit**	**n**	**Mean**	**SD**	**Mean**	**SD**
Total score	Placebo	Baseline	64	42.31	2.68		
		Week 4	64	40.91	3.46	−1.41	2.42
		Week 8	64	39.77	4.28	−2.55	3.29
		Week 16	63	37.46	5.89	−4.81	5.32
	Methylcobalamin	Baseline	65	42.40	2.58		
		Week 4	65	41.97	2.95	−0.43	1.05
		Week 8	64	40.78	3.59	−1.58	2.01
		Week 16	63	39.35	4.53	−2.94	3.20
Bulbar function	Placebo	Baseline	64	10.61	1.71		
		Week 4	64	10.39	1.89	−0.22	0.72
		Week 8	64	10.19	2.04	−0.42	1.00
		Week 16	63	9.75	2.32	−0.84	1.38
	Methylcobalamin	Baseline	65	10.48	2.18		
		Week 4	65	10.34	2.35	−0.14	0.46
		Week 8	64	10.05	2.41	−0.41	0.71
		Week 16	63	9.59	2.89	−0.84	1.31
Limb function (Total)	Placebo	Baseline	64	19.84	3.05		
		Week 4	64	18.70	3.95	−1.14	2.10
		Week 8	64	17.86	4.56	−1.98	2.68
		Week 16	63	16.37	5.50	−3.46	3.98
	Methylcobalamin	Baseline	65	20.05	2.68		
		Week 4	65	19.75	2.81	−0.29	0.72
		Week 8	64	18.97	3.43	−1.06	1.51
		Week 16	63	18.02	4.35	−1.97	2.49
Limb function (Fine)	Placebo	Baseline	64	9.88	1.82		
		Week 4	64	9.36	2.33	−0.52	1.11
		Week 8	64	8.81	2.63	−1.06	1.40
		Week 16	63	8.19	3.19	−1.68	2.04
	Methylcobalamin	Baseline	65	9.91	1.72		
		Week 4	65	9.81	1.84	−0.08	0.48
		Week 8	64	9.42	2.09	−0.63	1.58
		Week 16	63	8.98	2.49	−0.89	1.42
Limb function (Gross)	Placebo	Baseline	64	9.97	2.00		
		Week 4	64	9.34	2.42	−0.63	1.20
		Week 8	64	9.05	2.69	−0.92	1.53
		Week 16	63	8.17	3.08	−1.78	2.19

ALSFRS-R	Group	Visit	n	Mean	SD	Change from baseline	
						Mean	SD
	Methylcobalamin	Baseline	64	10.13	1.83		
		Week 4	64	9.92	1.95	−0.22	0.72
		Week 8	64	9.55	2.17	−0.74	1.53
		Week 16	63	9.04	2.55	−1.08	1.45
Respiratory function	Placebo	Baseline	64	11.86	0.47		
		Week 4	64	11.81	0.50	0.05	0.21
		Week 8	64	11.72	0.68	−0.14	0.53
		Week 16	63	11.35	1.59	−0.51	1.52
	Methylcobalamin	Baseline	65	11.88	0.38		
		Week 4	65	11.88	0.33	0.00	0.31
		Week 8	64	11.77	0.56	−0.11	0.48
		Week 16	63	11.75	0.51	−0.13	0.46

eTable 4. ALSFRS-R Total Score in Subset in the FAS

Visit	Placebo			Methylcobalamin			Difference (95% CI)	P value
	n	ALSFRS-R total score (Mean ± SD)	Change from baseline (LSMean ± SE)	n	ALSFRS-R total score (Mean ± SD)	Change from baseline (LSMean ± SE)		
Age <65 (years)								
Baseline	34	42.88 ± 1.56		33	42.41 ± 2.52			
Week 16	32	37.55 ± 6.45	−4.76 ± 0.94	33	40.03 ± 4.27	−1.74 ± 0.95	3.02 (0.60–5.45)	0.015
Age ≥65 (years)								
Baseline	31	41.71 ± 3.44		31	42.39 ± 2.69			
Week 16	30	37.37 ± 5.31	−3.38 ± 0.77	31	38.65 ± 4.75	−2.74 ± 0.78	0.65 (−1.23–2.52)	0.494
Sex—Male								
Baseline	40	42.23 ± 2.48		34	42.50 ± 2.43			
Week 16	39	36.44 ± 6.33	−5.03 ± 0.91	32	40.25 ± 3.89	−1.29 ± 0.97	3.74 (1.50–5.99)	0.001
Sex—Female								
Baseline	24	42.46 ± 3.05		31	42.29 ± 2.78			
Week 16	24	39.13 ± 4.76	−3.31 ± 0.77	31	38.42 ± 5.00	−3.95 ± 0.72	−0.64 (−2.57–1.30)	0.511
Initial symptom—Bulbar onset								
Baseline	19	43.11 ± 2.18		19	41.47 ± 2.29			
Week 16	19	39.68 ± 5.13	−3.50 ± 1.03	19	37.79 ± 4.47	−3.44 ± 1.02	0.06 (−2.71–2.82)	0.967
Initial symptom—Limb onset								
Baseline	45	41.98 ± 2.82		46	42.78 ± 2.62			
Week 16	44	36.50 ± 5.99	−5.43 ± 0.74	44	40.02 ± 4.44	−2.40 ± 0.75	3.02 (1.14–4.90)	0.002
Time from onset to registration at the observation period ≤9 (months)								
Baseline	32	42.28 ± 3.14		37	42.97 ± 2.52			
Week 16	31	37.03 ±6.38	−5.04 ± 0.85	36	39.58 ± 4.74	−3.08 ± 0.81	1.96 (−0.19–4.12)	0.074
Time from onset to registration at the observation period 9 to ≤12 (months)								
Baseline	32	42.34 ± 2.18		28	41.64 ± 2.51			
Week 16	32	37.88 ± 5.45	−4.10 ± 0.87	27	39.04 ± 4.31	−1.94 ± 0.95	2.16 (−0.12–4.43)	0.063
%FVC at baseline <90%								
Baseline	27	41.85 ± 2.74		28	41.68 ± 2.45			
Week 16	27	35.44 ± 6.17	−6.22 ± 1.05	28	37.18 ± 4.56	−4.26 ± 1.05	1.97 (−0.87–4.81)	0.171
%FVC at baseline ≥90%								
Baseline	37	42.65 ± 2.63		37	42.95 ± 2.58			
Week 16	36	38.97 ± 5.26	−3.51 ± 0.80	35	41.09 ± 3.73	−1.33 ± 0.83	2.18 (0.62–3.75)	0.007

Visit	Placebo			Methylcobalamin			Difference (95% CI)	P value
	n	ALSFRS-R total score (Mean ± SD)	Change from baseline (LSMean ± SE)	n	ALSFRS-R total score (Mean ± SD)	Change from baseline (LSMean ± SE)		
Concomitant use of riluzole—No								
Baseline	6	42.83 ± 2.86		7	44.00 ± 2.38			
Week 16	6	39.17 ± 5.46	−5.70 ± 1.97	6	39.83 ± 3.66	−5.93 ± 1.98	−0.23 (−6.04–5.58)	0.930
Concomitant use of riluzole—Yes								
Baseline	58	42.26 ± 2.69		58	42.21 ± 2.56			
Week 16	57	37.28 ± 5.95	−4.68 ± 0.67	57	39.30 ± 4.64	−2.57 ± 0.67	2.11 (0.46–3.76)	0.013
Edaravone use before registration—No								
Baseline	58	42.26 ± 2.71		61	42.48 ± 2.54			
Week 16	57	37.21 ± 5.92	−5.03 ± 0.59	59	39.59 ± 4.41	−2.73 ± 0.58	2.30 (0.69–3.91)	0.005
Edaravone use before registration—Yes								
Baseline	6	42.83 ± 2.56		4	41.25 ± 3.40			
Week 16	6	39.83 ± 5.49		4	35.75 ± 5.50			
BMI <18.5								
Baseline	9	41.89 ± 1.83		9	41.33 ± 2.65			
Week 16	8	36.88 ± 3.14	−5.80 ± 1.32	9	38.11 ± 4.88	−3.99 ± 1.49	1.81 (−2.09–5.71)	0.327
BMI ≥18.5								
Baseline	55	42.38 ± 2.81		56	42.57 ± 2.56			
Week 16	55	37.55 ± 6.21	−4.59 ± 0.69	54	39.56 ± 4.48	−2.58 ± 0.69	2.01 (0.30–3.72)	0.022
Diagnostic grade by the updated Awaji criteria—Definite								
Baseline	16	41.31 ± 3.70		23	41.52 ± 2.25			
Week 16	16	37.31 ± 5.20	−4.00 ± 1.03	23	38.65 ± 4.22	−2.94 ± 0.90	1.06 (−1.50–3.63)	0.404
Diagnostic grade by the updated Awaji criteria—Probable and Probable laboratory-supported								
Baseline	48	42.65 ± 2.20		42	42.88 ± 2.65			
Week 16	47	37.51 ± 6.16	−4.98 ± 0.76	40	39.75 ± 4.71	−2.72 ± 0.82	2.26 (0.33–4.19)	0.022
Diagnostic grade by the El Escorial revised Airlie House diagnostic criteria—Definite								
Baseline	10	41.10 ± 4.18		12	41.00 ± 2.34			
Week 16	10	36.60 ± 4.30	−5.00 ± 1.30	12	36.50 ± 4.46	−4.96 ± 1.19	0.04 (−3.42–3.50)	0.981
Diagnostic grade by the El Escorial revised Airlie House diagnostic criteria—Probable								
Baseline	30	42.47 ± 2.26		30	42.40 ± 2.71			
Week 16	30	38.10 ± 5.95	−4.08 ± 0.92	29	39.55 ± 4.03	−2.45 ± 0.93	1.63 (−0.68–3.94)	0.164
Diagnostic grade by the El Escorial revised Airlie House diagnostic criteria—Probable laboratory-supported								
Baseline	20	42.20 ± 2.38		19	43.26 ± 2.16			
Week 16	20	36.00 ± 6.41	−5.40 ± 1.15	18	40.83 ± 4.55	−1.64 ± 1.26	3.76 (0.79–6.73)	0.012

Visit	Placebo			Methylcobalamin			Difference (95% CI)	P value
	n	ALSFRS-R total score (Mean ± SD)	Change from baseline (LSMean ± SE)	n	ALSFRS-R total score (Mean ± SD)	Change from baseline (LSMean ± SE)		
MRC score in neck flexor at the end of the observation period 5								
Baseline	48	42.65 ± 2.55		40	42.85 ± 2.40			
Week 16	48	37.88 ± 5.83	−4.44 ± 0.70	39	40.51 ± 3.89	−1.88 ± 0.79	2.56 (0.68–4.44)	0.008
MRC score in neck flexor at the end of the observation period ≤4								
Baseline	16	41.31 ± 2.91		25	41.68 ± 2.75			
Week 16	15	36.13 ± 6.10	−5.16 ± 1.26	24	37.46 ± 4.93	−4.75 ± 1.10	0.41 (−2.46–3.28)	0.774
ALS severity grade (Japan ALS severity classification) at the end of the observation period—Grade 1								
Baseline	21	43.62 ± 1.28		21	44.10 ± 2.41			
Week 16	21	39.29 ± 4.85	−4.44 ± 0.86	20	42.15 ± 3.70	−1.68 ± 0.84	2.76 (0.70–4.82)	0.010
ALS severity grade (Japan ALS severity classification) at the end of the observation period—Grade 2								
Baseline	43	41.67 ± 2.96		44	41.59 ± 2.28			
Week 16	42	36.55 ± 6.20	−4.78 ± 0.79	43	38.05 ± 4.31	−3.05 ± 0.79	1.73 (−0.30–3.76)	0.094
Change in ALSFRS-R total score from baseline to the end of the observation period—2 points								
Baseline	28	41.32 ± 3.38		31	41.71 ± 2.49			
Week 16	27	34.43 ± 6.48	−6.11 ± 1.13	30	38.00 ± 4.40	−3.11 ± 1.09	3.01 (0.37–5.64)	0.026
Change in ALSFRS-R total score from baseline to the end of the observation period—1 point								
Baseline	36	43.08 ± 1.66		34	43.03 ± 2.54			
Week 16	36	39.72 ± 4.25	−3.27 ± 0.63	33	40.58 ± 4.35	−2.19 ± 0.65	1.09 (−0.63–2.81)	0.212
ALSFRS-R total score at the end of the observation period ≤37								
Baseline	3	34.00 ± 2.65		1	36.00			
Week 16	3	32.33 ± 5.13		1	29.00			
ALSFRS-R total score at the end of the observation period 38 to 42								
Baseline	24	40.79 ± 1.28		31	40.42 ± 1.52			
Week 16	24	35.50 ± 4.85	−5.40 ± 1.01	31	37.19 ± 4.02	−3.36 ± 0.92	2.05 (−0.26–4.35)	0.080
ALSFRS-R total score at the end of the observation period ≥43								
Baseline	37	43.97 ± 1.01		33	44.45 ± 1.33			
Week 16	36	39.19 ± 6.05	−4.54 ± 0.80	31	41.84 ± 3.39	−2.07 ± 0.89	2.47 (0.27–4.67)	0.029

eTable 5. Summary of Severe Adverse Events by System Organ Class and Preferred Term

	Placebo	Methylcobalamin
System organ class / Preferred term	(n = 64)	(n = 65)
Number of patients	2 (3)	1 (2)
Nervous system disorders	1 (2)	0 (0)
Cerebral infarction	1 (2)	0 (0)
Respiratory, thoracic, and mediastinal disorders	1 (2)	0 (0)
Tracheal stenosis	1 (2)	0 (0)
Surgical and medical procedures	0 (0)	1 (2)
Hemorrhoid operation	0 (0)	1 (2)

eTable 6. Summary of Treatment-emergent Adverse Events by System Organ Class and Preferred Term

	Placebo (n = 64)			
System Organ Class /Preferred Term	Mild	Moderate	Severe	Total
The number of patients	1 (2)	0 (0)	0 (0)	1 (2)
Gastrointestinal disorders	0 (0)	0 (0)	0 (0)	0 (0)
Constipation	0 (0)	0 (0)	0 (0)	0 (0)
General disorders and administration site conditions	0 (0)	0 (0)	0 (0)	0 (0)
Injection site pain	0 (0)	0 (0)	0 (0)	0 (0)
Pyrexia	0 (0)	0 (0)	0 (0)	0 (0)
Investigations	0 (0)	0 (0)	0 (0)	0 (0)
Electrocardiogram QT prolonged	0 (0)	0 (0)	0 (0)	0 (0)
Skin and subcutaneous tissue disorders	0 (0)	0 (0)	0 (0)	0 (0)
Rash	0 (0)	0 (0)	0 (0)	0 (0)
Nervous system disorders	1 (2)	0 (0)	0 (0)	1 (2)
Hypoesthesia	1 (2)	0 (0)	0 (0)	1 (2)

	Methylcobalamin (n = 65)			
System Organ Class /Preferred Term	Mild	Moderate	Severe	Total
The number of patients	5 (8)	0 (0)	0 (0)	5 (7.7)
Gastrointestinal disorders	1 (2)	0 (0)	0 (0)	1 (2)
Constipation	1 (2)	0 (0)	0 (0)	1 (2)
General disorders and administration site conditions	2 (3)	0 (0)	0 (0)	2 (3)
Injection site pain	1 (2)	0 (0)	0 (0)	1 (2)
Pyrexia	1 (2)	0 (0)	0 (0)	1 (2)
Investigations	1 (2)	0 (0)	0 (0)	1 (2)
Electrocardiogram QT prolonged	1 (2)	0 (0)	0 (0)	1 (2)
Skin and subcutaneous tissue disorders	1 (2)	0 (0)	0 (0)	1 (2)
Rash	1 (2)	0 (0)	0 (0)	1 (2)
Nervous system disorders	0 (0)	0 (0)	0 (0)	0 (0)
Hypoesthesia	0 (0)	0 (0)	0 (0)	0 (0)

eTable 7. Summary of Electrocardiogram Parameter Before and After Administration

Parameter	Analysis date	Period	Group	n	Mean	SD	Change from baseline		
							Mean	SD	*P* value
RR interval (msec)	Baseline	Before administration	Placebo	64	825.7	158.5			
			Methylcobalamin	65	870.1	138.1			
		2 hours after administration	Placebo	64	824.7	149.3	−1.0	108.8	0.776
			Methylcobalamin	65	863.5	148.3	−6.6	90.9	
	Week 8–≤16	Before administration	Placebo	64	812.7	157.8			
			Methylcobalamin	63	875.4	123.3			
		2 hours after administration	Placebo	64	843.7	149.6	31.0	96.4	0.087
			Methylcobalamin	63	893.1	140.4	17.8	87.9	
PR interval (msec)	Baseline	Before administration	Placebo	64	165.8	20.7			
			Methylcobalamin	65	162.5	18.4			
		2 hours after administration	Placebo	64	165.5	20.9	−0.3	8.6	0.818
			Methylcobalamin	65	163.0	19.0	0.6	7.1	
	Week 8–≤16	Before administration	Placebo	64	163.9	21.3			
			Methylcobalamin	63	167.8	41.1			
		2 hours after administration	Placebo	64	165.0	22.7	1.1	7.7	0.538
			Methylcobalamin	63	162.7	20.8	−5.1	40.3	
QRS width (msec)	Baseline	Before administration	Placebo	64	95.6	12.8			
			Methylcobalamin	65	97.9	18.2			
		2 hours after administration	Placebo	64	94.3	12.0	−1.3	8.0	0.079
			Methylcobalamin	65	97.9	17.8	0.1	3.3	
	Week 8–≤16	Before administration	Placebo	64	93.9	12.1			
			Methylcobalamin	63	99.4	19.7			
		2 hours after administration	Placebo	64	94.9	11.6	1.0	4.1	0.385
			Methylcobalamin	63	98.3	18.8	−1.1	7.2	
QT interval (msec)	Baseline	Before administration	Placebo	64	387.7	33.3			
			Methylcobalamin	65	394.2	28.6			
		2 hours after administration	Placebo	64	389.5	31.8	1.8	21.6	0.929
			Methylcobalamin	65	394.7	30.3	0.6	17.6	
	Week 8–≤16	Before administration	Placebo	64	385.9	32.2			
			Methylcobalamin	63	430.7	237.3			
		2 hours after administration	Placebo	64	391.9	33.2	6.0	18.2	0.054
			Methylcobalamin	63	403.5	34.1	−27.1	234.6	

Parameter	Analysis date	Period	Group	n	Mean	SD	Change from baseline		
							Mean	Parameter	Analysis date
QTcB (msec)	Baseline	Before administration	Placebo	64	428.8	18.2			
			Methylcobalamin	65	424.5	20.8			
		2 hours after administration	Placebo	64	429.4	19.4	0.6	10.3	0.687
			Methylcobalamin	65	426.4	22.6	1.9	9.8	
	Week 8–≤16	Before administration	Placebo	64	426.8	18.7			
			Methylcobalamin	63	428.1	24.3			
		2 hours after administration	Placebo	64	428.7	20.7	1.9	10.5	0.512
			Methylcobalamin	63	428.5	25.3	0.4	9.7	
QTcF (msec)	Baseline	Before administration	Placebo	64	414.2	16.9			
			Methylcobalamin	65	413.6	18.4			
		2 hours after administration	Placebo	64	415.3	17.7	1.1	10.7	0.885
			Methylcobalamin	65	415.2	19.3	1.6	7.9	
	Week 8–≤16	Before administration	Placebo	64	412.3	18.3			
			Methylcobalamin	63	418.3	23.1			
		2 hours after administration	Placebo	64	415.5	19.5	3.2	10.1	0.509
			Methylcobalamin	63	419.5	24.8	1.2	9.2	

eTable 8. Summary of the Patients Who Met Possible and Suspected Grade by the El Escorial Revised Airlie House Diagnostic Criteria

Age	Sex	Initial symptom	Time from onset (month)	Severity	ALSFRS-R	%FVC	UAC ¶	rEEC †
70	M	Upper limb	12	1	47	99.7	Pro-lab ‡	Possible
58	F	Lower limb	12	2	45	100.8	Pro-lab	Possible
75	F	Bulbar	10	1	43	82.9	Definite	Possible
54	F	Upper limb	11	1	46	132.9	Probable	Possible
85	F	Bulbar	5	2	40	80.3	Pro-lab	Possible
69	M	Upper limb	10	2	45	110.9	Pro-lab	Possible
70	M	Upper limb	8	2	47	93.5	Pro-lab	Possible
44	M	Upper limb	6	1	46	104.9	Definite	Possible
78	F	Bulbar	4	2	40	123.5	Definite	Possible
60	F	Upper limb	11	1	45	101.6	Pro-lab	Possible
67	M	Upper limb	8	1	43	102.2	Pro-lab	Possible
59	M	Upper limb	11	1	47	101.1	Probable	Suspected

Character of the patients who met possible and suspected grade by the El Escorial Revised Airlie House Diagnostic Criteria at the registration of the observation period were listed.

¶ UAC; The updated Awaji criteria

† rEEC; The El Escorial Revised Airlie House Diagnostic Criteria

Pro-lab: Probable laboratory-supported

eAppendix. Inclusion and Exclusion criteria

Inclusion Criteria

(1) Patients who provided written consent to participate in this study

(2) Patients aged ≥20 years at the time of providing informed consent

(3) Patients diagnosed with sporadic or familial ALS corresponding to the categories of definite, probable, or probable laboratory-supported in the updated Awaji criteria

(4) Patients who were within 1 year of symptom onset at the beginning of the observation period

(5) Patients whose ALSFRS-R total score decreased by 1 or 2 points during the observation period (12 weeks)

(6) Patients rated as Grade 1 or 2 according to the Japan ALS severity classification (Grades 1–5, with Grade 5 being most severe)

(7) Patients seen on an outpatient basis

Exclusion Criteria

(1) Patients who have undergone tracheostomy

(2) Patients who are using a noninvasive respiratory support device

(3) Patients with ≤60% FVC

(4) Patients with chronic obstructive pulmonary disorder (COPD)

(5) Patients with signs and symptoms of vitamin B12 deficiency

(6) Patients who have received edaravone within 4 weeks before the observation period registration

(7) Patients who have started riluzole or changed the dosage or discontinued it after giving informed consent

(8) Patients with cognitive impairment

(9) Patients who are or may be pregnant

(10) Patients with a serious respiratory disorder, cardiovascular disease, or liver or kidney disease

(11) Patients with a malignant tumor

(12) Patients who have participated in another trial within the 12 weeks prior to giving informed consent

(13) Patients with present illness or history of drug allergy or severe allergic disease (anaphylactic shock)

(14) Patients who are determined to be unsuitable for this study by the investigator or sub-investigator

eAppendix. Diagnostic Criteria

The conventional Airlie House criteria have been widely used in clinical trials (Brooks et al. 2000). We adopted the Airlie House criteria in the previous trial (Kaji et al. 2019). The Airlie House criteria evaluate clinical and neurophysiological upper and lower motor neuron (UMN and LMN) dysfunction in four body regions (cranial, cervical, thoracic, and lumbosacral) and the diagnostic categories depend on the distribution of UMN and LMN dysfunction. It comprises four categories (definite, probable, probable-laboratory supported, and possible) and most clinical trials required a category of definite, probable, or probable-laboratory supported for diagnosis. Although the conventional Airlie House criteria has shown a high specificity, their low diagnostic sensitivity, especially in early stages, has been considered an issue (Costa, Swash, and de Carvalho 2012). To facilitate early diagnosis, the original Awaji criteria proposed that 1) neurophysiological features of LMN dysfunction including chronic and ongoing neurogenic changes were equivalent to clinical LMN signs and 2) fasciculation potentials and unstable motor units on needle electromyography were deemed to be a biomarker of ongoing denervation when combined with chronic neurogenic changes (de Carvalho et al. 2008). In fact, the original Awaji criteria were reported to accelerate the diagnosis by an average of 6 months compared to the Airlie House criteria (Okita et al. 2011). On the other hand, other studies reported that the original Awaji criteria had a lower sensitivity, a finding attributed to the omission of a ‘‘probable-laboratory supported” diagnostic category, in which a clinical upper motor neuron sign is required in one region (Higashihara et al. 2012)(Jang, Ph, and Bae 2014). Thereafter, the novel updated Awaji criteria, which reinclude the category of probable-laboratory supported, were advocated as an algorithm for combining the advantages of the Airlie House and original Awaji criteria; the updated Awaji criteria have higher sensitivity than the Airlie House and original Awaji criteria (Geevasinga et al. 2016). Therefore, the Japanese

Pharmaceuticals and Medical Devices Agency approved the adoption of the updated Awaji criteria in this trial on the condition that we would also document the diagnostic categories of the Airlie House criteria to compare their diagnostic sensitivity.

References:

- Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler. 2000;1(5):293-299.
- Kaji R, Imai T, Iwasaki Y, et al. Ultra-high-dose methylcobalamin in amyotrophic lateral sclerosis: A long-term phase II/III randomised controlled study. J Neurol Neurosurg Psychiatry. 2019;90(4):451-457.
- de Carvalho M, Dengler R, Eisen A, et al. Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol. 2008;119(3):497-503.
- Costa J, Swash M, de Carvalho M. Awaji criteria for the diagnosis of amyotrophic lateral sclerosis: A systematic review. Arch Neurol. 2012;69(11):1410-1416.
- Okita T, Nodera H, Shibuta Y, et al. Can Awaji ALS criteria provide earlier diagnosis than the revised El Escorial criteria? J Neurol Sci. 2011;302(1-2):29-32.
- Higashihara M, Sonoo M, Imafuku I, et al. Fasciculation potentials in amyotrophic lateral sclerosis and the diagnostic yield of the awaji algorithm. Muscle and Nerve. 2012;45(2):175-182. doi:10.1002/mus.22299
- Jang J, Ph D, Bae JS. The Awaji Criteria are not Always Superior to the Previous Criteria: A Meta-Analysis. Muscle and Nerve. 2015; 51(6):822-829.
- Geevasinga N, Macaskill P, Schrooten M, et al. Awaji criteria improves the diagnostic sensitivity in

amyotrophic lateral sclerosis: A systematic review using individual patient data. Clin Neurophysiol. 2016;127(7):2684-2691.

eAppendix. Sample Size

To determine the target patient profile, we compared the effect size of the change in total ALSFRS-R score at week 16 between early-stage patients (enrolled within 1 year of onset) with 1–2 points reduction and those with 1–3 points reduction in ALSFRS-R total score during the 12-week observation period in the previous trial (Kaji et al. 2019); the effect size was larger in the patients with 1–2 points reduction than those with 1–3 points reduction (data not shown) and thus we set the former as the target profile. In the sub-analysis of 58 patients who met the target profile (the placebo group, n=32; methylcobalamin 50 mg group, n=26), the change in ALSFRS-R total score at 16 weeks of the treatment period was −3.23 ± 4.01 points in the mecobalamin group and −5.84 ± 4.95 points in the placebo group (difference 2.61, 95% CI 0.15–5.06, P = 0.008). Based on these results, we reasoned that the score of ALSFRS-R total score in the methylcobalamin group would exceed that in the placebo group by 2.6 points if with the target profile. The required number of patients to set the type I error probability to ≤2.5% in the one-sided tests and to set the statistical power to ≥80% was a minimum of 60 patients per group based on subgroup results. Considering that there would be discontinuations during the trial, the target number of patients for this trial was determined to be 64 patients per group.

References:

- Kaji R, Imai T, Iwasaki Y, et al. Ultra-high-dose methylcobalamin in amyotrophic lateral sclerosis: A long-term phase II/III randomised controlled study. J Neurol Neurosurg Psychiatry. 2019;90(4):451-457.

eAppendix. Rationale for the treatment period of 16 weeks

In the previous trial, the ALSFRS-R was evaluated at week 4 and thereafter every 12 weeks, i.e., week 16, week 28, and eventually week 182 of the treatment (double-blind) period. Therefore, unfortunately we had no data at week 24 to be validated. To strictly validate the findings of the post hoc analysis of the previous trial, we could have selected 16 weeks or 28 weeks for the double-blind period. Considering the feasibility, 16 weeks was selected. Alternatively, we might have been able to set 24 weeks as the double-blind period and evaluate the ALSFRS-R at both weeks 16 and 24. In this case, however, we could have had a problem of determining the treatment duration for the primary outcome. If the change at week 16 had been set as the primary outcome, week 24 would not have been necessary; if the change at week 24 had been set as the primary outcome, the trial would not have been a validation; and if the changes at both weeks 16 and 24 had been set as the primary outcomes, multiple comparisons problem should have been considered and the statistical power might have been reduced for each point.