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Supplementary information

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Oligonucleotide–siRNA conjugate for SOD1 amyotrophic lateral sclerosis: a phase 1 trial

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Table S1: Changes in white blood cell count (×10^9/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	8.85	6.68	6.22	6.06	6.08	3.89
2 days after 1st treatment (Day 3)	9.09	7.35	5.78	7.02	7.19	3.88
Prior to the 2nd treatment (Day 15)	8.64	7.20	4.80	6.96	4.88	4.42
2 days after the 2nd treatment (Day 17)	8.55	6.42	5.32	5.98	4.21	3.63
Prior to the 3rd treatment (Day 29)	8.64	7.33	6.41	4.89	5.60	3.37
2 days after the 3rd treatment (Day 31)	9.31	6.17	5.38	5.21	4.86	3.96
Prior to the 4th treatment (Day 60 / Day 90)	10.91	6.09	5.90	4.72	5.24	3.88
2 days after the 4th treatment (Day 62 / Day 92)	8.33	5.20	6.84	5.90	4.67	4.48
Prior to the 5th treatment (Day 120 / Day 150)	9.97	6.28	6.30	6.85	4.94	3.89
2 days after the 5th treatment (Day 122 / Day 152)	8.41	5.22	6.16	6.69	4.57	4.30
Prior to the 6th treatment (Day 180 / Day 210)	7.83	7.60	5.71	5.87	6.49	3.20
2 days after the 6th treatment (Day 182 / Day 212)	8.68	4.61	5.57	5.36	5.14	4.69
Prior to the 7th treatment (Day 240)	9.77	5.72	9.1	-	-	-
2 days after the 7th treatment (Day 242)	9.25	5.34	7.13	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S2: Changes in neutrophil count and percentage of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Neutrophil count ($\times 10^9$/L)						
Baseline	5.64	4.22	4.20	4.43	3.87	2.23
2 days after 1st treatment (Day 3)	5.89	5.27	3.83	4.53	5.53	2.43
Prior to the 2nd treatment (Day 15)	5.66	4.28	3.14	5.24	2.93	2.39
2 days after the 2nd treatment (Day 17)	5.67	3.32	3.57	3.44	2.34	1.99
Prior to the 3rd treatment (Day 29)	5.55	4.16	4.50	3.32	3.23	1.79
2 days after the 3rd treatment (Day 31)	6.35	3.66	3.82	3.07	2.59	2.01
Prior to the 4th treatment (Day 60 / Day 90)	7.39	3.42	3.86	2.97	3.24	2.36
2 days after the 4th treatment (Day 62 / Day 92)	5.60	2.96	5.3	4.29	2.56	2.57
Prior to the 5th treatment (Day 120 / Day 150)	6.60	3.74	4.41	4.87	3.23	2.33
2 days after the 5th treatment (Day 122 / Day 152)	5.50	3.35	4.13	5.22	2.70	2.09
Prior to the 6th treatment (Day 180 / Day 210)	5.04	5.39	3.63	2.91	3.89	1.77
2 days after the 6th treatment (Day 182 / Day 212)	5.44	2.27	3.56	3.08	3.14	2.53
Prior to the 7th treatment (Day 240)	6.58	2.90	7.01	-	-	-
2 days after the 7th treatment (Day 242)	5.75	2.39	4.99	-	-	-
Neutrophil percentage (%)						
Baseline	63.60	63.10	67.40	73.10	63.50	57.40
2 days after 1st treatment (Day 3)	64.80	71.60	66.40	64.50	76.80	62.50
Prior to the 2nd treatment (Day 15)	65.60	59.40	65.70	75.40	59.90	54.10
2 days after the 2nd treatment (Day 17)	66.20	51.70	67.10	57.50	55.60	54.70
Prior to the 3rd treatment (Day 29)	64.20	56.80	70.10	67.90	57.60	53.20
2 days after the 3rd treatment (Day 31)	68.20	59.40	71.10	59.00	53.30	50.90
Prior to the 4th treatment (Day 60 / Day 90)	67.80	56.00	65.50	62.90	61.90	60.80
2 days after the 4th treatment (Day 62 / Day 92)	67.30	56.90	82.30	72.70	54.80	57.40
Prior to the 5th treatment (Day 120 / Day 150)	66.10	59.70	69.90	71.00	65.50	59.90
2 days after the 5th treatment (Day 122 / Day 152)	65.50	64.00	67.20	78.10	59.10	48.60
Prior to the 6th treatment (Day 180 / Day 210)	64.40	71.00	63.70	49.50	60.10	55.20
2 days after the 6th treatment (Day 182 / Day 212)	62.70	49.30	63.90	57.50	61.00	53.90
Prior to the 7th treatment (Day 240)	67.40	50.60	77.00	-	-	-
2 days after the 7th treatment (Day 242)	62.20	44.90	69.90	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S3: Changes in lymphocyte count and percentage of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Lymphocyte count ($\times 10^9$/L)						
Baseline	2.39	1.60	1.47	1.33	1.60	1.26
2 days after 1st treatment (Day 3)	2.45	1.42	1.36	2.05	1.21	1.07
Prior to the 2nd treatment (Day 15)	2.31	1.96	1.21	1.44	1.48	1.56
2 days after the 2nd treatment (Day 17)	2.28	2.23	1.32	2.13	1.49	1.20
Prior to the 3rd treatment (Day 29)	2.38	2.29	1.40	1.30	1.90	1.23
2 days after the 3rd treatment (Day 31)	2.31	1.86	1.15	1.83	1.75	1.52
Prior to the 4th treatment (Day 60 / Day 90)	2.69	1.97	1.56	1.50	1.53	1.14
2 days after the 4th treatment (Day 62 / Day 92)	2.12	1.70	0.64	1.29	1.69	1.46
Prior to the 5th treatment (Day 120 / Day 150)	2.72	2.01	1.44	1.66	1.30	1.22
2 days after the 5th treatment (Day 122 / Day 152)	2.22	1.34	1.50	1.10	1.52	1.73
Prior to the 6th treatment (Day 180 / Day 210)	2.11	1.58	1.61	2.52	1.83	1.06
2 days after the 6th treatment (Day 182 / Day 212)	2.56	1.79	1.57	1.96	1.48	1.60
Prior to the 7th treatment (Day 240)	2.50	2.23	1.57	-	-	-
2 days after the 7th treatment (Day 242)	2.78	2.16	1.59	-	-	-
Lymphocyte percentage (%)						
Baseline	27.0	24.0	23.7	21.9	26.4	32.5
2 days after 1st treatment (Day 3)	27.0	19.4	23.5	29.2	16.9	27.5
Prior to the 2nd treatment (Day 15)	26.7	27.3	25.2	20.7	30.4	35.4
2 days after the 2nd treatment (Day 17)	26.7	34.7	24.8	35.6	35.4	33.3
Prior to the 3rd treatment (Day 29)	27.5	31.2	21.9	26.6	34.0	36.6
2 days after the 3rd treatment (Day 31)	24.9	30.1	21.3	35.2	36.1	38.2
Prior to the 4th treatment (Day 60 / Day 90)	24.6	32.4	26.4	31.8	29.2	29.3
2 days after the 4th treatment (Day 62 / Day 92)	25.4	32.7	9.3	21.9	36.3	32.6
Prior to the 5th treatment (Day 120 / Day 150)	27.3	32.0	22.9	24.3	26.3	31.3
2 days after the 5th treatment (Day 122 / Day 152)	26.4	25.6	24.3	16.5	33.2	40.2
Prior to the 6th treatment (Day 180 / Day 210)	26.9	20.7	28.2	43.0	28.2	33.0
2 days after the 6th treatment (Day 182 / Day 212)	29.4	38.9	28.2	36.5	28.8	34.1
Prior to the 7th treatment (Day 240)	25.5	38.9	17.3	-	-	-
2 days after the 7th treatment (Day 242)	30.0	40.4	22.4	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S4: Changes in monocyte count and percentage of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Monocyte count ($\times 10^9$/L)						
Baseline	0.50	0.50	0.33	0.26	0.36	0.29
2 days after 1st treatment (Day 3)	0.47	0.39	0.38	0.37	0.32	0.27
Prior to the 2nd treatment (Day 15)	0.40	0.39	0.33	0.24	0.30	0.32
2 days after the 2nd treatment (Day 17)	0.37	0.34	0.28	0.35	0.22	0.32
Prior to the 3rd treatment (Day 29)	0.40	0.46	0.39	0.23	0.26	0.23
2 days after the 3rd treatment (Day 31)	0.38	0.37	0.29	0.26	0.28	0.31
Prior to the 4th treatment (Day 60 / Day 90)	0.52	0.39	0.35	0.21	0.33	0.26
2 days after the 4th treatment (Day 62 / Day 92)	0.36	0.31	0.48	0.27	0.28	0.32
Prior to the 5th treatment (Day 120 / Day 150)	0.42	0.34	0.31	0.25	0.28	0.26
2 days after the 5th treatment (Day 122 / Day 152)	0.40	0.28	0.36	0.33	0.20	0.36
Prior to the 6th treatment (Day 180 / Day 210)	0.39	0.40	0.34	0.34	0.42	0.23
2 days after the 6th treatment (Day 182 / Day 212)	0.40	0.30	0.30	0.26	0.33	0.41
Prior to the 7th treatment (Day 240)	0.44	0.29	0.37	-	-	-
2 days after the 7th treatment (Day 242)	0.43	0.42	0.37	-	-	-
Monocyte percentage (%)						
Baseline	5.70	7.50	5.40	4.20	5.90	7.40
2 days after 1st treatment (Day 3)	5.10	5.30	6.50	5.30	5.90	7.10
Prior to the 2nd treatment (Day 15)	4.60	5.40	6.80	3.40	4.40	7.20
2 days after the 2nd treatment (Day 17)	4.30	5.30	5.30	5.90	6.20	8.90
Prior to the 3rd treatment (Day 29)	4.70	6.30	6.10	4.60	5.20	6.70
2 days after the 3rd treatment (Day 31)	4.10	5.90	5.40	4.90	4.60	7.80
Prior to the 4th treatment (Day 60 / Day 90)	4.80	6.40	5.90	4.50	6.20	6.80
2 days after the 4th treatment (Day 62 / Day 92)	4.30	6.00	7.00	4.60	5.90	7.20
Prior to the 5th treatment (Day 120 / Day 150)	4.30	5.30	5.00	3.70	5.70	6.60
2 days after the 5th treatment (Day 122 / Day 152)	4.70	5.50	5.80	4.90	4.30	8.40
Prior to the 6th treatment (Day 180 / Day 210)	5.00	5.30	5.90	5.80	6.40	7.20
2 days after the 6th treatment (Day 182 / Day 212)	4.70	6.50	5.40	4.80	6.40	8.80
Prior to the 7th treatment (Day 240)	4.50	5.20	4.00	-	-	-
2 days after the 7th treatment (Day 242)	4.70	7.80	5.20	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S5: Changes in eosinophil count and percentage of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Eosinophil count ($\times 10^9$/L)						
Baseline	0.29	0.28	0.04	0.01	0.21	0.08
2 days after 1st treatment (Day 3)	0.26	0.20	0.18	0.03	0.10	0.09
Prior to the 2nd treatment (Day 15)	0.25	0.51	0.08	0.01	0.14	0.12
2 days after the 2nd treatment (Day 17)	0.21	0.47	0.12	0.02	0.13	0.10
Prior to the 3rd treatment (Day 29)	0.28	0.35	0.08	0.02	0.16	0.09
2 days after the 3rd treatment (Day 31)	0.24	0.23	0.10	0.02	0.20	0.10
Prior to the 4th treatment (Day 60 / Day 90)	0.21	0.25	0.10	0.01	0.10	0.10
2 days after the 4th treatment (Day 62 / Day 92)	0.21	0.18	0.07	0.01	0.11	0.10
Prior to the 5th treatment (Day 120 / Day 150)	0.19	0.15	0.10	0.03	0.10	0.06
2 days after the 5th treatment (Day 122 / Day 152)	0.26	0.21	0.13	0.01	0.13	0.09
Prior to the 6th treatment (Day 180 / Day 210)	0.24	0.16	0.10	0.06	0.31	0.11
2 days after the 6th treatment (Day 182 / Day 212)	0.24	0.21	0.11	0.03	0.18	0.12
Prior to the 7th treatment (Day 240)	0.21	0.24	0.11	-	-	-
2 days after the 7th treatment (Day 242)	0.25	0.31	0.14	-	-	-
Eosinophil percentage (%)						
Baseline	3.30	4.10	2.90	0.20	3.50	2.00
2 days after 1st treatment (Day 3)	2.80	2.70	3.10	0.40	1.40	2.40
Prior to the 2nd treatment (Day 15)	2.90	7.10	1.60	0.10	2.90	2.70
2 days after the 2nd treatment (Day 17)	2.50	7.30	2.30	0.40	3.20	2.60
Prior to the 3rd treatment (Day 29)	3.20	4.70	1.30	0.40	2.90	2.70
2 days after the 3rd treatment (Day 31)	2.50	3.70	1.80	0.40	4.10	2.60
Prior to the 4th treatment (Day 60 / Day 90)	2.50	4.10	1.70	0.20	2.00	0.36
2 days after the 4th treatment (Day 62 / Day 92)	2.50	3.40	1.00	0.20	2.40	2.10
Prior to the 5th treatment (Day 120 / Day 150)	1.90	2.40	1.60	0.50	2.00	1.70
2 days after the 5th treatment (Day 122 / Day 152)	3.10	4.10	2.10	0.10	2.90	2.00
Prior to the 6th treatment (Day 180 / Day 210)	3.10	2.10	1.70	1.00	4.70	3.50
2 days after the 6th treatment (Day 182 / Day 212)	2.80	4.50	2.00	0.60	3.60	2.60
Prior to the 7th treatment (Day 240)	2.20	4.20	1.20	-	-	-
2 days after the 7th treatment (Day 242)	2.70	5.80	2.00	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S6: Changes in basophil count and percentage of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Basophil count ($\times 10^9$/L)						
Baseline	0.03	0.08	0.04	0.03	0.04	0.03
2 days after 1st treatment (Day 3)	0.02	0.07	0.03	0.04	0.03	0.02
Prior to the 2nd treatment (Day 15)	0.02	0.06	0.04	0.03	0.03	0.03
2 days after the 2nd treatment (Day 17)	0.02	0.06	0.03	0.04	0.03	0.02
Prior to the 3rd treatment (Day 29)	0.03	0.07	0.04	0.02	0.05	0.03
2 days after the 3rd treatment (Day 31)	0.03	0.05	0.02	0.03	0.04	0.02
Prior to the 4th treatment (Day 60 / Day 90)	0.04	0.06	0.03	0.03	0.04	0.02
2 days after the 4th treatment (Day 62 / Day 92)	0.04	0.05	0.02	0.04	0.03	0.03
Prior to the 5th treatment (Day 120 / Day 150)	0.04	0.04	0.04	0.04	0.03	0.02
2 days after the 5th treatment (Day 122 / Day 152)	0.03	0.04	0.04	0.03	0.02	0.03
Prior to the 6th treatment (Day 180 / Day 210)	0.05	0.07	0.03	0.04	0.04	0.03
2 days after the 6th treatment (Day 182 / Day 212)	0.04	0.04	0.03	0.03	0.01	0.03
Prior to the 7th treatment (Day 240)	0.04	0.06	0.04	-	-	-
2 days after the 7th treatment (Day 242)	0.04	0.06	0.04	-	-	-
Basophil percentage (%)						
Baseline	0.40	1.30	0.60	0.60	0.70	0.70
2 days after 1st treatment (Day 3)	0.30	1.00	0.50	0.60	0.50	0.50
Prior to the 2nd treatment (Day 15)	0.20	0.80	0.70	0.40	0.60	0.60
2 days after the 2nd treatment (Day 17)	0.30	1.00	0.50	0.60	0.60	0.50
Prior to the 3rd treatment (Day 29)	0.40	1.00	0.60	0.50	0.90	0.50
2 days after the 3rd treatment (Day 31)	0.30	0.90	0.40	0.50	0.70	0.50
Prior to the 4th treatment (Day 60 / Day 90)	0.30	1.10	0.50	0.60	0.70	0.40
2 days after the 4th treatment (Day 62 / Day 92)	0.50	1.00	0.40	0.60	0.60	0.70
Prior to the 5th treatment (Day 120 / Day 150)	0.40	0.60	0.60	0.50	0.50	0.50
2 days after the 5th treatment (Day 122 / Day 152)	0.30	0.80	0.60	0.40	0.50	0.80
Prior to the 6th treatment (Day 180 / Day 210)	0.60	0.90	0.50	0.70	0.60	1.10
2 days after the 6th treatment (Day 182 / Day 212)	0.40	0.80	0.50	0.60	0.20	0.60
Prior to the 7th treatment (Day 240)	0.40	1.10	0.50	-	-	-
2 days after the 7th treatment (Day 242)	0.40	1.10	0.50	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S7: Changes in red blood cell count ($\times 10^{12}$/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	5.52	4.16	3.45	4.26	4.58	3.99
2 days after 1st treatment (Day 3)	5.29	4.56	3.20	4.20	4.44	3.69
Prior to the 2nd treatment (Day 15)	5.41	4.10	3.58	4.34	4.22	4.12
2 days after the 2nd treatment (Day 17)	5.50	4.15	3.39	4.19	4.26	3.59
Prior to the 3rd treatment (Day 29)	5.07	4.68	3.61	4.20	4.61	3.94
2 days after the 3rd treatment (Day 31)	5.31	4.36	3.55	4.16	4.33	3.81
Prior to the 4th treatment (Day 60 / Day 90)	5.54	4.70	3.89	4.85	4.65	3.83
2 days after the 4th treatment (Day 62 / Day 92)	5.40	4.30	3.78	4.31	4.42	3.81
Prior to the 5th treatment (Day 120 / Day 150)	5.53	4.33	3.95	4.39	4.41	3.75
2 days after the 5th treatment (Day 122 / Day 152)	5.37	4.17	3.72	4.20	4.48	3.86
Prior to the 6th treatment (Day 180 / Day 210)	5.47	4.35	3.88	4.26	4.32	3.98
2 days after the 6th treatment (Day 182 / Day 212)	5.52	4.24	3.89	4.50	4.07	4.06
Prior to the 7th treatment (Day 240)	5.77	4.37	3.93	-	-	-
2 days after the 7th treatment (Day 242)	5.65	4.38	3.82	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S8: Changes in hemoglobin (g/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	149	129	113	131	137	128
2 days after 1st treatment (Day 3)	150	142	104	125	132	115
Prior to the 2nd treatment (Day 15)	148	128	119	129	127	128
2 days after the 2nd treatment (Day 17)	158	130	110	127	129	112
Prior to the 3rd treatment (Day 29)	138	147	117	125	138	123
2 days after the 3rd treatment (Day 31)	151	139	117	124	128	119
Prior to the 4th treatment (Day 60 / Day 90)	155	150	124	141	138	120
2 days after the 4th treatment (Day 62 / Day 92)	155	133	117	128	132	116
Prior to the 5th treatment (Day 120 / Day 150)	159	133	126	130	131	118
2 days after the 5th treatment (Day 122 / Day 152)	152	131	117	126	134	118
Prior to the 6th treatment (Day 180 / Day 210)	155	134	122	127	127	125
2 days after the 6th treatment (Day 182 / Day 212)	155	131	123	136	125	123
Prior to the 7th treatment (Day 240)	159	136	123	-	-	-
2 days after the 7th treatment (Day 242)	162	137	121	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S9: Changes in hematocrit (L/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	0.46	0.38	0.34	0.39	0.40	0.37
2 days after 1st treatment (Day 3)	0.44	0.42	0.31	0.38	0.39	0.34
Prior to the 2nd treatment (Day 15)	0.45	0.38	0.35	0.39	0.38	0.38
2 days after the 2nd treatment (Day 17)	0.46	0.38	0.32	0.38	0.38	0.34
Prior to the 3rd treatment (Day 29)	0.42	0.43	0.35	0.37	0.41	0.37
2 days after the 3rd treatment (Day 31)	0.44	0.40	0.34	0.37	0.39	0.36
Prior to the 4th treatment (Day 60 / Day 90)	0.47	0.43	0.37	0.42	0.40	0.36
2 days after the 4th treatment (Day 62 / Day 92)	0.45	0.39	0.35	0.38	0.39	0.35
Prior to the 5th treatment (Day 120 / Day 150)	0.46	0.40	0.36	0.39	0.39	0.35
2 days after the 5th treatment (Day 122 / Day 152)	0.45	0.38	0.34	0.37	0.39	0.36
Prior to the 6th treatment (Day 180 / Day 210)	0.45	0.40	0.35	0.37	0.38	0.37
2 days after the 6th treatment (Day 182 / Day 212)	0.46	0.39	0.35	0.40	0.36	0.38
Prior to the 7th treatment (Day 240)	0.48	0.39	0.36	-	-	-
2 days after the 7th treatment (Day 242)	0.47	0.40	0.35	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S10: Changes in mean corpuscular volume (fL) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	83.0	90.7	98.1	90.3	87.0	93.0
2 days after 1st treatment (Day 3)	82.8	92.1	97.5	90.0	88.6	92.1
Prior to the 2nd treatment (Day 15)	82.6	92.4	97.7	89.9	88.7	91.7
2 days after the 2nd treatment (Day 17)	83.2	91.3	95.6	90.0	88.7	93.2
Prior to the 3rd treatment (Day 29)	83.5	91.1	96.1	88.9	88.8	93.4
2 days after the 3rd treatment (Day 31)	83.3	92.1	95.6	88.7	89.3	93.4
Prior to the 4th treatment (Day 60 / Day 90)	84.0	90.5	94.0	86.4	86.8	93.1
2 days after the 4th treatment (Day 62 / Day 92)	83.1	90.0	93.2	82.7	87.4	92.1
Prior to the 5th treatment (Day 120 / Day 150)	83.7	91.2	91.5	88.4	88.6	92.2
2 days after the 5th treatment (Day 122 / Day 152)	83.1	91.0	91.8	87.8	87.5	92.2
Prior to the 6th treatment (Day 180 / Day 210)	82.8	90.0	91.5	88.0	88.3	92.2
2 days after the 6th treatment (Day 182 / Day 212)	82.7	91.7	90.9	88.6	88.8	93.2
Prior to the 7th treatment (Day 240)	82.8	89.8	91.5	-	-	-
2 days after the 7th treatment (Day 242)	83.1	90.5	91.9	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S11: Changes in mean corpuscular hemoglobin of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Mean corpuscular hemoglobin (pg)						
Baseline	27.1	31.1	32.9	30.8	29.9	32.0
2 days after 1st treatment (Day 3)	28.3	31.1	32.5	29.8	29.7	31.3
Prior to the 2nd treatment (Day 15)	27.3	31.2	33.4	29.7	30.2	31.2
2 days after the 2nd treatment (Day 17)	28.7	31.3	32.4	30.3	30.2	31.3
Prior to the 3rd treatment (Day 29)	27.3	31.4	32.4	29.9	29.9	31.1
2 days after the 3rd treatment (Day 31)	28.4	31.8	32.9	29.9	29.7	31.3
Prior to the 4th treatment (Day 60 / Day 90)	28.0	31.8	31.9	29.0	29.7	31.3
2 days after the 4th treatment (Day 62 / Day 92)	28.7	30.9	30.9	29.7	29.9	30.5
Prior to the 5th treatment (Day 120 / Day 150)	28.7	30.6	31.8	29.7	29.6	31.5
2 days after the 5th treatment (Day 122 / Day 152)	28.3	31.4	31.4	30.1	30.0	30.5
Prior to the 6th treatment (Day 180 / Day 210)	28.3	30.9	31.5	29.9	29.4	31.3
2 days after the 6th treatment (Day 182 / Day 212)	28.1	31.0	31.6	30.2	30.6	30.3
Prior to the 7th treatment (Day 240)	27.6	31.1	31.2	-	-	-
2 days after the 7th treatment (Day 242)	28.7	31.2	31.7	-	-	-
Mean corpuscular hemoglobin Concentration (g/L)						
Baseline	326.0	343.0	335.0	341.0	344.0	344.0
2 days after 1st treatment (Day 3)	342.0	338.0	333.0	331.0	335.0	339.0
Prior to the 2nd treatment (Day 15)	330.0	338.0	342.0	330.0	340.0	340.0
2 days after the 2nd treatment (Day 17)	345.0	343.0	339.0	337.0	341.0	335.0
Prior to the 3rd treatment (Day 29)	327.0	345.0	337.0	336.0	336.0	333.0
2 days after the 3rd treatment (Day 31)	341.0	346.0	344.0	337.0	332.0	335.0
Prior to the 4th treatment (Day 60 / Day 90)	333.0	351.0	339.0	336.0	342.0	336.0
2 days after the 4th treatment (Day 62 / Day 92)	345.0	343.0	332.0	341.0	342.0	331.0
Prior to the 5th treatment (Day 120 / Day 150)	342.0	336.0	348.0	336.0	334.0	342.0
2 days after the 5th treatment (Day 122 / Day 152)	341.0	345.0	342.0	343.0	343.0	331.0
Prior to the 6th treatment (Day 180 / Day 210)	341.0	340.0	344.0	340.0	332.0	340.0
2 days after the 6th treatment (Day 182 / Day 212)	340.0	337.0	348.0	341.0	345.0	326.0
Prior to the 7th treatment (Day 240)	333.0	346.0	342.0	-	-	-
2 days after the 7th treatment (Day 242)	346.0	345.0	345.0	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S12: Changes in red cell distribution width of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Red cell distribution width (fL)						
Baseline	41.40	38.00	40.00	40.80	39.90	43.70
2 days after 1st treatment (Day 3)	39.30	38.60	39.80	40.80	40.70	42.10
Prior to the 2nd treatment (Day 15)	39.70	39.40	41.40	41.10	41.00	42.10
2 days after the 2nd treatment (Day 17)	39.70	38.70	39.90	40.40	39.40	42.80
Prior to the 3rd treatment (Day 29)	39.90	40.00	40.70	40.20	40.60	43.70
2 days after the 3rd treatment (Day 31)	40.10	39.60	40.90	40.80	39.00	42.60
Prior to the 4th treatment (Day 60 / Day 90)	40.40	38.00	39.90	38.60	38.80	42.80
2 days after the 4th treatment (Day 62 / Day 92)	40.10	37.90	38.90	39.30	38.60	41.80
Prior to the 5th treatment (Day 120 / Day 150)	40.60	39.90	40.40	41.50	39.70	42.60
2 days after the 5th treatment (Day 122 / Day 152)	39.00	39.00	39.90	40.70	39.30	42.70
Prior to the 6th treatment (Day 180 / Day 210)	40.20	38.50	40.00	38.80	40.80	42.40
2 days after the 6th treatment (Day 182 / Day 212)	40.10	38.90	39.40	40.70	38.70	43.50
Prior to the 7th treatment (Day 240)	39.90	38.60	41.40	-	-	-
2 days after the 7th treatment (Day 242)	39.70	38.80	42.00	-	-	-
Red cell distribution width - coefficient of variation(%)						
Baseline	13.7	11.8	11.5	12.8	12.9	13.4
2 days after 1st treatment (Day 3)	13.5	12.0	11.6	12.8	13.0	12.9
Prior to the 2nd treatment (Day 15)	13.6	12.2	11.9	13.0	12.9	12.9
2 days after the 2nd treatment (Day 17)	13.6	12.1	11.8	12.7	12.6	13.0
Prior to the 3rd treatment (Day 29)	13.5	12.5	12.0	12.8	12.7	13.2
2 days after the 3rd treatment (Day 31)	13.6	12.3	12.2	13.0	12.4	12.9
Prior to the 4th treatment (Day 60 / Day 90)	13.7	12.0	12.0	12.6	12.7	13.0
2 days after the 4th treatment (Day 62 / Day 92)	13.7	11.9	11.7	12.7	12.5	12.9
Prior to the 5th treatment (Day 120 / Day 150)	13.7	12.3	12.4	13.3	12.9	13.3
2 days after the 5th treatment (Day 122 / Day 152)	13.4	12.0	12.3	13.1	12.8	13.0
Prior to the 6th treatment (Day 180 / Day 210)	13.8	12.1	12.4	12.6	12.7	13.0
2 days after the 6th treatment (Day 182 / Day 212)	13.8	12.1	12.3	12.9	12.6	13.2
Prior to the 7th treatment (Day 240)	13.6	12.2	13.0	-	-	-
2 days after the 7th treatment (Day 242)	13.5	12.2	13.0	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S13: Changes in platelet count ($\times 10^9$/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	244	298	222	404	323	211
2 days after 1st treatment (Day 3)	231	284	212	387	302	172
Prior to the 2nd treatment (Day 15)	239	227	239	354	331	224
2 days after the 2nd treatment (Day 17)	251	235	231	327	297	199
Prior to the 3rd treatment (Day 29)	226	295	265	371	339	206
2 days after the 3rd treatment (Day 31)	281	254	272	342	312	207
Prior to the 4th treatment (Day 60 / Day 90)	269	272	265	361	309	198
2 days after the 4th treatment (Day 62 / Day 92)	284	228	222	318	288	198
Prior to the 5th treatment (Day 120 / Day 150)	269	225	291	378	298	188
2 days after the 5th treatment (Day 122 / Day 152)	239	212	268	335	300	181
Prior to the 6th treatment (Day 180 / Day 210)	282	266	270	288	326	169
2 days after the 6th treatment (Day 182 / Day 212)	260	229	249	258	144	184
Prior to the 7th treatment (Day 240)	291	244	261	-	-	-
2 days after the 7th treatment (Day 242)	268	247	246	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S14: Changes in mean platelet volume (fL) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	9.7	10.3	9.1	8.7	8.1	8.1
2 days after 1st treatment (Day 3)	9.2	10.2	9.2	9.1	8.4	7.9
Prior to the 2nd treatment (Day 15)	9.4	10.9	8.6	9.5	8.2	8.0
2 days after the 2nd treatment (Day 17)	9.0	10.0	8.4	9.3	8.3	8.0
Prior to the 3rd treatment (Day 29)	9.5	9.9	8.8	9.3	8.6	8.2
2 days after the 3rd treatment (Day 31)	9.3	10.1	8.7	9.2	8.4	8.3
Prior to the 4th treatment (Day 60 / Day 90)	9.1	9.9	9.0	8.7	8.2	8.2
2 days after the 4th treatment (Day 62 / Day 92)	9.1	9.6	9.0	8.8	8.1	8.3
Prior to the 5th treatment (Day 120 / Day 150)	9.0	10.2	9.0	8.7	8.2	8.1
2 days after the 5th treatment (Day 122 / Day 152)	9.0	9.8	8.4	8.6	8.5	8.4
Prior to the 6th treatment (Day 180 / Day 210)	9.2	9.7	8.9	9.0	8.6	8.3
2 days after the 6th treatment (Day 182 / Day 212)	8.8	9.3	8.8	8.8	9.2	8.8
Prior to the 7th treatment (Day 240)	9.6	9.8	8.6	-	-	-
2 days after the 7th treatment (Day 242)	9.1	9.4	8.6	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S15: Changes in plateletcrit (%) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	0.24	0.31	0.20	0.35	0.26	0.17
2 days after 1st treatment (Day 3)	0.21	0.29	0.20	0.35	0.25	0.14
Prior to the 2nd treatment (Day 15)	0.23	0.25	0.20	0.34	0.27	0.18
2 days after the 2nd treatment (Day 17)	0.23	0.23	0.19	0.31	0.24	0.16
Prior to the 3rd treatment (Day 29)	0.21	0.29	0.23	0.35	0.29	0.17
2 days after the 3rd treatment (Day 31)	0.26	0.26	0.24	0.31	0.26	0.17
Prior to the 4th treatment (Day 60 / Day 90)	0.24	0.27	0.24	0.31	0.25	0.16
2 days after the 4th treatment (Day 62 / Day 92)	0.26	0.22	0.20	0.28	0.23	0.16
Prior to the 5th treatment (Day 120 / Day 150)	0.24	0.23	0.26	0.33	0.24	0.15
2 days after the 5th treatment (Day 122 / Day 152)	0.21	0.21	0.23	0.29	0.26	0.15
Prior to the 6th treatment (Day 180 / Day 210)	0.26	0.26	0.24	0.26	0.28	0.14
2 days after the 6th treatment (Day 182 / Day 212)	0.23	0.21	0.22	0.23	0.13	0.16
Prior to the 7th treatment (Day 240)	0.28	0.24	0.22	-	-	-
2 days after the 7th treatment (Day 242)	0.24	0.23	0.21	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S16: Changes in platelet distribution width (fL) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	16.4	16.3	15.6	15.8	15.6	15.6
2 days after 1st treatment (Day 3)	16.2	16.5	15.8	15.8	15.8	15.7
Prior to the 2nd treatment (Day 15)	16.3	16.0	15.7	16.1	15.6	15.5
2 days after the 2nd treatment (Day 17)	16.3	16.1	15.8	16.0	15.6	15.4
Prior to the 3rd treatment (Day 29)	16.2	16.1	15.8	16.0	15.8	15.5
2 days after the 3rd treatment (Day 31)	16.3	16.2	15.8	15.9	15.8	15.5
Prior to the 4th treatment (Day 60 / Day 90)	16.3	16.3	15.7	15.7	15.7	15.3
2 days after the 4th treatment (Day 62 / Day 92)	16.3	16.1	15.9	16.0	15.6	15.6
Prior to the 5th treatment (Day 120 / Day 150)	16.1	16.3	15.8	15.9	15.6	15.5
2 days after the 5th treatment (Day 122 / Day 152)	16.1	16.2	15.7	15.8	15.8	15.6
Prior to the 6th treatment (Day 180 / Day 210)	16.1	16.2	15.7	16.0	15.8	15.7
2 days after the 6th treatment (Day 182 / Day 212)	16.2	15.9	15.7	16.0	16.5	15.7
Prior to the 7th treatment (Day 240)	16.2	16.0	15.8	-	-	-
2 days after the 7th treatment (Day 242)	16.0	15.9	15.7	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S17: Changes in platelet-large cell ratio (%) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	25.2	28,2	18.3	17.3	13.4	12.9
2 days after 1st treatment (Day 3)	22.3	27.6	19.0	18.9	15.5	12.3
Prior to the 2nd treatment (Day 15)	23.3	32.1	15.8	22.3	14.6	12.1
2 days after the 2nd treatment (Day 17)	20.9	27.0	15.2	21.4	13.6	12.6
Prior to the 3rd treatment (Day 29)	24.1	25.5	17.3	20.8	16.1	21.4
2 days after the 3rd treatment (Day 31)	23.5	26.5	16.4	19.8	15.3	13.7
Prior to the 4th treatment (Day 60 / Day 90)	21.9	26.0	18.5	16.9	14.6	12.4
2 days after the 4th treatment (Day 62 / Day 92)	21.4	23.2	18.7	18.4	13.3	13.8
Prior to the 5th treatment (Day 120 / Day 150)	21.0	27.6	19.2	17.3	14.2	12.7
2 days after the 5th treatment (Day 122 / Day 152)	20.6	24.1	15.2	16.3	16.1	14.3
Prior to the 6th treatment (Day 180 / Day 210)	22.1	24.5	18.0	18.6	16.3	14.4
2 days after the 6th treatment (Day 182 / Day 212)	20.9	20.7	17.3	18.9	20.5	16.8
Prior to the 7th treatment (Day 240)	24.8	24.2	16.2	-	-	-
2 days after the 7th treatment (Day 242)	21.6	22.8	16.3	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S18: Changes in C-reactive protein (mg/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	5.78	0.31	0.33	1.27	0.31	1.25
2 days after 1st treatment (Day 3)	12.46	0.46	0.96	14.96	2.30	0.01
Prior to the 2nd treatment (Day 15)	6.21	3.55	0.83	2.39	0.07	0.22
2 days after the 2nd treatment (Day 17)	6.47	1.18	0.74	5.80	1.01	0.12
Prior to the 3rd treatment (Day 29)	4.06	0.00	0.40	1.46	0.39	0.40
2 days after the 3rd treatment (Day 31)	7.44	0.52	1.11	3.85	0.48	0.67
Prior to the 4th treatment (Day 60 / Day 90)	11.57	0.86	1.14	1.31	0.24	0.13
2 days after the 4th treatment (Day 62 / Day 92)	17.42	0.64	5.79	17.98	0.36	0.81
Prior to the 5th treatment (Day 120 / Day 150)	4.06	3.23	1.05	1.05	0.20	0.18
2 days after the 5th treatment (Day 122 / Day 152)	6.92	1.21	0.46	6.78	0.24	0.10
Prior to the 6th treatment (Day 180 / Day 210)	3.49	0.00	1.10	0.57	0.06	0.03
2 days after the 6th treatment (Day 182 / Day 212)	7.15	0.08	1.52	1.68	0.71	0.64
Prior to the 7th treatment (Day 240)	3.87	3.82	1.77	-	-	-
2 days after the 7th treatment (Day 242)	5.40	1.82	1.68	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S19: Changes in total protein (g/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	70.2	71.0	61.9	73.9	79.9	71.8
2 days after 1st treatment (Day 3)	71.7	75.6	56.8	69.6	74.4	62.8
Prior to the 2nd treatment (Day 15)	73.2	69.9	64.2	68.8	72.2	72.8
2 days after the 2nd treatment (Day 17)	77.5	68.0	61.4	70.4	74.4	61.3
Prior to the 3rd treatment (Day 29)	66.9	78.3	63.5	66.5	79.5	68.9
2 days after the 3rd treatment (Day 31)	76.1	74.4	65.0	65.2	71.5	66.2
Prior to the 4th treatment (Day 60 / Day 90)	79.7	78.2	66.5	75.8	77.2	66.5
2 days after the 4th treatment (Day 62 / Day 92)	75.5	72.6	65.0	66.2	75.6	65.1
Prior to the 5th treatment (Day 120 / Day 150)	78.8	69.9	67.7	73.4	74.3	66.5
2 days after the 5th treatment (Day 122 / Day 152)	74.4	70.0	61.8	69.3	77.2	68.8
Prior to the 6th treatment (Day 180 / Day 210)	77.2	71.9	66.0	66.9	69.8	67.0
2 days after the 6th treatment (Day 182 / Day 212)	79.1	68.5	68.1	74.4	72.1	69.6
Prior to the 7th treatment (Day 240)	77.4	71.6	63.9	-	-	-
2 days after the 7th treatment (Day 242)	78.1	70.6	62.2	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S20: Changes in albumin (g/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	42.8	43.2	41.6	43.7	46.8	43.5
2 days after 1st treatment (Day 3)	43.2	45.3	37.7	40.8	44.1	38.6
Prior to the 2nd treatment (Day 15)	43.2	41.8	42.5	40.6	43.2	44.2
2 days after the 2nd treatment (Day 17)	46.0	39.2	40.2	40.8	44.1	38.3
Prior to the 3rd treatment (Day 29)	41.0	46.7	41.7	38.5	46.8	42.3
2 days after the 3rd treatment (Day 31)	46.5	44.2	42.4	37.8	42.1	39.9
Prior to the 4th treatment (Day 60 / Day 90)	49.0	45.9	43.9	42.8	45.4	41.1
2 days after the 4th treatment (Day 62 / Day 92)	45.6	42.4	41.6	37.9	45.0	40.4
Prior to the 5th treatment (Day 120 / Day 150)	48.8	42.5	43.1	41.5	44.2	40.6
2 days after the 5th treatment (Day 122 / Day 152)	45.9	41.7	40.1	39.0	44.5	41.3
Prior to the 6th treatment (Day 180 / Day 210)	47.6	44.8	42.2	37.4	41.0	41.1
2 days after the 6th treatment (Day 182 / Day 212)	48.3	42.2	43.1	41.7	42.4	41.9
Prior to the 7th treatment (Day 240)	47.0	44.2	41.0	-	-	-
2 days after the 7th treatment (Day 242)	47.3	42.8	39.4	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S21: Changes in globulin (g/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	27.4	27.8	20.3	30.2	33.1	28.3
2 days after 1st treatment (Day 3)	28.5	30.3	19.1	28.8	30.3	24.2
Prior to the 2nd treatment (Day 15)	30.0	28.1	21.7	28.2	29.0	28.6
2 days after the 2nd treatment (Day 17)	31.5	28.8	21.2	29.6	30.3	23.0
Prior to the 3rd treatment (Day 29)	25.9	31.6	21.8	28.0	32.7	26.6
2 days after the 3rd treatment (Day 31)	29.6	30.2	22.6	27.4	29.4	26.3
Prior to the 4th treatment (Day 60 / Day 90)	30.7	32.3	22.6	33.0	31.8	25.4
2 days after the 4th treatment (Day 62 / Day 92)	29.9	30.2	23.4	28.3	30.6	24.7
Prior to the 5th treatment (Day 120 / Day 150)	30.0	27.4	24.6	31.9	30.1	25.9
2 days after the 5th treatment (Day 122 / Day 152)	28.5	28.3	21.7	30.3	32.7	27.5
Prior to the 6th treatment (Day 180 / Day 210)	29.6	27.1	23.8	29.5	28.8	25.9
2 days after the 6th treatment (Day 182 / Day 212)	30.8	26.3	25.0	32.7	29.7	27.7
Prior to the 7th treatment (Day 240)	30.4	27.4	22.9	-	-	-
2 days after the 7th treatment (Day 242)	30.8	27.8	22.8	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S22: Changes in albumin-to-globulin ratio of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	1.6	1.6	2.0	1.4	1.4	1.5
2 days after 1st treatment (Day 3)	1.5	1.5	2.0	1.4	1.5	1.6
Prior to the 2nd treatment (Day 15)	1.4	1.5	2.0	1.4	1.5	1.5
2 days after the 2nd treatment (Day 17)	1.5	1.4	1.9	1.4	1.5	1.7
Prior to the 3rd treatment (Day 29)	1.6	1.5	1.9	1.4	1.4	1.6
2 days after the 3rd treatment (Day 31)	1.6	1.5	1.9	1.4	1.4	1.5
Prior to the 4th treatment (Day 60 / Day 90)	1.6	1.4	1.9	1.3	1.4	1.6
2 days after the 4th treatment (Day 62 / Day 92)	1.5	1.4	1.8	1.3	1.5	1.6
Prior to the 5th treatment (Day 120 / Day 150)	1.6	1.6	1.8	1.3	1.5	1.6
2 days after the 5th treatment (Day 122 / Day 152)	1.6	1.5	1.8	1.3	1.4	1.5
Prior to the 6th treatment (Day 180 / Day 210)	1.6	1.7	1.8	1.3	1.4	1.6
2 days after the 6th treatment (Day 182 / Day 212)	1.6	1.6	1.7	1.3	1.4	1.5
Prior to the 7th treatment (Day 240)	1.5	1.6	1.8	-	-	-
2 days after the 7th treatment (Day 242)	1.5	1.5	1.7	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S23: Changes in alanine aminotransferase (U/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	72.5	9.0	19.2	8.0	50.2	32.5
2 days after 1st treatment (Day 3)	68.8	8.1	18.4	7.3	46.0	28.0
Prior to the 2nd treatment (Day 15)	74.1	8.0	23.5	7.2	36.4	37.9
2 days after the 2nd treatment (Day 17)	91.6	6.5	19.1	8.0	46.0	30.3
Prior to the 3rd treatment (Day 29)	92.0	8.1	23.2	8.0	67.2	32.7
2 days after the 3rd treatment (Day 31)	101.2	8.0	17.4	8.6	45.9	28.7
Prior to the 4th treatment (Day 60 / Day 90)	84.1	7.0	20.7	11.6	33.4	30.4
2 days after the 4th treatment (Day 62 / Day 92)	75.9	5.6	17.1	8.1	26.8	28.7
Prior to the 5th treatment (Day 120 / Day 150)	127.5	8.9	24.7	8.7	37.7	18.4
2 days after the 5th treatment (Day 122 / Day 152)	114.7	8.9	18.6	7.3	34.4	19.8
Prior to the 6th treatment (Day 180 / Day 210)	106.4	8.7	28.1	7.7	27.9	31.3
2 days after the 6th treatment (Day 182 / Day 212)	127.3	7.9	27.7	9.1	31.6	31.8
Prior to the 7th treatment (Day 240)	101.9	8.3	31.8	-	-	-
2 days after the 7th treatment (Day 242)	106.1	8.1	28.8	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S24: Changes in aspartate aminotransferase (U/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	33.5	15.5	23.3	13.8	42.9	33.1
2 days after 1st treatment (Day 3)	30.7	13.6	20.1	16.2	37.7	26.4
Prior to the 2nd treatment (Day 15)	35.8	14.5	21.5	13.4	32.0	30.6
2 days after the 2nd treatment (Day 17)	42.4	13.4	18.6	15.8	37.7	25.8
Prior to the 3rd treatment (Day 29)	39.8	12.5	21.2	13.5	38.9	28.6
2 days after the 3rd treatment (Day 31)	42.8	13.8	19.4	14.0	33.6	22.0
Prior to the 4th treatment (Day 60 / Day 90)	33.2	12.1	22.4	15.9	30.0	25.5
2 days after the 4th treatment (Day 62 / Day 92)	33.2	10.6	22.4	11.2	22.7	24.1
Prior to the 5th treatment (Day 120 / Day 150)	52.5	17.4	22.4	16.8	31.8	23.9
2 days after the 5th treatment (Day 122 / Day 152)	47.4	15.2	21.3	13.6	26.7	22.7
Prior to the 6th treatment (Day 180 / Day 210)	48.4	15.3	26.1	16.2	33.6	26.4
2 days after the 6th treatment (Day 182 / Day 212)	62.3	15.4	27.0	18.4	30.2	27.8
Prior to the 7th treatment (Day 240)	48.2	14.5	28.0	-	-	-
2 days after the 7th treatment (Day 242)	44.4	13.1	28.8	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S25: Changes in alkaline phosphatase (U/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	66.1	56.3	36.2	57.0	51.6	45.5
2 days after 1st treatment (Day 3)	76.4	56.2	31.6	55.0	53.3	40.3
Prior to the 2nd treatment (Day 15)	71.9	63.8	44.4	55.6	52.4	43.8
2 days after the 2nd treatment (Day 17)	76.6	53.7	36.5	59.7	53.3	39.1
Prior to the 3rd treatment (Day 29)	67.0	60.1	34.6	56.5	80.0	43.3
2 days after the 3rd treatment (Day 31)	77.9	57.3	40.1	56.4	67.7	41.5
Prior to the 4th treatment (Day 60 / Day 90)	76.2	62.0	41.9	57.7	51.8	45.7
2 days after the 4th treatment (Day 62 / Day 92)	76.5	54.6	42.4	49.9	59.2	47.7
Prior to the 5th treatment (Day 120 / Day 150)	71.5	52.4	45.0	64.9	51.6	41.6
2 days after the 5th treatment (Day 122 / Day 152)	73.9	52.8	39.4	63.3	55.5	45.1
Prior to the 6th treatment (Day 180 / Day 210)	76.6	52.9	48.8	58.4	54.3	47.5
2 days after the 6th treatment (Day 182 / Day 212)	78.3	50.6	42.3	68.6	63.4	51.2
Prior to the 7th treatment (Day 240)	77.6	56.2	46.1	-	-	-
2 days after the 7th treatment (Day 242)	77.6	61.0	40.9	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S26: Changes in γ-glutamyl transferase (U/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	73.5	13.7	13.6	18.5	35.2	26.6
2 days after 1st treatment (Day 3)	77.4	13.4	11.8	17.2	38.3	22.8
Prior to the 2nd treatment (Day 15)	88.4	12.6	12.9	14.7	38.9	31.9
2 days after the 2nd treatment (Day 17)	97.9	10.6	11.8	14.7	38.3	25.8
Prior to the 3rd treatment (Day 29)	73.1	13.9	14.2	12.0	77.3	32.3
2 days after the 3rd treatment (Day 31)	83.9	12.2	12.8	11.2	66.3	33.7
Prior to the 4th treatment (Day 60 / Day 90)	92.7	13.9	15.7	14.8	27.5	27.1
2 days after the 4th treatment (Day 62 / Day 92)	89.0	12.9	13.5	11.4	26.0	29.2
Prior to the 5th treatment (Day 120 / Day 150)	110.4	10.9	15.8	16.6	32.4	24.3
2 days after the 5th treatment (Day 122 / Day 152)	103.0	11.5	13.5	14.7	32.8	25.3
Prior to the 6th treatment (Day 180 / Day 210)	107.6	16.2	17.5	13.2	28.7	27.9
2 days after the 6th treatment (Day 182 / Day 212)	105.5	13.9	16.3	14.6	29.6	30.1
Prior to the 7th treatment (Day 240)	88.2	15.6	13.2	-	-	-
2 days after the 7th treatment (Day 242)	90.3	14.5	12.5	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S27: Changes in total bilirubin (μmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	9.94	10.07	8.94	12.41	8.31	11.48
2 days after 1st treatment (Day 3)	8.13	9.38	8.62	16.35	6.39	12.18
Prior to the 2nd treatment (Day 15)	10.14	9.53	8.02	10.66	5.76	8.71
2 days after the 2nd treatment (Day 17)	11.18	8.58	10.99	12.51	6.39	7.68
Prior to the 3rd treatment (Day 29)	9.65	15.02	11.28	8.84	6.90	8.47
2 days after the 3rd treatment (Day 31)	10.40	9.60	10.65	10.66	4.25	9.10
Prior to the 4th treatment (Day 60 / Day 90)	11.92	11.64	8.60	14.90	7.60	10.63
2 days after the 4th treatment (Day 62 / Day 92)	8.04	13.40	9.27	17.87	6.75	5.93
Prior to the 5th treatment (Day 120 / Day 150)	10.91	8.95	8.54	13.98	9.13	9.71
2 days after the 5th treatment (Day 122 / Day 152)	10.46	9.12	9.22	18.82	5.66	6.80
Prior to the 6th treatment (Day 180 / Day 210)	8.75	15.20	9.02	12.41	4.20	7.48
2 days after the 6th treatment (Day 182 / Day 212)	12.07	11.12	10.75	13.97	6.12	5.52
Prior to the 7th treatment (Day 240)	9.22	14.25	7.86	-	-	-
2 days after the 7th treatment (Day 242)	10.48	10.15	8.52	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S28: Changes in direct bilirubin (µmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	2.48	2.66	1.48	2.72	1.47	2.84
2 days after 1st treatment (Day 3)	1.86	2.42	1.70	4.16	1.09	2.68
Prior to the 2nd treatment (Day 15)	2.37	2.65	1.45	2.81	1.17	1.84
2 days after the 2nd treatment (Day 17)	2.88	2.52	2.10	2.98	1.09	1.81
Prior to the 3rd treatment (Day 29)	2.65	3.21	2.34	2.53	1.16	1.61
2 days after the 3rd treatment (Day 31)	2.60	2.60	2.52	2.77	1.02	1.98
Prior to the 4th treatment (Day 60 / Day 90)	3.50	2.98	1.90	3.50	1.12	1.99
2 days after the 4th treatment (Day 62 / Day 92)	1.98	3.60	2.02	4.13	1.18	0.83
Prior to the 5th treatment (Day 120 / Day 150)	2.73	2.04	1.24	3.21	1.87	2.31
2 days after the 5th treatment (Day 122 / Day 152)	2.59	2.15	1.74	4.16	1.08	1.10
Prior to the 6th treatment (Day 180 / Day 210)	2.48	3.80	2.04	3.11	0.56	1.35
2 days after the 6th treatment (Day 182 / Day 212)	2.87	2.68	2.50	3.02	0.92	1.36
Prior to the 7th treatment (Day 240)	2.09	3.35	1.65	-	-	-
2 days after the 7th treatment (Day 242)	2.40	2.41	1.62	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S29: Changes in indirect bilirubin (μmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	7.5	7.4	7.5	9.7	6.8	6.7
2 days after 1st treatment (Day 3)	6.3	7.0	6.9	12.2	5.3	9.5
Prior to the 2nd treatment (Day 15)	7.8	6.9	6.6	7.8	4.6	5.3
2 days after the 2nd treatment (Day 17)	8.3	6.1	8.9	9.5	5.3	5.9
Prior to the 3rd treatment (Day 29)	7.0	11.8	8.9	6.3	5.7	6.9
2 days after the 3rd treatment (Day 31)	7.8	7.0	8.1	7.9	3.2	7.6
Prior to the 4th treatment (Day 60 / Day 90)	8.9	8.7	6.7	11.4	6.5	8.6
2 days after the 4th treatment (Day 62 / Day 92)	6.1	9.8	7.3	13.7	5.6	5.1
Prior to the 5th treatment (Day 120 / Day 150)	8.2	6.9	7.3	10.8	7.3	7.4
2 days after the 5th treatment (Day 122 / Day 152)	7.9	7.0	7.5	14.7	4.6	5.7
Prior to the 6th treatment (Day 180 / Day 210)	6.3	11.4	7.0	9.3	3.6	6.1
2 days after the 6th treatment (Day 182 / Day 212)	9.2	8.4	8.3	10.9	5.2	4.2
Prior to the 7th treatment (Day 240)	7.1	10.9	6.2	-	-	-
2 days after the 7th treatment (Day 242)	8.1	7.7	6.9	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S30: Changes in total bile acids (µmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	3.4	14.1	10.0	7.9	1.4	3.1
2 days after 1st treatment (Day 3)	8.6	3.8	2.8	2.4	8.0	2.1
Prior to the 2nd treatment (Day 15)	3.0	5.7	7.1	16.1	2.0	2.4
2 days after the 2nd treatment (Day 17)	1.9	3.1	3.0	4.7	8.0	5.6
Prior to the 3rd treatment (Day 29)	2.1	4.2	4.3	28.1	2.0	1.1
2 days after the 3rd treatment (Day 31)	2.2	5.7	3.0	4.3	7.8	1.6
Prior to the 4th treatment (Day 60 / Day 90)	1.4	10.8	5.2	2.3	1.5	1.5
2 days after the 4th treatment (Day 62 / Day 92)	3.5	1.1	4.4	2.2	2.4	14.0
Prior to the 5th treatment (Day 120 / Day 150)	2.1	26.6	6.0	4.7	3.4	2.0
2 days after the 5th treatment (Day 122 / Day 152)	2.3	4.8	3.4	6.0	4.1	7.1
Prior to the 6th treatment (Day 180 / Day 210)	2.3	3.2	8.4	3.0	13.2	2.7
2 days after the 6th treatment (Day 182 / Day 212)	1.9	4.5	3.3	6.3	2.4	12.0
Prior to the 7th treatment (Day 240)	1.4	3.0	5.1	-	-	-
2 days after the 7th treatment (Day 242)	2.1	3.9	2.8	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S31: Changes in cholinesterase (U/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	12922	7117	6349	8579	10974	7389
2 days after 1st treatment (Day 3)	13045	7599	5650	7697	10018	6508
Prior to the 2nd treatment (Day 15)	13060	6295	5909	7772	10347	7781
2 days after the 2nd treatment (Day 17)	14327	6442	5671	7703	10018	6713
Prior to the 3rd treatment (Day 29)	11490	7781	5777	7119	11223	7474
2 days after the 3rd treatment (Day 31)	13591	7206	5842	6924	10119	7148
Prior to the 4th treatment (Day 60 / Day 90)	13722	7669	6557	8288	10392	7055
2 days after the 4th treatment (Day 62 / Day 92)	12840	6858	6042	6918	10147	6900
Prior to the 5th treatment (Day 120 / Day 150)	14803	6308	6758	7862	9781	6896
2 days after the 5th treatment (Day 122 / Day 152)	13847	6298	6066	7252	10586	7136
Prior to the 6th treatment (Day 180 / Day 210)	13461	7568	6939	7218	9603	7499
2 days after the 6th treatment (Day 182 / Day 212)	14273	6943	7098	7976	9505	7654
Prior to the 7th treatment (Day 240)	13479	7284	6449	-	-	-
2 days after the 7th treatment (Day 242)	13957	7251	6265	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S32: Changes in creatine kinase of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Creatine kinase(U/L)						
Baseline	453.0	74.0	174.0	102.4	606.1	300.9
2 days after 1st treatment (Day 3)	280.7	74.6	124.9	117.3	260.7	328.0
Prior to the 2nd treatment (Day 15)	237.5	57.2	128.3	87.5	370.0	266.7
2 days after the 2nd treatment (Day 17)	365.2	56.8	184.9	84.8	260.7	197.6
Prior to the 3rd treatment (Day 29)	371.8	87.4	155.2	97.7	279.5	268.4
2 days after the 3rd treatment (Day 31)	227.6	73.2	193.9	102.7	243.4	196.1
Prior to the 4th treatment (Day 60 / Day 90)	299.5	124.5	129.1	84.4	316.1	253.8
2 days after the 4th treatment (Day 62 / Day 92)	290.2	76.5	133.0	70.3	229.2	276.2
Prior to the 5th treatment (Day 120 / Day 150)	641.5	90.1	132.8	83.7	227.2	451.6
2 days after the 5th treatment (Day 122 / Day 152)	253.5	68.3	180.2	76.3	207.3	298.7
Prior to the 6th treatment (Day 180 / Day 210)	251.1	115.3	176.9	82.2	308.5	256.4
2 days after the 6th treatment (Day 182 / Day 212)	204.2	69.4	219.0	81.7	351.7	328.6
Prior to the 7th treatment (Day 240)	483.7	62.3	142.3	-	-	-
2 days after the 7th treatment (Day 242)	219.8	47.9	313.0	-	-	-
Creatine kinase-MB (Mass) (ng/mL)						
Baseline	4.50	2.80	3.30	2.20	4.80	5.70
2 days after 1st treatment (Day 3)	3.70	3.30	2.80	2.20	2.40	2.80
Prior to the 2nd treatment (Day 15)	3.30	2.40	2.60	4.10	4.20	3.50
2 days after the 2nd treatment (Day 17)	3.80	2.20	3.40	2.40	2.40	4.30
Prior to the 3rd treatment (Day 29)	3.60	2.90	2.60	3.90	4.40	4.30
2 days after the 3rd treatment (Day 31)	3.78	2.31	3.30	3.30	3.60	3.00
Prior to the 4th treatment (Day 60 / Day 90)	4.10	3.30	2.65	2.56	5.00	4.30
2 days after the 4th treatment (Day 62 / Day 92)	4.20	1.10	1.40	2.10	2.90	4.20
Prior to the 5th treatment (Day 120 / Day 150)	5.00	3.20	1.70	4.30	4.10	4.60
2 days after the 5th treatment (Day 122 / Day 152)	3.50	3.00	1.40	3.00	3.70	3.90
Prior to the 6th treatment (Day 180 / Day 210)	3.10	1.81	3.20	3.30	4.40	3.60
2 days after the 6th treatment (Day 182 / Day 212)	2.50	2.00	2.90	4.50	5.10	4.00
Prior to the 7th treatment (Day 240)	3.40	2.40	3.30	-	-	-
2 days after the 7th treatment (Day 242)	2.90	2.00	3.80	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S33: Changes in lactate dehydrogenase (U/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	201.6	210.1	172.3	160.5	245.7	218.3
2 days after 1st treatment (Day 3)	185.0	202.3	175.5	157.7	256.1	142.0
Prior to the 2nd treatment (Day 15)	198.1	189.4	171.6	156.3	196.4	184.9
2 days after the 2nd treatment (Day 17)	215.1	159.8	151.7	185.8	256.1	148.5
Prior to the 3rd treatment (Day 29)	229.1	189.1	161.8	166.6	210.8	175.3
2 days after the 3rd treatment (Day 31)	267.4	187.9	174.1	156.8	201.2	170.9
Prior to the 4th treatment (Day 60 / Day 90)	212.6	211.3	211.3	183.6	292.7	179.9
2 days after the 4th treatment (Day 62 / Day 92)	186.4	173.0	184.0	186.4	226.3	193.3
Prior to the 5th treatment (Day 120 / Day 150)	227.1	186.9	210.6	188.9	211.5	188.7
2 days after the 5th treatment (Day 122 / Day 152)	203.0	218.8	180.0	156.2	203.9	190.3
Prior to the 6th treatment (Day 180 / Day 210)	238.7	207.1	248.8	168.4	247.6	171.2
2 days after the 6th treatment (Day 182 / Day 212)	235.7	198.7	240.2	185.3	226.0	174.6
Prior to the 7th treatment (Day 240)	269.5	197.0	217.9	-	-	-
2 days after the 7th treatment (Day 242)	290.3	171.9	190.2	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S34: Changes in α-hydroxybutyrate dehydrogenase (U/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	137.2	156.2	140.1	122.0	178.3	162.7
2 days after 1st treatment (Day 3)	128.8	152.4	140.9	121.2	192.9	118.7
Prior to the 2nd treatment (Day 15)	136.7	148.4	135.8	117.2	145.5	135.6
2 days after the 2nd treatment (Day 17)	145.7	127.5	125.4	140.3	156.2	114.3
Prior to the 3rd treatment (Day 29)	156.0	157.7	133.5	122.5	156.2	131.4
2 days after the 3rd treatment (Day 31)	181.2	153.9	139.3	117.1	148.2	127.7
Prior to the 4th treatment (Day 60 / Day 90)	154.5	176.4	163.5	152.8	235.9	140.5
2 days after the 4th treatment (Day 62 / Day 92)	133.9	144.0	146.2	149.1	180.9	141.6
Prior to the 5th treatment (Day 120 / Day 150)	152.8	151.3	164.7	146.3	155.4	138.2
2 days after the 5th treatment (Day 122 / Day 152)	138.2	176.2	141.5	118.1	154.5	142.5
Prior to the 6th treatment (Day 180 / Day 210)	160.6	160.2	187.2	130.0	186.3	129.4
2 days after the 6th treatment (Day 182 / Day 212)	162.7	160.6	184.8	145.8	175.0	133.9
Prior to the 7th treatment (Day 240)	200.3	160.3	163.8	-	-	-
2 days after the 7th treatment (Day 242)	211.6	143.1	142.3	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S35: Changes in triglycerides (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	2.36	0.47	1.66	1.19	2.04	0.81
2 days after 1st treatment (Day 3)	4.17	0.77	1.22	0.69	1.69	0.91
Prior to the 2nd treatment (Day 15)	2.75	0.59	1.24	1.10	2.51	1.01
2 days after the 2nd treatment (Day 17)	2.99	1.05	1.76	0.82	1.69	1.33
Prior to the 3rd treatment (Day 29)	2.19	0.56	0.83	1.40	2.05	0.54
2 days after the 3rd treatment (Day 31)	3.81	1.05	1.13	0.91	1.78	0.87
Prior to the 4th treatment (Day 60 / Day 90)	1.91	1.00	1.15	0.66	1.52	0.88
2 days after the 4th treatment (Day 62 / Day 92)	2.80	0.54	1.98	0.62	2.13	1.78
Prior to the 5th treatment (Day 120 / Day 150)	2.22	0.38	1.44	0.87	1.26	0.56
2 days after the 5th treatment (Day 122 / Day 152)	2.54	0.56	1.45	0.73	1.70	1.89
Prior to the 6th treatment (Day 180 / Day 210)	2.47	0.33	1.48	0.65	2.66	0.72
2 days after the 6th treatment (Day 182 / Day 212)	2.64	0.43	1.68	0.68	2.54	1.70
Prior to the 7th treatment (Day 240)	2.46	0.37	1.39	-	-	-
2 days after the 7th treatment (Day 242)	2.41	0.89	2.21	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S36: Changes in total cholesterol (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	5.57	4.39	5.93	6.28	8.70	5.45
2 days after 1st treatment (Day 3)	5.50	4.41	5.47	5.75	7.99	4.69
Prior to the 2nd treatment (Day 15)	5.36	4.00	5.92	5.49	7.65	5.53
2 days after the 2nd treatment (Day 17)	5.65	4.19	5.70	5.38	7.99	4.63
Prior to the 3rd treatment (Day 29)	4.79	5.56	5.45	4.71	8.32	5.44
2 days after the 3rd treatment (Day 31)	5.92	4.69	5.06	4.77	6.89	5.11
Prior to the 4th treatment (Day 60 / Day 90)	5.99	5.07	6.17	6.09	7.76	5.50
2 days after the 4th treatment (Day 62 / Day 92)	6.03	4.42	4.88	5.40	6.86	5.07
Prior to the 5th treatment (Day 120 / Day 150)	6.05	4.24	6.16	5.47	6.86	4.94
2 days after the 5th treatment (Day 122 / Day 152)	5.80	4.14	5.40	5.15	6.33	5.10
Prior to the 6th treatment (Day 180 / Day 210)	5.88	4.82	5.48	5.14	6.25	4.88
2 days after the 6th treatment (Day 182 / Day 212)	6.42	4.45	5.71	5.59	6.18	4.75
Prior to the 7th treatment (Day 240)	5.72	4.87	5.07	-	-	-
2 days after the 7th treatment (Day 242)	6.30	4.50	5.11	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S37: Changes in high-density lipoprotein cholesterol (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	0.98	1.51	1.72	1.52	1.96	1.88
2 days after 1st treatment (Day 3)	0.92	1.62	1.59	1.49	1.82	1.47
Prior to the 2nd treatment (Day 15)	0.96	1.67	1.80	1.28	1.66	1.63
2 days after the 2nd treatment (Day 17)	0.98	1.35	1.59	1.35	1.77	1.34
Prior to the 3rd treatment (Day 29)	0.91	1.80	1.72	1.23	1.91	1.62
2 days after the 3rd treatment (Day 31)	0.98	1.57	1.58	1.22	1.61	1.57
Prior to the 4th treatment (Day 60 / Day 90)	1.15	1.61	1.95	1.63	1.74	1.60
2 days after the 4th treatment (Day 62 / Day 92)	1.00	1.57	1.43	1.42	1.60	1.51
Prior to the 5th treatment (Day 120 / Day 150)	1.10	1.65	1.86	1.45	1.76	1.50
2 days after the 5th treatment (Day 122 / Day 152)	1.02	1.54	1.58	1.40	1.61	1.48
Prior to the 6th treatment (Day 180 / Day 210)	1.09	2.39	1.68	1.34	1.35	1.44
2 days after the 6th treatment (Day 182 / Day 212)	1.11	1.99	1.53	1.48	1.37	1.44
Prior to the 7th treatment (Day 240)	1.08	1.91	1.55	-	-	-
2 days after the 7th treatment (Day 242)	1.15	1.69	1.42	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S38: Changes in low-density lipoprotein cholesterol (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	3.89	2.71	3.99	4.61	6.85	3.51
2 days after 1st treatment (Day 3)	3.86	2.48	3.59	4.10	6.29	3.12
Prior to the 2nd treatment (Day 15)	3.89	2.01	4.03	4.10	5.93	3.97
2 days after the 2nd treatment (Day 17)	4.26	2.49	3.84	3.81	6.29	3.14
Prior to the 3rd treatment (Day 29)	3.54	3.59	3.67	3.41	6.53	3.74
2 days after the 3rd treatment (Day 31)	4.36	3.02	3.34	3.29	5.06	3.39
Prior to the 4th treatment (Day 60 / Day 90)	4.50	3.11	4.26	4.48	6.05	3.71
2 days after the 4th treatment (Day 62 / Day 92)	4.64	2.54	3.03	3.63	5.14	3.20
Prior to the 5th treatment (Day 120 / Day 150)	4.69	2.37	4.08	3.72	4.95	3.12
2 days after the 5th treatment (Day 122 / Day 152)	4.54	2.39	3.55	3.41	4.26	3.15
Prior to the 6th treatment (Day 180 / Day 210)	4.60	2.34	3.52	3.38	4.30	3.16
2 days after the 6th treatment (Day 182 / Day 212)	5.10	2.23	3.84	3.70	4.09	2.93
Prior to the 7th treatment (Day 240)	4.41	2.65	3.07	-	-	-
2 days after the 7th treatment (Day 242)	4.87	2.57	3.11	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S39: Changes in apolipoprotein of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Apolipoprotein A1 (g/L)						
Baseline	1.17	1.30	1.61	1.55	1.88	1.67
2 days after 1st treatment (Day 3)	1.07	1.48	1.43	1.47	1.77	1.44
Prior to the 2nd treatment (Day 15)	1.12	1.41	1.63	1.35	1.67	1.59
2 days after the 2nd treatment (Day 17)	1.15	1.41	1.48	1.34	1.77	1.29
Prior to the 3rd treatment (Day 29)	1.07	1.47	1.62	1.34	1.86	1.60
2 days after the 3rd treatment (Day 31)	1.17	1.44	1.61	1.31	1.66	1.53
Prior to the 4th treatment (Day 60 / Day 90)	1.26	1.41	1.74	1.60	1.74	1.61
2 days after the 4th treatment (Day 62 / Day 92)	1.12	1.32	1.60	1.34	1.69	1.61
Prior to the 5th treatment (Day 120 / Day 150)	1.16	1.44	1.79	1.64	1.81	1.52
2 days after the 5th treatment (Day 122 / Day 152)	1.11	1.51	1.63	1.55	1.81	1.50
Prior to the 6th treatment (Day 180 / Day 210)	1.25	1.86	1.81	1.44	1.54	1.50
2 days after the 6th treatment (Day 182 / Day 212)	1.23	1.66	1.74	1.60	1.57	1.60
Prior to the 7th treatment (Day 240)	1.32	1.71	1.64	-	-	-
2 days after the 7th treatment (Day 242)	1.34	1.59	1.65	-	-	-
Apolipoprotein B (g/L)						
Baseline	1.16	0.77	0.91	1.31	1.81	0.88
2 days after 1st treatment (Day 3)	1.18	0.70	0.85	1.15	1.74	0.78
Prior to the 2nd treatment (Day 15)	1.19	0.61	0.96	1.15	1.46	0.99
2 days after the 2nd treatment (Day 17)	1.27	0.69	1.05	1.02	1.74	0.81
Prior to the 3rd treatment (Day 29)	1.07	0.83	0.95	0.92	1.55	0.94
2 days after the 3rd treatment (Day 31)	1.13	0.68	0.89	0.93	1.37	0.86
Prior to the 4th treatment (Day 60 / Day 90)	1.09	0.74	1.10	1.18	1.47	0.95
2 days after the 4th treatment (Day 62 / Day 92)	1.10	0.67	0.66	1.01	1.35	0.85
Prior to the 5th treatment (Day 120 / Day 150)	1.13	0.70	1.03	1.08	1.31	0.83
2 days after the 5th treatment (Day 122 / Day 152)	1.30	0.66	0.94	1.00	1.32	0.83
Prior to the 6th treatment (Day 180 / Day 210)	1.26	0.66	0.97	0.95	1.24	0.84
2 days after the 6th treatment (Day 182 / Day 212)	1.35	0.65	1.07	1.02	1.24	0.82
Prior to the 7th treatment (Day 240)	1.22	0.77	0.92	-	-	-
2 days after the 7th treatment (Day 242)	1.33	0.68	0.95	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S40: Changes in urea (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	5.0	6.7	5.2	5.7	4.3	4.6
2 days after 1st treatment (Day 3)	5.3	2.4	3.7	5.7	4.6	4.2
Prior to the 2nd treatment (Day 15)	4.9	4.0	5.5	5.3	5.3	6.4
2 days after the 2nd treatment (Day 17)	5.5	2.8	4.3	4.8	4.6	5.7
Prior to the 3rd treatment (Day 29)	4.7	4.7	6.0	5.2	4.4	5.9
2 days after the 3rd treatment (Day 31)	4.4	4.7	3.8	5.8	4.8	5.7
Prior to the 4th treatment (Day 60 / Day 90)	5.2	3.9	5.4	3.7	4.6	5.6
2 days after the 4th treatment (Day 62 / Day 92)	5.3	2.4	2.9	4.1	3.3	6.7
Prior to the 5th treatment (Day 120 / Day 150)	4.9	3.2	5.8	4.8	4.7	5.2
2 days after the 5th treatment (Day 122 / Day 152)	4.0	4.6	3.6	3.7	4.3	7.7
Prior to the 6th treatment (Day 180 / Day 210)	4.2	4.8	5.6	5.1	6.4	5.6
2 days after the 6th treatment (Day 182 / Day 212)	5.4	3.5	3.5	6.0	3.8	5.3
Prior to the 7th treatment (Day 240)	4.1	3.9	4.4	-	-	-
2 days after the 7th treatment (Day 242)	4.7	4.2	2.5	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S41: Changes in uric acid (μmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	559.9	266.6	191.6	353.0	204.2	342.7
2 days after 1st treatment (Day 3)	545.1	225.8	210.0	320.3	241.1	301.7
Prior to the 2nd treatment (Day 15)	598.3	307.7	214.9	328.6	276.6	310.2
2 days after the 2nd treatment (Day 17)	625.6	321.7	236.9	282.8	241.1	345.7
Prior to the 3rd treatment (Day 29)	606.5	304.6	198.1	336.1	245.0	341.7
2 days after the 3rd treatment (Day 31)	582.4	290.6	217.9	325.5	274.3	281.4
Prior to the 4th treatment (Day 60 / Day 90)	643.0	270.6	211.4	366.6	217.1	253.0
2 days after the 4th treatment (Day 62 / Day 92)	612.5	262.7	260.8	345.2	212.5	254.5
Prior to the 5th treatment (Day 120 / Day 150)	655.9	263.2	193.6	291.1	197.2	225.5
2 days after the 5th treatment (Day 122 / Day 152)	653.8	264.6	211.4	301.3	241.3	234.8
Prior to the 6th treatment (Day 180 / Day 210)	611.8	316.8	229.7	324.2	214.8	331.7
2 days after the 6th treatment (Day 182 / Day 212)	693.0	288.8	292.5	331.9	208.0	309.4
Prior to the 7th treatment (Day 240)	579.2	297.2	219.7	-	-	-
2 days after the 7th treatment (Day 242)	651.2	257.6	246.4	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S42: Changes in creatinine (μmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	57.4	21.6	26.5	29.5	31.3	73.1
2 days after 1st treatment (Day 3)	58.0	24.2	28.7	29.5	32.3	70.8
Prior to the 2nd treatment (Day 15)	53.8	29.2	27.3	29.7	34.8	73.1
2 days after the 2nd treatment (Day 17)	65.0	23.2	27.5	27.1	32.3	71.6
Prior to the 3rd treatment (Day 29)	55.2	26.6	27.1	27.7	30.3	68.9
2 days after the 3rd treatment (Day 31)	53.8	25.9	32.0	25.6	28.7	74.8
Prior to the 4th treatment (Day 60 / Day 90)	55.3	29.0	24.2	22.4	30.2	70.2
2 days after the 4th treatment (Day 62 / Day 92)	52.3	30.9	29.9	23.2	33.0	71.3
Prior to the 5th treatment (Day 120 / Day 150)	57.8	26.8	22.4	21.1	31.4	63.7
2 days after the 5th treatment (Day 122 / Day 152)	54.1	26.8	22.9	21.9	30.7	66.8
Prior to the 6th treatment (Day 180 / Day 210)	52.8	31.6	26.9	23.5	40.9	78.5
2 days after the 6th treatment (Day 182 / Day 212)	60.9	26.9	32.8	22.7	27.7	75.1
Prior to the 7th treatment (Day 240)	55.2	27.3	22.7	-	-	-
2 days after the 7th treatment (Day 242)	61.0	32.3	22.3	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S43: Changes in estimated glomerular filtration rate (mL/min) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	142.66	182.67	150.50	123.61	131.89	113.03
2 days after 1st treatment (Day 3)	142.05	175.97	146.61	123.61	130.53	114.52
Prior to the 2nd treatment (Day 15)	146.51	165.43	149.04	123.34	127.37	113.03
2 days after the 2nd treatment (Day 17)	135.56	178.43	148.68	127.11	130.53	114.00
Prior to the 3rd treatment (Day 29)	144.97	170.58	149.40	126.20	133.30	115.81
2 days after the 3rd treatment (Day 31)	146.51	172.08	141.45	129.52	135.71	111.97
Prior to the 4th treatment (Day 60 / Day 90)	144.87	165.80	155.07	135.33	133.45	114.93
2 days after the 4th treatment (Day 62 / Day 92)	148.22	162.37	144.64	133.78	129.61	114.19
Prior to the 5th treatment (Day 120 / Day 150)	142.26	168.97	159.06	137.05	131.75	119.61
2 days after the 5th treatment (Day 122 / Day 152)	146.18	168.97	157.91	135.39	132.73	117.29
Prior to the 6th treatment (Day 180 / Day 210)	146.61	160.05	148.72	132.28	119.93	109.77
2 days after the 6th treatment (Day 182 / Day 212)	138.26	168.76	139.32	133.80	136.34	111.78
Prior to the 7th treatment (Day 240)	143.96	167.94	157.26	-	-	-
2 days after the 7th treatment (Day 242)	138.17	158.90	158.18	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S44: Changes in homocysteine (µmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	9.78	7.72	5.16	13.51	8.36	7.56
2 days after 1st treatment (Day 3)	11.04	6.55	4.28	11.03	7.27	7.83
Prior to the 2nd treatment (Day 15)	12.04	8.85	4.68	10.91	8.61	9.84
2 days after the 2nd treatment (Day 17)	13.36	6.05	5.55	12.49	7.27	7.27
Prior to the 3rd treatment (Day 29)	11.17	9.52	5.68	11.92	9.31	9.36
2 days after the 3rd treatment (Day 31)	12.37	9.24	5.60	12.49	9.37	8.52
Prior to the 4th treatment (Day 60 / Day 90)	15.9	9.16	6.10	12.66	8.33	6.90
2 days after the 4th treatment (Day 62 / Day 92)	12.39	7.07	8.19	10.06	8.26	6.67
Prior to the 5th treatment (Day 120 / Day 150)	13.44	7.61	6.77	11.30	8.38	7.65
2 days after the 5th treatment (Day 122 / Day 152)	11.70	8.74	7.26	10.21	9.32	6.24
Prior to the 6th treatment (Day 180 / Day 210)	12.39	10.35	6.69	12.30	9.24	7.47
2 days after the 6th treatment (Day 182 / Day 212)	14.15	10.31	8.10	13.42	8.46	7.53
Prior to the 7th treatment (Day 240)	13.43	9.75	5.83	-	-	-
2 days after the 7th treatment (Day 242)	14.09	9.59	6.40	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S45: Changes in glucose (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	5.20	4.87	4.85	6.72	5.04	5.21
2 days after 1st treatment (Day 3)	6.75	4.98	4.67	4.78	5.20	4.93
Prior to the 2nd treatment (Day 15)	5.31	4.85	4.89	5.12	4.78	4.79
2 days after the 2nd treatment (Day 17)	4.56	4.99	4.70	5.06	5.20	6.04
Prior to the 3rd treatment (Day 29)	5.03	4.50	4.74	5.29	4.99	5.37
2 days after the 3rd treatment (Day 31)	6.91	4.38	4.86	4.93	4.75	5.08
Prior to the 4th treatment (Day 60 / Day 90)	4.69	4.80	4.99	4.95	5.18	5.20
2 days after the 4th treatment (Day 62 / Day 92)	6.35	5.09	4.85	5.39	5.38	5.51
Prior to the 5th treatment (Day 120 / Day 150)	4.85	4.88	4.88	5.16	5.14	5.50
2 days after the 5th treatment (Day 122 / Day 152)	4.38	4.54	4.94	6.47	5.11	4.58
Prior to the 6th treatment (Day 180 / Day 210)	4.72	4.91	4.87	4.74	5.04	5.45
2 days after the 6th treatment (Day 182 / Day 212)	4.72	4.66	5.07	5.11	4.92	5.10
Prior to the 7th treatment (Day 240)	4.55	4.52	5.06	-	-	-
2 days after the 7th treatment (Day 242)	4.69	4.33	5.04	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S46: Changes in total carbon dioxide (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	24.0	25.0	24.0	22.0	25.0	25.0
2 days after 1st treatment (Day 3)	27.0	26.0	26.0	23.0	26.0	26.0
Prior to the 2nd treatment (Day 15)	25.0	27.0	25.0	22.0	26.0	24.0
2 days after the 2nd treatment (Day 17)	25.0	25.0	26.0	23.0	26.0	24.0
Prior to the 3rd treatment (Day 29)	23.0	26.0	21.0	22.0	25.0	26.0
2 days after the 3rd treatment (Day 31)	25.4	24.1	25.0	25.0	26.0	28.0
Prior to the 4th treatment (Day 60 / Day 90)	24.0	27.0	23.6	23.1	25.0	26.0
2 days after the 4th treatment (Day 62 / Day 92)	24.0	27.5	25.0	23.0	30.0	26.0
Prior to the 5th treatment (Day 120 / Day 150)	25.0	24.0	24.0	24.0	28.0	23.0
2 days after the 5th treatment (Day 122 / Day 152)	26.0	27.0	24.0	22.0	27.0	24.0
Prior to the 6th treatment (Day 180 / Day 210)	22.0	25.0	26.0	23.0	24.0	23.0
2 days after the 6th treatment (Day 182 / Day 212)	25.0	26.0	26.0	24.0	26.0	27.0
Prior to the 7th treatment (Day 240)	27.0	24.0	22.0	-	-	-
2 days after the 7th treatment (Day 242)	27.0	29.0	26.0	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S47: Changes in total calcium (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	2.36	2.35	2.29	2.38	2.58	2.42
2 days after 1st treatment (Day 3)	2.44	2.47	2.22	2.36	2.49	2.31
Prior to the 2nd treatment (Day 15)	2.41	2.30	2.25	2.35	2.48	2.31
2 days after the 2nd treatment (Day 17)	2.55	2.28	2.31	2.42	2.49	2.23
Prior to the 3rd treatment (Day 29)	2.25	2.52	2.29	2.30	2.52	2.28
2 days after the 3rd treatment (Day 31)	2.46	2.48	2.30	2.33	2.36	2.34
Prior to the 4th treatment (Day 60 / Day 90)	2.53	2.57	2.40	2.46	2.35	2.27
2 days after the 4th treatment (Day 62 / Day 92)	2.47	2.47	2.38	2.30	2.47	2.30
Prior to the 5th treatment (Day 120 / Day 150)	2.48	2.36	2.26	2.39	2.44	2.31
2 days after the 5th treatment (Day 122 / Day 152)	2.41	2.36	2.24	2.29	2.51	2.31
Prior to the 6th treatment (Day 180 / Day 210)	2.48	2.42	2.31	2.31	2.40	2.28
2 days after the 6th treatment (Day 182 / Day 212)	2.56	2.29	2.33	2.40	2.40	2.40
Prior to the 7th treatment (Day 240)	2.46	2.28	2.29	-	-	-
2 days after the 7th treatment (Day 242)	2.47	2.37	2.30	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S48: Changes in sodium (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	141.7	140.8	140.1	141.3	141.3	141.6
2 days after 1st treatment (Day 3)	142.4	140.9	141.4	144.3	142.2	142.0
Prior to the 2nd treatment (Day 15)	141.8	143.5	140.2	141.8	139.5	139.2
2 days after the 2nd treatment (Day 17)	142.3	143.1	140.0	142.2	142.2	141.6
Prior to the 3rd treatment (Day 29)	140.6	139.3	140.8	141.0	141.8	141.3
2 days after the 3rd treatment (Day 31)	140.7	139.0	140.9	142.5	142.0	144.2
Prior to the 4th treatment (Day 60 / Day 90)	142.0	138.8	140.0	141.6	141.0	141.7
2 days after the 4th treatment (Day 62 / Day 92)	140.5	139.1	143.2	141.4	142.4	144.7
Prior to the 5th treatment (Day 120 / Day 150)	142.4	141.3	141.8	139.8	141.9	143.0
2 days after the 5th treatment (Day 122 / Day 152)	140.2	140.8	141.8	138.4	141.0	143.6
Prior to the 6th treatment (Day 180 / Day 210)	143.2	138.4	140.8	141.3	139.7	140.8
2 days after the 6th treatment (Day 182 / Day 212)	143.8	142.4	143.0	140.6	141.4	141.6
Prior to the 7th treatment (Day 240)	144.8	139.6	141.0	-	-	-
2 days after the 7th treatment (Day 242)	146.1	143.6	142.6	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S49: Changes in potassium (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	4.36	3.43	3.62	3.74	4.27	4.01
2 days after 1st treatment (Day 3)	4.12	4.11	3.33	3.96	4.49	4.16
Prior to the 2nd treatment (Day 15)	4.23	3.45	4.50	3.91	4.68	3.90
2 days after the 2nd treatment (Day 17)	5.13	3.87	4.07	4.30	4.49	3.92
Prior to the 3rd treatment (Day 29)	4.00	3.56	3.88	3.61	4.43	4.10
2 days after the 3rd treatment (Day 31)	3.86	3.68	3.94	3.81	4.35	4.26
Prior to the 4th treatment (Day 60 / Day 90)	4.36	4.12	3.67	4.24	4.36	4.15
2 days after the 4th treatment (Day 62 / Day 92)	4.07	3.78	3.81	3.61	5.10	4.06
Prior to the 5th treatment (Day 120 / Day 150)	4.43	3.43	3.73	4.12	4.60	3.91
2 days after the 5th treatment (Day 122 / Day 152)	4.22	3.68	3.79	3.79	4.68	3.86
Prior to the 6th treatment (Day 180 / Day 210)	4.36	3.82	3.68	3.77	5.14	4.24
2 days after the 6th treatment (Day 182 / Day 212)	4.53	3.67	3.96	4.18	4.26	4.17
Prior to the 7th treatment (Day 240)	4.58	3.82	3.51	-	-	-
2 days after the 7th treatment (Day 242)	4.80	3.82	3.59	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S50: Changes in chloride (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	104.7	105.1	106.5	106.0	102.7	106.0
2 days after 1st treatment (Day 3)	104.2	103.8	106.6	106.9	105.0	107.1
Prior to the 2nd treatment (Day 15)	104.2	106.0	106.2	107.4	102.2	104.8
2 days after the 2nd treatment (Day 17)	103.7	106.6	105.7	106.7	105.0	106.5
Prior to the 3rd treatment (Day 29)	105.1	102.2	107.2	107.7	103.4	106.0
2 days after the 3rd treatment (Day 31)	103.8	101.6	104.9	107.6	104.4	108.3
Prior to the 4th treatment (Day 60 / Day 90)	104.5	101.9	105.8	105.0	105.0	107.9
2 days after the 4th treatment (Day 62 / Day 92)	102.5	103.5	105.3	104.9	104.2	110.3
Prior to the 5th treatment (Day 120 / Day 150)	105.9	104.6	104.8	104.9	104.9	109.5
2 days after the 5th treatment (Day 122 / Day 152)	103.5	102.7	104.1	103.9	102.6	108.9
Prior to the 6th treatment (Day 180 / Day 210)	106.3	100.8	105.9	107.2	102.8	107.6
2 days after the 6th treatment (Day 182 / Day 212)	104.9	104.4	105.9	105.9	103.7	106.1
Prior to the 7th treatment (Day 240)	106.7	105.4	105.2	-	-	-
2 days after the 7th treatment (Day 242)	106.3	105.4	106.0	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S51: Changes in inorganic phosphate (mmol/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	1.28	1.20	1.08	1.28	1.28	1.09
2 days after 1st treatment (Day 3)	1.29	1.04	1.24	1.28	1.18	1.08
Prior to the 2nd treatment (Day 15)	1.40	1.14	1.18	1.23	1.25	0.94
2 days after the 2nd treatment (Day 17)	1.35	1.29	1.37	1.40	1.18	0.78
Prior to the 3rd treatment (Day 29)	1.25	1.38	1.17	1.14	1.37	0.95
2 days after the 3rd treatment (Day 31)	1.13	1.32	1.39	1.30	1.04	1.07
Prior to the 4th treatment (Day 60 / Day 90)	1.16	1.25	1.28	1.39	1.19	0.94
2 days after the 4th treatment (Day 62 / Day 92)	1.07	1.21	1.47	1.06	0.93	0.93
Prior to the 5th treatment (Day 120 / Day 150)	1.34	1.02	1.19	1.46	1.16	0.88
2 days after the 5th treatment (Day 122 / Day 152)	1.23	1.17	1.27	1.23	1.15	0.87
Prior to the 6th treatment (Day 180 / Day 210)	1.20	1.23	1.19	1.31	1.29	0.89
2 days after the 6th treatment (Day 182 / Day 212)	1.31	1.19	1.41	1.37	1.18	0.99
Prior to the 7th treatment (Day 240)	1.30	1.13	1.05	-	-	-
2 days after the 7th treatment (Day 242)	1.30	1.32	1.11	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S52: Changes in prothrombin time (sec) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	11.20	11.60	10.60	11.90	10.30	11.40
2 days after 1st treatment (Day 3)	11.90	11.40	11.70	11.80	10.10	11.70
Prior to the 2nd treatment (Day 15)	11.60	11.60	11.10	12.00	10.10	10.90
2 days after the 2nd treatment (Day 17)	12.00	11.00	11.20	12.00	10.10	11.80
Prior to the 3rd treatment (Day 29)	10.80	11.90	11.50	12.10	10.10	11.40
2 days after the 3rd treatment (Day 31)	11.90	12.00	11.30	12.20	10.10	11.40
Prior to the 4th treatment (Day 60 / Day 90)	11.60	12.20	10.90	11.90	10.70	11.60
2 days after the 4th treatment (Day 62 / Day 92)	12.20	11.70	10.90	12.50	10.20	10.90
Prior to the 5th treatment (Day 120 / Day 150)	11.90	11.50	11.20	12.10	10.40	11.50
2 days after the 5th treatment (Day 122 / Day 152)	12.10	11.00	10.60	12.00	10.00	11.80
Prior to the 6th treatment (Day 180 / Day 210)	11.30	10.90	10.40	12.40	10.40	12.10
2 days after the 6th treatment (Day 182 / Day 212)	12.00	11.20	11.30	11.80	10.70	10.90
Prior to the 7th treatment (Day 240)	11.50	11.10	11.10	-	-	-
2 days after the 7th treatment (Day 242)	11.50	11.40	11.30	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S53: Changes in thrombin time (sec) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	13.0	16.2	17.1	14.9	14.2	16.0
2 days after 1st treatment (Day 3)	12.7	13.8	16.9	14.1	13.9	17.4
Prior to the 2nd treatment (Day 15)	12.9	15.1	16.1	14.6	14.8	16.4
2 days after the 2nd treatment (Day 17)	12.8	14.2	15.7	14.7	13.9	16.0
Prior to the 3rd treatment (Day 29)	13.5	15.1	16.3	14.8	13.8	15.6
2 days after the 3rd treatment (Day 31)	12.3	13.5	15.8	14.4	14.1	15.3
Prior to the 4th treatment (Day 60 / Day 90)	12.9	14.6	15.9	14.6	14.7	17.1
2 days after the 4th treatment (Day 62 / Day 92)	12.4	15.0	14.7	14.4	14.0	15.8
Prior to the 5th treatment (Day 120 / Day 150)	13.0	15.8	15.3	14.8	13.9	16.2
2 days after the 5th treatment (Day 122 / Day 152)	13.3	13.7	15.3	13.6	13.1	16.9
Prior to the 6th treatment (Day 180 / Day 210)	13.9	16.3	15.9	14.9	15.0	16.1
2 days after the 6th treatment (Day 182 / Day 212)	13.5	16.0	15.9	12.9	14.0	15.3
Prior to the 7th treatment (Day 240)	13.6	14.8	15.5	-	-	-
2 days after the 7th treatment (Day 242)	12.8	15.3	15.5	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S54: Changes in activated partial thromboplastin time (sec) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	33.00	29.80	31.30	32.80	28.10	32.70
2 days after 1st treatment (Day 3)	34.50	30.80	31.30	31.30	24.40	29.70
Prior to the 2nd treatment (Day 15)	32.40	29.10	32.60	29.70	26.50	30.80
2 days after the 2nd treatment (Day 17)	33.60	29.20	31.80	31.20	24.40	30.30
Prior to the 3rd treatment (Day 29)	31.70	31.30	31.30	32.50	27.90	29.30
2 days after the 3rd treatment (Day 31)	33.20	29.10	29.90	30.10	26.20	29.90
Prior to the 4th treatment (Day 60 / Day 90)	32.50	31.50	32.20	31.70	26.40	30.10
2 days after the 4th treatment (Day 62 / Day 92)	33.20	29.80	31.70	28.60	27.00	29.60
Prior to the 5th treatment (Day 120 / Day 150)	33.90	29.80	31.20	32.40	27.10	30.70
2 days after the 5th treatment (Day 122 / Day 152)	32.70	29.70	30.00	29.50	27.60	29.30
Prior to the 6th treatment (Day 180 / Day 210)	34.00	29.10	30.70	32.40	26.50	30.70
2 days after the 6th treatment (Day 182 / Day 212)	33.50	28.90	31.40	30.00	28.40	31.10
Prior to the 7th treatment (Day 240)	34.40	29.30	30.60	-	-	-
2 days after the 7th treatment (Day 242)	33.20	30.30	29.90	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S55: Changes in international normalized ratio of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	0.98	1.02	0.93	1.04	0.94	1.04
2 days after 1st treatment (Day 3)	1.04	1.00	1.03	1.04	0.92	1.06
Prior to the 2nd treatment (Day 15)	1.02	1.02	0.97	1.05	0.92	0.99
2 days after the 2nd treatment (Day 17)	1.05	0.97	0.98	1.09	0.92	1.07
Prior to the 3rd treatment (Day 29)	0.95	1.04	1.01	1.10	0.89	1.04
2 days after the 3rd treatment (Day 31)	1.04	1.05	0.99	1.11	0.89	1.04
Prior to the 4th treatment (Day 60 / Day 90)	1.02	1.07	0.99	1.08	0.97	1.05
2 days after the 4th treatment (Day 62 / Day 92)	1.07	1.03	0.99	1.10	0.93	0.99
Prior to the 5th treatment (Day 120 / Day 150)	1.04	1.01	1.02	1.10	0.95	1.03
2 days after the 5th treatment (Day 122 / Day 152)	1.06	0.97	0.96	1.09	0.91	1.06
Prior to the 6th treatment (Day 180 / Day 210)	1.03	0.99	0.95	1.11	0.93	1.08
2 days after the 6th treatment (Day 182 / Day 212)	1.09	1.02	1.03	1.06	0.95	0.97
Prior to the 7th treatment (Day 240)	1.05	1.01	0.99	-	-	-
2 days after the 7th treatment (Day 242)	1.05	1.04	1.01	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S56: Changes in fibrinogen (g/L) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	3.81	2.13	2.19	3.23	3.74	2.59
2 days after 1st treatment (Day 3)	4.32	2.76	2.44	3.71	4.20	2.24
Prior to the 2nd treatment (Day 15)	3.55	2.42	2.62	3.00	3.32	2.80
2 days after the 2nd treatment (Day 17)	4.32	2.90	2.52	3.64	4.20	2.67
Prior to the 3rd treatment (Day 29)	3.44	2.52	2.39	3.28	3.71	2.48
2 days after the 3rd treatment (Day 31)	4.07	2.77	2.40	3.32	3.54	3.21
Prior to the 4th treatment (Day 60 / Day 90)	4.47	2.70	2.54	3.45	3.33	2.45
2 days after the 4th treatment (Day 62 / Day 92)	5.92	2.90	3.04	3.50	3.84	2.69
Prior to the 5th treatment (Day 120 / Day 150)	4.05	2.58	2.57	3.45	3.29	2.41
2 days after the 5th treatment (Day 122 / Day 152)	3.85	3.07	2.54	3.70	3.75	2.83
Prior to the 6th treatment (Day 180 / Day 210)	3.75	2.41	2.30	2.82	3.47	2.32
2 days after the 6th treatment (Day 182 / Day 212)	4.04	2.36	2.95	3.32	3.89	3.25
Prior to the 7th treatment (Day 240)	3.80	2.80	2.89	-	-	-
2 days after the 7th treatment (Day 242)	4.39	3.25	2.83	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S57: Changes in fibrin degradation products (µg/mL) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	0.91	1.27	0.96	1.49	1.50	1.20
2 days after 1st treatment (Day 3)	0.63	0.64	1.50	2.02	1.10	1.19
Prior to the 2nd treatment (Day 15)	0.91	0.90	1.50	1.92	0.56	1.11
2 days after the 2nd treatment (Day 17)	0.85	0.77	1.21	1.62	1.10	0.77
Prior to the 3rd treatment (Day 29)	1.96	1.00	1.97	2.73	0.72	0.73
2 days after the 3rd treatment (Day 31)	0.70	0.08	1.20	2.34	1.11	1.50
Prior to the 4th treatment (Day 60 / Day 90)	1.20	0.68	1.00	1.17	0.75	0.70
2 days after the 4th treatment (Day 62 / Day 92)	0.89	0.60	0.99	1.87	0.90	0.51
Prior to the 5th treatment (Day 120 / Day 150)	1.45	0.48	1.31	1.17	1.60	1.38
2 days after the 5th treatment (Day 122 / Day 152)	1.06	0.80	0.74	2.44	1.53	1.48
Prior to the 6th treatment (Day 180 / Day 210)	0.89	0.95	0.97	2.25	1.12	1.70
2 days after the 6th treatment (Day 182 / Day 212)	1.10	0.41	0.67	3.32	1.39	1.58
Prior to the 7th treatment (Day 240)	0.95	0.61	1.78	-	-	-
2 days after the 7th treatment (Day 242)	0.34	0.90	1.82	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S58: Changes in D-Dimer (µg/mL) of lab assessments of the patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	0.34	0.24	0.59	0.50	0.90	0.90
2 days after 1st treatment (Day 3)	0.33	0.36	0.40	0.81	0.40	0.68
Prior to the 2nd treatment (Day 15)	0.23	0.00	0.73	0.62	0.50	0.38
2 days after the 2nd treatment (Day 17)	0.41	0.55	0.70	0.46	0.40	0.56
Prior to the 3rd treatment (Day 29)	0.29	0.10	0.50	1.05	0.47	0.52
2 days after the 3rd treatment (Day 31)	0.33	0.34	0.73	0.80	0.18	0.50
Prior to the 4th treatment (Day 60 / Day 90)	0.50	0.29	0.64	0.76	0.64	0.44
2 days after the 4th treatment (Day 62 / Day 92)	0.52	0.46	0.51	0.49	0.65	0.24
Prior to the 5th treatment (Day 120 / Day 150)	0.29	0.62	0.34	0.49	0.40	0.34
2 days after the 5th treatment (Day 122 / Day 152)	0.64	0.20	0.11	0.72	0.56	0.33
Prior to the 6th treatment (Day 180 / Day 210)	0.62	0.59	0.29	0.48	0.04	0.66
2 days after the 6th treatment (Day 182 / Day 212)	1.00	0.28	0.33	0.71	0.48	0.33
Prior to the 7th treatment (Day 240)	0.42	0.34	0.69	-	-	-
2 days after the 7th treatment (Day 242)	0.31	0.60	0.24	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S59: Changes in patient blood pressure (mmHg) during hospitalization in the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	147/90	111/63	129/84	140/89	133/80	103/69
The day of 1st treatment (Day 1)	148/88	110/60	115/74	149/83	110/75	91/61
1 day after 1st treatment (Day 2)	126/88	90/60	101/63	120/70	102/65	85/53
The day of 2nd treatment (Day 15)	143/88	106/71	112/69	121/75	108/72	97/70
1 day after 2nd treatment (Day 16)	129/77	110/63	104/62	120/61	119/68	89/57
The day of 3rd treatment (Day 29)	130/78	105/64	112/66	159/80	103/83	NA
1 day after the 3rd treatment (Day 30)	143/82	99/54	101/60	110/60	92/61	NA
The day of 4th treatment (Day 60 / Day 90)	141/81	105/61	117/71	161/95	112/81	104/68
1 day after 4th treatment (Day 61 / Day 91)	138/88	124/74	110/69	117/59	120/77	108/70
The day of 5th treatment (Day 120 / Day 150)	150/77	111/57	120/78	158/94	110/82	105/62
1 days after 5th treatment (Day 121 / Day 151)	116/61	106/60	103/60	113/67	106/78	91/59
The day of 6th treatment (Day 180 / Day 210)	141/69	99/52	115/66	135/80	110/73	109/65
1 day after 6th treatment (Day 181 / Day 211)	136/78	105/55	122/74	128/78	97/69	113/63
The day of 7th treatment (Day 240)	132/72	105/61	122/74	-	-	-
1 day after 7th treatment (Day 241)	125/74	96/58	109/73	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210). NA=Not Assessed (the examination was not conducted).

Table S60: Changes in patient heart rate (bpm) during hospitalization in the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	72	76	66	90	72	70
The day of 1st treatment (Day 1)	74	62	64	70	74	76
1 day after 1st treatment (Day 2)	78	76	76	70	68	72
The day of 2nd treatment (Day 15)	80	70	74	80	76	76
1 day after 2nd treatment (Day 16)	NA	70	60	72	78	62
The day of 3rd treatment (Day 29)	78	66	84	72	70	NA
1 day after the 3rd treatment (Day 30)	80	62	66	76	70	NA
The day of 4th treatment (Day 60 / Day 90)	84	68	62	64	70	76
1 day after 4th treatment (Day 61 / Day 91)	80	74	76	72	66	70
The day of 5th treatment (Day 120 / Day 150)	80	NA	76	64	70	68
1 days after 5th treatment (Day 121 / Day 151)	66	68	86	68	86	72
The day of 6th treatment (Day 180 / Day 210)	72	70	68	72	76	74
1 day after 6th treatment (Day 181 / Day 211)	76	78	76	70	78	76
The day of 7th treatment (Day 240)	74	78	66	-	-	-
1 day after 7th treatment (Day 241)	76	80	68	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210). NA=Not Assessed (the examination was not conducted).

Table S61: Changes in patient respiratory rate (/min) during hospitalization in the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	19	18	18	18	18	18
The day of 1st treatment (Day 1)	20	18	18	18	20	16
1 day after 1st treatment (Day 2)	16	20	18	20	16	18
The day of 2nd treatment (Day 15)	14	20	20	18	16	20
1 day after 2nd treatment (Day 16)	20	18	16	20	20	18
The day of 3rd treatment (Day 29)	20	18	18	20	18	NA
1 day after the 3rd treatment (Day 30)	20	16	20	18	16	NA
The day of 4th treatment (Day 60 / Day 90)	20	18	18	18	16	20
1 day after 4th treatment (Day 61 / Day 91)	16	16	18	16	20	18
The day of 5th treatment (Day 120 / Day 150)	16	16	18	18	18	16
1 days after 5th treatment (Day 121 / Day 151)	18	20	20	18	18	20
The day of 6th treatment (Day 180 / Day 210)	19	18	20	18	16	18
1 day after 6th treatment (Day 181 / Day 211)	18	16	18	16	18	18
The day of 7th treatment (Day 240)	20	18	18	-	-	-
1 day after 7th treatment (Day 241)	18	16	16	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210). NA=Not Assessed (the examination was not conducted).

Table S62: Changes in patient temperature (℃) during hospitalization in the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	36.2	36.6	36.5	36.7	36.2	36.5
The day of 1st treatment (Day 1) (AM 8:00)	36.1	36.1	36.0	36.2	36.1	36.0
The day of 1st treatment (Day 1) (PM 8:00)	36.5	36.6	36.3	36.6	36.6	36.5
1 day after 1st treatment (Day 2) (AM 8:00)	36.5	36.3	36.3	36.5	36.7	36.5
1 day after 1st treatment (Day 2) (PM 8:00)	36.3	36.9	36.1	36.5	36.2	36.5
The day of 2nd treatment (Day 15) (AM 8:00)	36.5	36.2	36.2	36.2	36.3	36.5
The day of 2nd treatment (Day 15) (PM 8:00)	37.0	36.9	36.3	36.6	36.6	36.6
1 day after 2nd treatment (Day 16) (AM 8:00)	36.6	36.6	36.2	36.8	36.4	36.5
1 day after 2nd treatment (Day 16) (PM 8:00)	36.4	36.6	36.5	36.8	37.0	36.2
The day of 3rd treatment (Day 29) (AM 8:00)	36.2	36.0	36.5	36.5	36.3	NA
The day of 3rd treatment (Day 29) (PM 8:00)	36.8	36.4	36.7	36.8	36.1	NA
1 day after the 3rd treatment (Day 30) (AM 8:00)	36.4	36.1	36.7	36.7	36.8	NA
1 day after the 3rd treatment (Day 30) (PM 8:00)	36.8	36.6	36.7	36.7	36.3	NA
The day of 4th treatment (Day 60 / Day 90) (AM 8:00)	36.2	36.4	36.2	36.0	36.1	36.2
The day of 4th treatment (Day 60 / Day 90) (PM 8:00)	37.0	37.8	36.8	36.7	36.2	36.3
1 day after 4th treatment (Day 61 / Day 91) (AM 8:00)	36.5	37.2	36.5	37.1	36.7	36.1
1 day after 4th treatment (Day 61 / Day 91) (PM 8:00)	36.8	36.8	36.5	36.8	36.0	36.2
The day of 5th treatment (Day 120 / Day 150) (AM 8:00)	36.9	36.6	36.5	36.2	36.4	36.7
The day of 5th treatment (Day 120 / Day 150) (PM 8:00)	36.9	37.0	36.8	36.9	36.5	36.2
1 days after 5th treatment (Day 121 / Day 151) (AM 8:00)	36.5	36.7	36.1	36.4	36.4	36.4
1 days after 5th treatment (Day 121 / Day 151) (PM 8:00)	36.4	37.3	36.8	37.4	NA	36.4
The day of 6th treatment (Day 180 / Day 210) (AM 8:00)	36.6	36.2	36.9	36.5	36.1	36.4
The day of 6th treatment (Day 180 / Day 210) (PM 8:00)	37.0	36.9	36.7	36.9	36.6	36.5
1 day after 6th treatment (Day 181 / Day 211) (AM 8:00)	36.7	36.9	36.8	37.0	36.5	36.1
1 day after 6th treatment (Day 181 / Day 211) (PM 8:00)	36.1	36.0	37.0	36.0	36.4	36.0
The day of 7th treatment (Day 240) (AM 8:00)	36.2	37.0	36.4	-	-	-
The day of 7th treatment (Day 240) (PM 8:00)	36.9	36.7	36.9	-	-	-
1 day after 7th treatment (Day 241) (AM 8:00)	37.0	36.7	36.7	-	-	-
1 day after 7th treatment (Day 241) (PM 8:00)	36.6	36.5	36.7	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210). NA=Not Assessed (the examination was not conducted); AM=Ante Meridiem (before noon); PM=Post Meridiem (after noon).

Table S63: Physical examination of the heart of patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 15	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 29	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 60 / Day 90	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 120 / Day 150	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 180 / Day 210	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 240	Unremarkable	Unremarkable	Unremarkable	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S64: Physical examination of the lungs of patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 15	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 29	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 60 / Day 90	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 120 / Day 150	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 180 / Day 210	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 240	Unremarkable	Unremarkable	Unremarkable	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S65: Physical examination of the abdomen of patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 15	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 29	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 60 / Day 90	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 120 / Day 150	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 180 / Day 210	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 240	Unremarkable	Unremarkable	Unremarkable	-	-	-

Cases 1-3 received 7 doses (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 doses (at Days 1, 15, 29, 90, 150, 210).

Table S66: Clinical vignette of patients in the trial

Participants	Characteristics
Case 1	**(1) Initial Presentation & Course** • A 31-year-old male presented with a 6-year history of progressive weakness and muscle atrophy in his right lower limb. Over the past year, the symptoms had worsened and involved both upper limbs. The progression was chronic and insidious.
	(2) Key Diagnostic Findings • Examination: Neurological examination revealed a combination of upper and lower motor neuron signs. There was muscle wasting in the shoulder girdle, hands (first dorsal interosseous and thenar muscles), and lower limbs. Muscle strength was reduced, particularly in distal limb muscles. Deep tendon reflexes were pathologically brisk at the right knee, but absent at the ankles. The plantar responses were extensor (Babinski sign positive). Sensation and coordination were intact. Cranial nerve examination, including tongue inspection, was normal. • Electromyography (EMG): Demonstrated active and chronic neurogenic changes in the cervical, thoracic, and lumbosacral segments, supporting widespread lower motor neuron involvement. • Family History: Significant for ALS in his uncle and cousin. His paternal grandfather was reported to have had muscle atrophy. • Genetic Testing: Identified a heterozygous mutation (c.125G>A, p.Gly42Asp) in the *SOD1* gene.
	(3) Diagnosis • Based on the progressive painless weakness, the combination of upper and lower motor neuron signs on examination, the EMG findings, and the positive *SOD1* mutation, the patient met the revised El Escorial criteria for clinically definite familial ALS.
	(4) Baseline Status & Treatment • At enrollment, his ALSFRS-R score was 43 and FVC was 115.6%. He provided informed consent and received a total of 7 doses of intrathecal therapy as per the study protocol.
	(5) Follow-up & Notable Course • During treatment, he experienced fasciculations after the second dose, which did not worsen. A transient, modest elevation in alanine aminotransferase occurred, which was attributed to a combination of underlying fatty liver disease and possible treatment effect; it stabilized with continued hepatoprotective therapy. At day 240, muscle strength showed a mixed pattern of mild improvement in some proximal muscles and slight decline in one hand muscle. Subjectively, he reported improved lower limb strength but increased clumsiness in fine hand movements. His ALSFRS-R score was 40 and FVC was 112.9% at the last follow-up.
Case 2	**(1) Initial Presentation & Course** • A 26-year-old female presented with a 3-year history of progressive weakness and muscle atrophy in her left lower limb. The symptoms had worsened over the past year, involving her right lower limb. The disease followed a chronic, progressive course.
	(2) Key Diagnostic Findings • Examination: Neurological examination revealed lower motor neuron signs including tongue fasciculations and asymmetric muscle atrophy, most pronounced in the lower limbs and gluteal muscles. Muscle strength assessment showed significant weakness in both lower limbs (proximal and distal, left worse than right) and mild weakness in the distal upper limbs. Deep tendon reflexes were markedly brisk in the upper limbs but absent in the lower limbs. Ankle clonus was present bilaterally. Plantar responses were flexor. Sensation was intact. • Electromyography (EMG): Demonstrated active and chronic neurogenic changes in the bulbar, cervical, thoracic, and lumbosacral segments, confirming widespread lower motor neuron involvement. • Family History: Her father was an asymptomatic carrier of a *SOD1* gene mutation (c.260A>G). • Genetic Testing: Identified the same heterozygous mutation (c.260A>G, p.Asn87Ser) in the *SOD1* gene.
	(3) Diagnosis • Based on the progressive weakness without sensory loss, the combination of clinical signs (lower motor neuron signs with upper motor neuron features evidenced by hyperreflexia and clonus), widespread denervation on EMG, and the identified *SOD1* mutation, she met the revised El Escorial criteria for clinically definite familial ALS.
	(4) Baseline Status & Treatment • At enrollment, her ALSFRS-R score was 41 and FVC was 84.2%. After providing informed consent, she received a total of 7 intrathecal injections per the study protocol.
	(5) Follow-up & Notable Course • Following the first dose, she experienced fasciculations which did not worsen with subsequent treatments. No clinically significant laboratory abnormalities were observed. At the 240-day follow-up, muscle strength exhibited a mixed pattern: mild improvement in some lower limb muscles (notably from 0 to 2 grades in the most severely affected left distal muscles) accompanied by mild decline in some upper limb and contralateral lower limb muscles. Subjectively, she reported improved ability in gross motor tasks like turning in bed but increased clumsiness in fine motor activities. Her ALSFRS-R score was 39 and FVC was 86.8% at the last assessment.

Participants	Characteristics
Case 3	**(1) Initial Presentation & Course** • A 44-year-old female presented with a 10-month history of progressive weakness and muscle atrophy in her left lower limb. The disease followed a chronic, progressive course.
	(2) Key Diagnostic Findings • Examination: Neurological examination revealed asymmetric muscle atrophy involving the thenar eminences (left > right), left first dorsal interosseous, and left thigh/calf muscles. Muscle strength was severely and asymmetrically reduced, with profound weakness (grade 0) in multiple muscle groups of the left lower limb, and milder weakness in the distal left upper limb. Muscle tone was decreased on the left. Deep tendon reflexes were brisk in the upper limbs but absent at the left knee and both ankles. Pathological reflexes (Hoffmann and Babinski signs) were positive on the left. • Electromyography (EMG): Demonstrated neurogenic changes consistent with involvement across cervical, thoracic, and lumbosacral segments. • Family History: Denied. • Genetic Testing: Identified a heterozygous mutation (c.125G>A, p.Gly42Asp) in the *SOD1* gene.
	(3) Diagnosis • Based on the progressive, painless weakness, the combination of upper (hyperreflexia, positive pathological reflexes) and lower (atrophy, severe weakness, areflexia) motor neuron signs on examination, corresponding EMG findings, and the identified *SOD1* mutation, she met the revised El Escorial criteria for clinically definite ALS.
	(4) Baseline Status & Treatment • At enrollment, her ALSFRS-R score was 36 and FVC was 84.7%. After providing informed consent, she received a total of 7 intrathecal injections per the study protocol.
	(5) Follow-up & Notable Course • She experienced a post-lumbar puncture headache after the first dose, which was considered procedure-related. At the 240-day follow-up, muscle strength showed a mixed pattern of mild improvement in some right-sided limb muscles and slight decline in the already severely affected left lower limb. Subjectively, she reported no significant change in functional strength, remaining non-ambulatory and dependent for daily activities. Her ALSFRS-R score was 40 and FVC was 105.4% at the last assessment.
Case 4	**(1) Initial Presentation & Course** • A 67-year-old female presented with an over 3-year history (44-month) of progressive dysarthria, followed by bilateral limb weakness over the past 2 years, and worsening dysphagia for 3 months. The disease followed a chronic, progressive course.
	(2) Key Diagnostic Findings • Examination: Neurological examination revealed prominent bulbar signs, including dysarthria, tongue atrophy, and reduced gag reflex. There was widespread, symmetric muscle atrophy involving the shoulder girdle, hands, and gluteal muscles. Muscle strength was diffusely reduced to grades 2-4 in both proximal and distal limb muscles, with greater weakness distally. The plantar response was extensor on the left (Babinski sign positive). • Electromyography (EMG): Demonstrated active and chronic neurogenic changes in the bulbar, cervical, thoracic, and lumbosacral segments. • Family History: Highly significant, with her father and three siblings (two brothers and one sister) diagnosed with ALS. • Genetic Testing: Identified a heterozygous mutation (c.425G>C, p.Gly142Ala) in the *SOD1* gene.
	(3) Diagnosis • Based on the progressive weakness without sensory loss, the combination of bulbar and limb lower motor neuron signs with an upper motor neuron sign (extensor plantar response), widespread denervation on EMG, and the strong family history with a confirmed *SOD1* mutation, she met the revised El Escorial criteria for clinically definite familial ALS.
	(4) Baseline Status & Treatment • At enrollment, her ALSFRS-R score was 25 and FVC was 63.1%, indicating significant functional and respiratory involvement. After providing informed consent, she received a total of 6 intrathecal injections per the study protocol.
	(5) Follow-up & Notable Course • The treatment was well-tolerated without significant adverse events or laboratory abnormalities. At the last follow-up, neurological examination revealed a mixed but overall stable pattern of muscle strength compared to baseline. Notably, her ALSFRS-R score declined to 20, while her FVC improved to 73.3%.

Participants	Characteristics
Case 5	**(1) Initial Presentation & Course** • A 55-year-old female presented with an over 1.5-year (21-month) history of progressive weakness in her left lower limb, followed by right hand weakness over the past year. The disease followed a chronic, progressive course.
	(2) Key Diagnostic Findings • Examination: Neurological examination revealed bulbar signs (dysarthria, tongue fasciculations). Muscle strength was mildly reduced (grade 4) in the distal limbs, with relative preservation of proximal strength. An upper motor neuron sign was present (extensor plantar response on the right). • Electromyography (EMG): Demonstrated active and chronic neurogenic changes in the bulbar, cervical, thoracic, and lumbosacral segments. • Family History: Positive, with her younger brother diagnosed with ALS. • Genetic Testing: Identified a heterozygous mutation (c.317C>T, p.Ser106Leu) in the *SOD1* gene.
	(3) Diagnosis • Based on the progressive weakness without sensory loss, the combination of lower motor neuron (fasciculations) and upper motor neuron (Babinski sign) signs, widespread denervation on EMG, and the positive family history with a confirmed *SOD1* mutation, she met the revised El Escorial criteria for clinically definite familial ALS.
	(4) Baseline Status & Treatment • At enrollment, her ALSFRS-R score was 38 and FVC was 114.8%. After providing informed consent, she received a total of 6 intrathecal injections per the study protocol.
	(5) Follow-up & Notable Course • The treatment was well-tolerated without significant adverse events or laboratory abnormalities. At the last follow-up, her neurological examination and ALSFRS-R score (38) remained largely stable, with only a mild decrement noted in left distal lower limb strength. Her FVC was 112.4%.
Case 6	**(1) Initial Presentation & Course** • A 50-year-old male presented with an over 1-year history (17-month) of progressive stiffness and impaired mobility in both lower limbs. The disease followed a chronic, progressive course.
	(2) Key Diagnostic Findings • Examination: Neurological examination revealed subtle muscle wasting in the right calf and left posterior arm. Muscle strength was largely preserved (grade 5), with only mild, asymmetric weakness (grade 4) noted in a few distal hand muscles. A key finding was increased muscle tone in the left lower limb. Deep tendon reflexes were brisk in the upper limbs but absent at the left knee and both ankles. • Electromyography (EMG): Demonstrated neurogenic changes in the cervical, thoracic, and lumbosacral segments. • Family History: Denied. • Genetic Testing: Identified a heterozygous mutation (c.146A>G, p.His49Arg) in the *SOD1* gene.
	(3) Diagnosis • Based on the progressive motor symptoms without sensory loss, the combination of upper motor neuron signs (spasticity, hyperreflexia) and lower motor neuron signs (mild atrophy, areflexia), corresponding EMG findings, and the identified SOD1 mutation, he met the revised El Escorial criteria for clinically definite ALS.
	(4) Baseline Status & Treatment • At enrollment, his ALSFRS-R score was 46 and FVC was 92.5%. After providing informed consent, he received a total of 6 intrathecal injections per the study protocol.
	(5) Follow-up & Notable Course • The treatment was well-tolerated. He reported leg pain during the later stages of treatment, which was managed with pregabalin and attributed to the underlying disease. A mild, stable anemia was noted and considered unrelated to the study drug. At the last follow-up, neurological examination showed minimal change in muscle strength. His ALSFRS-R score was 45 and FVC was 95.1%.

Table S67: Electrocardiogram (ECG) Results in patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	Nonspecific ST-segment elevation	Short PR interval; Right axis deviation	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Day 15	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	NA
Day 29	Nonspecific ST-segment elevation	Unremarkable	Unremarkable	Unremarkable	NA	NA
Day 60	Nonspecific ST-segment elevation	Unremarkable	Unremarkable	NA	-	-
Day 90	Unremarkable	Unremarkable	Unremarkable	NA	NA	NA
Day 120	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable	NA
Day 180 / Day 150	Nonspecific ST-segment elevation; Prolonged QTc interval	Unremarkable	Unremarkable	Unremarkable	Unremarkable	NA
Day 240 / Day 210	Nonspecific ST-segment elevation	Unremarkable	NA	Unremarkable	Unremarkable	Unremarkable

Cases 1-3 underwent 8 assessments (at baseline, Days 15, 29, 60, 90, 120, 180, 240), while Cases 4-6 underwent 7 assessments (at baseline, Days 15, 29, 90, 120, 150, 210). NA=Not Assessed (the examination was not conducted).

Table S68: Changes in CSF Proteins during the trial

Participants	Time	ADA	NGLU	C-Pro	NCL	LAC
Case 1	Baseline	0.2	3.82	27.03	125	1.4
	Prior to the 2nd treatment	0.3	3.48	27.81	127	1.5
	Prior to the 3rd treatment	0.2	3.42	30.13	125	1.5
	Prior to the 4th treatment	0.3	3.62	31.91	126	1.4
	Prior to the 5th treatment	0.1	3.35	31.1	126	1.3
	Prior to the 6th treatment	0.4	3.34	33.03	126	1.4
	Prior to the 7th treatment	0.1	3.6	42.77	131	2.6
Case2	Baseline	NA	2.9	41.22	121	1.4
	Prior to the 2nd treatment	0.2	3.21	40.99	126	1.6
	Prior to the 3rd treatment	0.2	3.04	40.89	124	1.4
	Prior to the 4th treatment	0.1	3	38.89	121	1.3
	Prior to the 5th treatment	0.1	2.9	41.73	123	1.3
	Prior to the 6th treatment	0	3.03	37.55	121	1.2
	Prior to the 7th treatment	0.2	3.12	34.88	121	1.3
Case3	Baseline	0.1	3.75	45.32	122	1.5
	Prior to the 2nd treatment	0	3.24	47.62	123	1.3
	Prior to the 3rd treatment	0.3	3.15	48.83	121	1.3
	Prior to the 5th treatment	NA	3.37	42.99	124	1.5
	Prior to the 6th treatment	NA	3.57	38.66	123	1.3
	Prior to the 7th treatment	NA	3.26	41.02	120	1.4
Case4	Baseline	0.2	3.99	34.66	127	1.8
	Prior to the 2nd treatment	0.1	3.53	37.5	126	1.6
	Prior to the 3rd treatment	0.1	3.66	36.12	126	1.7
	Prior to the 4th treatment	0.1	3.38	34.9	122	1.5
	Prior to the 5th treatment	0.2	3.3	31.42	124	1.7
	Prior to the 6th treatment	0.2	3.52	46.08	125	1.6
Case5	Baseline	0	4.08	40.84	123	1.7
	Prior to the 2nd treatment	0.1	3.14	46.75	105	1.4
	Prior to the 3rd treatment	0.2	3.66	49.7	123	1.6
	Prior to the 4th treatment	0.1	3.54	58.47	123	1.5
	Prior to the 5th treatment	0.2	3.63	61.52	121	1.6
	Prior to the 6th treatment	0.2	3.69	56.17	120	1.6

Participants	Time	ADA	NGLU	C-Pro	NCL	LAC
Case6	Baseline	NA	3.76	37.3	119	1.4
	Prior to the 2nd treatment	NA	3.65	50.16	124	1.4
	Prior to the 3rd treatment	0.1	3.62	43.62	124	1.5
	Prior to the 4th treatment	NA	3.54	39.62	122	1.4
	Prior to the 5th treatment	NA	3.27	47.87	123	1.4
	Prior to the 6th treatment	0.1	3.21	60.59	121	1.3

ADA=Adenosine Deaminase (U/L); NGLU=Glucose in Cerebrospinal Fluid (mmol/L); C-Pro=Cerebrospinal Fluid Total Protein (mg/dL); NCL=Chloride in Cerebrospinal Fluid (mmol/L); LAC=Lactate (mmol/L); NA=Below the lower limit of quantification.

Table S69: ALSFRS-R changes in patients before and after treatment

Characteristics	Case 1	Case 2	Case 6	Case 3	Case 5	Case 4
Baseline ALSFRS-R (points)	43	41	46	36	38	25
Declined score before baseline (points)	5	7	2	12	10	23
Disease duration (months)	72	36	17	10	21	44
Progression before baseline (points/month)	0.069	0.194	0.118	1.200	0.476	0.523
Mean±SD before baseline (subgroup) (points/month)	0.127±0.036			0.838±0.362		0.523
Mean±SD before baseline (subgroup) (points/month)	0.430±0.172					
ALSFRS-R at 7/8 months after treatment (points)	40	35	45	40	36	20
Declined score at 7/8 months after treatment (points)	3	6	1	-4	2	5
Progression after treatment (points/month)	0.375	0.750	0.143	-0.500	0.286	0.714
Mean±SD after treatment (subgroup) (points/month)	0.423±0.177			-0.107±0.393		0.714
Mean±SD after treatment (subgroup) (points/month)	0.295±0.187					

The final follow-up visit occurred at Day 240 for Cases 1-3 and at Day 210 for Cases 4-6. All pre- and post-treatment calculations for progression rates use the respective observation periods for each case. Disease duration is measured from the reported date of symptom onset. SD=Standard Deviation.

Table S70: CSF NfL levels (pg/mL) in patients during the trial

Timeline	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Baseline	4764.7	7373.4	667762.5	21889.0	31230.9	6383.8
Day 15	4304.3	7775.5	628309.0	16070.5	31737.0	10438.9
Day 29	3977.0	6411.9	263741.7	9701.3	25966.2	7658.5
Day 60 / Day 90	2529.8	3226.3	NA	5666.8	7103.3	1374.7
Day 120 / Day 150	1900.0	1416.6	5250.7	5181.9	7931.9	1970.2
Day 180 / Day 210	7124.9	3345.6	5299.5	6953.8	10846.9	3484.3
Day 240	12737.7	7177.0	9576.3	-	-	-

Cases 1-3 received 7 lumbar punctures (at Days 1, 15, 29, 60, 120, 180, 240), while Cases 4-6 received 6 lumbar punctures (at Days 1, 15, 29, 90, 150, 210). NA=No additional sample available for this assay.

Table S71: Changes in muscle strength of sternocleidomastoid muscle of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 2	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 3	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 4	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 5	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 6	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S72: Changes in muscle strength of deltoid muscle of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	4	4	4	5	5	5
	R	4	4	4	5	5	5
Case 2	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 3	**L**	4	5	5	4	5	5
	R	5	5	5	5	5	5
Case 4	**L**	2	2	2	2	3	3
	R	3	3	3	3	2	2
Case 5	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 6	**L**	5	5	5	5	5	5
	R	5	5	4	4	5	5

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S73: Changes in muscle strength of biceps brachii of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 2	**L**	5	5	5	5	5	5-
	R	5	5	5	5	5	5-
Case 3	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 4	**L**	4	3	3	4	2	3
	R	4	3	3	4	4	4
Case 5	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 6	**L**	5	5	5	5	5	5
	R	5	4	5	5	5	5

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S74: Changes in muscle strength of triceps brachii of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 2	**L**	5	5	5	5	5	5-
	R	5	5	5	5	5	5-
Case 3	**L**	5	5	4	4	5	5
	R	5	5	5	5	4	5
Case 4	**L**	4	4	4	4	4	4
	R	4	4	4	4	4	3
Case 5	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 6	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S75: Changes in muscle strength of extensor digitorum of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	4	4	4	5	5	5
	R	3	3	4	5	5	5
Case 2	**L**	4	4	4	4	5	4
	R	4	4	4	5	5	5
Case 3	**L**	4	4	4	4	4	4
	R	5	5	5	4	5	5
Case 4	**L**	3	3	3	4	4	3
	R	0	0	0	0	0	2
Case 5	**L**	4	4	4	4	4	4
	R	4	4	4	4	4	4
Case 6	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S76: Changes in muscle strength of abductor pollicis brevis of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	4	4	4	4	4	4
	R	3	3	4	4	4	4
Case 2	**L**	5	5	5	5	4	4
	R	5	5	5	5	5	5
Case 3	**L**	4	4	4	4	4	4
	R	5	5	5	5	5	5
Case 4	**L**	3	3	3	3	3	4
	R	3	3	3	3	3	3
Case 5	**L**	4	4	4	4	4	4
	R	3	4	4	4	4	4
Case 6	**L**	4	5	5	5	5	5
	R	4	5	5	5	5	5

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S77: Changes in muscle strength of extensor digiti minimi of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	3	3	3	3	3	3
	R	3	3	4	3	3	4
Case 2	**L**	4	4	4	5	4	4
	R	4	4	4	5	4	5
Case 3	**L**	4	4	5	5	4	4
	R	4	4	5	5	4	5
Case 4	**L**	4	3	3	3	3	1
	R	4	4	3	3	3	1
Case 5	**L**	0	-	-	4	4	4
	R	4	4	4	-	-	-
Case 6	**L**	3	5	5	5	5	5
	R	4	5	5	5	5	5

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S78: Changes in muscle strength of first dorsal interosseous muscle of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	4	4	4	3	3	3
	R	3	3	3	3	3	3
Case 2	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 3	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 4	**L**	2	1	0	2	1	0
	R	0	0	0	0	0	0
Case 5	**L**	3	3	4	4	4	4
	R	2	2	4	4	3	3
Case 6	**L**	3	5	5	5	5	5
	R	4	5	4	4	5	5

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S79: Changes in muscle strength of iliopsoas muscle of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	4	4	4	4	4	4
	R	4	4	4	4	4	4
Case 2	**L**	3	3	3	3	3	4
	R	4	4	4	4	4	4
Case 3	**L**	0	1	1	0	0	0
	R	4	4	4	4	4	4
Case 4	**L**	2	1	2	3	2	2
	R	3	3	3	3	3	3
Case 5	**L**	4	4	4	4	4	5
	R	4	4	4	4	4	5
Case 6	**L**	4	5	4	4	5	5
	R	5	4	3	3	5	5

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S80: Changes in muscle strength of quadriceps femoris of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	4	4	4	4	4	4
	R	4	4	4	4	4	4
Case 2	**L**	2	2	2	3	2	2
	R	3	4	4	4	4	4
Case 3	**L**	2	2	2	1	1	1
	R	4	4	4	4	4	5
Case 4	**L**	3	3	3	3	2	2
	R	3	3	3	3	3	4
Case 5	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 6	**L**	5	5	5	5	5	4+
	R	5	4	4	4	5	4+

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S81: Changes in muscle strength of hamstrings of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	4	4	4	4	4	4
	R	4	4	4	4	4	4
Case 2	**L**	0	0	0	2	2	2
	R	4	3	3	4	4	4
Case 3	**L**	0	0	0	1	0	1
	R	4	4	4	4	4	4
Case 4	**L**	3	3	3	3	3	3
	R	3	4	4	4	4	2
Case 5	**L**	4	5	4	4	4	4
	R	4	5	5	5	4	4
Case 6	**L**	5	5	5	5	5	5-
	R	5	5	3	3	5	4+

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S82: Changes in muscle strength of anterior tibial muscle of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	4	4	4	5	5	5
	R	4	4	4	5	5	5
Case 2	**L**	0	0	0	0	0	0
	R	4	3	4	3	3	3
Case 3	**L**	0	0	0	0	0	0
	R	4	4	5	5	5	5
Case 4	**L**	2	2	2	2	0	0
	R	2	2	3	3	3	3
Case 5	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 6	**L**	5	5	5	5	5	5
	R	5	5	3	3	5	5

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S83: Changes in muscle strength of gastrocnemius of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	3	3	3	3	3	3
	R	3	3	3	3	3	3
Case 2	**L**	0	0	1	1	2	2
	R	3	3	3	3	4	4
Case 3	**L**	0	0	0	0	0	0
	R	5	5	5	5	5	5
Case 4	**L**	2	2	2	2	2	2
	R	2	2	3	3	3	3
Case 5	**L**	5	5	5	5	5	5
	R	5	5	5	5	5	5
Case 6	**L**	5	5	5	5	5	5
	R	5	5	3	3	4	4

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S84: Changes in muscle strength of flexors of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	2	2	2	3	2	2
	R	2	2	2	3	2	2
Case 2	**L**	0	0	1	1	0	0
	R	4	3	4	3	3	3
Case 3	**L**	0	0	0	0	0	1
	R	5	5	4	4	4	4
Case 4	**L**	2	2	2	2	2	2
	R	4	4	4	4	2	2
Case 5	**L**	5	5	5	5	4	4
	R	5	5	5	5	5	5
Case 6	**L**	5	5	5	5	5	5
	R	5	3	3	3	4	4

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S85: Changes in muscle strength of extensors of the patients during the trial

Patients		Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	4	4	4	5	4	4
	R	4	4	4	5	4	4
Case 2	**L**	0	0	0	0	2	2
	R	3	3	3	4	4	4
Case 3	**L**	0	0	0	0	0	0
	R	5	5	5	4	5	5
Case 4	**L**	2	2	2	2	1	0
	R	4	4	4	4	3	2
Case 5	**L**	5	5	5	5	4	4
	R	5	5	5	5	5	5
Case 6	**L**	5	5	5	5	5	5
	R	5	5	3	3	4+	5

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). L=left. R=right.

Table S86: Changes in compound muscle action potential (CMAP) of abductor pollicis brevis of the patients during the trial

Patients	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	100%	100%	100%	133.33%
Case 2	95.24%	80.95%	101.59%	92.06%
Case 3	102.86%	128.57%	125.71%	142.86%
Case 4	100%	100%	100%	100%
Case 5	91.34%	68.50%	79.53%	88.19%
Case 6	104.07%	90.24%	23.58%	121.14%

Cases 1-3 underwent 5 assessments (at baseline, Days 29, 90, 180, 240), while Cases 4-6 underwent 5 assessments (at baseline, Days 29, 90, 150, 210). Values in table represent the ratio of the CMAP amplitude at follow-up to that at baseline.

Table S87: Changes in compound muscle action potential (CMAP) of tibialis anterior of the patients during the trial

Patients	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	87.23%	82.98%	108.51%	104.26%
Case 2	116.16%	117.17%	120.20%	118.18%
Case 3	130.70%	129.82%	135.96%	121.92%
Case 4	102.94%	85.29%	35.29%	41.18%
Case 5	81.90%	79.05%	82.86%	78.10%
Case 6	105.35%	100.53%	111.76%	110.70%

Cases 1-3 underwent 5 assessments (at baseline, Days 29, 90, 180, 240), while Cases 4-6 underwent 5 assessments (at baseline, Days 29, 90, 150, 210). Values in table represent the ratio of the CMAP amplitude at follow-up to that at baseline.

Table S88: Changes in compound muscle action potential (CMAP) of abductor digiti minimi of the patients during the trial

Patients	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	100%	94.12%	123.53%	158.82%
Case 2	300.00%	300.00%	500.00%	200.00%
Case 3	No definite waveform observed	No definite waveform observed	No definite waveform observed	No definite waveform observed
Case 4	166.67%	100.00%	150.00%	150.00%
Case 5	101.23%	101.23%	111.11%	111.11%
Case 6	113.43%	104.48%	108.96%	114.93%

Cases 1-3 underwent 5 assessments (at baseline, Days 29, 90, 180, 240), while Cases 4-6 underwent 5 assessments (at baseline, Days 29, 90, 150, 210). Values in table represent the ratio of the CMAP amplitude at follow-up to that at baseline.

Table S89: Changes in motor unit number index (MUNIX) of abductor pollicis brevis of the patients during the trial

Patients		baseline	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	-	-	-	-	-
	R	3	3	3	3	2
Case 2	**L**	17	17	13	18	17
	R	43	40	41	50	51
Case 3	**L**	23	11	14	20	26
	R	84	96	108	102	86
Case 4	**L**	-	4	4	-	-
	R	-	-	-	-	-
Case 5	**L**	74	78	61	84	83
	R	21	12	16	16	16
Case 6	**L**	167	163	154	168	190
	R	117	91	95	89	105

Cases 1-3 underwent 5 assessments (at baseline, Days 29, 90, 180, 240), while Cases 4-6 underwent 5 assessments (at baseline, Days 29, 90, 150, 210). L=left. R=right.

Table S90: Changes in motor unit number index (MUNIX) of tibialis anterior of the patients during the trial

Patients		baseline	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	45	22	9	6	6
	R	29	20	16	20	20
Case 2	**L**	71	84	96	80	63
	R	113	80	88	88	86
Case 3	**L**	132	151	138	127	132
	R	144	150	178	154	152
Case 4	**L**	27	16	17	11	5
	R	55	15	9	5	3
Case 5	**L**	76	60	49	56	45
	R	45	17	32	37	46
Case 6	**L**	215	244	204	214	215
	R	246	230	228	233	256

Cases 1-3 underwent 5 assessments (at baseline, Days 29, 90, 180, 240), while Cases 4-6 underwent 5 assessments (at baseline, Days 29, 90, 150, 210). L=left. R=right.

Table S91: Changes in motor unit number index (MUNIX) of abductor digiti minimi of the patients during the trial

Patients		baseline	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	32	31	28	39	35
	R	26	18	16	18	21
Case 2	**L**	-	-	-	-	-
	R	7	12	11	13	11
Case 3	**L**	-	-	-	-	-
	R	125	109	142	136	119
Case 4	**L**	3	3	7	7	6
	R	14	13	16	13	15
Case 5	**L**	49	62	70	73	72
	R	86	76	76	98	82
Case 6	**L**	209	197	205	200	206
	R	88	82	81	84	86

Cases 1-3 underwent 5 assessments (at baseline, Days 29, 90, 180, 240), while Cases 4-6 underwent 5 assessments (at baseline, Days 29, 90, 150, 210). L=left. R=right.

Table S92: Changes in motor unit size index(MUSIX) of abductor pollicis brevis of the patients during the trial

Patients		baseline	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	-	-	-	-	-
	R	95	98	74	122	103
Case 2	**L**	224	218	241	226	223
	R	168	186	194	159	157
Case 3	**L**	118	20	168	162	133
	R	93	80	84	88	103
Case 4	**L**	-	328	276	-	-
	R	-	-	-	-	-
Case 5	**L**	96	92	88	82	93
	R	109	235	135	148	150
Case 6	**L**	71	83	74	77	77
	R	91	108	96	123	112

Cases 1-3 underwent 5 assessments (at baseline, Days 29, 90, 180, 240), while Cases 4-6 underwent 5 assessments (at baseline, Days 29, 90, 150, 210). L=left. R=right.

Table S93: Changes in motor unit size index(MUSIX) of tibialis anterior of the patients during the trial

Patients		baseline	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	80	94	110	160	182
	R	86	142	123	123	114
Case 2	**L**	101	106	93	104	129
	R	75	120	107	108	100
Case 3	**L**	68	63	71	85	70
	R	63	56	55	59	64
Case 4	**L**	194	257	221	186	385
	R	38	115	180	146	265
Case 5	**L**	81	95	107	95	107
	R	114	284	92	115	94
Case 6	**L**	60	49	52	57	53
	R	59	59	59	63	61

Cases 1-3 underwent 5 assessments (at baseline, Days 29, 90, 180, 240), while Cases 4-6 underwent 5 assessments (at baseline, Days 29, 90, 150, 210). L=left. R=right.

Table S94: Changes in motor unit size index(MUSIX) of abductor digiti minimi of the patients during the trial

Patients		baseline	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	**L**	49	46	49	42	44
	R	49	53	55	71	48
Case 2	**L**	-	-	-	-	-
	R	69	48	50	69	72
Case 3	**L**	-	-	-	-	-
	R	38	45	37	40	45
Case 4	**L**	77	85	55	61	84
	R	44	63	44	49	51
Case 5	**L**	91	72	70	80	98
	R	59	64	64	62	59
Case 6	**L**	45	47	47	49	47
	R	49	50	47	51	55

Cases 1-3 underwent 5 assessments (at baseline, Days 29, 90, 180, 240), while Cases 4-6 underwent 5 assessments (at baseline, Days 29, 90, 150, 210). L=left. R=right.

Table S95: Changes in balance score assessed by short physical performance battery of three patients during the trial

Patients	Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	2	2	1	1	1	0
Case 5	2	4	3	3	3	3
Case 6	4	4	4	4	4	4

Case 1 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 5-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210).

Table S96: Changes in gait speed score assessed by short physical performance battery of three patients during the trial

Patients	Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	4	4	4	4	4	3
Case 5	4	4	4	4	4	4
Case 6	3	4	4	4	4	4

Case 1 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 5-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210).

Table S97: Changes in sit-to-Stand score assessed by short physical performance battery of three patients during the trial

Patients	Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	3	3	3	4	3	3
Case 5	4	4	4	4	4	4
Case 6	4	4	4	4	4	4

Case 1 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 5-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210).

Table S98: Changes in total score assessed by short physical performance battery of three patients during the trial

Patients	Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	9	9	8	9	8	6
Case 5	10	12	11	11	11	11
Case 6	11	12	12	12	12	12

Case 1 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 5-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210).

Table S99: Changes in score of tandem walk of three patients during the trial

Patients	Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	2	2	2	2	2	2
Case 5	2	1	2	2	2	2
Case 6	0	0	0	0	0	0

Case 1 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 5-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210).

Table S100: Changes in mean time (s) of 6-meter walk test of three patients during the trial

Patients	Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	6.14	6.85	6.83	6.67	7.48	7.27
Case 5	5.5	5.74	5.67	6.66	5.76	5.77
Case 6	7.18	6.75	6.65	6.97	6.35	6.71

Case 1 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 5-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210).

Table S101: Changes in mean speed (m/s) of 6-meter walk test of three patients during the trial

Patients	Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	0.98	0.88	0.88	0.9	0.8	0.83
Case 5	1.09	1.05	1.06	0.9	1.04	1.04
Case 6	0.84	0.89	0.9	0.86	0.94	0.89

Case 1 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 5-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210).

Table S102: Changes in London ALS stage of six patients during the trial

Patients	Baseline	Day 15	Day 29	Day 90	Day 180 / Day 150	Day 240 / Day 210
Case 1	3	3	3	3	3	3
Case 2	3	3	3	3	3	3
Case 3	3	3	3	3	3	3
Case 4	3	3	3	3	3	3
Case 5	3	3	3	3	3	3
Case 6	2 - A	2 - B	2 - B	NA	2 - B	2 - B

Cases 1-3 underwent 6 assessments (at baseline, Days 15, 29, 90, 180, 240), while Cases 4-6 underwent 6 assessments (at baseline, Days 15, 29, 90, 150, 210). NA=Not Assessed (the examination was not conducted).

Supplementary Information_Protocol

This clinical trial protocol has been provided by the authors to give readers additional information about the work.

This supplement contains the following items:

No.	Item	Version	Date	Page No.
1	Original protocol*	V1.1	Feb. 14th, 2023	2 to 57
2	First amendment protocol	V1.2	Apr. 26th, 2023	58 to 113
3	Second amendment protocol	V1.3	Jun. 1st, 2023	114 to 169
4	Third amendment protocol	V2.0	Aug. 9th, 2023	170 to 225
5	Final protocol	V2.1	Oct 12th, 2023	226 to 281
6	Summary of protocol amendments	-	-	282 to 288

*The protocol (Version 1.1) was drafted and approved by the ethics committees before the trial began.

Safety and Tolerance of RAG-17 in Amyotrophic Lateral Sclerosis Patients With *SOD1* Gene Mutation (CREATION)

Study Protocol

Principal Investigator: Yilong Wang

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Protocol Version: 1.1

Feb. 14th, 2023

Summary of the Trial Protocol

Study Title	Safety and tolerance of RAG-17 in amyotrophic lateral sclerosis patients with *SOD1* gene mutation
Study Purposes	To evaluate the safety, tolerance and pharmacokinetics (PK) of RAG-17 in adult ALS subjects with *SOD1* gene mutation. **Primary purposes：** 1. To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17. 2. To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17. **Secondary purposes：** 1. To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2. To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3. To evaluate the pharmacokinetics of RAG-17 in plasma and cerebrospinal fluid on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 4. To evaluate the time to invasive mechanical ventilation or death

	after the first injection of RAG-17 in ALS patients. **Exploratory purposes：** 1. To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180. 2. To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180. 3. To evaluate the changes in muscle strength on day 15, day 29, day 90, and day 180. 4. To evaluate the changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180. 5. To evaluate the changes in 7T MRI imaging features on day 29, day 90, and day 180. 6. To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180. 7. To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, and day 180. 8. To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, and day 180. 9. To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.
Study	Ongoing open-label, single-center, first-in-human and dose escalation

Design	clinical study
Total number of cases	Six ALS patients with *SOD1* gene mutations
Subjects Selection	**Inclusion Criteria** (1) Patients permitted by medical personnel who could complete the clinical trial. (2) Age 18~75 years, gender unlimited. (3) Clearly identify ALS patients with *SOD1* gene mutations (known *SOD1* mutation sites and reported disease progression). (4) Forced vital capacity during screening period ≥ 80% of expected lung capacity. (5) Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology. (6) The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form. (7) The subjects (including male subjects) have no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum. **Exclusion Criteria** (1) The *SOD1* mutation site occurs in patients with nucleotides 44-66 (calculated from the translation of SOD1 protein) and P.F21C mutations. (2) Patients who have previously received or are currently receiving Tofersen treatment.

	(3) HIV test positive or positive test history. (4) Hepatitis C virus antibody positive or positive test history. (5) Active hepatitis B infection (hepatitis B surface antigen positive and/or hepatitis B core antibody positive). (6) Other experimental drugs used within one month or within five drug half-lives. (7) Lumbar diseases and deformities. (8) Suffering from other diseases known to be related to motor neuron dysfunction, which may confuse or blur the diagnosis of ALS. (9) Other psychiatric disorders diagnosed according to DSM-V diagnostic criteria, or obvious suicidal intentions. (10) Combined with severe liver dysfunction, renal dysfunction, or severe heart dysfunction (severe liver dysfunction refers to ALT value ≥ 2.0 times the upper limit of normal value or AST value ≥ 2.0 times the upper limit of normal value; severe renal dysfunction refers to CRE ≥ 1.5 times the upper limit of normal value or eGFR < 40mL/min/1.73m^2; severe heart dysfunction refers to NYHA 3-4 levels). (11) Permanent and sustained dependence on ventilator assisted ventilation. (12) History of combined alcohol and drug abuse. (13) Pregnant, lactating, or potentially pregnant patients, as well as those planning to conceive. (14) Patients participating in other clinical trials or applying other biologics, drugs, or devices under study. (15) Patients who have received any vaccination within 28 days. (16) Contraindications for MRI examination (ie. claustrophobia). (17) Those who can't complete follow-up due to other reasons.

Therapeutic Regimen

Study drug：Aseptic aqueous solution of the RAG-17 formulation (sodium salt)

Storage condition：Stored at room temperature

Specification：75 mg (2.5 mL)

Route, dosage and schedule of administration: Intrathecal administration. The safety, tolerance, and pharmacokinetics of the RAG-17 drug will be evaluated by a dose-escalation approach. The dose of RAG-17 will range from a minimum of 60 mg to the maximum tolerated dose (MTD). Administration will be conducted once every two weeks, starting at 60 mg, for dose escalation. Once the tolerated dose is reached, a fixed dose of the drug will be administered every two months for continued treatment, with a total treatment duration of 8 months. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD. For the subsequent maintenance treatment phase, the optimal dose between the safe dose and the MTD will be selected as the dose for continued treatment.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	DLT/dose	Intrathecal injection	6
5th Administration	D120	Optimal dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

Dose escalation criteria: If no AEs or SAEs occur within 14 days after the first administration, the subject proceed with a dose

	escalation of 30 mg every 14 days during the observation period. It is planned to achieve DLT through 3 to 4 dose escalations, followed by continuous treatment with the optimal dose for a 6-month treatment period. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD.
Study Outcomes	**Primary outcomes** 1) Incidence of AEs and SAEs within 180 days after the first injection RAG-17. 2) Changes in laboratory assessments, vital signs, ALSFRS-R scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection RAG-17. **Second outcomes：** 1) Changes in SOD1 protein levels in cerebrospinal fluid, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2) Changes in plasma NFL levels from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3) Changes in the pharmacokinetics of RAG-17 in plasma and cerebrospinal fluid on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 4) Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

	Exploratory outcomes： 1) Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180. 2) Changes in fatigue severity (Fatigue Severity Scale, FSS) at on day 15, day 29, day 90, and day 180. 3) Changes in muscle strength on day 15, day 29, day 90, and day 180. 4) Changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180. 5) Changes in 7T MRI imaging features on day 29, day 90, and day 180. 6) Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180. 7) Changes in modified Norris scale scores on day 15, day 29, day 90, and day 180. 8) Changes in quality of life (including ALSAQ-40、EQ-5D-5L、HAMA and HAMD) on day 15, day 29, day 90, and day 180. 9) Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.
Statistical Analysis	• Statistical analysis will be performed with SAS software. • Demographic, medical, histories and baseline characteristics information will be listed and summarized. • The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after

	treatment. Safety evaluation will be analyzed using safety data set. The number of people of events should be recorded in detail and showing the event rate in summary statement. The event rate will be calculated as the number patients experiencing the event / the number of patients enrolled. • Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 180-day follow-up assessments for all participants, the data were analyzed.
Study Duration	2 years

1. Study Background

Amyotrophic lateral sclerosis (ALS) is a chronic, progressive neurodegenerative disease characterized by prominent damage to both upper and lower motor neurons, manifesting in symptoms such as muscle weakness, muscle atrophy, bulbar paralysis, and pyramidal signs. ALS is a rare disease, with a global standardized annual incidence rate of only 1.68 cases per 100,000 person-years[1]. The exact incidence rate of ALS in China still not well-established, but it is estimated that there are at least 100,000 patients [2]. The peak age of onset for ALS is around 50 years, with a growing trend toward earlier onset ages[3]. ALS is incurable, with a typical survival period of 3-5 years, and only approximately 10% of patients survive for more than 10 years[4]. As a result, it is listed by the World Health Organization as one of the "five major incurable diseases in the world" alongside cancer, acquired immunodeficiency syndrome, leukemia and rheumatoid arthritis.

Currently, the pathogenesis of ALS remains unclear, and available treatments are limited, primarily focusing on symptomatic relief rather than a cure. Since 1980, more than 80 randomized controlled trials (RCTs) have been conducted globally. Only three drugs for the treatment of ALS have been approved by the U.S. Food and Drug Administration (FDA): the neuroprotective drug riluzole, which inhibits excessive neurotransmission; the antioxidant edaravone; and Relyvrio (sodium phenylbutyrate and taurursodiol)[5-7]. Drug interventions for ALS include anti-apoptosis, anti-inflammation, anti-excitotoxicity, anti-oxidation, anti-aggregation, neurotrophic growth factors and neuroprotection, among others. In 2017, riluzole, the primary drug for ALS, became the first FDA-approved drug treatment for the disease. However, it cannot halt the progression of ALS, let alone reverse it, and only extends patient survival by 2 to 3 months. The riluzole costs about 1,800~4,000 RMB per month. However, it is only covered by medical insurance in certain provinces and cities. Edaravone is indicated for cerebral infarction and was approved for the treatment of ALS in the United States in 2019. It was approved for use in China in September 2020, specifically for patients with acceptable respiratory function in the early stage (within 2 years) of the disease. In September 2022, the FDA approved Relyvrio, a

combination drug composed of sodium phenylbutrate and taurursodiol, for the treatment of adult patients with ALS. The approval process for Relyvrio drug at the FDA has been marked by numerous challenges. At the first Peripheral and Central Nervous System Drug Advisory Committee (PCNSDAC) review meeting organized by the FDA in March 2022, the expert panel expressed concerns regarding issues such as the small scale of the Relyvri clinical trialo, data deficiencies, and the applicability of statistical methods, with a voting result of 6:4. In response to this, Amylyx reanalyzed the survival data of the CENTAUR trial of Relyvrio and submitted it, along with the biomarker data from another phase II clinical trial for Alzheimer's disease to the FDA for re-evaluation. At the second PCNSDAC meeting in September, the new drug application of Relyvrio was approved with a voting result of 7:2, and it was subsequently approved by the FDA. Currently, this drug has not been approved in China. As a result, existing therapies for ALS remain limited, and the demand for effective ALS treatments remains unmet.

Currently, the pathogenesis of ALS remains unclear, and available treatment are limited, mainly focusing on symptomatic relief rather than a cure. ALS can be divided into sporadic ALS (sALS) and familial ALS (fALS), with sALS accounting for the majority of cases (approximately 90%) and fALS accounting for 5-10%[8]. fALS can result from mutations in multiple genes, with the most common being a mutation in the *superoxide dismutase 1* (*SOD1*), which occurs in 2% to 7% of cases[9]. Approximately 20% of fALS and 5% of sALS cases are associated with the *SOD1* gene. Asia has the highest number of ALS, with Asian patients typically exhibiting a younger age of onset, a lower proportion of bulbar-onset ALS, and more pronounced cognitive dysfunction[10]. In Asia, fALS is mainly caused by *SOD1* gene mutations, whereas in Europe, mutations in the *C9ORF72* gene are more common. The frequency of the *SOD1* gene mutations is highest among ALS patient in central and southern China[11]. SOD1 is a potent antioxidant enzyme that, when combined with copper and zinc ions, helps convert superoxide into oxygen molecules and hydrogen peroxide, thereby protecting cells from damage[12-14]. Mutations in the *SOD1* gene in ALS patients lead to misfolding and accumulation of the SOD1 protein within

spinal motor neurons. While several mutations in SOD1 have been identified, not all of them result in ALS. Harmful mutations alter the functional activity of SOD1, with loss-of-function mutations impairing degradation of reactive oxygen species (ROS), which damages motor neurons, and gain-of-function mutations increasing oxidative activity, resulting in excessive production of hydrogen peroxide and enhancing protein-protein interactions[15-17]. These effects disrupt SOD1 protein level and activity, inhibit axonal transport, and cause mitochondrial dysfunction[15], ultimately leading to neuronal degeneration and death. Therefore, reducing the level of toxic SOD1 protein may offer an effective therapeutic approach for ALS caused by *SOD1* mutations. Researches have indicated that knockdown or knockout of normal SOD1 expression does not result in significant side effects[18-19].

Currently, several drugs under investigation have entered clinical studies, including small molecule drugs, antibody drugs, antisense oligonucleotides (ASO) drugs and cell therapy. Among these, except for small nucleic acid drugs (antisense oligonucleotides, small interfering RNA) which directly target pathogenic genes, the others are non-disease-modifying therapies. Tofersen, an antisense oligonucleotide drug developed by Biogen for the treatment of *SOD1*-ALS, binds to the mRNA encoding SOD1 and is degraded by ribonuclease H (RNase-H), thereby reducing the production of mutant SOD1 protein[20]. Tofersen is currently undergoing a New Drug Application (NDA) review by the U.S. FDA. The NDA submission for Tofersen is based on data from a Phase 1 study in healthy volunteers, a dose-escalation Phase 1/2 clinical study, a Phase 3 VALOR clinical study, and an open-label extension (OLE) study. While the Phase III of Tofersen for the treatment of did not meet the primary endpoint, Biogen has observed indications of reduced disease progression in several secondary and exploratory endpoints. Biogen's integration of 12-month data shows that earlier use of Tofersen, compared to delayed initiation, significantly reduces the neurofilament levels and slows the decline of various efficacy endpoints. In terms of adverse events, the most common side effects in patients were headache, falls, back pain and limb pain. About 6.7% of patients had serious neurological-related events, including myelitis, radiculitis, aseptic meningitis and optic disc edema[21-22].

RNA interference (RNAi) is a biopharmaceutical technology that utilizes double-stranded RNA molecules, known as small interfering RNA (siRNA), to target and silence specific genes at the mRNA level. Based on this mechanism, five drugs have been approved by the U.S. FDA, with nearly a hundred others currently in clinical development. RAG-17 is a 20 kDa siRNA targeting the *SOD1* gene. Through the RNAi mechanism, RAG-17 binds to *SOD1* mRNA, leading to the degradation of the target mRNA by the RNA-induced silencing complex (RISC). By recognizing and mediating the degradation of the target mRNA, RAG-17 silences the *SOD1* gene and downregulates the expression of the SOD-1 protein. RAG-17 is administered via intrathecal injection and is intended for the treatment of ALS patients with *SOD1* mutations. Compared to single-stranded self-delivering ASO drugs, siRNA offers a cascade amplification effect on the target gene and provides a higher gene silencing efficiency. This drug leverages a unique delivery technology for small nucleic acid drugs, enabling efficient siRNA delivery to the central nervous system and offering the potential for treatment of neurological diseases. The schematic diagram of the mechanism of RAG-17 in the treatment of ALS is shown below.

The *in vitro* pharmacodynamic results indicate that RAG-17 shows a concentration-dependent knockdown of *hSOD1* mRNA, with a half-maximal inhibitory concentration (IC50) of 0.011 nM. Compared to the control drug Tofersen, the IC50 of RAG-17 is 100 times lower, and the maximum inhibition rate reaches more than 90%. Cell apoptosis assays show that RAG-17 has no toxic response of cell apoptosis. Off-target effect analysis results show that RAG-17 dose not significantly affect the mRNA expression levels of non-target genes, demonstrating its specificity for *SOD1* gene knockdown.

The h*SOD1*^{G93A} transgenic mice exhibit progressive motor neuron degeneration, which closely resembles human ALS and is a widely recognized animal model for studying ALS. It has been extensively used in ALS pathogenesis research and drug screening [23-25]. To evaluated the inhibitory activity of RAG-17 on the target gene *in vivo*, a single-dose proof of concept (POC) experiment was conducted using the h*SOD1*^{G93A} ALS disease model mice. At 60 days of age, 200 μg of the siRNA drug

RAG-17 (~10 nmol) and the control drug Tofersen (28 nmol) were injected into the lateral ventricle. Tissue RNA was extracted 14 days post-administration, and RT-PCR was performed to assess the target gene, human *SOD1*. The results indicated that the mRNA expression of *SOD1* in both brain and spinal cord tissues was reduced by approximately 80%, with RAG-17 demonstrating superior gene down-regulation compared to the control drug.

Subsequently, a multi-dose pharmacodynamics (PD) experiment was conducted. Doses of 50 μg, 100 μg, 200 μg, and 400 μg of the siRNA drug RAG-17 were injected into the lateral ventricle. The PD indicators (relative expression levels of human *SOD1* mRNA) in the target tissues were assessed at 14 days and 56 days post-injection to determine the pharmacodynamic activity, dose response and the duration of effect at the respective doses. The results showed that gene knockdown was dose-dependent in three different regions of the brain (prefrontal cortex, cerebellum, and other brain tissues) and the spinal cord. Notably, only the high-dose group (400 μg) maintained 50% gene knockdown (KD) activity 56 days after injection, although this activity was weaker compared to 14 days post-injection. Compared to small molecule drugs, the significant advantage of small nucleic acid drugs is the extended duration of their therapeutic effect. Further testing at an extended time point (8 weeks) revealed that RAG-17 sustained the pharmacological activity with gene knockdown for at least 8 weeks in the model mice, providing valuable data to support the potential frequency of clinical administration.

To further verify the correlation between drug exposure in tissues and drug efficacy, the stem-loop PCR method was applied to detect the siRNA concentration in tissues at 14 days and 56 days post-administration. The results demonstrated a clear correlation between the drug concentration in tissues and PD effects.

To further explore the pharmacodynamics of RAG-17, human *SOD1*^{G93A} transgenic ALS disease model mice were used. Both male and female ALS disease model mice were administered intrathecally twice on the 68th and 100th days of life, with doses of the RAG-17 (RD-12500) at 75 μg, 150 μg, and 300 μg. Two negative control groups were included: a vehicle group and an inactvice (scrambled siRNA) group. The

scrambled siRNA served as a control for the target siRNA, being non-functional, and used to assess the potential inhibitory effects of the target siRNA. Additionally, 150 ug of Tofersen (RD-10748) was included as the positive control group. This study conducted pharmacodynamic evaluations based on multiple indicators, including animal body weight, motor function (grip strength in the fatigue rotating rod test, rotarod test, and open field test), disease onset time, lifespan, and neurological function score (neuroscore).

The results of animal weight changes indicated that both the vehicle group and the inactive group began to show a significant decrease in body weight around 130 days, with a more pronounced decrease in male mice. This suggests that the onset of h*SOD1*^{G93A} ALS disease model occurs earlier in males than in females. Among the groups, the positive group (RD-10748) showed improvements in body weight, with more significant weight maintenance in male mice. The body weight data from the drug treatment groups demonstrated a dose-dependent alleviation of weight loss, especially in female mice. As the disease progresses in ALS mice, their body weight gradually decreases. Compared to the vehicle control group, all dose groups of RD-12500 (RAG-17) and ASO (RD-10748) demonstrated a delayed onset of disease.

After two intrathecal administrations in the ALS disease model mice, all treatment groups of both male and female mice showed an extension in the median survival period. The low-dose group of of 75 μg RAG-17 and the medium-dose of 150 μg ASO (RD-10748) demonstrated comparable efficacy. The survival periods for male mice were 207 days (75 μg RAG-17) and 191 days (150 μg ASO), respectively. The survival periods of the female group were 198.5 days (75 μg RAG-17) and 189 days (150 μg ASO), respectively. In the female high-dose group (300 μg), the survival period was significantly extended to 227 days, representing a 54-day increase compared to the vehicle control group (173 days). However, the high-dose group (300 μg) in the male mice did not show a marked superiority. Female animals exhibited greater sensitive to drug treatment, and the results aligned with the trend of weight change.

The typical pathological feature of h*SOD1*^{G93A} ALS disease model mice is the

progressive loss of motor function. The Rotarod test for motor function fatigue and the Grip strength test were used to evaluate the therapeutic effect of the drug on the ALS model. The results showed that, in the Rotarod test, the drug-treated group demonstrated a significant increase in the time ALS mice were able to maintain their position on the accelerating rotating rod at 0-30 rpm, compared to both the vehicle and the inactive control groups. The Grip strength test revealed that the drug treatment group significantly improved the hindlimb muscle function of the disease model mice. Additionally, the open field test, which evaluates autonomous behavior, exploratory behavior and anxiety in a novel environment, showed that the drug-treatment group exhibited improved autonomous motor ability, with a dose-dependence effect.

Before performing the Rotarod and hindlimb grip strength behavioral tests, the neurological function of ALS mice was scored on a scale of 0 to 4 points. This scoring system objectively evaluates the common hindlimb dysfunction in h*SOD1*^{G93A} transgenic mice. The results showed that mice in the vehicle (aCSF) and inactive control groups exhibited abnormal tilting (NS2 symptoms) at PND130 days, with the severity progressively worsening until hindlimb paralysis (i.e., ≥ NS3). In contrast, at all time points during the study, the average score of all RAG-17 treatment doses never exceeded NS2. These results indicated that RAG-17 treatment, regardless of the dose, improved motor function in the animals compared with the control group.

In conclusion, RAG-17 treatment can effectively inhibit the gene expression of h*SOD1* in h*SOD1*^{G93A} transgenic mice, significantly delay the disease onset, extend the survival time of affected mice, enhance their grip strength , and improve the motor function.

Currently, the non-clinical safety evaluation of RAG-17 is ongoing. The safety dose exploration experiments have been completed in both rats (following two intrathecal administrations, a non-relevant species) and cynomolgus monkeys (a relevant species). In the rat safety dose exploration experiment, the main findings included transient neurological functions such as tremors, abnormal gait, and hindlimb weakness, in some animals of the medium-high dose group (2.5mg/dose) and high dose group (4.5mg/dose) after intrathecal drug injection. In the safety dose exploration

experiment with cynomolgus monkeys, one male monkey (3001) died after administration of the high dose of 200mg (equivalent to 2g in human dose), while another female monkey (3501) showed severe toxic reactions but gradually recovered over days 17-18. The two animals in the low-dose group of 30mg showed no abnormalities after the first administration, but tremors occurred after the second administration. As a result, animals in the vehicle group were used as alternatives and received the medium-dose experiment of 50mg. The 50mg dose group (equivalent to 500mg in human dose) showed transient tremors, body stiffness, and reduced activity after administration, but these symptoms resolved within approximately 2 to 4 hours.

In terms of histopathology, axonal degeneration of the sciatic nerve, unrelated to the dose, was observed in both species. In the cynomolgus monkey study (H59-0025-TX), mild to mild multifocal axonal degeneration was observed in both bilateral and unilateral sciatic nerves of the male in the 50 mg/dose and 30 mg/dose groups. Additionally, mild to mild multifocal axonal degeneration was observed in the female monkeys across all dose groups. This alteration is characterized by a "bubbly" appearance of multiple affected fibers, axonal rupture (aggregating into eosinophilic masses), and some cellular debris surrounding the vacuoles. Although there was no control group in the cynomolgus monkey study, the changes observed in the sciatic nerve were still considered related to the test drug, consistent with the findings from the rat study. In each dose group, mild multifocal axonal degeneration was observed at the injection site in the female monkeys. The characteristics of this alteration are similar to those observed in the sciatic nerve and may be due to the administration procedure. In addition, mild to mild diffuse vacuolation of macrophages in the medulla of mesenteric lymph nodes was observed in all dose groups. Since these adverse reactions were not dose-dependent, their correlation with the test drug cannot be determined at this time.

Based on the results of the safety dose exploration experiments, formal GLP toxicology experiments are currently underway. These formal GLP toxicology tests have reduced the administration doses for rats and cynomolgus monkeys, as well as optimized the intrathecal injection procedure. The preliminary safety dose exploration

experiment results of rats and cynomolgus monkeys can also provide insight into the potential clinical risks of this test drug.

This project conducts a single-center, dose-escalation clinical study, aiming to evaluate the safety, tolerance and pharmacokinetics of RAG-17 in adult ALS patients carrying the *SOD1* mutation, in preparation for subsequent efficacy clinical trial.

2. Study Purposes

This study primarily aims to evaluate the safety, tolerance and pharmacokinetics of RAG-17 in adult ALS patients with *SOD1* gene mutation.

2.1 Primary Purposes：

(1) To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17.

(2) To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

2.2 Second Purposes：

(1) To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(2) To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(3) To evaluate the pharmacokinetics of RAG-17 in plasma and cerebrospinal fluid on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(4) To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

2.3 Exploratory Purposes：

(1) To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180.

(2) To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180.

(3) To evaluate the changes in muscle strength on day 15, day 29, day 90, and day 180.

(4) To evaluate the changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180.

(5) To evaluate the changes in 7T MRI imaging features on day 29, day 90, and day 180.

(6) To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180.

(7) To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, and day 180.

(8) To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, and day 180.

(9) To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.

3. Study Design Type, Principles and Experimental Procedures

3.1 Study Design

The study is an ongoing open-label, single-center, first-in-human, and dose escalation clinical trial.

Adult patients with a confirmed or probable familial or sporadic of ALS with confirmed *SOD1* gene mutations (forced vital capacity ≥ 80% of predicted value) will be included according to the revised El Escorial criteria.

1) **Administration：** A total of 7 doses will be administered on Day 1, Day 15, Day 29, Day 60, Day 120, Day 180, and Day 240. The drug will be administered via

intrathecal injection of RAG-17, with doses of 60 mg per administration, 90 mg per administration, 120 mg per administration, DLT per administration, with the last three doses being the optimal dose. The treatment period of this study is 8 months. Various indicators, adverse events, and concomitant medications will be collected on Day 1, Day 15, Day 29, Day 60, Day 90, and Day 180 post-treatment. The study is expected to be conducted at a single center, Beijing Tiantan Hospital, with the recruitment of 6 participants, aiming to complete the study within 2 years.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	DLT/dose	Intrathecal injection	6
5th Administration	D120	Optimal dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

2) **Dose Escalation Criteria:** If no AEs or SAEs occur within 14 days after the first administration, the subject proceed with a dose escalation of 30 mg every 14 days during the observation period. It is planned to achieve DLT through 3 to 4 dose escalations, followed by continuous treatment with the optimal dose for a 6-month treatment period. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD.

3) **Dose Escalation Stopping Criteria：** 'Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0' will be used as the toxicity evaluation standard for this protocol.

If a dose meets any of the following criteria, dose escalation will be considered for stopping or the trial will be continued at an additional lower dose level, unless it can

be clearly confirmed that the adverse event is unrelated to the drug (e.g., skin abrasions, hospitalization or death caused from a car accident).

① At the same dose level (within the same administration group), if ≥ 1 subject experiences an AE of grade ≥ 2;

② Other situations that may increase the risk of subjects, which will be discussed by the investigator to decide whether to terminate dose escalation.

4) **Individual Drug Withdrawal Criteria:** A subject will no longer participate in the subsequent administration, if they meet any of the following criteria:

① During the trial, the subject experiences a SAE or an AE of grade ≥ 3 (including laboratory abnormalities) , regardless of whether it is related to the drug;

② The subject experiences an event that, in the investigator's assessment, may significantly increase the risk to the subject.

3.2 Sample Size

This is an exploratory, hypothesis-generating study and a dose escalation clinical study to explore the maximum tolerated dose, so a total of 6 cases are required for sample size estimation.

3.3 Study Period

Work Progress	Timeline
Study Design, Study Approval Review, Ethical Review, Contract Signing, Human Genetic Resources Approval, Study Registration	2022.12-2023.05
Project Official Launch, Subject Enrollment and Follow-up	2023.06-2024.06
Data Cleaning and Analysis, Manuscript Writing	2024.06-2024.12
Total Duration	2 Years

3.4 Subject Selection

During the entire study period, it is planned to enroll 6 subjects. Ethical approval must be obtained before patient recruitment. The ethics committee will have access to all study documents and training materials.

- **Inclusion Criteria**

(1) Patients permitted by medical personnel who could complete the clinical trial.

(2) Age 18–75 years, gender unlimited.

(3) Clearly identify ALS patients with SOD1 gene mutations (known *SOD1* mutation sites and reported disease progression).

(4) Forced vital capacity during screening period ≥ 80% of expected lung capacity.

(5) Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology.

(6) The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form.

(7) The subjects (including male subjects) have no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum.

- **Exclusion Criteria**

(1) The *SOD1* mutation site occurs in patients with nucleotides 44-66 (calculated from the translation of SOD1 protein) and P.F21C mutations.

(2) Patients who have previously received or are currently receiving Tofersen treatment.

(3) HIV test positive or positive test history.

(4) Hepatitis C virus antibody positive or positive test history.

(5) Active hepatitis B infection (hepatitis B surface antigen positive and/or hepatitis B core antibody positive).

(6) Other experimental drugs used within one month or within five drug half-lives.

(7) Lumbar diseases and deformities.

(8) Suffering from other diseases known to be related to motor neuron dysfunction, which may confuse or blur the diagnosis of ALS.

(9) Other psychiatric disorders diagnosed according to DSM-V diagnostic criteria, or obvious suicidal intentions.

(10) Combined with severe liver dysfunction, renal dysfunction, or severe heart dysfunction (severe liver dysfunction refers to ALT value ≥ 2.0 times the upper

limit of normal value or AST value ≥ 2.0 times the upper limit of normal value; severe renal dysfunction refers to CRE ≥ 1.5 times the upper limit of normal value or eGFR < 40mL/min/1.73m^2; severe heart dysfunction refers to NYHA 3-4 levels).

(11) Permanent and sustained dependence on ventilator assisted ventilation.

(12) History of combined alcohol and drug abuse.

(13) Pregnant, lactating, or potentially pregnant patients, as well as those planning to conceive.

(14) Patients participating in other clinical trials or applying other biologics, drugs, or devices under study.

(15) Patients who have received any vaccination within 28 days.

(16) Contraindications for MRI examination (ie. claustrophobia).

(17) Those who can't complete follow-up due to other reasons.

4. Study Method

(1) Drug Dosage Form

The aseptic aqueous solution of the RAG-17 preparation (sodium salt) used in the study is stored at room temperature, with a specification of 75 mg (2.5 mL).

(2) Intervention Measures

1) Basic Treatment

All enrolled subjects will maintain basic medication treatment, and the dosage and type of medications will remain unchanged during the trial period.

Subjects who have already received riluzole treatment before enrollment may continue using it during the study period, maintaining a stable dosage (50mg per dose, twice daily). Throughout the study, discontinuation of riluzole for reasons other than medical necessity, as determined by the investigator, is not recommended.

The concomitant medications situation of the patients should be recorded in detail in the CRF form, including the name of the medication, the dosage, and frequency. If during the study, a change in the existing medication is deemed necessary by the investigator due to the patient's condition, it will be considered a protocol deviation. An adverse event record report and a concomitant medication form should be

completed. The investigator will then assess whether the subject can continue participating in the study. If not, the subject will be treated as a withdrawn case.

2) **RAG-17 Treatment**

The administration begins with an initial intrathecal injection dose of 60 mg. Within the first 14 days following the initial dose, if no AEs or SAEs are observed, the dose may be escalated by 30mg at 14-day intervals. After reaching the maximum tolerated dose (MTD), an optimal dose between the safe dose and the MTD will be selected as the dose for continued treatment. Continuous treatment will be administered every 2 months, with a total treatment duration of 8 months.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	DLT/dose	Intrathecal injection	6
5th Administration	D120	Optimal dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

5. Concomitant Medications

(1) Prohibited Concomitant Medications

Subjects must contact their investigators before taking any new medications, including over-the-counter drugs and herbal preparations.

• The use of any antiplatelet or anticoagulant drugs (e.g. aspirin, clopidogrel) is prohibited within 7 days prior to each lumbar puncture and until 48 hours after the procedure.

• The use of DPS, copper (II) (diacetylbis (N4-methylaminothiourea)), or pyrimethamine is prohibited during the study.

• The use of gamma globulin is not recommended during the study.

(2) Permitted Concomitant Medications

If subjects are taking concomitant vitamins, minerals and supplements, they should avoid exceeding the recommended daily dose within 14 days prior to the first administration of the RAG-17 and during the study period (e.g. no more than 5 grams of creatine per day and no more than 1000 IU of vitamin E per day). These daily limits include the total dose obtained from the combined use of daily multivitamins and supplements.

Medications not listed in the prohibited concomitant medications section above are considered permitted concomitant medications. Concomitant medications for symptom management during the study are determined by the investigator. All enrolled cases must maintain basic medication treatment, with no changes to the dosage or type of medications during the trial. If subjects have questions regarding permitted concomitant treatments, they should seek medical guidance from the investigator. The concomitant medications of the patients must be documented in detail in the CRF form, including the name of the medication, the dosage, and frequency. If a change or adjustment to the existing medications become necessary due to the subject's condition during the study, and the investigator deems it essential, this will be considered a protocol deviation. The adverse event record form and the concomitant medication form should be promptly completed. The investigator will assess whether the subject can continue participating in the study. If continuation is not feasible, the case will be treated as an excluded case. In special circumstances (e.g. the subjects suffers from other diseases during the study), when additional treatments are necessary, these must be explained in the CRF form, including the disease diagnosis, medications, dosage, and usage details.

6. Observation Indicators and Examination Timeline

6.1 General Demographic Indicators and Clinical Data

(1) Collection time point: baseline

(2) Collection content: Basic demographic characteristics include gender, age, ethnicity, height, weight, education level, marital status; vital signs include body temperature, pulse, respiration rate, blood pressure; 12-lead electrocardiogram (ECG);

waist circumference, physical examination; clinical data include relevant medical history, past medical history, medication used prior to enrollment, smoking and drinking history, and family medical history.

6.2 Daily Life Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo assessments using the ALSFRS-R and Modified Norris Scale before and after treatment. The results after treatment intervention will be compared with baseline data to evaluate the effect of RAG-17 on improving daily living function of the subjects.

(3) Observation indicators:

1) ALSFRS-R: This is a rapidly administered ordinal rating scale (grade 0-4) used to assess the subjects' ability and independence in 12 functional activities. ALSFRS-R measures 4 functional domains, including respiratory, bulbar, gross motor, and fine motor functions. The scale includes 12 items, each scored from 0 to 4, with a total score of 48. Higher scores indicate better function, while lower scores reflect more severe neurological impairment.

2) Modified Norris Scale: This assessment tool for ALS consists of two parts: the Limb Norris Scale (comprises 21 items to evaluate limb function) and the Norris Bulbar Scale (includes 13 items to assess bulbar function). Lower scores indicate more severe neurological impairment.

6.3 Respiratory Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo forced vital capacity (FVC) testing before and after treatment. The results post-treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving the respiratory function.

(3) Contents of observation indicators:

FVC: The subjects are measured in an upright position. Measured as the maximum volume of air that can be forcefully exhaled as quickly as possible after a maximal inhalation.

6.4 Fatigue Severity Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements with the Fatigue Severity Scale (FSS) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving the severity of fatigue in subjects.

(3) Observation indicators:

Fatigue severity: The FSS consists of 9 items, designed to measure the degree of fatigue and its impact on a person's activities and lifestyle across various diseases. The evaluation covers areas such as daily activity ability, participation, and sleep. Each question is scored on a 7-point scale: 1 = strongly disagree and 7 = strongly agree. The minimum possible score = 9 and the maximum possible score = 63. Higher scores indicate the greater severity of fatigue.

6.5 Muscle Strength Test

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurement using the Medical Research Council (MRC) Scale (sternocleidomastoid muscle, deltoid muscle, biceps brachii, triceps brachii, common digital extensor muscle, abductor pollicis brevis muscle, abductor digiti minimi muscle, first dorsal interosseous muscle, and iliopsoas muscle, quadriceps femoris, biceps femoris, tibialis anterior, gastrocnemius muscle, flexor muscle, extensor muscle) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving muscle strength.

(3) Observation indicators:

MRC scale: The MRC muscle strength grading method, is an enhanced version of the Lovett grading method, further subdivided by the range of motion and the level of resistance applied. If the measured muscle strength is slightly stronger than a certain level, a "+" is added to the upper right corner of this level. If it is slightly weaker, a "-" is added to the upper right corner. The refinement addresses the limitation of Lovett grading method, providing a more precise assessment of muscle

strength.

In this study, the MRC grading is used to measure the muscle strength of the abductor pollicis brevis muscle, abductor digiti minimi muscle and tibialis anterior muscle.

6.6 Electromyography Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements of compound muscle action potential (CMAP), motor unit number index (MUNIX), and motor unit size index (MUSIX) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the electromyographic parameters of the subjects.

(3) Observation indicators:

CMAP: CAMP represents the summated action potentials of muscle fibers that are almost simultaneously activated when a certain nerve is directly or indirectly stimulated. The amplitude from baseline to peak, duration, latency of the negative phase of the potential, as well as the details of the stimulation method and recording method should be recorded.

MUNIX: A motor unit is a functional unit located in the anterior horn of the spinal cord, consisting of an alpha motor neuron and all the muscle fibers it innervates. It is the basic unit of motor function, with the minimum unit of normal muscle contraction being the contraction of one motor unit. MUNIX is a sensitive biomarker for monitoring the nerve innervation status of patients with neurodegenerative diseases and is a highly sensitive and effective marker for monitoring the loss of motor units in ALS. The method uses CMAP and the surface electromyographic interference pattern (SIP) to evaluate the number and size of motor units (MUs).

MUSIX: The size of a motor unit is determined by the number of nerve endings and branches of the neuron axon. Generally, larger muscles have larger motor unit. Each muscle contains a certain number of motor units. The calculation method is to divide the amplitude of the compound muscle action potential (CMAP) by the MUNIX value.

6.7 Imaging Indicators

(1) Collection time points: baseline, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurement with 7T MRI imaging before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the imaging indicators of the subjects.

(3) Observation indicators:

7T MRI imaging indicators: 7T ultra-high field magnetic resonance is an advanced magnetic resonance imaging system currently available for human body scanning. Compared with the traditional 1.5T and 3T magnetic resonances, 7T magnetic resonance can provide a doubled signal-to-noise ratio, finer spatial resolution, and more excellent tissue contrast. It is more suitable for observing the fine structure of tissues and physiological changes in functional metabolism and other information, thereby revealing physiological and pathological mechanisms that are difficult to be discovered by traditional platforms.

6.8 Gait Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo the following assessments: three-dimensional gait analysis under multitasks, Short Physical Performance Battery (SPPB), item 1 (Tandem Walking) of the Scale for the Assessment and Rating of Ataxia (SARA), and 6-meter walking test before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the gait function of the subjects.

(3) Observation indicators: Semi-quantitative scales include the Short Physical Performance Battery (SPPB) and item 1 (Tandem Walking) of the Scale for the Assessment and Rating of Ataxia (SARA); Quantitative gait assessment includes 6-meter walking test.

SPPB: The SPPB is a widely used method for evaluating muscle function. It consists of 3 items: balance test, 4-meter timed walking test, and timed sit-to-stand test. Each individual test can score a maximum of 4 points, giving a total score of 12

points. To improve the accuracy of the test, each test is usually repeated 2 to 3 times, with the shortest time recorded for scoring.

6-meter normal walking test: It is conducted on an 8-meter walkway with a timing taken over a 6-meter distance. Landmark markers are placed at the start of the walkway, at the 1-meter, the 7-meter, and the 8-meter points, for a total of 4 markers. Timing starts at the 1-meter and stops at the 7-meter mark to avoid the influence of markers on the subject's behavior, such as intentional changes in walking pace or nervousness. The test is performed 4 times; the first trial serves as a practice and is not recorded. The times from the subsequent 3 trials are recorded, and the average of these times is used for analysis.

Item 1: SARA is a clinical scale used for the semi-quantitative assessment of cerebellar ataxia severity. It can also serve as a rehabilitation indicator for gait and independence in daily activities for ataxic stroke patients. In this study, the gait part (Item 1) is selected. It consists of 8 levels corresponding to scores ranging from 1 to 8. Grade 1 is a mild abnormality corresponding to 1 point, up to grade 8 is unable to walk even with assistance corresponding to 8 points.

6.9 Quality of Life Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements with the Amyotrophic Lateral Sclerosis Self-Assessment Questionnaire (ALSAQ-40), EQ-5D-5L scale, and HAMA and HAMD-17 scales before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the quality of life of the subjects.

(3) Observation indicators:

ALSAQ-40: ALSAQ-40 is a commonly used quality-of-life scale for ALS. The sections on social interaction and eating ability provide subjective insights into articulation and swallowing functions. ALSAQ-40 consists of 40 items involving the patient's condition, requiring a 5-point scale rating. The questions cover five health status dimensions affected by the disease: physical activity (including 10 items), daily living activities and independence (10 items), eating (3 items), communication (7

items), and emotional function (10 items). Each dimension is scored on a scale from 0 to 100: 0 represents the possible best health status and 100 represents the possible worst health status.

EQ-5D-5L: EQ-5D-5L is a standardized scale for measuring health status, that is an updated version of the EQ-5D scale. The scale consists of two parts: the EQ-5D-5L descriptive system and the visual analogue scale (EQ-VAS). The EQ-5D-5L descriptive system describes five dimensions: Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression. Each dimension contains five levels: 1=No difficulty, 2=Slight difficulty, 3=Moderate difficulty, 4=Severe difficulty, 5=Unable to perform/very severe difficulty. The responses of the five dimensions can generate a five-digit code to describe the health status of the respondent. The health status 1,1,1,1,1 indicates no problems in all five dimensions. EQ-VAS records the self-rated health status of the respondent on a vertical visual analogue scale, with the ends of the scale marked as "The best health status you can imagine" and "The worst health status you can imagine".

6.10 Clinical Staging Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo assessment with the London ALS staging and the Milan-Turin staging (ALS-MITOS) before and after treatment. The results after treatment intervention will be compared with the baseline data to assess the disease progression of ALS patients and evaluate the effect of RAG-17 on the condition of the subjects.

(3) Observation indicators:

London ALS staging: According to the most recently affected region in the course of the disease, ALS is divided into 5 stages: Stage 1 = symptom onset (involvement of the first area); Stage 2A = confirmed diagnosis, and Stage 2B = involvement of the second area; Stage 3 = involvement of the third area; Stage 4A = the patient requires percutaneous endoscopic gastrostomy (PEG), and Stage 4B = the patient requires non-invasive ventilation; Stage 5 = death or mechanical ventilation. The average survival time and 5-year survival rate vary across stages.

Milan-Turin staging: The patient's functions are divided into 4 domains by assessing 4 key points (including mobility, swallowing, communication, and respiration). Each domain is scored as either 0 (below the threshold) or 1 (above the threshold). The total score across these domains determines the stage: Stage 0 = no functional loss; Stage 1 = loss of one functional domain; Stage 2 = loss of two functional domains; Stage 3 = loss of three functional domains; Stage 4 = loss of four functional domains; Stage 5 = death or mechanical ventilation.

6.11 Laboratory Examination Data Collection

(1) Collection time points: baseline, day 1, day 15, day 29, day 60, day 120, day 180, day 240

(2) Collection content: All subjects will undergo laboratory tests including blood routine, urine routine, liver function, kidney function, and coagulation tests before and after treatment respectively. The results after treatment intervention will be compared with the baseline data to evaluate the impact of RAG-17 on the general physical condition of the subjects and assist in determining the occurrence of adverse events and other related events.

All subjects will undergo the detection of SOD1 protein in cerebrospinal fluid, plasma neurofilament light chain protein (NFL), and the pharmacokinetics of RAG-17 in plasma and cerebrospinal fluid before and after treatment.

Additionally, during the dose-escalation phase, the plasma samples will be collected on the 4th day after treatment to detect PK and NFL levels. In the fixed-dose phase, the sampling time will be adjusted based on the PK and PD data from the dose escalation stage.

6.12 Other Information Collection

(1) Collection time points: baseline, day 1, day 15, day 29, day 60, day 120, day 180, day 240

(2) Collection content: During the study period, all the medications taken by the subjects will be recorded, including the reasons for use, drug names, dosages, and duration of use. All AEs, adverse reactions and their handling processes will also be documented; The timing of tracheostomy, invasive mechanical ventilation, death, and

the duration of invasive mechanical ventilation will be recorded; Subjects' medication adherence, cases of withdrawal or exclusion from the study will be recorded.

7. Follow-up Time

Follow-up plan: For all subjects, face-to-face visits will be conducted at baseline, day 1, day 15, day 29, day 60, day 120, day 180, and day 240. They will undergo tests including daily living function, respiratory function, muscle strength, quality of life, gait function, and clinical staging, as well as the collection of adverse events and concomitant medications use. The study is expected to be conducted at Beijing Tiantan Hospital, with a target recruitment of 6 participants, and is planned to be completed within 2 years.

Follow-up content: To evaluate the differences in clinical manifestations of the participants after receiving the RAG-17 intervention at baseline, day 1, day 15, day 29, day 60, day 120, and day 180, including:

- Laboratory examinations: tests including blood routine, urine routine, liver function, kidney function, coagulation, SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma and CSF will be conducted at baseline, day 1, day 15, day 29, day 60, day 120, day 180, and day 240.
- Daily living functions: ALSFRS-R scoring and Modified Norris Scale scoring will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Respiratory function: FVC evaluations will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Fatigue severity: FSS assessment will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Muscle strength test: MRC assessment will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Electromyogram indicators (abductor pollicis brevis, abductor digiti minimi, and anterior tibial muscle): CMAP, MUNIX, and MUSIX will be collected at baseline, day 15, day 29, day 90, and day 180.
- Imaging examinations: 7T magnetic resonance imaging will be conducted at baseline, day 29, day 90, and day 180.

- Gait function: SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Quality of life: assessments of ALSAQ-40, EQ-5D-5L, HAMA, and HAMD-17 will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Clinical staging: London ALS staging and Milan-Turin staging assessments will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Other indicators: information such as vital signs, abnormalities in heart/lung/abdomen examination, neurological examination, electrocardiogram, adverse events, concomitant medications, the time to tracheostomy, invasive mechanical ventilation, death, and the duration of invasive mechanical ventilation of the patients will be collected at baseline, day 1, day 15, day 29, day 60, day 90, day 120, day 180, and day 240.

8. Study Process

8.1 Information Collection and Informed Consent (Visit 1)

Patients who meet the inclusion criteria but do not meet the exclusion criteria will be introduced to the study protocol and purposes by investigator. The patient or their legal representatives will be provided with complete information about the study, accompanied by corresponding written materials, and a written informed consent form will be signed.

- Collection of medical history: including current medical history, past medical history, history of concomitant medication, history of smoking and alcohol consumption, family history;
- Physical examination: including vital signs, heart/lung/abdomen examination, neurological examination, 12-lead electrocardiogram;
- Laboratory examinations: including blood routine, urine routine, liver function, kidney function, coagulation function, BNP; Blood sample collection for verification of *SOD1* gene mutation sites, plasma neurofilament light chain protein (NFL);
- 7T magnetic resonance imaging;

- Assessment of respiratory function (including FVC)
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Assessment of daily living functions: ALSFRS-R score, Modified Norris Scale score;
- Assessment of fatigue severity: FSS scale score;
- Assessment of muscle strength and electromyogram indicators: MRC assessment (sternocleidomastoid muscle, deltoid muscle, biceps brachii, triceps brachii, common digital extensor muscle, abductor pollicis brevis, abductor digiti minimi, first dorsal interosseous muscle and iliopsoas muscle, quadriceps femoris, biceps femoris, anterior tibial muscle, gastrocnemius muscle, flexor muscle, extensor muscle grading); CMAP, MUNIX, MUSIX;
- Assessment of gait function: SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking;
- Assessment of quality of life: ALSAQ-40 and EQ-5D-5L scale scores;
- Assessment of emotional scale: HAMA and HAMD-17 scores.

8.2 Days 1-4 Post-Enrollment (First Treatment) (Visit 2)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect CSF 15 minutes after the treatment; Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma and CSF;
- Record adverse events and concomitant medications:
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver

function, kidney function, and coagulation function.

- Arrange the follow-up appointment for day 15, face-to-face follow-up.

8.3 Days 15-18 Post-Enrollment (Second Treatment) (Visit 3)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect CSF 15 minutes after the treatment; Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma and CSF;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- Arrange the follow-up appointment for day 29, face-to-face follow-up.

8.4 Days 29-32 Post-Enrollment (Third Treatment) (Visit 4)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These

samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;

- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect CSF 15 minutes after the treatment; Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma and CSF;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 60, face-to-face follow-up.

8.5 Days 60-63 Post-Enrollment (Fourth Treatment) (Visit 5)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;

- Post-Treatment Sampling: Collect CSF 15 minutes after the treatment; Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma and CSF;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 90, face-to-face follow-up.

8.6 Days 90-93 Post-Enrollment (Visit 6)

- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 120, face-to-face follow-up.

8.7 Days 120-123 after enrollment (Fifth treatment) (Visit 7)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment

protocol;

- Post-Treatment Sampling: Collect CSF 15 minutes after the treatment; Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma and CSF;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 180, face-to-face follow-up.

8.8 Days 180-183 Post-Enrollment (Sixth Treatment) (Visit 8)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect CSF 15 minutes after the treatment; Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma and CSF;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);

- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 240, face-to-face follow-up.

8.9 Days 240-243 after enrollment (Seventh treatment) (Visit 9)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect CSF 15 minutes after the treatment; Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma and CSF;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.

8.10 Study Flowchart

Examination / Treatment	**Screening / Baseline**	Treatment and Follow-up							
		1st Administration	2nd Administration	3rd Administration	4th Administration		5th Administration	6th Administration	6th Administration
		Day 1-4	Day 15-18	Day 29-32	Day 60-63	Day 90-93	Day 120-123	Day 180-183	Day 240-243
Verification of inclusion / exclusion criteria	√								

Sign the informed consent form	√								
Demographic data	√								
Medical history / Personal history / Family history	√								
Height / Weight / Waist circumference / Physical examination	√	√	√	√	√	√	√	√	√
Concomitant medication	√	√	√	√	√	√	√	√	√
Blood pressure / Respiration / Heart rate / Body temperature	√	√	√	√	√	√	√	√	√
Neurological examination	√	√	√	√	√	√	√	√	√
12-lead electrocardiogram	√	√	√	√	√	√	√	√	√
Laboratory examination[1]	√	√	√	√	√		√	√	√
ALSFRS-R score	√		√	√		√		√	
Modified Norris Scale	√		√	√		√		√	
Forced vital capacity	√		√	√		√		√	
Fatigue Severity Scale	√		√	√		√		√	
Muscle strength / Electromyogram	√		√	√		√		√	
7T MRI	√			√		√		√	
SPPB	√		√	√		√		√	
Tandem Walking	√		√	√		√		√	
6-meter walking	√		√	√		√		√	
Three-dimensional gait analysis under multitasking	√		√	√		√		√	
ALSAQ-40	√		√	√		√		√	
EQ-5D-5L	√		√	√		√		√	
Anxiety Scale (HAMA)	√		√	√		√		√	
Depression Scale (HAMD-17)	√		√	√		√		√	
London ALS staging	√		√	√		√		√	
Milan-Turin staging (AKS-MITOS)	√		√	√		√		√	
Intrathecal administration of RAG-17	√	√	√	√	√		√	√	√

Blood sample collection [2]	√	√	√	√	√		√	√	√
Cerebrospinal fluid sample collection [3]	√	√	√	√	√		√	√	√
Adverse events / Severe adverse events	√	√	√	√	√	√	√	√	√
Study drug adherence	√	√	√	√	√		√	√	√

[1] Laboratory examinations: blood routine, urine routine, liver and kidney functions, renal function, coagulation function, BNP (BNP is only examined at baseline).
[2] Samples are collected at baseline; during the treatments 1 to 7 , samples are collected at 4 hours before each treatment, and 15 minutes, 4 hours, 48 hours, and 4 days after each treatment.
[3] During treatments 1 to 7, samples are collected 15 minutes before and 15 minutes after each treatment.

9. Clinical Evaluation

9.1 Primary Outcomes：

1) Incidence of AEs and SAEs within 180 days after the first injection of RAG-17.
2) Changes in laboratory assessments, vital signs, ALSFRS-R scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

9.2 Second Outcomes：

1) Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
2) Changes in plasma NFL levels from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

Note: During the dose-escalation phase, evaluate the plasma NFL levels on day 4 after each administration. In the fixed-dose phase, consider adjusting the currently set PD sampling times based on the changes in the plasma NFL levels observed in the dose-escalation phase. This adjustment aims to ensure that pharmacodynamic characteristics of RAG-17 are accurately reflected in subsequent participants receiving the same dose of investigational drug.

3) Changes in the pharmacokinetics of RAG-17 in plasma and cerebrospinal fluid on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first

injection of RAG-17 in adult ALS patients.

Note: During the dose-escalation phase, evaluate the drug concentration in plasma on day 4 after administration. If RAG-17 cannot be detected in the plasma at the specific time points by the determined detection method, adjustments to the currently set PK sampling times may be considered during the fixed-dose phase. This adjustment aims to ensure that the pharmacokinetic characteristics of RAG-17 are accurately reflected in subsequent participants receiving the same dose of the investigational drug.

4) Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

9.3 Exploratory outcomes：

1) Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180.
2) Changes in fatigue severity (Fatigue Severity Scale, FSS) at on day 15, day 29, day 90, and day 180.
3) Changes in muscle strength on day 15, day 29, day 90, and day 180.
4) Changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180.
5) Changes in 7T MRI imaging features on day 29, day 90, and day 180.
6) Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180.
7) Changes in modified Norris scale scores on day 15, day 29, day 90, and day 180.
8) Changes in quality of life (including ALSAQ-40、EQ-5D-5L、HAMA and HAMD) on day 15, day 29, day 90, and day 180.
9) Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.

10. Known and Potential Risks and Benefits, and Risk Management Plan

10.1 Adverse Event Monitoring

All information related to adverse events, whether reported by the participants, identified by the investigators, or detected through physical examinations, laboratory tests, or other methods, should be recorded on the adverse event page of the case report form. These events will be managed and reported in accordance with the relevant regulations. A comprehensive adverse events report will include in the final trial report.

10.2 Adverse Event Definition

10.2.1 Adverse Event (AE)

An adverse event refers to any unfavorable medical occurrence or worsening of a pre-existing condition that arises in a participant during the use of the investigational drug, regardless of whether it has a causal relationship with the investigational drug treatment. Adverse medical occurrences can manifest as clinical symptoms/signs, diseases, or abnormal laboratory findings. AEs include any unfavorable medical occurrences that occur at any stage of the clinical study, including the lead-in or washout periods, even before the investigational drug has been administered to the participant. AEs include both serious and non-serious adverse events.

10.2.2 Serious Adverse Event

An adverse event during the study will be classified as serious, if it meets one or more of the following criteria:

- Results in death
- Immediately life-threatening

Note: Life-threatening refers to an event that poses an immediate risk to the patient's life at the time it occurs, not an event that could result in death if it were to worsen.

- Requires hospitalization or prolongs the current hospitalization
- Results in permanent or severe disability/incapacity, or loss of essential abilities required for normal living
- Leads to congenital anomaly, birth defects, or reproductive issues
- Constitutes a significant medical event that may endanger the subject or necessitate medical intervention to prevent the outcomes listed above.

10.3 Adverse Events Documentation

Non-serious AE: Non-serious AEs of interest occurring during the study period from the start until the end of the study (e.g. renal function impairment/increased serum creatinine, elevated liver function test indicators, upper gastrointestinal discomfort, muscle/bone pain, hypocalcemia, ocular inflammation, arrhythmia, fractures, etc.) and information about drug discontinuation due to SAEs must be documented. Documentation of other non-serious AEs is at the discretion of the investigator .

Serious AE: All SAEs must be documented.

10.4 Assessment of the Relationship Between AEs and the Investigational Drug

The relationship between AEs and the investigational drug will be assessed using a 5-level standard: ①Definitely related; ②Highly likely related; ③Possibly related; ④Unlikely related; ⑤Definitely unrelated; ⑥Unable to determine.

①Definitely related: Matches the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or disappears after dose reduction or drug withdrawal. Reaction reappears upon re-administration.

②Highly likely related: Matches to the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or disappears after dose reduction or drug withdrawal. However, the clinical status of the participants or other reasons may also cause the reaction.

③Possibly related: Matches the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or is not obvious after dose reduction or drug withdrawal. However, the clinical status of the subject or other reasons can equally explain the reaction.

④Unlikely related: Does not match the known reaction type of the suspected drug. Dose not align with the reasonable time frame after administration. The clinical status of the subject or other reasons may also cause the reaction.

⑤Definitely unrelated: Does not match the known reaction type of the suspected drug. Dose not align with the reasonable time frame after administration. The clinical

status of the subject or other reasons can also explain the reaction. Reaction decreases or disappears when clinical symptoms or other causes are ruled out..

⑥Unable to determine.

The incidence of AEs will be calculated and summarized for classifications ① + ② + ③ from each case report form.

10.5 Unexpected Adverse Event

An unexpected adverse event refers to any event not mentioned in the investigator's brochure, or differs in nature and severity from what is described in the investigator's brochure. In the absence of an investigator's brochure, the event is inconsistent with the risk information described in the study protocol or application materials.

10.6 Reporting of Serious Adverse Events

Notify the designated representative of the sponsor immediately. Send the signed and dated relevant pages of the case report form to the sponsor’s representative, along with copies of all examination results and their completion dates. At the same time, ensure the patient privacy is protected by removing all identifiable information from the clinical source document copies provided to the sponsor. Laboratory results should include normal range values.

All SAEs must be reported within 24 hours of occurrence. Record the SAE in the designed section of the CRF. After signing, report it preferably via fax to the monitoring center. The name, address and fax number of the monitoring center will be specified in the clinical trial protocol.

10.7 Risk Control and Management

In this study, the investigational drug RAG-17 may cause adverse reactions such as tremors, abnormal gait, and hind limb weakness. If such actions occur and are deemed related to the drug, it is recommended to stop the administration, monitor vital signs, conduct auxiliary examinations such as blood routine, coagulation profile, muscle enzymes, liver function, kidney function, blood gas analysis, ultrasound, CT scans. Provide timely symptomatic and supportive treatment based on clinical findings. If necessary, consult with relevant specialists to manage the adverse events effectively. For serious adverse events, it is necessary to ensure airway patency, autonomous

respiration, stable blood pressure and heart rate. If necessary, treatment should be carried out in the intensive care unit.

For all adverse events, the investigators should continue to follow up with patients and track any related outcomes (clinical symptoms, signs, laboratory test results) until the related clinical symptoms are completely resolved, the test results return to normal or the condition is stable. This follow-up should persist even if the patient withdraws from the clinical trial. Additionally, the monitoring team may request further investigations as needed. After the termination of the study, the investigators should still pay attention to any serious adverse events that may occur.

10.8 Subjects Benefits

Participation in this clinical study may help alleviate the subject's condition, but it is also possible that the investigational drug will not provide the expected therapeutic effect. In other words, subjects may not gain any direct benefits from participating in this study. However, this study may contribute to the development of new treatment options for patients with similar conditions, offering more choices to others. In addition, subjects may gain valuable information about their conditions through the physical examinations and medical tests conducted during the study.

11. Quality Control and Quality Assurance of the Study

Representatives of the sponsor or collaborators will regularly monitor the clinical study. The scope and nature of supervision will be determined based on considerations such as the clinical study's objectives, purposes, design, complexity, scale, and endpoints.

11.1 Protocol Adherence Management

- Careful selection of participants: When selecting subjects, investigators should confirm accurate diagnoses, understand common factors leading to dropout, conduct a comprehensive assessment of participants, and perform a pre-evaluation of adherence. Participants with high compliance potential should be carefully screened and selectively included.
- Informed consent process: Before signing the informed consent form, investigators should provide detailed and clear explanations to the subjects and

their families in an easy-to-understand manner. This ensure subjects fully understand the investigational drug and the treatment process. Investigators must thoroughly address participants' questions, respect their choices, and encourage confidence in completing the study, which fosters better adherence.

- Optimizing the protocol: The study protocol should be designed to minimize complexity, favoring simple and practical approaches that align with participants' daily lives. A well-designed, scientifically sound, and highly operable protocol will enhance adherence.
- Managing adverse events: If participants experience adverse reactions, adverse events, or serious adverse events during the clinical study, regardless of whether they are related to the investigational drug, researchers must promptly take appropriate treatment measures. Ensuring timely medical intervention safeguards participants' safety, builds trust in the study, and improves adherence.
- Follow-up and reminders: A contact information sheet and visit schedule should be established to remind participants of follow-up visits. For those who miss scheduled visits, researchers should promptly follow up via phone. These efforts help ensure participants remain engaged and adhere to the study protocol.

11.2 Study Monitoring

The primary responsibility of the monitoring team of the collaborators is to assist investigators and the sponsor or collaborators in ensuring the clinical study adheres to the highest standards of ethics, scientific rigor, professionalism, and regulatory compliance. According to the Good Clinical Practice (GCP) principle, the monitoring team should verify the CRF against the original source documents.

The monitoring team will regularly assess the progress of the study, through on-site visits, letters, or telephone communication. This includes evaluating adherence to the clinical protocol by researchers and participants and addressing urgent issues. Medical monitors will also periodically review participant safety. During these monitoring visits, the monitors will collaborate with the investigators to focus on the following key points (not exhaustive): informed consent of subjects, recruitment and follow-up of subjects, documentation and reporting of serious adverse event, concomitant

treatment and data quality.

11.3 Data Verification

The following measures will be taken to ensure the accuracy, consistency, completeness and reliability of the data:

- Regular on-site monitoring of the study;
- CRF summary based on source documents;
- Data management quality control checks;
- Medical review by the sponsor.

The sponsor or designated personnel (such as CRO) will be responsible for the data management in this clinical study. The responsible party will design and distribute CRFs and oversee their monitoring. If the data are incomplete or inconsistent, the responsible party will issue queries for site staff to address and make necessary corrections.

11.4 Personnel Training

The investigators shall ensure adherence to the clinical trial protocol, compliance with the Good Clinical Practice (GCP) guidelines outlined in the ICH, and conformity with applicable laws and regulations during the conduct of the clinical trial.

The investigators should ensure adherence to all study procedures outlined in the clinical trial protocol (including safety principles). The investigator should accurately and clearly provide reliable data and all information required by the clinical trial protocol (e.g., drug overdose, pregnancy) through appropriate methods, such as CRFs, deviation resolution forms (DRFs), and others, in accordance with relevant requirements. Additionally, the investigator must ensure that the monitoring team has direct access to the source documents.

The investigators may appoint individuals deemed suitable to assist in conducting the study. These assistants will help manage the clinical study in accordance with the study protocol. All assistants will be appointed and documented in a timely manner. They will work under the supervision and management of the investigator, who will provide them with a copy of the clinical study protocol and all necessary information. The sponsor or collaborator of the clinical study are accountable to the health

authority for ensuring the proper management of the clinical study by employing all appropriate means, such as ethics, compliance with the clinical study protocol, completeness and validity of the data recorded in the CRF.

11.5 Data Management

During the clinical study, the data of the subjects should be collected anonymously in the CRF, with subjects identified only by their number and the initials of their name. In cases where participant identity must be disclosed due to safety or administrative reasons, both the investigator and any involved parties share the responsibility for maintaining confidentiality. The informed consent form allows the subject to agree to authorized personnel from the sponsor or collaborators, ethics committees, and regulatory authorities directly accessing relevant source documents associated with the CRF (e.g., medical records, appointment logs, original laboratory records). These individuals must adhere to professional confidentiality standards and ensure the privacy of all participant personal or medical information.

The medical information obtained in the study should remain confidential and may only be disclosed to third parties with the permission of the informed consent form and the subject's signed authorization form (or separate authorization for the use and disclosure of personal health information) or when permitted or required by law.

For treatment purposes, medical information may be shared with the participant's personal physician or other appropriate healthcare professionals responsible for the participant's well-being.

11.6 Handling of Biological Samples

The biological samples, such as blood and cerebrospinal fluid collected in this study, will be processed (e.g. centrifugation and aliquoting) at Beijing Tiantan Hospital, Capital Medical University. Samples designed for evaluation will be analyzed at a centralized clinical laboratory with recognized accredited certification. All biological including blood and cerebrospinal fluid will be stored at Beijing Tiantan Hospital, Capital Medical University for 10 years.

12. Data Retention

The reviewed CRF and imaging data will be carefully entered and processed by the

data management center and properly stored. Once data entry is completed, the CRF will be stored by the research center.

The study monitor will perform ongoing source data verification to ensure that the key study protocol data (i.e., source data) transcribed on the CRF by authorized on-site personnel are accurate and complete, and that they can be verified against the original source documents.

Source documents are the original records where subject data is first recorded and stored. These include, but are not limited to hospital records, clinical and office charts, laboratory records, memos, subject diaries or assessment checklists, pharmacy dispensing records, data from automated instrument records, verified and certified as accurate and complete copies of audio transcriptions, microfilms, photographic negatives, microfiche or magnetic media, computed tomography, subject files, and records kept in pharmacies, laboratories and medical technology departments related to clinical studies.

The source documents required to verify the validity and completeness of the data transcribed on the CRF must never be deleted or destroyed.

To facilitate source data verification, the investigator and the institution must allow the sponsor to access applicable source documents and reports for direct inspection. This is to support monitoring, sponsor audits, clinical research reviews, and summaries by the management committees or ethics committees. Additionally, appropriate regulatory agencies must be allowed to inspect the study site.

13. Data Safety Monitoring

The clinical study will develop a data safety monitoring plan based on the level of risk involved. All adverse events will be thoroughly documented, appropriately managed, and followed up until resolved or the condition stabilizes. Serious adverse events and unexpected events will be promptly reported to the ethics committee, regulatory authorities, the sponsor, and the drug regulatory agency in accordance with relevant regulations. The principal investigator will regularly conduct cumulative reviews of all adverse events and, when necessary, convene investigator meetings to assess the risks and benefits of the study. For studies with risks exceeding the minimal

threshold, independent data monitors will be assigned to oversee the study data. For high-risk studies, an independent data safety monitoring board will be established to review the accumulated safety and efficacy data and provide recommendations on whether the study should continue.

During the clinical trial, participant data will be collected anonymously in the CRF, with participants identified only by their participant number and the initials of their name. In cases where participant identity must be disclosed due to safety or administrative reasons, both the investigator and the involved parties share the responsibility for maintaining confidentiality. The informed consent form allows participants to consent to the authorized sponsor, ethics committee, and regulatory authorities directly accessing relevant source documents on the CRFs (e.g., medical records, appointment logs, original laboratory records). These individuals must adhere to professional confidentiality standards and maintain the privacy of all participant personal or medical information.

14. Statistical Analysis

- Statistical analysis will be performed with SAS software.
- Demographic, medical, histories and baseline characteristics information will be listed and summarized.
- The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after treatment. Safety evaluation will be analyzed using safety data set. The number of people of events should be recorded in detail and showing the event rate in summary statement. The event rate will be calculated as the number patients experiencing the event / the number of patients enrolled.
- Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis.

Following the completion of the 180-day follow-up assessments for all participants, the data were analyzed.

15. Ethical Considerations

The clinical study will adhere to the World Medical Association's Declaration of Helsinki and other relevant regulations. Before the study begins, the trial protocol will be approved by the ethics committee. Before enrollment, the investigator is responsible for providing a complete and comprehensive explanation of the study's purpose, procedures, and potential risks to the participant or their representative. A written informed consent form must be signed, ensuring that participants are aware of their right to withdraw from the study at any time. The informed consent form will be retained as part of the clinical study documentation for future reference. Throughout the study, the personal privacy and data confidentiality of participants will be safeguarded.

Screening and informed consent: The investigator (or designated personnel authorized and responsible by the investigator, in compliance with relevant regulations) will recruit participants in an independent consultation room at Beijing Tiantan Hospital, Capital Medical University, ensuring the privacy and confidentiality of the participants. Participants and their legal representatives should be fully informed about all relevant aspects of the clinical trial, including written information approved by the ethics committee. The investigator should use language or terms that the participant can understand, providing as much information as possible about the study, and allow sufficient time for the participant or their legal representative to consider whether to participate. Before participating in the clinical trial, the patient or their legal representative must discuss the informed consent with the investigator and sign the informed consent form (including the participant's name and the date). The investigator should provide the participant with a copy of the signed informed consent form.

16. Data Confidentiality

Throughout the entire research and data analysis process, participant information will be strictly protected. The executive committee will disclose the trial results in

accordance with management regulations and procedures. Once the database is locked, the trial results will be published as soon as possible. Biostatistical experts involved in the study have the right to access the dataset but will not be able to identify any individual participants. In line with the GCP principles outlined in the ICH guidelines, the monitoring team will verify the CRFs against the original source documents. The informed consent form will include a statement that allows participants to consent to authorized personnel from the sponsor, ethics committee, and regulatory authorities directly accessing relevant source documents on the CRFs (e.g., medical records, appointment logs, original laboratory records). Researchers must adhere to professional confidentiality standards and ensure the confidentiality of all personal and medical information of the participants.

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Safety and Tolerance of RAG-17 in Amyotrophic Lateral Sclerosis Patients With *SOD1* Gene Mutation (CREATION)

Study Protocol

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Protocol Version: 1.2

Apr. 26th, 2023

Summary of the Trial Protocol

Study Title	Safety and tolerance of RAG-17 in amyotrophic lateral sclerosis patients with *SOD1* gene mutation
Study Purposes	To evaluate the safety, tolerance and pharmacokinetics (PK) of RAG-17 in adult ALS subjects with *SOD1* gene mutation. **Primary purposes：** 1. To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17. 2. To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17. **Secondary purposes：** 1. To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2. To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3. To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 4. To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

	Exploratory purposes： 1. To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180. 2. To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180. 3. To evaluate the changes in muscle strength on day 15, day 29, day 90, and day 180. 4. To evaluate the changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180. 5. To evaluate the changes in 7T MRI imaging features on day 29, day 90, and day 180. 6. To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180. 7. To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, and day 180. 8. To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, and day 180. 9. To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.
Study Design	Ongoing open-label, single-center, first-in-human and dose escalation clinical study

Total number of cases	Six ALS patients with *SOD1* gene mutations
Subjects Selection	**Inclusion Criteria** (1) Patients permitted by medical personnel who could complete the clinical trial. (2) Age 18~75 years, gender unlimited. (3) Clearly identify ALS patients with *SOD1* gene mutations (known *SOD1* mutation sites and reported disease progression). (4) Forced vital capacity during screening period ≥ 80% of expected lung capacity. (5) Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology. (6) The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form. (7) The subjects (including male subjects) have no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum. **Exclusion Criteria** (1) The *SOD1* mutation site occurs in patients with nucleotides 44-66 (calculated from the translation of SOD1 protein) and P.F21C mutations. (2) Patients who have previously received or are currently receiving Tofersen treatment. (3) HIV test positive or positive test history.

	(4) Hepatitis C virus antibody positive or positive test history. (5) Active hepatitis B infection (hepatitis B surface antigen positive and/or hepatitis B core antibody positive). (6) Other experimental drugs used within one month or within five drug half-lives. (7) Lumbar diseases and deformities. (8) Suffering from other diseases known to be related to motor neuron dysfunction, which may confuse or blur the diagnosis of ALS. (9) Other psychiatric disorders diagnosed according to DSM-V diagnostic criteria, or obvious suicidal intentions. (10) Combined with severe liver dysfunction, renal dysfunction, or severe heart dysfunction (severe liver dysfunction refers to ALT value ≥ 2.0 times the upper limit of normal value or AST value ≥ 2.0 times the upper limit of normal value; severe renal dysfunction refers to CRE ≥ 1.5 times the upper limit of normal value or eGFR < 40mL/min/1.73m^2; severe heart dysfunction refers to NYHA 3-4 levels). (11) Permanent and sustained dependence on ventilator assisted ventilation. (12) History of combined alcohol and drug abuse. (13) Pregnant, lactating, or potentially pregnant patients, as well as those planning to conceive. (14) Patients participating in other clinical trials or applying other biologics, drugs, or devices under study. (15) Patients who have received any vaccination within 28 days. (16) Contraindications for MRI examination (ie. claustrophobia). (17) Those who can’t complete follow-up due to other reasons.

Therapeutic Regimen

Study drug：Aseptic aqueous solution of the RAG-17 formulation (sodium salt)

Storage condition：-20°C ± 5°C, stored in a frozen state

Specification：75 mg (2.5 mL)

Route, dosage and schedule of administration: Intrathecal administration. The safety, tolerance, and pharmacokinetics of the RAG-17 drug will be evaluated by a dose-escalation approach. The dose of RAG-17 will range from a minimum of 60 mg to the maximum tolerated dose (MTD). Administration will be conducted once every two weeks, starting at 60 mg, for dose escalation. Once the tolerated dose is reached, a fixed dose of the drug will be administered every two months for continued treatment, with a total treatment duration of 8 months. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD. For the subsequent maintenance treatment phase, the optimal dose between the safe dose and the MTD will be selected as the dose for continued treatment.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	DLT/dose	Intrathecal injection	6
5th Administration	D120	Optimal dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

Dose escalation criteria: If no AEs or SAEs occur within 14 days after the first administration, the subject proceed with a dose

	escalation of 30 mg every 14 days during the observation period. It is planned to achieve DLT through 3 to 4 dose escalations, followed by continuous treatment with the optimal dose for a 6-month treatment period. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD.
Study Outcomes	**Primary outcomes** 1) Incidence of AEs and SAEs within 180 days after the first injection RAG-17. 2) Changes in laboratory assessments, vital signs, ALSFRS-R scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection RAG-17. **Second outcomes：** 1) Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2) Changes in plasma NFL levels from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3) Changes in the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 4) Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

	Exploratory outcomes： 1) Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180. 2) Changes in fatigue severity (Fatigue Severity Scale, FSS) at on day 15, day 29, day 90, and day 180. 3) Changes in muscle strength on day 15, day 29, day 90, and day 180. 4) Changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180. 5) Changes in 7T MRI imaging features on day 29, day 90, and day 180. 6) Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180. 7) Changes in modified Norris scale scores on day 15, day 29, day 90, and day 180. 8) Changes in quality of life (including ALSAQ-40、EQ-5D-5L、HAMA and HAMD) on day 15, day 29, day 90, and day 180. 9) Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.
Statistical Analysis	• Statistical analysis will be performed with SAS software. • Demographic, medical, histories and baseline characteristics information will be listed and summarized. • The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after

	treatment. Safety evaluation will be analyzed using safety data set. The number of people of events should be recorded in detail and showing the event rate in summary statement. The event rate will be calculated as the number patients experiencing the event / the number of patients enrolled. • Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 180-day follow-up assessments for all participants, the data were analyzed.
Study Duration	2 years

1. Study Background

Amyotrophic lateral sclerosis (ALS) is a chronic, progressive neurodegenerative disease characterized by prominent damage to both upper and lower motor neurons, manifesting in symptoms such as muscle weakness, muscle atrophy, bulbar paralysis, and pyramidal signs. ALS is a rare disease, with a global standardized annual incidence rate of only 1.68 cases per 100,000 person-years[1]. The exact incidence rate of ALS in China still not well-established, but it is estimated that there are at least 100,000 patients [2]. The peak age of onset for ALS is around 50 years, with a growing trend toward earlier onset ages[3]. ALS is incurable, with a typical survival period of 3-5 years, and only approximately 10% of patients survive for more than 10 years[4]. As a result, it is listed by the World Health Organization as one of the "five major incurable diseases in the world" alongside cancer, acquired immunodeficiency syndrome, leukemia and rheumatoid arthritis.

Currently, the pathogenesis of ALS remains unclear, and available treatments are limited, primarily focusing on symptomatic relief rather than a cure. Since 1980, more than 80 randomized controlled trials (RCTs) have been conducted globally. Only three drugs for the treatment of ALS have been approved by the U.S. Food and Drug Administration (FDA): the neuroprotective drug riluzole, which inhibits excessive neurotransmission; the antioxidant edaravone; and Relyvrio (sodium phenylbutyrate and taurursodiol)[5-7]. Drug interventions for ALS include anti-apoptosis, anti-inflammation, anti-excitotoxicity, anti-oxidation, anti-aggregation, neurotrophic growth factors and neuroprotection, among others. In 2017, riluzole, the primary drug for ALS, became the first FDA-approved drug treatment for the disease. However, it cannot halt the progression of ALS, let alone reverse it, and only extends patient survival by 2 to 3 months. The riluzole costs about 1,800~4,000 RMB per month. However, it is only covered by medical insurance in certain provinces and cities. Edaravone is indicated for cerebral infarction and was approved for the treatment of ALS in the United States in 2019. It was approved for use in China in September 2020, specifically for patients with acceptable respiratory function in the early stage (within 2 years) of the disease. In September 2022, the FDA approved Relyvrio, a

combination drug composed of sodium phenylbutrate and taurursodiol, for the treatment of adult patients with ALS. The approval process for Relyvrio drug at the FDA has been marked by numerous challenges. At the first Peripheral and Central Nervous System Drug Advisory Committee (PCNSDAC) review meeting organized by the FDA in March 2022, the expert panel expressed concerns regarding issues such as the small scale of the Relyvri clinical trialo, data deficiencies, and the applicability of statistical methods, with a voting result of 6:4. In response to this, Amylyx reanalyzed the survival data of the CENTAUR trial of Relyvrio and submitted it, along with the biomarker data from another phase II clinical trial for Alzheimer's disease to the FDA for re-evaluation. At the second PCNSDAC meeting in September, the new drug application of Relyvrio was approved with a voting result of 7:2, and it was subsequently approved by the FDA. Currently, this drug has not been approved in China. As a result, existing therapies for ALS remain limited, and the demand for effective ALS treatments remains unmet.

Currently, the pathogenesis of ALS remains unclear, and available treatment are limited, mainly focusing on symptomatic relief rather than a cure. ALS can be divided into sporadic ALS (sALS) and familial ALS (fALS), with sALS accounting for the majority of cases (approximately 90%) and fALS accounting for 5-10%[8]. fALS can result from mutations in multiple genes, with the most common being a mutation in the *superoxide dismutase 1* (*SOD1*), which occurs in 2% to 7% of cases[9]. Approximately 20% of fALS and 5% of sALS cases are associated with the *SOD1* gene. Asia has the highest number of ALS, with Asian patients typically exhibiting a younger age of onset, a lower proportion of bulbar-onset ALS, and more pronounced cognitive dysfunction[10]. In Asia, fALS is mainly caused by *SOD1* gene mutations, whereas in Europe, mutations in the *C9ORF72* gene are more common. The frequency of the *SOD1* gene mutations is highest among ALS patient in central and southern China[11]. SOD1 is a potent antioxidant enzyme that, when combined with copper and zinc ions, helps convert superoxide into oxygen molecules and hydrogen peroxide, thereby protecting cells from damage[12-14]. Mutations in the *SOD1* gene in ALS patients lead to misfolding and accumulation of the SOD1 protein within

spinal motor neurons. While several mutations in SOD1 have been identified, not all of them result in ALS. Harmful mutations alter the functional activity of SOD1, with loss-of-function mutations impairing degradation of reactive oxygen species (ROS), which damages motor neurons, and gain-of-function mutations increasing oxidative activity, resulting in excessive production of hydrogen peroxide and enhancing protein-protein interactions[15-17]. These effects disrupt SOD1 protein level and activity, inhibit axonal transport, and cause mitochondrial dysfunction[15], ultimately leading to neuronal degeneration and death. Therefore, reducing the level of toxic SOD1 protein may offer an effective therapeutic approach for ALS caused by *SOD1* mutations. Researches have indicated that knockdown or knockout of normal SOD1 expression does not result in significant side effects[18-19].

Currently, several drugs under investigation have entered clinical studies, including small molecule drugs, antibody drugs, antisense oligonucleotides (ASO) drugs and cell therapy. Among these, except for small nucleic acid drugs (antisense oligonucleotides, small interfering RNA) which directly target pathogenic genes, the others are non-disease-modifying therapies. Tofersen, an antisense oligonucleotide drug developed by Biogen for the treatment of *SOD1*-ALS, binds to the mRNA encoding SOD1 and is degraded by ribonuclease H (RNase-H), thereby reducing the production of mutant SOD1 protein[20]. Tofersen is currently undergoing a New Drug Application (NDA) review by the U.S. FDA. The NDA submission for Tofersen is based on data from a Phase 1 study in healthy volunteers, a dose-escalation Phase 1/2 clinical study, a Phase 3 VALOR clinical study, and an open-label extension (OLE) study. While the Phase III of Tofersen for the treatment of did not meet the primary endpoint, Biogen has observed indications of reduced disease progression in several secondary and exploratory endpoints. Biogen's integration of 12-month data shows that earlier use of Tofersen, compared to delayed initiation, significantly reduces the neurofilament levels and slows the decline of various efficacy endpoints. In terms of adverse events, the most common side effects in patients were headache, falls, back pain and limb pain. About 6.7% of patients had serious neurological-related events, including myelitis, radiculitis, aseptic meningitis and optic disc edema[21-22].

RNA interference (RNAi) is a biopharmaceutical technology that utilizes double-stranded RNA molecules, known as small interfering RNA (siRNA), to target and silence specific genes at the mRNA level. Based on this mechanism, five drugs have been approved by the U.S. FDA, with nearly a hundred others currently in clinical development. RAG-17 is a 20 kDa siRNA targeting the *SOD1* gene. Through the RNAi mechanism, RAG-17 binds to *SOD1* mRNA, leading to the degradation of the target mRNA by the RNA-induced silencing complex (RISC). By recognizing and mediating the degradation of the target mRNA, RAG-17 silences the *SOD1* gene and downregulates the expression of the SOD-1 protein. RAG-17 is administered via intrathecal injection and is intended for the treatment of ALS patients with *SOD1* mutations. Compared to single-stranded self-delivering ASO drugs, siRNA offers a cascade amplification effect on the target gene and provides a higher gene silencing efficiency. This drug leverages a unique delivery technology for small nucleic acid drugs, enabling efficient siRNA delivery to the central nervous system and offering the potential for treatment of neurological diseases. The schematic diagram of the mechanism of RAG-17 in the treatment of ALS is shown below.

The *in vitro* pharmacodynamic results indicate that RAG-17 shows a concentration-dependent knockdown of *hSOD1* mRNA, with a half-maximal inhibitory concentration (IC50) of 0.011 nM. Compared to the control drug Tofersen, the IC50 of RAG-17 is 100 times lower, and the maximum inhibition rate reaches more than 90%. Cell apoptosis assays show that RAG-17 has no toxic response of cell apoptosis. Off-target effect analysis results show that RAG-17 dose not significantly affect the mRNA expression levels of non-target genes, demonstrating its specificity for *SOD1* gene knockdown.

The h*SOD1*^{G93A} transgenic mice exhibit progressive motor neuron degeneration, which closely resembles human ALS and is a widely recognized animal model for studying ALS. It has been extensively used in ALS pathogenesis research and drug screening [23-25]. To evaluated the inhibitory activity of RAG-17 on the target gene *in vivo*, a single-dose proof of concept (POC) experiment was conducted using the h*SOD1*^{G93A} ALS disease model mice. At 60 days of age, 200 μg of the siRNA drug

RAG-17 (~10 nmol) and the control drug Tofersen (28 nmol) were injected into the lateral ventricle. Tissue RNA was extracted 14 days post-administration, and RT-PCR was performed to assess the target gene, human *SOD1*. The results indicated that the mRNA expression of *SOD1* in both brain and spinal cord tissues was reduced by approximately 80%, with RAG-17 demonstrating superior gene down-regulation compared to the control drug.

Subsequently, a multi-dose pharmacodynamics (PD) experiment was conducted. Doses of 50 μg, 100 μg, 200 μg, and 400 μg of the siRNA drug RAG-17 were injected into the lateral ventricle. The PD indicators (relative expression levels of human *SOD1* mRNA) in the target tissues were assessed at 14 days and 56 days post-injection to determine the pharmacodynamic activity, dose response and the duration of effect at the respective doses. The results showed that gene knockdown was dose-dependent in three different regions of the brain (prefrontal cortex, cerebellum, and other brain tissues) and the spinal cord. Notably, only the high-dose group (400 μg) maintained 50% gene knockdown (KD) activity 56 days after injection, although this activity was weaker compared to 14 days post-injection. Compared to small molecule drugs, the significant advantage of small nucleic acid drugs is the extended duration of their therapeutic effect. Further testing at an extended time point (8 weeks) revealed that RAG-17 sustained the pharmacological activity with gene knockdown for at least 8 weeks in the model mice, providing valuable data to support the potential frequency of clinical administration.

To further verify the correlation between drug exposure in tissues and drug efficacy, the stem-loop PCR method was applied to detect the siRNA concentration in tissues at 14 days and 56 days post-administration. The results demonstrated a clear correlation between the drug concentration in tissues and PD effects.

To further explore the pharmacodynamics of RAG-17, human *SOD1*^{G93A} transgenic ALS disease model mice were used. Both male and female ALS disease model mice were administered intrathecally twice on the 68th and 100th days of life, with doses of the RAG-17 (RD-12500) at 75 μg, 150 μg, and 300 μg. Two negative control groups were included: a vehicle group and an inactvice (scrambled siRNA) group. The

scrambled siRNA served as a control for the target siRNA, being non-functional, and used to assess the potential inhibitory effects of the target siRNA. Additionally, 150 ug of Tofersen (RD-10748) was included as the positive control group. This study conducted pharmacodynamic evaluations based on multiple indicators, including animal body weight, motor function (grip strength in the fatigue rotating rod test, rotarod test, and open field test), disease onset time, lifespan, and neurological function score (neuroscore).

The results of animal weight changes indicated that both the vehicle group and the inactive group began to show a significant decrease in body weight around 130 days, with a more pronounced decrease in male mice. This suggests that the onset of h*SOD1*^{G93A} ALS disease model occurs earlier in males than in females. Among the groups, the positive group (RD-10748) showed improvements in body weight, with more significant weight maintenance in male mice. The body weight data from the drug treatment groups demonstrated a dose-dependent alleviation of weight loss, especially in female mice. As the disease progresses in ALS mice, their body weight gradually decreases. Compared to the vehicle control group, all dose groups of RD-12500 (RAG-17) and ASO (RD-10748) demonstrated a delayed onset of disease.

After two intrathecal administrations in the ALS disease model mice, all treatment groups of both male and female mice showed an extension in the median survival period. The low-dose group of of 75 μg RAG-17 and the medium-dose of 150 μg ASO (RD-10748) demonstrated comparable efficacy. The survival periods for male mice were 207 days (75 μg RAG-17) and 191 days (150 μg ASO), respectively. The survival periods of the female group were 198.5 days (75 μg RAG-17) and 189 days (150 μg ASO), respectively. In the female high-dose group (300 μg), the survival period was significantly extended to 227 days, representing a 54-day increase compared to the vehicle control group (173 days). However, the high-dose group (300 μg) in the male mice did not show a marked superiority. Female animals exhibited greater sensitive to drug treatment, and the results aligned with the trend of weight change.

The typical pathological feature of h*SOD1*^{G93A} ALS disease model mice is the

progressive loss of motor function. The Rotarod test for motor function fatigue and the Grip strength test were used to evaluate the therapeutic effect of the drug on the ALS model. The results showed that, in the Rotarod test, the drug-treated group demonstrated a significant increase in the time ALS mice were able to maintain their position on the accelerating rotating rod at 0-30 rpm, compared to both the vehicle and the inactive control groups. The Grip strength test revealed that the drug treatment group significantly improved the hindlimb muscle function of the disease model mice. Additionally, the open field test, which evaluates autonomous behavior, exploratory behavior and anxiety in a novel environment, showed that the drug-treatment group exhibited improved autonomous motor ability, with a dose-dependence effect.

Before performing the Rotarod and hindlimb grip strength behavioral tests, the neurological function of ALS mice was scored on a scale of 0 to 4 points. This scoring system objectively evaluates the common hindlimb dysfunction in h*SOD1*^{G93A} transgenic mice. The results showed that mice in the vehicle (aCSF) and inactive control groups exhibited abnormal tilting (NS2 symptoms) at PND130 days, with the severity progressively worsening until hindlimb paralysis (i.e., ≥ NS3). In contrast, at all time points during the study, the average score of all RAG-17 treatment doses never exceeded NS2. These results indicated that RAG-17 treatment, regardless of the dose, improved motor function in the animals compared with the control group.

In conclusion, RAG-17 treatment can effectively inhibit the gene expression of h*SOD1* in h*SOD1*^{G93A} transgenic mice, significantly delay the disease onset, extend the survival time of affected mice, enhance their grip strength , and improve the motor function.

Currently, the non-clinical safety evaluation of RAG-17 is ongoing. The safety dose exploration experiments have been completed in both rats (following two intrathecal administrations, a non-relevant species) and cynomolgus monkeys (a relevant species). In the rat safety dose exploration experiment, the main findings included transient neurological functions such as tremors, abnormal gait, and hindlimb weakness, in some animals of the medium-high dose group (2.5mg/dose) and high dose group (4.5mg/dose) after intrathecal drug injection. In the safety dose exploration

experiment with cynomolgus monkeys, one male monkey (3001) died after administration of the high dose of 200mg (equivalent to 2g in human dose), while another female monkey (3501) showed severe toxic reactions but gradually recovered over days 17-18. The two animals in the low-dose group of 30mg showed no abnormalities after the first administration, but tremors occurred after the second administration. As a result, animals in the vehicle group were used as alternatives and received the medium-dose experiment of 50mg. The 50mg dose group (equivalent to 500mg in human dose) showed transient tremors, body stiffness, and reduced activity after administration, but these symptoms resolved within approximately 2 to 4 hours.

In terms of histopathology, axonal degeneration of the sciatic nerve, unrelated to the dose, was observed in both species. In the cynomolgus monkey study (H59-0025-TX), mild to mild multifocal axonal degeneration was observed in both bilateral and unilateral sciatic nerves of the male in the 50 mg/dose and 30 mg/dose groups. Additionally, mild to mild multifocal axonal degeneration was observed in the female monkeys across all dose groups. This alteration is characterized by a "bubbly" appearance of multiple affected fibers, axonal rupture (aggregating into eosinophilic masses), and some cellular debris surrounding the vacuoles. Although there was no control group in the cynomolgus monkey study, the changes observed in the sciatic nerve were still considered related to the test drug, consistent with the findings from the rat study. In each dose group, mild multifocal axonal degeneration was observed at the injection site in the female monkeys. The characteristics of this alteration are similar to those observed in the sciatic nerve and may be due to the administration procedure. In addition, mild to mild diffuse vacuolation of macrophages in the medulla of mesenteric lymph nodes was observed in all dose groups. Since these adverse reactions were not dose-dependent, their correlation with the test drug cannot be determined at this time.

Based on the results of the safety dose exploration experiments, formal GLP toxicology experiments are currently underway. These formal GLP toxicology tests have reduced the administration doses for rats and cynomolgus monkeys, as well as optimized the intrathecal injection procedure. The preliminary safety dose exploration

experiment results of rats and cynomolgus monkeys can also provide insight into the potential clinical risks of this test drug.

This project conducts a single-center, dose-escalation clinical study, aiming to evaluate the safety, tolerance and pharmacokinetics of RAG-17 in adult ALS patients carrying the *SOD1* mutation, in preparation for subsequent efficacy clinical trial.

2. Study Purposes

This study primarily aims to evaluate the safety, tolerance and pharmacokinetics of RAG-17 in adult ALS patients with *SOD1* gene mutation.

2.1 Primary Purposes：

(1) To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17.

(2) To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

2.2 Second Purposes：

(1) To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(2) To evaluate the changes in neurofilament light chain (NFL) levels in plasma frombaseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(3) To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(4) To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

2.3 Exploratory Purposes：

(1) To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180.

(2) To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180.

(3) To evaluate the changes in muscle strength on day 15, day 29, day 90, and day 180.

(4) To evaluate the changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180.

(5) To evaluate the changes in 7T MRI imaging features on day 29, day 90, and day 180.

(6) To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180.

(7) To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, and day 180.

(8) To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, and day 180.

(9) To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.

3. Study Design Type, Principles and Experimental Procedures

3.1 Study Design

The study is an ongoing open-label, single-center, first-in-human, and dose escalation clinical trial.

Adult patients with a confirmed or probable familial or sporadic of ALS with confirmed *SOD1* gene mutations (forced vital capacity ≥ 80% of predicted value) will be included according to the revised El Escorial criteria.

1) **Administration：** A total of 7 doses will be administered on Day 1, Day 15, Day 29, Day 60, Day 120, Day 180, and Day 240. The drug will be administered via

intrathecal injection of RAG-17, with doses of 60 mg per administration, 90 mg per administration, 120 mg per administration, DLT per administration, with the last three doses being the optimal dose. The treatment period of this study is 8 months. Various indicators, adverse events, and concomitant medications will be collected on Day 1, Day 15, Day 29, Day 60, Day 90, and Day 180 post-treatment. The study is expected to be conducted at a single center, Beijing Tiantan Hospital, with the recruitment of 6 participants, aiming to complete the study within 2 years.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	DLT/dose	Intrathecal injection	6
5th Administration	D120	Optimal dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

2) **Dose Escalation Criteria:** If no AEs or SAEs occur within 14 days after the first administration, the subject proceed with a dose escalation of 30 mg every 14 days during the observation period. It is planned to achieve DLT through 3 to 4 dose escalations, followed by continuous treatment with the optimal dose for a 6-month treatment period. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD.

3) **Dose Escalation Stopping Criteria：** 'Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0' will be used as the toxicity evaluation standard for this protocol.

If a dose meets any of the following criteria, dose escalation will be considered for stopping or the trial will be continued at an additional lower dose level, unless it can

be clearly confirmed that the adverse event is unrelated to the drug (e.g., skin abrasions, hospitalization or death caused from a car accident).

① At the same dose level (within the same administration group), if ≥ 1 subject experiences an AE of grade ≥ 2;

② Other situations that may increase the risk of subjects, which will be discussed by the investigator to decide whether to terminate dose escalation.

4) **Individual Drug Withdrawal Criteria:** A subject will no longer participate in the subsequent administration, if they meet any of the following criteria:

① During the trial, the subject experiences a SAE or an AE of grade ≥ 3 (including laboratory abnormalities) , regardless of whether it is related to the drug;

② The subject experiences an event that, in the investigator's assessment, may significantly increase the risk to the subject.

3.2 Sample Size

This is an exploratory, hypothesis-generating study and a dose escalation clinical study to explore the maximum tolerated dose, so a total of 6 cases are required for sample size estimation.

3.3 Study Period

Work Progress	Timeline
Study Design, Study Approval Review, Ethical Review, Contract Signing, Human Genetic Resources Approval, Study Registration	2022.12-2023.05
Project Official Launch, Subject Enrollment and Follow-up	2023.06-2024.06
Data Cleaning and Analysis, Manuscript Writing	2024.06-2024.12
Total Duration	2 Years

3.4 Subject Selection

During the entire study period, it is planned to enroll 6 subjects. Ethical approval must be obtained before patient recruitment. The ethics committee will have access to all study documents and training materials.

- **Inclusion Criteria**

(1) Patients permitted by medical personnel who could complete the clinical trial.

(2) Age 18–75 years, gender unlimited.

(3) Clearly identify ALS patients with SOD1 gene mutations (known *SOD1* mutation sites and reported disease progression).

(4) Forced vital capacity during screening period ≥ 80% of expected lung capacity.

(5) Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology.

(6) The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form.

(7) The subjects (including male subjects) have no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum.

- **Exclusion Criteria**

(1) The *SOD1* mutation site occurs in patients with nucleotides 44-66 (calculated from the translation of SOD1 protein) and P.F21C mutations.

(2) Patients who have previously received or are currently receiving Tofersen treatment.

(3) HIV test positive or positive test history.

(4) Hepatitis C virus antibody positive or positive test history.

(5) Active hepatitis B infection (hepatitis B surface antigen positive and/or hepatitis B core antibody positive).

(6) Other experimental drugs used within one month or within five drug half-lives.

(7) Lumbar diseases and deformities.

(8) Suffering from other diseases known to be related to motor neuron dysfunction, which may confuse or blur the diagnosis of ALS.

(9) Other psychiatric disorders diagnosed according to DSM-V diagnostic criteria, or obvious suicidal intentions.

(10) Combined with severe liver dysfunction, renal dysfunction, or severe heart dysfunction (severe liver dysfunction refers to ALT value ≥ 2.0 times the upper

limit of normal value or AST value ≥ 2.0 times the upper limit of normal value; severe renal dysfunction refers to CRE ≥ 1.5 times the upper limit of normal value or eGFR < 40mL/min/1.73m^2; severe heart dysfunction refers to NYHA 3-4 levels).

(11) Permanent and sustained dependence on ventilator assisted ventilation.

(12) History of combined alcohol and drug abuse.

(13) Pregnant, lactating, or potentially pregnant patients, as well as those planning to conceive.

(14) Patients participating in other clinical trials or applying other biologics, drugs, or devices under study.

(15) Patients who have received any vaccination within 28 days.

(16) Contraindications for MRI examination (ie. claustrophobia).

(17) Those who can't complete follow-up due to other reasons.

4. Study Method

(1) Drug Dosage Form

The aseptic aqueous solution of the RAG-17 preparation (sodium salt) used in the study is stored at -20℃±5℃, with a specification of 75 mg (2.5 mL).

(2) Intervention Measures

1) Basic Treatment

All enrolled subjects will maintain basic medication treatment, and the dosage and type of medications will remain unchanged during the trial period.

Subjects who have already received riluzole treatment before enrollment may continue using it during the study period, maintaining a stable dosage (50mg per dose, twice daily). Throughout the study, discontinuation of riluzole for reasons other than medical necessity, as determined by the investigator, is not recommended.

The concomitant medications situation of the patients should be recorded in detail in the CRF form, including the name of the medication, the dosage, and frequency. If during the study, a change in the existing medication is deemed necessary by the investigator due to the patient's condition, it will be considered a protocol deviation. An adverse event record report and a concomitant medication form should be

completed. The investigator will then assess whether the subject can continue participating in the study. If not, the subject will be treated as a withdrawn case.

2) **RAG-17 Treatment**

The administration begins with an initial intrathecal injection dose of 60 mg. Within the first 14 days following the initial dose, if no AEs or SAEs are observed, the dose may be escalated by 30mg at 14-day intervals. After reaching the maximum tolerated dose (MTD), an optimal dose between the safe dose and the MTD will be selected as the dose for continued treatment. Continuous treatment will be administered every 2 months, with a total treatment duration of 8 months.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	DLT/dose	Intrathecal injection	6
5th Administration	D120	Optimal dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

5. Concomitant Medications

(1) Prohibited Concomitant Medications

Subjects must contact their investigators before taking any new medications, including over-the-counter drugs and herbal preparations.

• The use of any antiplatelet or anticoagulant drugs (e.g. aspirin, clopidogrel) is prohibited within 7 days prior to each lumbar puncture and until 48 hours after the procedure.

• The use of DPS, copper (II) (diacetylbis (N4-methylaminothiourea)), or pyrimethamine is prohibited during the study.

• The use of gamma globulin is not recommended during the study.

(2) Permitted Concomitant Medications

If subjects are taking concomitant vitamins, minerals and supplements, they should avoid exceeding the recommended daily dose within 14 days prior to the first administration of the RAG-17 and during the study period (e.g. no more than 5 grams of creatine per day and no more than 1000 IU of vitamin E per day). These daily limits include the total dose obtained from the combined use of daily multivitamins and supplements.

Medications not listed in the prohibited concomitant medications section above are considered permitted concomitant medications. Concomitant medications for symptom management during the study are determined by the investigator. All enrolled cases must maintain basic medication treatment, with no changes to the dosage or type of medications during the trial. If subjects have questions regarding permitted concomitant treatments, they should seek medical guidance from the investigator. The concomitant medications of the patients must be documented in detail in the CRF form, including the name of the medication, the dosage, and frequency. If a change or adjustment to the existing medications become necessary due to the subject's condition during the study, and the investigator deems it essential, this will be considered a protocol deviation. The adverse event record form and the concomitant medication form should be promptly completed. The investigator will assess whether the subject can continue participating in the study. If continuation is not feasible, the case will be treated as an excluded case. In special circumstances (e.g. the subjects suffers from other diseases during the study), when additional treatments are necessary, these must be explained in the CRF form, including the disease diagnosis, medications, dosage, and usage details.

6. Observation Indicators and Examination Timeline

6.1 General Demographic Indicators and Clinical Data

(1) Collection time point: baseline

(2) Collection content: Basic demographic characteristics include gender, age, ethnicity, height, weight, education level, marital status; vital signs include body temperature, pulse, respiration rate, blood pressure; 12-lead electrocardiogram (ECG);

waist circumference, physical examination; clinical data include relevant medical history, past medical history, medication used prior to enrollment, smoking and drinking history, and family medical history.

6.2 Daily Life Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo assessments using the ALSFRS-R and Modified Norris Scale before and after treatment. The results after treatment intervention will be compared with baseline data to evaluate the effect of RAG-17 on improving daily living function of the subjects.

(3) Observation indicators:

1) ALSFRS-R: This is a rapidly administered ordinal rating scale (grade 0-4) used to assess the subjects' ability and independence in 12 functional activities. ALSFRS-R measures 4 functional domains, including respiratory, bulbar, gross motor, and fine motor functions. The scale includes 12 items, each scored from 0 to 4, with a total score of 48. Higher scores indicate better function, while lower scores reflect more severe neurological impairment.

2) Modified Norris Scale: This assessment tool for ALS consists of two parts: the Limb Norris Scale (comprises 21 items to evaluate limb function) and the Norris Bulbar Scale (includes 13 items to assess bulbar function). Lower scores indicate more severe neurological impairment.

6.3 Respiratory Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo forced vital capacity (FVC) testing before and after treatment. The results post-treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving the respiratory function.

(3) Contents of observation indicators:

FVC: The subjects are measured in an upright position. Measured as the maximum volume of air that can be forcefully exhaled as quickly as possible after a maximal inhalation.

6.4 Fatigue Severity Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements with the Fatigue Severity Scale (FSS) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving the severity of fatigue in subjects.

(3) Observation indicators:

Fatigue severity: The FSS consists of 9 items, designed to measure the degree of fatigue and its impact on a person's activities and lifestyle across various diseases. The evaluation covers areas such as daily activity ability, participation, and sleep. Each question is scored on a 7-point scale: 1 = strongly disagree and 7 = strongly agree. The minimum possible score = 9 and the maximum possible score = 63. Higher scores indicate the greater severity of fatigue.

6.5 Muscle Strength Test

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurement using the Medical Research Council (MRC) Scale (sternocleidomastoid muscle, deltoid muscle, biceps brachii, triceps brachii, common digital extensor muscle, abductor pollicis brevis muscle, abductor digiti minimi muscle, first dorsal interosseous muscle, and iliopsoas muscle, quadriceps femoris, biceps femoris, tibialis anterior, gastrocnemius muscle, flexor muscle, extensor muscle) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving muscle strength.

(3) Observation indicators:

MRC scale: The MRC muscle strength grading method, is an enhanced version of the Lovett grading method, further subdivided by the range of motion and the level of resistance applied. If the measured muscle strength is slightly stronger than a certain level, a "+" is added to the upper right corner of this level. If it is slightly weaker, a "-" is added to the upper right corner. The refinement addresses the limitation of Lovett grading method, providing a more precise assessment of muscle

strength.

In this study, the MRC grading is used to measure the muscle strength of the abductor pollicis brevis muscle, abductor digiti minimi muscle and tibialis anterior muscle.

6.6 Electromyography Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements of compound muscle action potential (CMAP), motor unit number index (MUNIX), and motor unit size index (MUSIX) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the electromyographic parameters of the subjects.

(3) Observation indicators:

CMAP: CAMP represents the summated action potentials of muscle fibers that are almost simultaneously activated when a certain nerve is directly or indirectly stimulated. The amplitude from baseline to peak, duration, latency of the negative phase of the potential, as well as the details of the stimulation method and recording method should be recorded.

MUNIX: A motor unit is a functional unit located in the anterior horn of the spinal cord, consisting of an alpha motor neuron and all the muscle fibers it innervates. It is the basic unit of motor function, with the minimum unit of normal muscle contraction being the contraction of one motor unit. MUNIX is a sensitive biomarker for monitoring the nerve innervation status of patients with neurodegenerative diseases and is a highly sensitive and effective marker for monitoring the loss of motor units in ALS. The method uses CMAP and the surface electromyographic interference pattern (SIP) to evaluate the number and size of motor units (MUs).

MUSIX: The size of a motor unit is determined by the number of nerve endings and branches of the neuron axon. Generally, larger muscles have larger motor unit. Each muscle contains a certain number of motor units. The calculation method is to divide the amplitude of the compound muscle action potential (CMAP) by the MUNIX value.

6.7 Imaging Indicators

(1) Collection time points: baseline, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurement with 7T MRI imaging before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the imaging indicators of the subjects.

(3) Observation indicators:

7T MRI imaging indicators: 7T ultra-high field magnetic resonance is an advanced magnetic resonance imaging system currently available for human body scanning. Compared with the traditional 1.5T and 3T magnetic resonances, 7T magnetic resonance can provide a doubled signal-to-noise ratio, finer spatial resolution, and more excellent tissue contrast. It is more suitable for observing the fine structure of tissues and physiological changes in functional metabolism and other information, thereby revealing physiological and pathological mechanisms that are difficult to be discovered by traditional platforms.

6.8 Gait Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo the following assessments: three-dimensional gait analysis under multitasks, Short Physical Performance Battery (SPPB), item 1 (Tandem Walking) of the Scale for the Assessment and Rating of Ataxia (SARA), and 6-meter walking test before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the gait function of the subjects.

(3) Observation indicators: Semi-quantitative scales include the Short Physical Performance Battery (SPPB) and item 1 (Tandem Walking) of the Scale for the Assessment and Rating of Ataxia (SARA); Quantitative gait assessment includes 6-meter walking test.

SPPB: The SPPB is a widely used method for evaluating muscle function. It consists of 3 items: balance test, 4-meter timed walking test, and timed sit-to-stand test. Each individual test can score a maximum of 4 points, giving a total score of 12

points. To improve the accuracy of the test, each test is usually repeated 2 to 3 times, with the shortest time recorded for scoring.

6-meter normal walking test: It is conducted on an 8-meter walkway with a timing taken over a 6-meter distance. Landmark markers are placed at the start of the walkway, at the 1-meter, the 7-meter, and the 8-meter points, for a total of 4 markers. Timing starts at the 1-meter and stops at the 7-meter mark to avoid the influence of markers on the subject's behavior, such as intentional changes in walking pace or nervousness. The test is performed 4 times; the first trial serves as a practice and is not recorded. The times from the subsequent 3 trials are recorded, and the average of these times is used for analysis.

Item 1: SARA is a clinical scale used for the semi-quantitative assessment of cerebellar ataxia severity. It can also serve as a rehabilitation indicator for gait and independence in daily activities for ataxic stroke patients. In this study, the gait part (Item 1) is selected. It consists of 8 levels corresponding to scores ranging from 1 to 8. Grade 1 is a mild abnormality corresponding to 1 point, up to grade 8 is unable to walk even with assistance corresponding to 8 points.

6.9 Quality of Life Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements with the Amyotrophic Lateral Sclerosis Self-Assessment Questionnaire (ALSAQ-40), EQ-5D-5L scale, and HAMA and HAMD-17 scales before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the quality of life of the subjects.

(3) Observation indicators:

ALSAQ-40: ALSAQ-40 is a commonly used quality-of-life scale for ALS. The sections on social interaction and eating ability provide subjective insights into articulation and swallowing functions. ALSAQ-40 consists of 40 items involving the patient's condition, requiring a 5-point scale rating. The questions cover five health status dimensions affected by the disease: physical activity (including 10 items), daily living activities and independence (10 items), eating (3 items), communication (7

items), and emotional function (10 items). Each dimension is scored on a scale from 0 to 100: 0 represents the possible best health status and 100 represents the possible worst health status.

EQ-5D-5L: EQ-5D-5L is a standardized scale for measuring health status, that is an updated version of the EQ-5D scale. The scale consists of two parts: the EQ-5D-5L descriptive system and the visual analogue scale (EQ-VAS). The EQ-5D-5L descriptive system describes five dimensions: Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression. Each dimension contains five levels: 1=No difficulty, 2=Slight difficulty, 3=Moderate difficulty, 4=Severe difficulty, 5=Unable to perform/very severe difficulty. The responses of the five dimensions can generate a five-digit code to describe the health status of the respondent. The health status 1,1,1,1,1 indicates no problems in all five dimensions. EQ-VAS records the self-rated health status of the respondent on a vertical visual analogue scale, with the ends of the scale marked as "The best health status you can imagine" and "The worst health status you can imagine".

6.10 Clinical Staging Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo assessment with the London ALS staging and the Milan-Turin staging (ALS-MITOS) before and after treatment. The results after treatment intervention will be compared with the baseline data to assess the disease progression of ALS patients and evaluate the effect of RAG-17 on the condition of the subjects.

(3) Observation indicators:

London ALS staging: According to the most recently affected region in the course of the disease, ALS is divided into 5 stages: Stage 1 = symptom onset (involvement of the first area); Stage 2A = confirmed diagnosis, and Stage 2B = involvement of the second area; Stage 3 = involvement of the third area; Stage 4A = the patient requires percutaneous endoscopic gastrostomy (PEG), and Stage 4B = the patient requires non-invasive ventilation; Stage 5 = death or mechanical ventilation. The average survival time and 5-year survival rate vary across stages.

Milan-Turin staging: The patient's functions are divided into 4 domains by assessing 4 key points (including mobility, swallowing, communication, and respiration). Each domain is scored as either 0 (below the threshold) or 1 (above the threshold). The total score across these domains determines the stage: Stage 0 = no functional loss; Stage 1 = loss of one functional domain; Stage 2 = loss of two functional domains; Stage 3 = loss of three functional domains; Stage 4 = loss of four functional domains; Stage 5 = death or mechanical ventilation.

6.11 Laboratory Examination Data Collection

(1) Collection time points: baseline, day 1, day 15, day 29, day 60, day 120, day 180, day 240

(2) Collection content: All subjects will undergo laboratory tests including blood routine, urine routine, liver function, kidney function, and coagulation tests before and after treatment respectively. The results after treatment intervention will be compared with the baseline data to evaluate the impact of RAG-17 on the general physical condition of the subjects and assist in determining the occurrence of adverse events and other related events.

All subjects will undergo the detection of SOD1 protein in cerebrospinal fluid, plasma neurofilament light chain protein (NFL), and the pharmacokinetics of RAG-17 in plasma before and after treatment.

Additionally, during the dose-escalation phase, the plasma samples will be collected on the 4th day after treatment to detect PK and NFL levels. In the fixed-dose phase, the sampling time will be adjusted based on the PK and PD data from the dose escalation stage.

6.12 Other Information Collection

(1) Collection time points: baseline, day 1, day 15, day 29, day 60, day 120, day 180, day 240

(2) Collection content: During the study period, all the medications taken by the subjects will be recorded, including the reasons for use, drug names, dosages, and duration of use. All AEs, adverse reactions and their handling processes will also be documented; The timing of tracheostomy, invasive mechanical ventilation, death, and

the duration of invasive mechanical ventilation will be recorded; Subjects' medication adherence, cases of withdrawal or exclusion from the study will be recorded.

7. Follow-up Time

Follow-up plan: For all subjects, face-to-face visits will be conducted at baseline, day 1, day 15, day 29, day 60, day 120, day 180, and day 240. They will undergo tests including daily living function, respiratory function, muscle strength, quality of life, gait function, and clinical staging, as well as the collection of adverse events and concomitant medications use. The study is expected to be conducted at Beijing Tiantan Hospital, with a target recruitment of 6 participants, and is planned to be completed within 2 years.

Follow-up content: To evaluate the differences in clinical manifestations of the participants after receiving the RAG-17 intervention at baseline, day 1, day 15, day 29, day 60, day 120, and day 180, including:

- Laboratory examinations: tests including blood routine, urine routine, liver function, kidney function, coagulation, SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma will be conducted at baseline, day 1, day 15, day 29, day 60, day 120, day 180, and day 240.
- Daily living functions: ALSFRS-R scoring and Modified Norris Scale scoring will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Respiratory function: FVC evaluations will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Fatigue severity: FSS assessment will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Muscle strength test: MRC assessment will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Electromyogram indicators (abductor pollicis brevis, abductor digiti minimi, and anterior tibial muscle): CMAP, MUNIX, and MUSIX will be collected at baseline, day 15, day 29, day 90, and day 180.
- Imaging examinations: 7T magnetic resonance imaging will be conducted at baseline, day 29, day 90, and day 180.

- Gait function: SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Quality of life: assessments of ALSAQ-40, EQ-5D-5L, HAMA, and HAMD-17 will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Clinical staging: London ALS staging and Milan-Turin staging assessments will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Other indicators: information such as vital signs, abnormalities in heart/lung/abdomen examination, neurological examination, electrocardiogram, adverse events, concomitant medications, the time to tracheostomy, invasive mechanical ventilation, death, and the duration of invasive mechanical ventilation of the patients will be collected at baseline, day 1, day 15, day 29, day 60, day 90, day 120, day 180, and day 240.

8. Study Process

8.1 Information Collection and Informed Consent (Visit 1)

Patients who meet the inclusion criteria but do not meet the exclusion criteria will be introduced to the study protocol and purposes by investigator. The patient or their legal representatives will be provided with complete information about the study, accompanied by corresponding written materials, and a written informed consent form will be signed.

- Collection of medical history: including current medical history, past medical history, history of concomitant medication, history of smoking and alcohol consumption, family history;
- Physical examination: including vital signs, heart/lung/abdomen examination, neurological examination, 12-lead electrocardiogram;
- Laboratory examinations: including blood routine, urine routine, liver function, kidney function, coagulation function, BNP; Blood sample collection for verification of *SOD1* gene mutation sites, plasma neurofilament light chain protein (NFL);
- 7T magnetic resonance imaging;

- Assessment of respiratory function (including FVC)
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Assessment of daily living functions: ALSFRS-R score, Modified Norris Scale score;
- Assessment of fatigue severity: FSS scale score;
- Assessment of muscle strength and electromyogram indicators: MRC assessment (sternocleidomastoid muscle, deltoid muscle, biceps brachii, triceps brachii, common digital extensor muscle, abductor pollicis brevis, abductor digiti minimi, first dorsal interosseous muscle and iliopsoas muscle, quadriceps femoris, biceps femoris, anterior tibial muscle, gastrocnemius muscle, flexor muscle, extensor muscle grading); CMAP, MUNIX, MUSIX;
- Assessment of gait function: SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking;
- Assessment of quality of life: ALSAQ-40 and EQ-5D-5L scale scores;
- Assessment of emotional scale: HAMA and HAMD-17 scores.

8.2 Days 1-4 Post-Enrollment (First Treatment) (Visit 2)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications:
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.

- Arrange the follow-up appointment for day 15, face-to-face follow-up.

8.3 Days 15-18 Post-Enrollment (Second Treatment) (Visit 3)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- Arrange the follow-up appointment for day 29, face-to-face follow-up.

8.4 Days 29-32 Post-Enrollment (Third Treatment) (Visit 4)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;

- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 60, face-to-face follow-up.

8.5 Days 60-63 Post-Enrollment (Fourth Treatment) (Visit 5)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;

- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 90, face-to-face follow-up.

8.6 Days 90-93 Post-Enrollment (Visit 6)

- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 120, face-to-face follow-up.

8.7 Days 120-123 after enrollment (Fifth treatment) (Visit 7)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;

- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 180, face-to-face follow-up.

8.8 Days 180-183 Post-Enrollment (Sixth Treatment) (Visit 8)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- 7T magnetic resonance imaging;

- Arrange the follow-up appointment for day 240, face-to-face follow-up.

8.9 Days 240-243 after enrollment (Seventh treatment) (Visit 9)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.

8.10 Study Flowchart

Examination / Treatment	Screening / Baseline	Treatment and Follow-up							
		1st Administration	2nd Administration	3rd Administration	4th Administration		5th Administration	6th Administration	6th Administration
		Day 1-4	Day 15-18	Day 29-32	Day 60-63	Day 90-93	Day 120-123	Day 180-183	Day 240-243
Verification of inclusion / exclusion criteria	√								
Sign the informed consent form	√								
Demographic data	√								
Medical history / Personal history / Family history	√								
Height / Weight / Waist circumference / Physical examination	√	√	√	√	√	√	√	√	√
Concomitant medication	√	√	√	√	√	√	√	√	√
Blood pressure / Respiration / Heart rate / Body temperature	√	√	√	√	√	√	√	√	√
Neurological examination	√	√	√	√	√	√	√	√	√
12-lead electrocardiogram	√	√	√	√	√	√	√	√	√
Laboratory examination[1]	√	√	√	√	√		√	√	√
ALSFRS-R score	√		√	√		√		√	
Modified Norris Scale	√		√	√		√		√	
Forced vital capacity	√		√	√		√		√	
Fatigue Severity Scale	√		√	√		√		√	
Muscle strength / Electromyogram	√		√	√		√		√	
7T MRI	√			√		√		√	
SPPB	√		√	√		√		√	
Tandem Walking	√		√	√		√		√	
6-meter walking	√		√	√		√		√	
Three-dimensional gait analysis under multitasking	√		√	√		√		√	
ALSAQ-40	√		√	√		√		√	

EQ-5D-5L	√		√	√		√		√	
Anxiety Scale (HAMA)	√		√	√		√		√	
Depression Scale (HAMD-17)	√		√	√		√		√	
London ALS staging	√		√	√		√		√	
Milan-Turin staging (AKS-MITOS)	√		√	√		√		√	
Intrathecal administration of RAG-17	√	√	√	√	√		√	√	√
Blood sample collection [2]	√	√	√	√	√		√	√	√
Cerebrospinal fluid sample collection [3]	√	√	√	√	√		√	√	√
Adverse events / Severe adverse events	√	√	√	√	√	√	√	√	√
Study drug adherence	√	√	√	√	√		√	√	√

[1] Laboratory examinations: blood routine, urine routine, liver and kidney functions, renal function, coagulation function, BNP (BNP is only examined at baseline).
[2] Samples are collected at baseline; during the treatments 1 to 7 , samples are collected at 4 hours before each treatment, and 15 minutes, 4 hours, 48 hours, and 4 days after each treatment.
[3] During treatments 1 to 7, samples are collected 15 minutes before each treatment (Collection before drug administration via lumbar puncture, not restricted to 15 minutes).

9. Clinical Evaluation

9.1 Primary Outcomes：

1) Incidence of AEs and SAEs within 180 days after the first injection of RAG-17.
2) Changes in laboratory assessments, vital signs, ALSFRS-R scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

9.2 Second Outcomes：

1) Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
2) Changes in plasma NFL levels from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

Note: During the dose-escalation phase, evaluate the plasma NFL levels on day 4 after each administration. In the fixed-dose phase, consider adjusting the currently set

PD sampling times based on the changes in the plasma NFL levels observed in the dose-escalation phase. This adjustment aims to ensure that pharmacodynamic characteristics of RAG-17 are accurately reflected in subsequent participants receiving the same dose of investigational drug.

3) Changes in the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

Note: During the dose-escalation phase, evaluate the drug concentration in plasma on day 4 after administration. If RAG-17 cannot be detected in the plasma at the specific time points by the determined detection method, adjustments to the currently set PK sampling times may be considered during the fixed-dose phase. This adjustment aims to ensure that the pharmacokinetic characteristics of RAG-17 are accurately reflected in subsequent participants receiving the same dose of the investigational drug.

4) Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

9.3 Exploratory outcomes：

1) Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180.
2) Changes in fatigue severity (Fatigue Severity Scale, FSS) at on day 15, day 29, day 90, and day 180.
3) Changes in muscle strength on day 15, day 29, day 90, and day 180.
4) Changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180.
5) Changes in 7T MRI imaging features on day 29, day 90, and day 180.
6) Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180.
7) Changes in modified Norris scale scores on day 15, day 29, day 90, and day 180.

8) Changes in quality of life (including ALSAQ-40、EQ-5D-5L、HAMA and HAMD) on day 15, day 29, day 90, and day 180.
9) Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.

10. Known and Potential Risks and Benefits, and Risk Management Plan

10.1 Adverse Event Monitoring

All information related to adverse events, whether reported by the participants, identified by the investigators, or detected through physical examinations, laboratory tests, or other methods, should be recorded on the adverse event page of the case report form. These events will be managed and reported in accordance with the relevant regulations. A comprehensive adverse events report will include in the final trial report.

10.2 Adverse Event Definition

10.2.1 Adverse Event (AE)

An adverse event refers to any unfavorable medical occurrence or worsening of a pre-existing condition that arises in a participant during the use of the investigational drug, regardless of whether it has a causal relationship with the investigational drug treatment. Adverse medical occurrences can manifest as clinical symptoms/signs, diseases, or abnormal laboratory findings. AEs include any unfavorable medical occurrences that occur at any stage of the clinical study, including the lead-in or washout periods, even before the investigational drug has been administered to the participant. AEs include both serious and non-serious adverse events.

10.2.2 Serious Adverse Event

An adverse event during the study will be classified as serious, if it meets one or more of the following criteria:

- Results in death
- Immediately life-threatening

Note: Life-threatening refers to an event that poses an immediate risk to the patient's life at the time it occurs, not an event that could result in death if it were to worsen.

• Requires hospitalization or prolongs the current hospitalization

• Results in permanent or severe disability/incapacity, or loss of essential abilities required for normal living

• Leads to congenital anomaly, birth defects, or reproductive issues

• Constitutes a significant medical event that may endanger the subject or necessitate medical intervention to prevent the outcomes listed above.

10.3 Adverse Events Documentation

Non-serious AE: Non-serious AEs of interest occurring during the study period from the start until the end of the study (e.g. renal function impairment/increased serum creatinine, elevated liver function test indicators, upper gastrointestinal discomfort, muscle/bone pain, hypocalcemia, ocular inflammation, arrhythmia, fractures, etc.) and information about drug discontinuation due to SAEs must be documented. Documentation of other non-serious AEs is at the discretion of the investigator .

Serious AE: All SAEs must be documented.

10.4 Assessment of the Relationship Between AEs and the Investigational Drug

The relationship between AEs and the investigational drug will be assessed using a 5-level standard: ①Definitely related; ②Highly likely related; ③Possibly related; ④Unlikely related; ⑤Definitely unrelated; ⑥Unable to determine.

①Definitely related: Matches the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or disappears after dose reduction or drug withdrawal. Reaction reappears upon re-administration.

②Highly likely related: Matches to the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or disappears after dose reduction or drug withdrawal. However, the clinical status of the participants or other reasons may also cause the reaction.

③Possibly related: Matches the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or is not obvious after dose reduction or drug withdrawal. However, the clinical status of the

subject or other reasons can equally explain the reaction.

④Unlikely related: Does not match the known reaction type of the suspected drug. Dose not align with the reasonable time frame after administration. The clinical status of the subject or other reasons may also cause the reaction.

⑤Definitely unrelated: Does not match the known reaction type of the suspected drug. Dose not align with the reasonable time frame after administration. The clinical status of the subject or other reasons can also explain the reaction. Reaction decreases or disappears when clinical symptoms or other causes are ruled out..

⑥Unable to determine.

The incidence of AEs will be calculated and summarized for classifications ① + ② + ③ from each case report form.

10.5 Unexpected Adverse Event

An unexpected adverse event refers to any event not mentioned in the investigator's brochure, or differs in nature and severity from what is described in the investigator's brochure. In the absence of an investigator's brochure, the event is inconsistent with the risk information described in the study protocol or application materials.

10.6 Reporting of Serious Adverse Events

Notify the designated representative of the sponsor immediately. Send the signed and dated relevant pages of the case report form to the sponsor’s representative, along with copies of all examination results and their completion dates. At the same time, ensure the patient privacy is protected by removing all identifiable information from the clinical source document copies provided to the sponsor. Laboratory results should include normal range values.

All SAEs must be reported within 24 hours of occurrence. Record the SAE in the designed section of the CRF. After signing, report it preferably via fax to the monitoring center. The name, address and fax number of the monitoring center will be specified in the clinical trial protocol.

10.7 Risk Control and Management

In this study, the investigational drug RAG-17 may cause adverse reactions such as tremors, abnormal gait, and hind limb weakness. If such actions occur and are deemed

related to the drug, it is recommended to stop the administration, monitor vital signs, conduct auxiliary examinations such as blood routine, coagulation profile, muscle enzymes, liver function, kidney function, blood gas analysis, ultrasound, CT scans. Provide timely symptomatic and supportive treatment based on clinical findings. If necessary, consult with relevant specialists to manage the adverse events effectively. For serious adverse events, it is necessary to ensure airway patency, autonomous respiration, stable blood pressure and heart rate. If necessary, treatment should be carried out in the intensive care unit.

For all adverse events, the investigators should continue to follow up with patients and track any related outcomes (clinical symptoms, signs, laboratory test results) until the related clinical symptoms are completely resolved, the test results return to normal or the condition is stable. This follow-up should persist even if the patient withdraws from the clinical trial. Additionally, the monitoring team may request further investigations as needed. After the termination of the study, the investigators should still pay attention to any serious adverse events that may occur.

10.8 Subjects Benefits

Participation in this clinical study may help alleviate the subject's condition, but it is also possible that the investigational drug will not provide the expected therapeutic effect. In other words, subjects may not gain any direct benefits from participating in this study. However, this study may contribute to the development of new treatment options for patients with similar conditions, offering more choices to others. In addition, subjects may gain valuable information about their conditions through the physical examinations and medical tests conducted during the study.

11. Quality Control and Quality Assurance of the Study

Representatives of the sponsor or collaborators will regularly monitor the clinical study. The scope and nature of supervision will be determined based on considerations such as the clinical study's objectives, purposes, design, complexity, scale, and endpoints.

11.1 Protocol Adherence Management

- Careful selection of participants: When selecting subjects, investigators should

confirm accurate diagnoses, understand common factors leading to dropout, conduct a comprehensive assessment of participants, and perform a pre-evaluation of adherence. Participants with high compliance potential should be carefully screened and selectively included.

- Informed consent process: Before signing the informed consent form, investigators should provide detailed and clear explanations to the subjects and their families in an easy-to-understand manner. This ensure subjects fully understand the investigational drug and the treatment process. Investigators must thoroughly address participants' questions, respect their choices, and encourage confidence in completing the study, which fosters better adherence.
- Optimizing the protocol: The study protocol should be designed to minimize complexity, favoring simple and practical approaches that align with participants' daily lives. A well-designed, scientifically sound, and highly operable protocol will enhance adherence.
- Managing adverse events: If participants experience adverse reactions, adverse events, or serious adverse events during the clinical study, regardless of whether they are related to the investigational drug, researchers must promptly take appropriate treatment measures. Ensuring timely medical intervention safeguards participants' safety, builds trust in the study, and improves adherence.
- Follow-up and reminders: A contact information sheet and visit schedule should be established to remind participants of follow-up visits. For those who miss scheduled visits, researchers should promptly follow up via phone. These efforts help ensure participants remain engaged and adhere to the study protocol.

11.2 Study Monitoring

The primary responsibility of the monitoring team of the collaborators is to assist investigators and the sponsor or collaborators in ensuring the clinical study adheres to the highest standards of ethics, scientific rigor, professionalism, and regulatory compliance. According to the Good Clinical Practice (GCP) principle, the monitoring team should verify the CRF against the original source documents.

The monitoring team will regularly assess the progress of the study, through on-site

visits, letters, or telephone communication. This includes evaluating adherence to the clinical protocol by researchers and participants and addressing urgent issues. Medical monitors will also periodically review participant safety. During these monitoring visits, the monitors will collaborate with the investigators to focus on the following key points (not exhaustive): informed consent of subjects, recruitment and follow-up of subjects, documentation and reporting of serious adverse event, concomitant treatment and data quality.

11.3 Data Verification

The following measures will be taken to ensure the accuracy, consistency, completeness and reliability of the data:

- Regular on-site monitoring of the study;
- CRF summary based on source documents;
- Data management quality control checks;
- Medical review by the sponsor.

The sponsor or designated personnel (such as CRO) will be responsible for the data management in this clinical study. The responsible party will design and distribute CRFs and oversee their monitoring. If the data are incomplete or inconsistent, the responsible party will issue queries for site staff to address and make necessary corrections.

11.4 Personnel Training

The investigators shall ensure adherence to the clinical trial protocol, compliance with the Good Clinical Practice (GCP) guidelines outlined in the ICH, and conformity with applicable laws and regulations during the conduct of the clinical trial.

The investigators should ensure adherence to all study procedures outlined in the clinical trial protocol (including safety principles). The investigator should accurately and clearly provide reliable data and all information required by the clinical trial protocol (e.g., drug overdose, pregnancy) through appropriate methods, such as CRFs, deviation resolution forms (DRFs), and others, in accordance with relevant requirements. Additionally, the investigator must ensure that the monitoring team has direct access to the source documents.

The investigators may appoint individuals deemed suitable to assist in conducting the study. These assistants will help manage the clinical study in accordance with the study protocol. All assistants will be appointed and documented in a timely manner. They will work under the supervision and management of the investigator, who will provide them with a copy of the clinical study protocol and all necessary information. The sponsor or collaborator of the clinical study are accountable to the health authority for ensuring the proper management of the clinical study by employing all appropriate means, such as ethics, compliance with the clinical study protocol, completeness and validity of the data recorded in the CRF.

11.5 Data Management

During the clinical study, the data of the subjects should be collected anonymously in the CRF, with subjects identified only by their number and the initials of their name. In cases where participant identity must be disclosed due to safety or administrative reasons, both the investigator and any involved parties share the responsibility for maintaining confidentiality. The informed consent form allows the subject to agree to authorized personnel from the sponsor or collaborators, ethics committees, and regulatory authorities directly accessing relevant source documents associated with the CRF (e.g., medical records, appointment logs, original laboratory records). These individuals must adhere to professional confidentiality standards and ensure the privacy of all participant personal or medical information.

The medical information obtained in the study should remain confidential and may only be disclosed to third parties with the permission of the informed consent form and the subject's signed authorization form (or separate authorization for the use and disclosure of personal health information) or when permitted or required by law.

For treatment purposes, medical information may be shared with the participant's personal physician or other appropriate healthcare professionals responsible for the participant's well-being.

11.6 Handling of Biological Samples

The biological samples, such as blood and cerebrospinal fluid collected in this study, will be processed (e.g. centrifugation and aliquoting) at Beijing Tiantan Hospital,

Capital Medical University. Samples designed for evaluation will be analyzed at a centralized clinical laboratory with recognized accredited certification. All biological including blood and cerebrospinal fluid will be stored at Beijing Tiantan Hospital, Capital Medical University for 10 years.

12. Data Retention

The reviewed CRF and imaging data will be carefully entered and processed by the data management center and properly stored. Once data entry is completed, the CRF will be stored by the research center.

The study monitor will perform ongoing source data verification to ensure that the key study protocol data (i.e., source data) transcribed on the CRF by authorized on-site personnel are accurate and complete, and that they can be verified against the original source documents.

Source documents are the original records where subject data is first recorded and stored. These include, but are not limited to hospital records, clinical and office charts, laboratory records, memos, subject diaries or assessment checklists, pharmacy dispensing records, data from automated instrument records, verified and certified as accurate and complete copies of audio transcriptions, microfilms, photographic negatives, microfiche or magnetic media, computed tomography, subject files, and records kept in pharmacies, laboratories and medical technology departments related to clinical studies.

The source documents required to verify the validity and completeness of the data transcribed on the CRF must never be deleted or destroyed.

To facilitate source data verification, the investigator and the institution must allow the sponsor to access applicable source documents and reports for direct inspection. This is to support monitoring, sponsor audits, clinical research reviews, and summaries by the management committees or ethics committees. Additionally, appropriate regulatory agencies must be allowed to inspect the study site.

13. Data Safety Monitoring

The clinical study will develop a data safety monitoring plan based on the level of risk involved. All adverse events will be thoroughly documented, appropriately

managed, and followed up until resolved or the condition stabilizes. Serious adverse events and unexpected events will be promptly reported to the ethics committee, regulatory authorities, the sponsor, and the drug regulatory agency in accordance with relevant regulations. The principal investigator will regularly conduct cumulative reviews of all adverse events and, when necessary, convene investigator meetings to assess the risks and benefits of the study. For studies with risks exceeding the minimal threshold, independent data monitors will be assigned to oversee the study data. For high-risk studies, an independent data safety monitoring board will be established to review the accumulated safety and efficacy data and provide recommendations on whether the study should continue.

During the clinical trial, participant data will be collected anonymously in the CRF, with participants identified only by their participant number and the initials of their name. In cases where participant identity must be disclosed due to safety or administrative reasons, both the investigator and the involved parties share the responsibility for maintaining confidentiality. The informed consent form allows participants to consent to the authorized sponsor, ethics committee, and regulatory authorities directly accessing relevant source documents on the CRFs (e.g., medical records, appointment logs, original laboratory records). These individuals must adhere to professional confidentiality standards and maintain the privacy of all participant personal or medical information.

14. Statistical Analysis

- Statistical analysis will be performed with SAS software.
- Demographic, medical, histories and baseline characteristics information will be listed and summarized.
- The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after treatment. Safety evaluation will be analyzed using safety data set. The number of people of events should be recorded in detail and showing the event rate in summary statement. The event rate will be calculated

as the number patients experiencing the event / the number of patients enrolled.

- Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 180-day follow-up assessments for all participants, the data were analyzed.

15. Ethical Considerations

The clinical study will adhere to the World Medical Association's Declaration of Helsinki and other relevant regulations. Before the study begins, the trial protocol will be approved by the ethics committee. Before enrollment, the investigator is responsible for providing a complete and comprehensive explanation of the study's purpose, procedures, and potential risks to the participant or their representative. A written informed consent form must be signed, ensuring that participants are aware of their right to withdraw from the study at any time. The informed consent form will be retained as part of the clinical study documentation for future reference. Throughout the study, the personal privacy and data confidentiality of participants will be safeguarded.

Screening and informed consent: The investigator (or designated personnel authorized and responsible by the investigator, in compliance with relevant regulations) will recruit participants in an independent consultation room at Beijing Tiantan Hospital, Capital Medical University, ensuring the privacy and confidentiality of the participants. Participants and their legal representatives should be fully informed about all relevant aspects of the clinical trial, including written information approved by the ethics committee. The investigator should use language or terms that the participant can understand, providing as much information as possible about the study, and allow sufficient time for the participant or their legal representative to consider whether to participate. Before participating in the clinical trial, the patient or their legal representative must discuss the informed consent with the investigator and

sign the informed consent form (including the participant's name and the date). The investigator should provide the participant with a copy of the signed informed consent form.

16. Data Confidentiality

Throughout the entire research and data analysis process, participant information will be strictly protected. The executive committee will disclose the trial results in accordance with management regulations and procedures. Once the database is locked, the trial results will be published as soon as possible. Biostatistical experts involved in the study have the right to access the dataset but will not be able to identify any individual participants. In line with the GCP principles outlined in the ICH guidelines, the monitoring team will verify the CRFs against the original source documents. The informed consent form will include a statement that allows participants to consent to authorized personnel from the sponsor, ethics committee, and regulatory authorities directly accessing relevant source documents on the CRFs (e.g., medical records, appointment logs, original laboratory records). Researchers must adhere to professional confidentiality standards and ensure the confidentiality of all personal and medical information of the participants.

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Safety and Tolerance of RAG-17 in Amyotrophic Lateral Sclerosis Patients With *SOD1* Gene Mutation (CREATION)

Study Protocol

(Protocol Number HX-A-2023004)

Principal Investigator: Yilong Wang

Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Protocol Version: 1.3

Jun. 1st, 2023

Summary of the Trial Protocol

Study Title	Safety and tolerance of RAG-17 in amyotrophic lateral sclerosis patients with *SOD1* gene mutation
Study Purposes	To evaluate the safety, tolerance and pharmacokinetics (PK) of RAG-17 in adult ALS subjects with *SOD1* gene mutation. **Primary purposes：** 1. To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17. 2. To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17. **Secondary purposes：** 1. To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2. To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3. To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 4. To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

	Exploratory purposes： 1. To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180. 2. To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180. 3. To evaluate the changes in muscle strength on day 15, day 29, day 90, and day 180. 4. To evaluate the changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180. 5. To evaluate the changes in 7T MRI imaging features on day 29, day 90, and day 180. 6. To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180. 7. To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, and day 180. 8. To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, and day 180. 9. To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.
Study Design	Ongoing open-label, single-center, first-in-human and dose escalation clinical study

Total number of cases	Six ALS patients with *SOD1* gene mutations
Subjects Selection	**Inclusion Criteria** (1) Patients permitted by medical personnel who could complete the clinical trial. (2) Age 18~75 years, gender unlimited. (3) Clearly identify ALS patients with *SOD1* gene mutations (known *SOD1* mutation sites and reported disease progression). (4) Forced vital capacity during screening period ≥ 80% of expected lung capacity. (5) Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology. (6) The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form. (7) The subjects (including male subjects) have no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum. **Exclusion Criteria** (1) The *SOD1* mutation site occurs in patients with nucleotides 44-66 (calculated from the translation of SOD1 protein) and P.F21C mutations. (2) Patients who have previously received or are currently receiving Tofersen treatment. (3) HIV test positive or positive test history.

	(4) Hepatitis C virus antibody positive or positive test history. (5) Active hepatitis B infection (hepatitis B surface antigen positive and/or hepatitis B core antibody positive). (6) Other experimental drugs used within one month or within five drug half-lives. (7) Lumbar diseases and deformities. (8) Suffering from other diseases known to be related to motor neuron dysfunction, which may confuse or blur the diagnosis of ALS. (9) Other psychiatric disorders diagnosed according to DSM-V diagnostic criteria, or obvious suicidal intentions. (10) Combined with severe liver dysfunction, renal dysfunction, or severe heart dysfunction (severe liver dysfunction refers to ALT value ≥ 2.0 times the upper limit of normal value or AST value ≥ 2.0 times the upper limit of normal value; severe renal dysfunction refers to CRE ≥ 1.5 times the upper limit of normal value or eGFR < 40mL/min/1.73m^2; severe heart dysfunction refers to NYHA 3-4 levels). (11) Permanent and sustained dependence on ventilator assisted ventilation. (12) History of combined alcohol and drug abuse. (13) Pregnant, lactating, or potentially pregnant patients, as well as those planning to conceive. (14) Patients participating in other clinical trials or applying other biologics, drugs, or devices under study. (15) Patients who have received any vaccination within 28 days. (16) Contraindications for MRI examination (ie. claustrophobia). (17) Those who can’t complete follow-up due to other reasons.

<table>
<tr><td>Therapeutic Regimen</td><td>
Study drug： Aseptic aqueous solution of the RAG-17 formulation (sodium salt)
Storage condition： -20°C ± 5°C, stored in a frozen state
Specification： 75 mg (2.5 mL)
Route, dosage and schedule of administration: Intrathecal administration. The safety, tolerance, and pharmacokinetics of the RAG-17 drug will be evaluated by a dose-escalation approach. The dose of RAG-17 will range from a minimum of 60 mg to the maximum tolerated dose (MTD). Administration will be conducted once every two weeks, starting at 60 mg, for dose escalation. Once the tolerated dose is reached, a fixed dose of the drug will be administered every two months for continued treatment, with a total treatment duration of 8 months. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD. For the subsequent maintenance treatment phase, the optimal dose between the safe dose and the MTD will be selected as the dose for continued treatment.
<table>
<tr><th>Treatment</th><th>Administration Day</th><th>Dose Level</th><th>Administration Route</th><th>Number of Subjects</th></tr>
<tr><td>1st Administration</td><td>D1</td><td>60 mg/dose</td><td>Intrathecal injection</td><td>3</td></tr>
<tr><td>2nd Administration</td><td>D15</td><td>90 mg/dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>3rd Administration</td><td>D29</td><td>120 mg/dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>4th Administration</td><td>D60</td><td>DLT/dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>5th Administration</td><td>D120</td><td>Optimal dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>6th Administration</td><td>D180</td><td>Optimal dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>7th Administration</td><td>D240</td><td>Optimal dose</td><td>Intrathecal injection</td><td>6</td></tr>
</table>
Dose escalation criteria: If no AEs or SAEs occur within 14 days after the first administration, the subject proceed with a dose
</td></tr>
</table>

	escalation of 30 mg every 14 days during the observation period. It is planned to achieve DLT through 3 to 4 dose escalations, followed by continuous treatment with the optimal dose for a 6-month treatment period. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD.
Study Outcomes	**Primary outcomes** 1) Incidence of AEs and SAEs within 180 days after the first injection RAG-17. 2) Changes in laboratory assessments, vital signs, ALSFRS-R scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection RAG-17. **Second outcomes：** 1) Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2) Changes in plasma NFL levels from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3) Changes in the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 4) Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

	Exploratory outcomes： 1) Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180. 2) Changes in fatigue severity (Fatigue Severity Scale, FSS) at on day 15, day 29, day 90, and day 180. 3) Changes in muscle strength on day 15, day 29, day 90, and day 180. 4) Changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180. 5) Changes in 7T MRI imaging features on day 29, day 90, and day 180. 6) Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180. 7) Changes in modified Norris scale scores on day 15, day 29, day 90, and day 180. 8) Changes in quality of life (including ALSAQ-40、EQ-5D-5L、HAMA and HAMD) on day 15, day 29, day 90, and day 180. 9) Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.
Statistical Analysis	• Statistical analysis will be performed with SAS software. • Demographic, medical, histories and baseline characteristics information will be listed and summarized. • The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after

	treatment. Safety evaluation will be analyzed using safety data set. The number of people of events should be recorded in detail and showing the event rate in summary statement. The event rate will be calculated as the number patients experiencing the event / the number of patients enrolled. • Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 180-day follow-up assessments for all participants, the data were analyzed.
Study Duration	2 years

1. Study Background

Amyotrophic lateral sclerosis (ALS) is a chronic, progressive neurodegenerative disease characterized by prominent damage to both upper and lower motor neurons, manifesting in symptoms such as muscle weakness, muscle atrophy, bulbar paralysis, and pyramidal signs. ALS is a rare disease, with a global standardized annual incidence rate of only 1.68 cases per 100,000 person-years[1]. The exact incidence rate of ALS in China still not well-established, but it is estimated that there are at least 100,000 patients [2]. The peak age of onset for ALS is around 50 years, with a growing trend toward earlier onset ages[3]. ALS is incurable, with a typical survival period of 3-5 years, and only approximately 10% of patients survive for more than 10 years[4]. As a result, it is listed by the World Health Organization as one of the "five major incurable diseases in the world" alongside cancer, acquired immunodeficiency syndrome, leukemia and rheumatoid arthritis.

Currently, the pathogenesis of ALS remains unclear, and available treatments are limited, primarily focusing on symptomatic relief rather than a cure. Since 1980, more than 80 randomized controlled trials (RCTs) have been conducted globally. Only three drugs for the treatment of ALS have been approved by the U.S. Food and Drug Administration (FDA): the neuroprotective drug riluzole, which inhibits excessive neurotransmission; the antioxidant edaravone; and Relyvrio (sodium phenylbutyrate and taurursodiol)[5-7]. Drug interventions for ALS include anti-apoptosis, anti-inflammation, anti-excitotoxicity, anti-oxidation, anti-aggregation, neurotrophic growth factors and neuroprotection, among others. In 2017, riluzole, the primary drug for ALS, became the first FDA-approved drug treatment for the disease. However, it cannot halt the progression of ALS, let alone reverse it, and only extends patient survival by 2 to 3 months. The riluzole costs about 1,800~4,000 RMB per month. However, it is only covered by medical insurance in certain provinces and cities. Edaravone is indicated for cerebral infarction and was approved for the treatment of ALS in the United States in 2019. It was approved for use in China in September 2020, specifically for patients with acceptable respiratory function in the early stage (within 2 years) of the disease. In September 2022, the FDA approved Relyvrio, a

combination drug composed of sodium phenylbutrate and taurursodiol, for the treatment of adult patients with ALS. The approval process for Relyvrio drug at the FDA has been marked by numerous challenges. At the first Peripheral and Central Nervous System Drug Advisory Committee (PCNSDAC) review meeting organized by the FDA in March 2022, the expert panel expressed concerns regarding issues such as the small scale of the Relyvri clinical trialo, data deficiencies, and the applicability of statistical methods, with a voting result of 6:4. In response to this, Amylyx reanalyzed the survival data of the CENTAUR trial of Relyvrio and submitted it, along with the biomarker data from another phase II clinical trial for Alzheimer's disease to the FDA for re-evaluation. At the second PCNSDAC meeting in September, the new drug application of Relyvrio was approved with a voting result of 7:2, and it was subsequently approved by the FDA. Currently, this drug has not been approved in China. As a result, existing therapies for ALS remain limited, and the demand for effective ALS treatments remains unmet.

Currently, the pathogenesis of ALS remains unclear, and available treatment are limited, mainly focusing on symptomatic relief rather than a cure. ALS can be divided into sporadic ALS (sALS) and familial ALS (fALS), with sALS accounting for the majority of cases (approximately 90%) and fALS accounting for 5-10%[8]. fALS can result from mutations in multiple genes, with the most common being a mutation in the *superoxide dismutase 1* (*SOD1*), which occurs in 2% to 7% of cases[9]. Approximately 20% of fALS and 5% of sALS cases are associated with the *SOD1* gene. Asia has the highest number of ALS, with Asian patients typically exhibiting a younger age of onset, a lower proportion of bulbar-onset ALS, and more pronounced cognitive dysfunction[10]. In Asia, fALS is mainly caused by *SOD1* gene mutations, whereas in Europe, mutations in the *C9ORF72* gene are more common. The frequency of the *SOD1* gene mutations is highest among ALS patient in central and southern China[11]. SOD1 is a potent antioxidant enzyme that, when combined with copper and zinc ions, helps convert superoxide into oxygen molecules and hydrogen peroxide, thereby protecting cells from damage[12-14]. Mutations in the *SOD1* gene in ALS patients lead to misfolding and accumulation of the SOD1 protein within

spinal motor neurons. While several mutations in SOD1 have been identified, not all of them result in ALS. Harmful mutations alter the functional activity of SOD1, with loss-of-function mutations impairing degradation of reactive oxygen species (ROS), which damages motor neurons, and gain-of-function mutations increasing oxidative activity, resulting in excessive production of hydrogen peroxide and enhancing protein-protein interactions[15-17]. These effects disrupt SOD1 protein level and activity, inhibit axonal transport, and cause mitochondrial dysfunction[15], ultimately leading to neuronal degeneration and death. Therefore, reducing the level of toxic SOD1 protein may offer an effective therapeutic approach for ALS caused by *SOD1* mutations. Researches have indicated that knockdown or knockout of normal SOD1 expression does not result in significant side effects[18-19].

Currently, several drugs under investigation have entered clinical studies, including small molecule drugs, antibody drugs, antisense oligonucleotides (ASO) drugs and cell therapy. Among these, except for small nucleic acid drugs (antisense oligonucleotides, small interfering RNA) which directly target pathogenic genes, the others are non-disease-modifying therapies. Tofersen, an antisense oligonucleotide drug developed by Biogen for the treatment of *SOD1*-ALS, binds to the mRNA encoding SOD1 and is degraded by ribonuclease H (RNase-H), thereby reducing the production of mutant SOD1 protein[20]. Tofersen is currently undergoing a New Drug Application (NDA) review by the U.S. FDA. The NDA submission for Tofersen is based on data from a Phase 1 study in healthy volunteers, a dose-escalation Phase 1/2 clinical study, a Phase 3 VALOR clinical study, and an open-label extension (OLE) study. While the Phase III of Tofersen for the treatment of did not meet the primary endpoint, Biogen has observed indications of reduced disease progression in several secondary and exploratory endpoints. Biogen's integration of 12-month data shows that earlier use of Tofersen, compared to delayed initiation, significantly reduces the neurofilament levels and slows the decline of various efficacy endpoints. In terms of adverse events, the most common side effects in patients were headache, falls, back pain and limb pain. About 6.7% of patients had serious neurological-related events, including myelitis, radiculitis, aseptic meningitis and optic disc edema[21-22].

RNA interference (RNAi) is a biopharmaceutical technology that utilizes double-stranded RNA molecules, known as small interfering RNA (siRNA), to target and silence specific genes at the mRNA level. Based on this mechanism, five drugs have been approved by the U.S. FDA, with nearly a hundred others currently in clinical development. RAG-17 is a 20 kDa siRNA targeting the *SOD1* gene. Through the RNAi mechanism, RAG-17 binds to *SOD1* mRNA, leading to the degradation of the target mRNA by the RNA-induced silencing complex (RISC). By recognizing and mediating the degradation of the target mRNA, RAG-17 silences the *SOD1* gene and downregulates the expression of the SOD-1 protein. RAG-17 is administered via intrathecal injection and is intended for the treatment of ALS patients with *SOD1* mutations. Compared to single-stranded self-delivering ASO drugs, siRNA offers a cascade amplification effect on the target gene and provides a higher gene silencing efficiency. This drug leverages a unique delivery technology for small nucleic acid drugs, enabling efficient siRNA delivery to the central nervous system and offering the potential for treatment of neurological diseases. The schematic diagram of the mechanism of RAG-17 in the treatment of ALS is shown below.

The *in vitro* pharmacodynamic results indicate that RAG-17 shows a concentration-dependent knockdown of *hSOD1* mRNA, with a half-maximal inhibitory concentration (IC50) of 0.011 nM. Compared to the control drug Tofersen, the IC50 of RAG-17 is 100 times lower, and the maximum inhibition rate reaches more than 90%. Cell apoptosis assays show that RAG-17 has no toxic response of cell apoptosis. Off-target effect analysis results show that RAG-17 dose not significantly affect the mRNA expression levels of non-target genes, demonstrating its specificity for *SOD1* gene knockdown.

The h*SOD1*^{G93A} transgenic mice exhibit progressive motor neuron degeneration, which closely resembles human ALS and is a widely recognized animal model for studying ALS. It has been extensively used in ALS pathogenesis research and drug screening [23-25]. To evaluated the inhibitory activity of RAG-17 on the target gene *in vivo*, a single-dose proof of concept (POC) experiment was conducted using the h*SOD1*^{G93A} ALS disease model mice. At 60 days of age, 200 μg of the siRNA drug

RAG-17 (~10 nmol) and the control drug Tofersen (28 nmol) were injected into the lateral ventricle. Tissue RNA was extracted 14 days post-administration, and RT-PCR was performed to assess the target gene, human *SOD1*. The results indicated that the mRNA expression of *SOD1* in both brain and spinal cord tissues was reduced by approximately 80%, with RAG-17 demonstrating superior gene down-regulation compared to the control drug.

Subsequently, a multi-dose pharmacodynamics (PD) experiment was conducted. Doses of 50 μg, 100 μg, 200 μg, and 400 μg of the siRNA drug RAG-17 were injected into the lateral ventricle. The PD indicators (relative expression levels of human *SOD1* mRNA) in the target tissues were assessed at 14 days and 56 days post-injection to determine the pharmacodynamic activity, dose response and the duration of effect at the respective doses. The results showed that gene knockdown was dose-dependent in three different regions of the brain (prefrontal cortex, cerebellum, and other brain tissues) and the spinal cord. Notably, only the high-dose group (400 μg) maintained 50% gene knockdown (KD) activity 56 days after injection, although this activity was weaker compared to 14 days post-injection. Compared to small molecule drugs, the significant advantage of small nucleic acid drugs is the extended duration of their therapeutic effect. Further testing at an extended time point (8 weeks) revealed that RAG-17 sustained the pharmacological activity with gene knockdown for at least 8 weeks in the model mice, providing valuable data to support the potential frequency of clinical administration.

To further verify the correlation between drug exposure in tissues and drug efficacy, the stem-loop PCR method was applied to detect the siRNA concentration in tissues at 14 days and 56 days post-administration. The results demonstrated a clear correlation between the drug concentration in tissues and PD effects.

To further explore the pharmacodynamics of RAG-17, human *SOD1*^{G93A} transgenic ALS disease model mice were used. Both male and female ALS disease model mice were administered intrathecally twice on the 68th and 100th days of life, with doses of the RAG-17 (RD-12500) at 75 μg, 150 μg, and 300 μg. Two negative control groups were included: a vehicle group and an inactvice (scrambled siRNA) group. The

scrambled siRNA served as a control for the target siRNA, being non-functional, and used to assess the potential inhibitory effects of the target siRNA. Additionally, 150 ug of Tofersen (RD-10748) was included as the positive control group. This study conducted pharmacodynamic evaluations based on multiple indicators, including animal body weight, motor function (grip strength in the fatigue rotating rod test, rotarod test, and open field test), disease onset time, lifespan, and neurological function score (neuroscore).

The results of animal weight changes indicated that both the vehicle group and the inactive group began to show a significant decrease in body weight around 130 days, with a more pronounced decrease in male mice. This suggests that the onset of h*SOD1*^{G93A} ALS disease model occurs earlier in males than in females. Among the groups, the positive group (RD-10748) showed improvements in body weight, with more significant weight maintenance in male mice. The body weight data from the drug treatment groups demonstrated a dose-dependent alleviation of weight loss, especially in female mice. As the disease progresses in ALS mice, their body weight gradually decreases. Compared to the vehicle control group, all dose groups of RD-12500 (RAG-17) and ASO (RD-10748) demonstrated a delayed onset of disease.

After two intrathecal administrations in the ALS disease model mice, all treatment groups of both male and female mice showed an extension in the median survival period. The low-dose group of of 75 μg RAG-17 and the medium-dose of 150 μg ASO (RD-10748) demonstrated comparable efficacy. The survival periods for male mice were 207 days (75 μg RAG-17) and 191 days (150 μg ASO), respectively. The survival periods of the female group were 198.5 days (75 μg RAG-17) and 189 days (150 μg ASO), respectively. In the female high-dose group (300 μg), the survival period was significantly extended to 227 days, representing a 54-day increase compared to the vehicle control group (173 days). However, the high-dose group (300 μg) in the male mice did not show a marked superiority. Female animals exhibited greater sensitive to drug treatment, and the results aligned with the trend of weight change.

The typical pathological feature of h*SOD1*^{G93A} ALS disease model mice is the

progressive loss of motor function. The Rotarod test for motor function fatigue and the Grip strength test were used to evaluate the therapeutic effect of the drug on the ALS model. The results showed that, in the Rotarod test, the drug-treated group demonstrated a significant increase in the time ALS mice were able to maintain their position on the accelerating rotating rod at 0-30 rpm, compared to both the vehicle and the inactive control groups. The Grip strength test revealed that the drug treatment group significantly improved the hindlimb muscle function of the disease model mice. Additionally, the open field test, which evaluates autonomous behavior, exploratory behavior and anxiety in a novel environment, showed that the drug-treatment group exhibited improved autonomous motor ability, with a dose-dependence effect.

Before performing the Rotarod and hindlimb grip strength behavioral tests, the neurological function of ALS mice was scored on a scale of 0 to 4 points. This scoring system objectively evaluates the common hindlimb dysfunction in h*SOD1*^{G93A} transgenic mice. The results showed that mice in the vehicle (aCSF) and inactive control groups exhibited abnormal tilting (NS2 symptoms) at PND130 days, with the severity progressively worsening until hindlimb paralysis (i.e., ≥ NS3). In contrast, at all time points during the study, the average score of all RAG-17 treatment doses never exceeded NS2. These results indicated that RAG-17 treatment, regardless of the dose, improved motor function in the animals compared with the control group.

In conclusion, RAG-17 treatment can effectively inhibit the gene expression of h*SOD1* in h*SOD1*^{G93A} transgenic mice, significantly delay the disease onset, extend the survival time of affected mice, enhance their grip strength , and improve the motor function.

Currently, the non-clinical safety evaluation of RAG-17 is ongoing. The safety dose exploration experiments have been completed in both rats (following two intrathecal administrations, a non-relevant species) and cynomolgus monkeys (a relevant species). In the rat safety dose exploration experiment, the main findings included transient neurological functions such as tremors, abnormal gait, and hindlimb weakness, in some animals of the medium-high dose group (2.5mg/dose) and high dose group (4.5mg/dose) after intrathecal drug injection. In the safety dose exploration

experiment with cynomolgus monkeys, one male monkey (3001) died after administration of the high dose of 200mg (equivalent to 2g in human dose), while another female monkey (3501) showed severe toxic reactions but gradually recovered over days 17-18. The two animals in the low-dose group of 30mg showed no abnormalities after the first administration, but tremors occurred after the second administration. As a result, animals in the vehicle group were used as alternatives and received the medium-dose experiment of 50mg. The 50mg dose group (equivalent to 500mg in human dose) showed transient tremors, body stiffness, and reduced activity after administration, but these symptoms resolved within approximately 2 to 4 hours.

In terms of histopathology, axonal degeneration of the sciatic nerve, unrelated to the dose, was observed in both species. In the cynomolgus monkey study (H59-0025-TX), mild to mild multifocal axonal degeneration was observed in both bilateral and unilateral sciatic nerves of the male in the 50 mg/dose and 30 mg/dose groups. Additionally, mild to mild multifocal axonal degeneration was observed in the female monkeys across all dose groups. This alteration is characterized by a "bubbly" appearance of multiple affected fibers, axonal rupture (aggregating into eosinophilic masses), and some cellular debris surrounding the vacuoles. Although there was no control group in the cynomolgus monkey study, the changes observed in the sciatic nerve were still considered related to the test drug, consistent with the findings from the rat study. In each dose group, mild multifocal axonal degeneration was observed at the injection site in the female monkeys. The characteristics of this alteration are similar to those observed in the sciatic nerve and may be due to the administration procedure. In addition, mild to mild diffuse vacuolation of macrophages in the medulla of mesenteric lymph nodes was observed in all dose groups. Since these adverse reactions were not dose-dependent, their correlation with the test drug cannot be determined at this time.

Based on the results of the safety dose exploration experiments, formal GLP toxicology experiments are currently underway. These formal GLP toxicology tests have reduced the administration doses for rats and cynomolgus monkeys, as well as optimized the intrathecal injection procedure. The preliminary safety dose exploration

experiment results of rats and cynomolgus monkeys can also provide insight into the potential clinical risks of this test drug.

This project conducts a single-center, dose-escalation clinical study, aiming to evaluate the safety, tolerance and pharmacokinetics of RAG-17 in adult ALS patients carrying the *SOD1* mutation, in preparation for subsequent efficacy clinical trial.

2. Study Purposes

This study primarily aims to evaluate the safety, tolerance and pharmacokinetics of RAG-17 in adult ALS patients with *SOD1* gene mutation.

2.1 Primary Purposes：

(1) To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17.

(2) To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

2.2 Second Purposes：

(1) To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(2) To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(3) To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(4) To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

2.3 Exploratory Purposes：

(1) To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180.

(2) To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180.

(3) To evaluate the changes in muscle strength on day 15, day 29, day 90, and day 180.

(4) To evaluate the changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180.

(5) To evaluate the changes in 7T MRI imaging features on day 29, day 90, and day 180.

(6) To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180.

(7) To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, and day 180.

(8) To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, and day 180.

(9) To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.

3. Study Design Type, Principles and Experimental Procedures

3.1 Study Design

The study is an ongoing open-label, single-center, first-in-human, and dose escalation clinical trial.

Adult patients with a confirmed or probable familial or sporadic of ALS with confirmed *SOD1* gene mutations (forced vital capacity ≥ 80% of predicted value) will be included according to the revised El Escorial criteria.

1) **Administration：** A total of 7 doses will be administered on Day 1, Day 15, Day 29, Day 60, Day 120, Day 180, and Day 240. The drug will be administered via

intrathecal injection of RAG-17, with doses of 60 mg per administration, 90 mg per administration, 120 mg per administration, DLT per administration, with the last three doses being the optimal dose. The treatment period of this study is 8 months. Various indicators, adverse events, and concomitant medications will be collected on Day 1, Day 15, Day 29, Day 60, Day 90, and Day 180 post-treatment. The study is expected to be conducted at a single center, Beijing Tiantan Hospital, with the recruitment of 6 participants, aiming to complete the study within 2 years.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	DLT/dose	Intrathecal injection	6
5th Administration	D120	Optimal dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

2) **Dose Escalation Criteria:** If no AEs or SAEs occur within 14 days after the first administration, the subject proceed with a dose escalation of 30 mg every 14 days during the observation period. It is planned to achieve DLT through 3 to 4 dose escalations, followed by continuous treatment with the optimal dose for a 6-month treatment period. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD.

3) **Dose Escalation Stopping Criteria：** 'Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0' will be used as the toxicity evaluation standard for this protocol.

If a dose meets any of the following criteria, dose escalation will be considered for stopping or the trial will be continued at an additional lower dose level, unless it can

be clearly confirmed that the adverse event is unrelated to the drug (e.g., skin abrasions, hospitalization or death caused from a car accident).

① At the same dose level (within the same administration group), if ≥ 1 subject experiences an AE of grade ≥ 2;

② Other situations that may increase the risk of subjects, which will be discussed by the investigator to decide whether to terminate dose escalation.

4) **Individual Drug Withdrawal Criteria:** A subject will no longer participate in the subsequent administration, if they meet any of the following criteria:

① During the trial, the subject experiences a SAE or an AE of grade ≥ 3 (including laboratory abnormalities) , regardless of whether it is related to the drug;

② The subject experiences an event that, in the investigator's assessment, may significantly increase the risk to the subject.

3.2 Sample Size

This is an exploratory, hypothesis-generating study and a dose escalation clinical study to explore the maximum tolerated dose, so a total of 6 cases are required for sample size estimation.

3.3 Study Period

Work Progress	Timeline
Study Design, Study Approval Review, Ethical Review, Contract Signing, Human Genetic Resources Approval, Study Registration	2022.12-2023.05
Project Official Launch, Subject Enrollment and Follow-up	2023.06-2024.06
Data Cleaning and Analysis, Manuscript Writing	2024.06-2024.12
Total Duration	2 Years

3.4 Subject Selection

During the entire study period, it is planned to enroll 6 subjects. Ethical approval must be obtained before patient recruitment. The ethics committee will have access to all study documents and training materials.

- **Inclusion Criteria**

(1) Patients permitted by medical personnel who could complete the clinical trial.

(2) Age 18–75 years, gender unlimited.

(3) Clearly identify ALS patients with SOD1 gene mutations (known *SOD1* mutation sites and reported disease progression).

(4) Forced vital capacity during screening period ≥ 80% of expected lung capacity.

(5) Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology.

(6) The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form.

(7) The subjects (including male subjects) have no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum.

- **Exclusion Criteria**

(1) The *SOD1* mutation site occurs in patients with nucleotides 44-66 (calculated from the translation of SOD1 protein) and P.F21C mutations.

(2) Patients who have previously received or are currently receiving Tofersen treatment.

(3) HIV test positive or positive test history.

(4) Hepatitis C virus antibody positive or positive test history.

(5) Active hepatitis B infection (hepatitis B surface antigen positive and/or hepatitis B core antibody positive).

(6) Other experimental drugs used within one month or within five drug half-lives.

(7) Lumbar diseases and deformities.

(8) Suffering from other diseases known to be related to motor neuron dysfunction, which may confuse or blur the diagnosis of ALS.

(9) Other psychiatric disorders diagnosed according to DSM-V diagnostic criteria, or obvious suicidal intentions.

(10) Combined with severe liver dysfunction, renal dysfunction, or severe heart dysfunction (severe liver dysfunction refers to ALT value ≥ 2.0 times the upper

limit of normal value or AST value ≥ 2.0 times the upper limit of normal value; severe renal dysfunction refers to CRE ≥ 1.5 times the upper limit of normal value or eGFR < 40mL/min/1.73m^2; severe heart dysfunction refers to NYHA 3-4 levels).

(11) Permanent and sustained dependence on ventilator assisted ventilation.

(12) History of combined alcohol and drug abuse.

(13) Pregnant, lactating, or potentially pregnant patients, as well as those planning to conceive.

(14) Patients participating in other clinical trials or applying other biologics, drugs, or devices under study.

(15) Patients who have received any vaccination within 28 days.

(16) Contraindications for MRI examination (ie. claustrophobia).

(17) Those who can't complete follow-up due to other reasons.

4. Study Method

(1) Drug Dosage Form

The aseptic aqueous solution of the RAG-17 preparation (sodium salt) used in the study is stored at -20℃±5℃, with a specification of 75 mg (2.5 mL).

(2) Intervention Measures

1) Basic Treatment

All enrolled subjects will maintain basic medication treatment, and the dosage and type of medications will remain unchanged during the trial period.

Subjects who have already received riluzole treatment before enrollment may continue using it during the study period, maintaining a stable dosage (50mg per dose, twice daily). Throughout the study, discontinuation of riluzole for reasons other than medical necessity, as determined by the investigator, is not recommended.

The concomitant medications situation of the patients should be recorded in detail in the CRF form, including the name of the medication, the dosage, and frequency. If during the study, a change in the existing medication is deemed necessary by the investigator due to the patient's condition, it will be considered a protocol deviation. An adverse event record report and a concomitant medication form should be

completed. The investigator will then assess whether the subject can continue participating in the study. If not, the subject will be treated as a withdrawn case.

2) **RAG-17 Treatment**

The administration begins with an initial intrathecal injection dose of 60 mg. Within the first 14 days following the initial dose, if no AEs or SAEs are observed, the dose may be escalated by 30mg at 14-day intervals. After reaching the maximum tolerated dose (MTD), an optimal dose between the safe dose and the MTD will be selected as the dose for continued treatment. Continuous treatment will be administered every 2 months, with a total treatment duration of 8 months.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	DLT/dose	Intrathecal injection	6
5th Administration	D120	Optimal dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

5. Concomitant Medications

(1) Prohibited Concomitant Medications

Subjects must contact their investigators before taking any new medications, including over-the-counter drugs and herbal preparations.

• The use of any antiplatelet or anticoagulant drugs (e.g. aspirin, clopidogrel) is prohibited within 7 days prior to each lumbar puncture and until 48 hours after the procedure.

• The use of DPS, copper (II) (diacetylbis (N4-methylaminothiourea)), or pyrimethamine is prohibited during the study.

• The use of gamma globulin is not recommended during the study.

(2) Permitted Concomitant Medications

If subjects are taking concomitant vitamins, minerals and supplements, they should avoid exceeding the recommended daily dose within 14 days prior to the first administration of the RAG-17 and during the study period (e.g. no more than 5 grams of creatine per day and no more than 1000 IU of vitamin E per day). These daily limits include the total dose obtained from the combined use of daily multivitamins and supplements.

Medications not listed in the prohibited concomitant medications section above are considered permitted concomitant medications. Concomitant medications for symptom management during the study are determined by the investigator. All enrolled cases must maintain basic medication treatment, with no changes to the dosage or type of medications during the trial. If subjects have questions regarding permitted concomitant treatments, they should seek medical guidance from the investigator. The concomitant medications of the patients must be documented in detail in the CRF form, including the name of the medication, the dosage, and frequency. If a change or adjustment to the existing medications become necessary due to the subject's condition during the study, and the investigator deems it essential, this will be considered a protocol deviation. The adverse event record form and the concomitant medication form should be promptly completed. The investigator will assess whether the subject can continue participating in the study. If continuation is not feasible, the case will be treated as an excluded case. In special circumstances (e.g. the subjects suffers from other diseases during the study), when additional treatments are necessary, these must be explained in the CRF form, including the disease diagnosis, medications, dosage, and usage details.

6. Observation Indicators and Examination Timeline

6.1 General Demographic Indicators and Clinical Data

(1) Collection time point: baseline

(2) Collection content: Basic demographic characteristics include gender, age, ethnicity, height, weight, education level, marital status; vital signs include body temperature, pulse, respiration rate, blood pressure; 12-lead electrocardiogram (ECG);

waist circumference, physical examination; clinical data include relevant medical history, past medical history, medication used prior to enrollment, smoking and drinking history, and family medical history.

6.2 Daily Life Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo assessments using the ALSFRS-R and Modified Norris Scale before and after treatment. The results after treatment intervention will be compared with baseline data to evaluate the effect of RAG-17 on improving daily living function of the subjects.

(3) Observation indicators:

1) ALSFRS-R: This is a rapidly administered ordinal rating scale (grade 0-4) used to assess the subjects' ability and independence in 12 functional activities. ALSFRS-R measures 4 functional domains, including respiratory, bulbar, gross motor, and fine motor functions. The scale includes 12 items, each scored from 0 to 4, with a total score of 48. Higher scores indicate better function, while lower scores reflect more severe neurological impairment.

2) Modified Norris Scale: This assessment tool for ALS consists of two parts: the Limb Norris Scale (comprises 21 items to evaluate limb function) and the Norris Bulbar Scale (includes 13 items to assess bulbar function). Lower scores indicate more severe neurological impairment.

6.3 Respiratory Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo forced vital capacity (FVC) testing before and after treatment. The results post-treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving the respiratory function.

(3) Contents of observation indicators:

FVC: The subjects are measured in an upright position. Measured as the maximum volume of air that can be forcefully exhaled as quickly as possible after a maximal inhalation.

6.4 Fatigue Severity Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements with the Fatigue Severity Scale (FSS) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving the severity of fatigue in subjects.

(3) Observation indicators:

Fatigue severity: The FSS consists of 9 items, designed to measure the degree of fatigue and its impact on a person's activities and lifestyle across various diseases. The evaluation covers areas such as daily activity ability, participation, and sleep. Each question is scored on a 7-point scale: 1 = strongly disagree and 7 = strongly agree. The minimum possible score = 9 and the maximum possible score = 63. Higher scores indicate the greater severity of fatigue.

6.5 Muscle Strength Test

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurement using the Medical Research Council (MRC) Scale (sternocleidomastoid muscle, deltoid muscle, biceps brachii, triceps brachii, common digital extensor muscle, abductor pollicis brevis muscle, abductor digiti minimi muscle, first dorsal interosseous muscle, and iliopsoas muscle, quadriceps femoris, biceps femoris, tibialis anterior, gastrocnemius muscle, flexor muscle, extensor muscle) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving muscle strength.

(3) Observation indicators:

MRC scale: The MRC muscle strength grading method, is an enhanced version of the Lovett grading method, further subdivided by the range of motion and the level of resistance applied. If the measured muscle strength is slightly stronger than a certain level, a "+" is added to the upper right corner of this level. If it is slightly weaker, a "-" is added to the upper right corner. The refinement addresses the limitation of Lovett grading method, providing a more precise assessment of muscle

strength.

In this study, the MRC grading is used to measure the muscle strength of the abductor pollicis brevis muscle, abductor digiti minimi muscle and tibialis anterior muscle.

6.6 Electromyography Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements of compound muscle action potential (CMAP), motor unit number index (MUNIX), and motor unit size index (MUSIX) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the electromyographic parameters of the subjects.

(3) Observation indicators:

CMAP: CAMP represents the summated action potentials of muscle fibers that are almost simultaneously activated when a certain nerve is directly or indirectly stimulated. The amplitude from baseline to peak, duration, latency of the negative phase of the potential, as well as the details of the stimulation method and recording method should be recorded.

MUNIX: A motor unit is a functional unit located in the anterior horn of the spinal cord, consisting of an alpha motor neuron and all the muscle fibers it innervates. It is the basic unit of motor function, with the minimum unit of normal muscle contraction being the contraction of one motor unit. MUNIX is a sensitive biomarker for monitoring the nerve innervation status of patients with neurodegenerative diseases and is a highly sensitive and effective marker for monitoring the loss of motor units in ALS. The method uses CMAP and the surface electromyographic interference pattern (SIP) to evaluate the number and size of motor units (MUs).

MUSIX: The size of a motor unit is determined by the number of nerve endings and branches of the neuron axon. Generally, larger muscles have larger motor unit. Each muscle contains a certain number of motor units. The calculation method is to divide the amplitude of the compound muscle action potential (CMAP) by the MUNIX value.

6.7 Imaging Indicators

(1) Collection time points: baseline, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurement with 7T MRI imaging before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the imaging indicators of the subjects.

(3) Observation indicators:

7T MRI imaging indicators: 7T ultra-high field magnetic resonance is an advanced magnetic resonance imaging system currently available for human body scanning. Compared with the traditional 1.5T and 3T magnetic resonances, 7T magnetic resonance can provide a doubled signal-to-noise ratio, finer spatial resolution, and more excellent tissue contrast. It is more suitable for observing the fine structure of tissues and physiological changes in functional metabolism and other information, thereby revealing physiological and pathological mechanisms that are difficult to be discovered by traditional platforms.

6.8 Gait Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo the following assessments: three-dimensional gait analysis under multitasks, Short Physical Performance Battery (SPPB), item 1 (Tandem Walking) of the Scale for the Assessment and Rating of Ataxia (SARA), and 6-meter walking test before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the gait function of the subjects.

(3) Observation indicators: Semi-quantitative scales include the Short Physical Performance Battery (SPPB) and item 1 (Tandem Walking) of the Scale for the Assessment and Rating of Ataxia (SARA); Quantitative gait assessment includes 6-meter walking test.

SPPB: The SPPB is a widely used method for evaluating muscle function. It consists of 3 items: balance test, 4-meter timed walking test, and timed sit-to-stand test. Each individual test can score a maximum of 4 points, giving a total score of 12

points. To improve the accuracy of the test, each test is usually repeated 2 to 3 times, with the shortest time recorded for scoring.

6-meter normal walking test: It is conducted on an 8-meter walkway with a timing taken over a 6-meter distance. Landmark markers are placed at the start of the walkway, at the 1-meter, the 7-meter, and the 8-meter points, for a total of 4 markers. Timing starts at the 1-meter and stops at the 7-meter mark to avoid the influence of markers on the subject's behavior, such as intentional changes in walking pace or nervousness. The test is performed 4 times; the first trial serves as a practice and is not recorded. The times from the subsequent 3 trials are recorded, and the average of these times is used for analysis.

Item 1: SARA is a clinical scale used for the semi-quantitative assessment of cerebellar ataxia severity. It can also serve as a rehabilitation indicator for gait and independence in daily activities for ataxic stroke patients. In this study, the gait part (Item 1) is selected. It consists of 8 levels corresponding to scores ranging from 1 to 8. Grade 1 is a mild abnormality corresponding to 1 point, up to grade 8 is unable to walk even with assistance corresponding to 8 points.

6.9 Quality of Life Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements with the Amyotrophic Lateral Sclerosis Self-Assessment Questionnaire (ALSAQ-40), EQ-5D-5L scale, and HAMA and HAMD-17 scales before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the quality of life of the subjects.

(3) Observation indicators:

ALSAQ-40: ALSAQ-40 is a commonly used quality-of-life scale for ALS. The sections on social interaction and eating ability provide subjective insights into articulation and swallowing functions. ALSAQ-40 consists of 40 items involving the patient's condition, requiring a 5-point scale rating. The questions cover five health status dimensions affected by the disease: physical activity (including 10 items), daily living activities and independence (10 items), eating (3 items), communication (7

items), and emotional function (10 items). Each dimension is scored on a scale from 0 to 100: 0 represents the possible best health status and 100 represents the possible worst health status.

EQ-5D-5L: EQ-5D-5L is a standardized scale for measuring health status, that is an updated version of the EQ-5D scale. The scale consists of two parts: the EQ-5D-5L descriptive system and the visual analogue scale (EQ-VAS). The EQ-5D-5L descriptive system describes five dimensions: Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression. Each dimension contains five levels: 1=No difficulty, 2=Slight difficulty, 3=Moderate difficulty, 4=Severe difficulty, 5=Unable to perform/very severe difficulty. The responses of the five dimensions can generate a five-digit code to describe the health status of the respondent. The health status 1,1,1,1,1 indicates no problems in all five dimensions. EQ-VAS records the self-rated health status of the respondent on a vertical visual analogue scale, with the ends of the scale marked as "The best health status you can imagine" and "The worst health status you can imagine".

6.10 Clinical Staging Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo assessment with the London ALS staging and the Milan-Turin staging (ALS-MITOS) before and after treatment. The results after treatment intervention will be compared with the baseline data to assess the disease progression of ALS patients and evaluate the effect of RAG-17 on the condition of the subjects.

(3) Observation indicators:

London ALS staging: According to the most recently affected region in the course of the disease, ALS is divided into 5 stages: Stage 1 = symptom onset (involvement of the first area); Stage 2A = confirmed diagnosis, and Stage 2B = involvement of the second area; Stage 3 = involvement of the third area; Stage 4A = the patient requires percutaneous endoscopic gastrostomy (PEG), and Stage 4B = the patient requires non-invasive ventilation; Stage 5 = death or mechanical ventilation. The average survival time and 5-year survival rate vary across stages.

Milan-Turin staging: The patient's functions are divided into 4 domains by assessing 4 key points (including mobility, swallowing, communication, and respiration). Each domain is scored as either 0 (below the threshold) or 1 (above the threshold). The total score across these domains determines the stage: Stage 0 = no functional loss; Stage 1 = loss of one functional domain; Stage 2 = loss of two functional domains; Stage 3 = loss of three functional domains; Stage 4 = loss of four functional domains; Stage 5 = death or mechanical ventilation.

6.11 Laboratory Examination Data Collection

(1) Collection time points: baseline, day 1, day 15, day 29, day 60, day 120, day 180, day 240

(2) Collection content: All subjects will undergo laboratory tests including blood routine, urine routine, liver function, kidney function, and coagulation tests before and after treatment respectively. The results after treatment intervention will be compared with the baseline data to evaluate the impact of RAG-17 on the general physical condition of the subjects and assist in determining the occurrence of adverse events and other related events.

All subjects will undergo the detection of SOD1 protein in cerebrospinal fluid, plasma neurofilament light chain protein (NFL), and the pharmacokinetics of RAG-17 in plasma before and after treatment.

6.12 Other Information Collection

(1) Collection time points: baseline, day 1, day 15, day 29, day 60, day 120, day 180, day 240

(2) Collection content: During the study period, all the medications taken by the subjects will be recorded, including the reasons for use, drug names, dosages, and duration of use. All AEs, adverse reactions and their handling processes will also be documented; The timing of tracheostomy, invasive mechanical ventilation, death, and the duration of invasive mechanical ventilation will be recorded; Subjects' medication adherence, cases of withdrawal or exclusion from the study will be recorded.

7. Follow-up Time

Follow-up plan: For all subjects, face-to-face visits will be conducted at baseline, day 1, day 15, day 29, day 60, day 120, day 180, and day 240. They will undergo tests including daily living function, respiratory function, muscle strength, quality of life, gait function, and clinical staging, as well as the collection of adverse events and concomitant medications use. The study is expected to be conducted at Beijing Tiantan Hospital, with a target recruitment of 6 participants, and is planned to be completed within 2 years.

Follow-up content: To evaluate the differences in clinical manifestations of the participants after receiving the RAG-17 intervention at baseline, day 1, day 15, day 29, day 60, day 120, and day 180, including:

- Laboratory examinations: tests including blood routine, urine routine, liver function, kidney function, coagulation, SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma will be conducted at baseline, day 1, day 15, day 29, day 60, day 120, day 180, and day 240.
- Daily living functions: ALSFRS-R scoring and Modified Norris Scale scoring will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Respiratory function: FVC evaluations will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Fatigue severity: FSS assessment will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Muscle strength test: MRC assessment will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Electromyogram indicators (abductor pollicis brevis, abductor digiti minimi, and anterior tibial muscle): CMAP, MUNIX, and MUSIX will be collected at baseline, day 15, day 29, day 90, and day 180.
- Imaging examinations: 7T magnetic resonance imaging will be conducted at baseline, day 29, day 90, and day 180.
- Gait function: SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking will be conducted at baseline, day 15, day 29, day 90, and day 180.

- Quality of life: assessments of ALSAQ-40, EQ-5D-5L, HAMA, and HAMD-17 will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Clinical staging: London ALS staging and Milan-Turin staging assessments will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Other indicators: information such as vital signs, abnormalities in heart/lung/abdomen examination, neurological examination, electrocardiogram, adverse events, concomitant medications, the time to tracheostomy, invasive mechanical ventilation, death, and the duration of invasive mechanical ventilation of the patients will be collected at baseline, day 1, day 15, day 29, day 60, day 90, day 120, day 180, and day 240.

8. Study Process

8.1 Information Collection and Informed Consent (Visit 1)

Patients who meet the inclusion criteria but do not meet the exclusion criteria will be introduced to the study protocol and purposes by investigator. The patient or their legal representatives will be provided with complete information about the study, accompanied by corresponding written materials, and a written informed consent form will be signed.

- Collection of medical history: including current medical history, past medical history, history of concomitant medication, history of smoking and alcohol consumption, family history;
- Physical examination: including vital signs, heart/lung/abdomen examination, neurological examination, 12-lead electrocardiogram;
- Laboratory examinations: including blood routine, urine routine, liver function, kidney function, coagulation function, BNP; Blood sample collection for verification of *SOD1* gene mutation sites, plasma neurofilament light chain protein (NFL);
- 7T magnetic resonance imaging;
- Assessment of respiratory function (including FVC)
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Assessment of daily living functions: ALSFRS-R score, Modified Norris Scale

score;

- Assessment of fatigue severity: FSS scale score;
- Assessment of muscle strength and electromyogram indicators: MRC assessment (sternocleidomastoid muscle, deltoid muscle, biceps brachii, triceps brachii, common digital extensor muscle, abductor pollicis brevis, abductor digiti minimi, first dorsal interosseous muscle and iliopsoas muscle, quadriceps femoris, biceps femoris, anterior tibial muscle, gastrocnemius muscle, flexor muscle, extensor muscle grading); CMAP, MUNIX, MUSIX;
- Assessment of gait function: SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking;
- Assessment of quality of life: ALSAQ-40 and EQ-5D-5L scale scores;
- Assessment of emotional scale: HAMA and HAMD-17 scores.

8.2 Days 1-4 Post-Enrollment (First Treatment) (Visit 2)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications:
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 15, face-to-face follow-up.

8.3 Days 15-18 Post-Enrollment (Second Treatment) (Visit 3)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal

injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;

- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- Arrange the follow-up appointment for day 29, face-to-face follow-up.

8.4 Days 29-32 Post-Enrollment (Third Treatment) (Visit 4)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6

hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;

- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 60, face-to-face follow-up.

8.5 Days 60-63 Post-Enrollment (Fourth Treatment) (Visit 5)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;

- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 90, face-to-face follow-up.

8.6 Days 90-93 Post-Enrollment (Visit 6)

- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 120, face-to-face follow-up.

8.7 Days 120-123 after enrollment (Fifth treatment) (Visit 7)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;

- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 180, face-to-face follow-up.

8.8 Days 180-183 Post-Enrollment (Sixth Treatment) (Visit 8)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 240, face-to-face follow-up.

8.9 Days 240-243 after enrollment (Seventh treatment) (Visit 9)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal

injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;

- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.

8.10 Study Flowchart

Examination / Treatment	Screening / Baseline	Treatment and Follow-up							
		1st Administration	2nd Administration	3rd Administration	4th Administration		5th Administration	6th Administration	6th Administration
		Day 1-4	Day 15-18	Day 29-32	Day 60-63	Day 90-93	Day 120-123	Day 180-183	Day 240-243
Verification of inclusion / exclusion criteria	√								
Sign the informed consent form	√								
Demographic data	√								
Medical history / Personal history / Family history	√								
Height / Weight / Waist circumference / Physical examination	√	√	√	√	√	√	√	√	√
Concomitant medication	√	√	√	√	√	√	√	√	√
Blood pressure / Respiration / Heart rate / Body temperature	√	√	√	√	√	√	√	√	√
Neurological examination	√	√	√	√	√	√	√	√	√
12-lead electrocardiogram	√	√	√	√	√	√	√	√	√
Laboratory examination[1]	√	√	√	√	√		√	√	√
ALSFRS-R score	√		√	√		√		√	
Modified Norris Scale	√		√	√		√		√	
Forced vital capacity	√		√	√		√		√	
Fatigue Severity Scale	√		√	√		√		√	
Muscle strength / Electromyogram	√		√	√		√		√	
7T MRI	√			√		√		√	
SPPB	√		√	√		√		√	
Tandem Walking	√		√	√		√		√	
6-meter walking	√		√	√		√		√	
Three-dimensional gait analysis under multitasking	√		√	√		√		√	
ALSAQ-40	√		√	√		√		√	

EQ-5D-5L	√		√	√		√		√	
Anxiety Scale (HAMA)	√		√	√		√		√	
Depression Scale (HAMD-17)	√		√	√		√		√	
London ALS staging	√		√	√		√		√	
Milan-Turin staging (AKS-MITOS)	√		√	√		√		√	
Intrathecal administration of RAG-17	√	√	√	√	√		√	√	√
Blood sample collection [2]	√	√	√	√	√		√	√	√
Cerebrospinal fluid sample collection [3]	√	√	√	√	√		√	√	√
Adverse events / Severe adverse events	√	√	√	√	√	√	√	√	√
Study drug adherence	√	√	√	√	√		√	√	√

[1] Laboratory examinations: blood routine, urine routine, liver functions, renal function, coagulation function, BNP (BNP is only examined at baseline). Laboratory tests during treatments 1-7: Conduct laboratory tests before each treatment and 48 hours after each treatment.
[2] Samples are collected at baseline; during the treatments 1 to 7 , samples are collected at 4 hours before each treatment, and 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, and 48 hours after each treatment.
[3] During treatments 1 to 7, samples are collected 15 minutes before each treatment (Collection before drug administration via lumbar puncture, not restricted to 15 minutes).

9. Clinical Evaluation

9.1 Primary Outcomes：

1) Incidence of AEs and SAEs within 180 days after the first injection of RAG-17.
2) Changes in laboratory assessments, vital signs, ALSFRS-R scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

9.2 Second Outcomes：

1) Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
2) Changes in plasma NFL levels from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
3) Changes in the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29,

day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

4) Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

9.3 Exploratory outcomes：

1) Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180.
2) Changes in fatigue severity (Fatigue Severity Scale, FSS) at on day 15, day 29, day 90, and day 180.
3) Changes in muscle strength on day 15, day 29, day 90, and day 180.
4) Changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180.
5) Changes in 7T MRI imaging features on day 29, day 90, and day 180.
6) Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180.
7) Changes in modified Norris scale scores on day 15, day 29, day 90, and day 180.
8) Changes in quality of life (including ALSAQ-40、EQ-5D-5L、HAMA and HAMD) on day 15, day 29, day 90, and day 180.
9) Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.

10. Known and Potential Risks and Benefits, and Risk Management Plan

10.1 Adverse Event Monitoring

All information related to adverse events, whether reported by the participants, identified by the investigators, or detected through physical examinations, laboratory tests, or other methods, should be recorded on the adverse event page of the case report form. These events will be managed and reported in accordance with the relevant regulations. A comprehensive adverse events report will include in the final trial report.

10.2 Adverse Event Definition

10.2.1 Adverse Event (AE)

An adverse event refers to any unfavorable medical occurrence or worsening of a pre-existing condition that arises in a participant during the use of the investigational drug, regardless of whether it has a causal relationship with the investigational drug treatment. Adverse medical occurrences can manifest as clinical symptoms/signs, diseases, or abnormal laboratory findings. AEs include any unfavorable medical occurrences that occur at any stage of the clinical study, including the lead-in or washout periods, even before the investigational drug has been administered to the participant. AEs include both serious and non-serious adverse events.

10.2.2 Serious Adverse Event

An adverse event during the study will be classified as serious, if it meets one or more of the following criteria:

- Results in death
- Immediately life-threatening

Note: Life-threatening refers to an event that poses an immediate risk to the patient's life at the time it occurs, not an event that could result in death if it were to worsen.

- Requires hospitalization or prolongs the current hospitalization
- Results in permanent or severe disability/incapacity, or loss of essential abilities required for normal living
- Leads to congenital anomaly, birth defects, or reproductive issues
- Constitutes a significant medical event that may endanger the subject or necessitate medical intervention to prevent the outcomes listed above.

10.3 Adverse Events Documentation

Non-serious AE: Non-serious AEs of interest occurring during the study period from the start until the end of the study (e.g. renal function impairment/increased serum creatinine, elevated liver function test indicators, upper gastrointestinal discomfort, muscle/bone pain, hypocalcemia, ocular inflammation, arrhythmia, fractures, etc.) and information about drug discontinuation due to SAEs must be

documented. Documentation of other non-serious AEs is at the discretion of the investigator .

Serious AE: All SAEs must be documented.

10.4 Assessment of the Relationship Between AEs and the Investigational Drug

The relationship between AEs and the investigational drug will be assessed using a 5-level standard: ①Definitely related; ②Highly likely related; ③Possibly related; ④Unlikely related; ⑤Definitely unrelated; ⑥Unable to determine.

①Definitely related: Matches the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or disappears after dose reduction or drug withdrawal. Reaction reappears upon re-administration.

②Highly likely related: Matches to the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or disappears after dose reduction or drug withdrawal. However, the clinical status of the participants or other reasons may also cause the reaction.

③Possibly related: Matches the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or is not obvious after dose reduction or drug withdrawal. However, the clinical status of the subject or other reasons can equally explain the reaction.

④Unlikely related: Does not match the known reaction type of the suspected drug. Dose not align with the reasonable time frame after administration. The clinical status of the subject or other reasons may also cause the reaction.

⑤Definitely unrelated: Does not match the known reaction type of the suspected drug. Dose not align with the reasonable time frame after administration. The clinical status of the subject or other reasons can also explain the reaction. Reaction decreases or disappears when clinical symptoms or other causes are ruled out..

⑥Unable to determine.

The incidence of AEs will be calculated and summarized for classifications ① + ② + ③ from each case report form.

10.5 Unexpected Adverse Event

An unexpected adverse event refers to any event not mentioned in the investigator's brochure, or differs in nature and severity from what is described in the investigator's brochure. In the absence of an investigator's brochure, the event is inconsistent with the risk information described in the study protocol or application materials.

10.6 Reporting of Serious Adverse Events

Notify the designated representative of the sponsor immediately. Send the signed and dated relevant pages of the case report form to the sponsor’s representative, along with copies of all examination results and their completion dates. At the same time, ensure the patient privacy is protected by removing all identifiable information from the clinical source document copies provided to the sponsor. Laboratory results should include normal range values.

All SAEs must be reported within 24 hours of occurrence. Record the SAE in the designed section of the CRF. After signing, report it preferably via fax to the monitoring center. The name, address and fax number of the monitoring center will be specified in the clinical trial protocol.

10.7 Risk Control and Management

In this study, the investigational drug RAG-17 may cause adverse reactions such as tremors, abnormal gait, and hind limb weakness. If such actions occur and are deemed related to the drug, it is recommended to stop the administration, monitor vital signs, conduct auxiliary examinations such as blood routine, coagulation profile, muscle enzymes, liver function, kidney function, blood gas analysis, ultrasound, CT scans. Provide timely symptomatic and supportive treatment based on clinical findings. If necessary, consult with relevant specialists to manage the adverse events effectively. For serious adverse events, it is necessary to ensure airway patency, autonomous respiration, stable blood pressure and heart rate. If necessary, treatment should be carried out in the intensive care unit.

For all adverse events, the investigators should continue to follow up with patients and track any related outcomes (clinical symptoms, signs, laboratory test results) until the related clinical symptoms are completely resolved, the test results return to normal or the condition is stable. This follow-up should persist even if the patient withdraws

from the clinical trial. Additionally, the monitoring team may request further investigations as needed. After the termination of the study, the investigators should still pay attention to any serious adverse events that may occur.

10.8 Subjects Benefits

Participation in this clinical study may help alleviate the subject's condition, but it is also possible that the investigational drug will not provide the expected therapeutic effect. In other words, subjects may not gain any direct benefits from participating in this study. However, this study may contribute to the development of new treatment options for patients with similar conditions, offering more choices to others. In addition, subjects may gain valuable information about their conditions through the physical examinations and medical tests conducted during the study.

11. Quality Control and Quality Assurance of the Study

Representatives of the sponsor or collaborators will regularly monitor the clinical study. The scope and nature of supervision will be determined based on considerations such as the clinical study's objectives, purposes, design, complexity, scale, and endpoints.

11.1 Protocol Adherence Management

- Careful selection of participants: When selecting subjects, investigators should confirm accurate diagnoses, understand common factors leading to dropout, conduct a comprehensive assessment of participants, and perform a pre-evaluation of adherence. Participants with high compliance potential should be carefully screened and selectively included.
- Informed consent process: Before signing the informed consent form, investigators should provide detailed and clear explanations to the subjects and their families in an easy-to-understand manner. This ensure subjects fully understand the investigational drug and the treatment process. Investigators must thoroughly address participants' questions, respect their choices, and encourage confidence in completing the study, which fosters better adherence.
- Optimizing the protocol: The study protocol should be designed to minimize complexity, favoring simple and practical approaches that align with participants'

daily lives. A well-designed, scientifically sound, and highly operable protocol will enhance adherence.

- Managing adverse events: If participants experience adverse reactions, adverse events, or serious adverse events during the clinical study, regardless of whether they are related to the investigational drug, researchers must promptly take appropriate treatment measures. Ensuring timely medical intervention safeguards participants' safety, builds trust in the study, and improves adherence.
- Follow-up and reminders: A contact information sheet and visit schedule should be established to remind participants of follow-up visits. For those who miss scheduled visits, researchers should promptly follow up via phone. These efforts help ensure participants remain engaged and adhere to the study protocol.

11.2 Study Monitoring

The primary responsibility of the monitoring team of the collaborators is to assist investigators and the sponsor or collaborators in ensuring the clinical study adheres to the highest standards of ethics, scientific rigor, professionalism, and regulatory compliance. According to the Good Clinical Practice (GCP) principle, the monitoring team should verify the CRF against the original source documents.

The monitoring team will regularly assess the progress of the study, through on-site visits, letters, or telephone communication. This includes evaluating adherence to the clinical protocol by researchers and participants and addressing urgent issues. Medical monitors will also periodically review participant safety. During these monitoring visits, the monitors will collaborate with the investigators to focus on the following key points (not exhaustive): informed consent of subjects, recruitment and follow-up of subjects, documentation and reporting of serious adverse event, concomitant treatment and data quality.

11.3 Data Verification

The following measures will be taken to ensure the accuracy, consistency, completeness and reliability of the data:

- Regular on-site monitoring of the study;
- CRF summary based on source documents;

- Data management quality control checks;
- Medical review by the sponsor.

The sponsor or designated personnel (such as CRO) will be responsible for the data management in this clinical study. The responsible party will design and distribute CRFs and oversee their monitoring. If the data are incomplete or inconsistent, the responsible party will issue queries for site staff to address and make necessary corrections.

11.4 Personnel Training

The investigators shall ensure adherence to the clinical trial protocol, compliance with the Good Clinical Practice (GCP) guidelines outlined in the ICH, and conformity with applicable laws and regulations during the conduct of the clinical trial.

The investigators should ensure adherence to all study procedures outlined in the clinical trial protocol (including safety principles). The investigator should accurately and clearly provide reliable data and all information required by the clinical trial protocol (e.g., drug overdose, pregnancy) through appropriate methods, such as CRFs, deviation resolution forms (DRFs), and others, in accordance with relevant requirements. Additionally, the investigator must ensure that the monitoring team has direct access to the source documents.

The investigators may appoint individuals deemed suitable to assist in conducting the study. These assistants will help manage the clinical study in accordance with the study protocol. All assistants will be appointed and documented in a timely manner. They will work under the supervision and management of the investigator, who will provide them with a copy of the clinical study protocol and all necessary information. The sponsor or collaborator of the clinical study are accountable to the health authority for ensuring the proper management of the clinical study by employing all appropriate means, such as ethics, compliance with the clinical study protocol, completeness and validity of the data recorded in the CRF.

11.5 Data Management

During the clinical study, the data of the subjects should be collected anonymously in the CRF, with subjects identified only by their number and the initials of their name.

In cases where participant identity must be disclosed due to safety or administrative reasons, both the investigator and any involved parties share the responsibility for maintaining confidentiality. The informed consent form allows the subject to agree to authorized personnel from the sponsor or collaborators, ethics committees, and regulatory authorities directly accessing relevant source documents associated with the CRF (e.g., medical records, appointment logs, original laboratory records). These individuals must adhere to professional confidentiality standards and ensure the privacy of all participant personal or medical information.

The medical information obtained in the study should remain confidential and may only be disclosed to third parties with the permission of the informed consent form and the subject's signed authorization form (or separate authorization for the use and disclosure of personal health information) or when permitted or required by law.

For treatment purposes, medical information may be shared with the participant's personal physician or other appropriate healthcare professionals responsible for the participant's well-being.

11.6 Handling of Biological Samples

The biological samples, such as blood and cerebrospinal fluid collected in this study, will be processed (e.g. centrifugation and aliquoting) at Beijing Tiantan Hospital, Capital Medical University. Samples designed for evaluation will be analyzed at a centralized clinical laboratory with recognized accredited certification. All biological including blood and cerebrospinal fluid will be stored at Beijing Tiantan Hospital, Capital Medical University for 10 years.

12. Data Retention

The reviewed CRF and imaging data will be carefully entered and processed by the data management center and properly stored. Once data entry is completed, the CRF will be stored by the research center.

The study monitor will perform ongoing source data verification to ensure that the key study protocol data (i.e., source data) transcribed on the CRF by authorized on-site personnel are accurate and complete, and that they can be verified against the original source documents.

Source documents are the original records where subject data is first recorded and stored. These include, but are not limited to hospital records, clinical and office charts, laboratory records, memos, subject diaries or assessment checklists, pharmacy dispensing records, data from automated instrument records, verified and certified as accurate and complete copies of audio transcriptions, microfilms, photographic negatives, microfiche or magnetic media, computed tomography, subject files, and records kept in pharmacies, laboratories and medical technology departments related to clinical studies.

The source documents required to verify the validity and completeness of the data transcribed on the CRF must never be deleted or destroyed.

To facilitate source data verification, the investigator and the institution must allow the sponsor to access applicable source documents and reports for direct inspection. This is to support monitoring, sponsor audits, clinical research reviews, and summaries by the management committees or ethics committees. Additionally, appropriate regulatory agencies must be allowed to inspect the study site.

13. Data Safety Monitoring

The clinical study will develop a data safety monitoring plan based on the level of risk involved. All adverse events will be thoroughly documented, appropriately managed, and followed up until resolved or the condition stabilizes. Serious adverse events and unexpected events will be promptly reported to the ethics committee, regulatory authorities, the sponsor, and the drug regulatory agency in accordance with relevant regulations. The principal investigator will regularly conduct cumulative reviews of all adverse events and, when necessary, convene investigator meetings to assess the risks and benefits of the study. For studies with risks exceeding the minimal threshold, independent data monitors will be assigned to oversee the study data. For high-risk studies, an independent data safety monitoring board will be established to review the accumulated safety and efficacy data and provide recommendations on whether the study should continue.

During the clinical trial, participant data will be collected anonymously in the CRF, with participants identified only by their participant number and the initials of their

name. In cases where participant identity must be disclosed due to safety or administrative reasons, both the investigator and the involved parties share the responsibility for maintaining confidentiality. The informed consent form allows participants to consent to the authorized sponsor, ethics committee, and regulatory authorities directly accessing relevant source documents on the CRFs (e.g., medical records, appointment logs, original laboratory records). These individuals must adhere to professional confidentiality standards and maintain the privacy of all participant personal or medical information.

14. Statistical Analysis

- Statistical analysis will be performed with SAS software.
- Demographic, medical, histories and baseline characteristics information will be listed and summarized.
- The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after treatment. Safety evaluation will be analyzed using safety data set. The number of people of events should be recorded in detail and showing the event rate in summary statement. The event rate will be calculated as the number patients experiencing the event / the number of patients enrolled.
- Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 180-day follow-up assessments for all participants, the data were analyzed.

15. Ethical Considerations

The clinical study will adhere to the World Medical Association's Declaration of Helsinki and other relevant regulations. Before the study begins, the trial protocol will be approved by the ethics committee. Before enrollment, the investigator is

responsible for providing a complete and comprehensive explanation of the study's purpose, procedures, and potential risks to the participant or their representative. A written informed consent form must be signed, ensuring that participants are aware of their right to withdraw from the study at any time. The informed consent form will be retained as part of the clinical study documentation for future reference. Throughout the study, the personal privacy and data confidentiality of participants will be safeguarded.

Screening and informed consent: The investigator (or designated personnel authorized and responsible by the investigator, in compliance with relevant regulations) will recruit participants in an independent consultation room at Beijing Tiantan Hospital, Capital Medical University, ensuring the privacy and confidentiality of the participants. Participants and their legal representatives should be fully informed about all relevant aspects of the clinical trial, including written information approved by the ethics committee. The investigator should use language or terms that the participant can understand, providing as much information as possible about the study, and allow sufficient time for the participant or their legal representative to consider whether to participate. Before participating in the clinical trial, the patient or their legal representative must discuss the informed consent with the investigator and sign the informed consent form (including the participant's name and the date). The investigator should provide the participant with a copy of the signed informed consent form.

16. Data Confidentiality

Throughout the entire research and data analysis process, participant information will be strictly protected. The executive committee will disclose the trial results in accordance with management regulations and procedures. Once the database is locked, the trial results will be published as soon as possible. Biostatistical experts involved in the study have the right to access the dataset but will not be able to identify any individual participants. In line with the GCP principles outlined in the ICH guidelines, the monitoring team will verify the CRFs against the original source documents. The informed consent form will include a statement that allows participants to consent to

authorized personnel from the sponsor, ethics committee, and regulatory authorities directly accessing relevant source documents on the CRFs (e.g., medical records, appointment logs, original laboratory records). Researchers must adhere to professional confidentiality standards and ensure the confidentiality of all personal and medical information of the participants.

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Safety and Tolerance of RAG-17 in Amyotrophic Lateral Sclerosis Patients With *SOD1* Gene Mutation

(CREATION)

Study Protocol

(Protocol Number HX-A-2023004)

Principal Investigator: Yilong Wang

Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Protocol Version: 2.0

Aug. 9th, 2023

Summary of the Trial Protocol

Study Title	Safety and tolerance of RAG-17 in amyotrophic lateral sclerosis patients with *SOD1* gene mutation
Study Purposes	To evaluate the safety, tolerance and pharmacokinetics (PK) of RAG-17 in adult ALS subjects with *SOD1* gene mutation. **Primary purposes：** 1. To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17. 2. To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17. **Secondary purposes：** 1. To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2. To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3. To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 4. To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

	Exploratory purposes： 1. To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180. 2. To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180. 3. To evaluate the changes in muscle strength on day 15, day 29, day 90, and day 180. 4. To evaluate the changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180. 5. To evaluate the changes in 7T MRI imaging features on day 29, day 90, and day 180. 6. To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180. 7. To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, and day. 8. To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, and day 180. 9. To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.
Study Design	Ongoing open-label, single-center, first-in-human and dose escalation clinical study

Total number of cases	Six ALS patients with *SOD1* gene mutations
Subjects Selection	**Inclusion Criteria** (1) Patients permitted by medical personnel who could complete the clinical trial. (2) Age 18~75 years, gender unlimited. (3) Clearly identify ALS patients with *SOD1* gene mutations (known *SOD1* mutation sites and reported disease progression). (4) Forced vital capacity during screening period ≥ 50% of expected lung capacity. (5) Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology. (6) The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form. (7) The subjects (including male subjects) have no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum. **Exclusion Criteria** (1) The *SOD1* mutation site occurs in patients with nucleotides 44-66 (calculated from the translation of SOD1 protein) and P.F21C mutations. (2) Patients who have previously received or are currently receiving Tofersen treatment. (3) HIV test positive or positive test history.

	(4) Hepatitis C virus antibody positive or positive test history. (5) Active hepatitis B infection (hepatitis B surface antigen positive and/or hepatitis B core antibody positive). (6) Other experimental drugs used within one month or within five drug half-lives. (7) Lumbar diseases and deformities. (8) Suffering from other diseases known to be related to motor neuron dysfunction, which may confuse or blur the diagnosis of ALS. (9) Other psychiatric disorders diagnosed according to DSM-V diagnostic criteria, or obvious suicidal intentions. (10) Combined with severe liver dysfunction, renal dysfunction, or severe heart dysfunction (severe liver dysfunction refers to ALT value ≥ 2.0 times the upper limit of normal value or AST value ≥ 2.0 times the upper limit of normal value; severe renal dysfunction refers to CRE ≥ 1.5 times the upper limit of normal value or eGFR < 40mL/min/1.73m^2; severe heart dysfunction refers to NYHA 3-4 levels). (11) Permanent and sustained dependence on ventilator assisted ventilation. (12) History of combined alcohol and drug abuse. (13) Pregnant, lactating, or potentially pregnant patients, as well as those planning to conceive. (14) Patients participating in other clinical trials or applying other biologics, drugs, or devices under study. (15) Patients who have received any vaccination within 28 days. (16) Contraindications for MRI examination (ie. claustrophobia). (17) Those who can’t complete follow-up due to other reasons.

<table>
<tr><td>Therapeutic Regimen</td><td>
Study drug： Aseptic aqueous solution of the RAG-17 formulation (sodium salt)
Storage condition： -20°C ± 5°C, stored in a frozen state
Specification： 75 mg (2.5 mL)
Route, dosage and schedule of administration: Intrathecal administration. The safety, tolerance, and pharmacokinetics of the RAG-17 drug will be evaluated by a dose-escalation approach. The dose of RAG-17 will range from a minimum of 60 mg to the maximum tolerated dose (MTD). Administration will be conducted once every two weeks, starting at 60 mg, for dose escalation. Once the tolerated dose is reached, a fixed dose of the drug will be administered every two months for continued treatment, with a total treatment duration of 8 months. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD. For the subsequent maintenance treatment phase, the optimal dose between the safe dose and the MTD will be selected as the dose for continued treatment.
<table>
<tr><th>Treatment</th><th>Administration Day</th><th>Dose Level</th><th>Administration Route</th><th>Number of Subjects</th></tr>
<tr><td>1st Administration</td><td>D1</td><td>60 mg/dose</td><td>Intrathecal injection</td><td>3</td></tr>
<tr><td>2nd Administration</td><td>D15</td><td>90 mg/dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>3rd Administration</td><td>D29</td><td>120 mg/dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>4th Administration</td><td>D60</td><td>120 mg/dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>5th Administration</td><td>D120</td><td>150/180 (no more than 180) mg/dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>6th Administration</td><td>D180</td><td>Optimal dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>7th Administration</td><td>D240</td><td>Optimal dose</td><td>Intrathecal injection</td><td>6</td></tr>
</table>
Dose escalation criteria: If no AEs or SAEs occur within 14 days
</td></tr>
</table>

	after the first administration, the subject proceed with a dose escalation of 30 mg every 14 days during the observation period. It is planned to achieve DLT through 3 to 4 dose escalations, followed by continuous treatment with the optimal dose for a 6-month treatment period. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD.
Study Outcomes	**Primary outcomes** 1) Incidence of AEs and SAEs within 180 days after the first injection RAG-17. 2) Changes in laboratory assessments, vital signs, ALSFRS-R scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection RAG-17. **Second outcomes：** 1) Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2) Changes in plasma NFL levels from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3) Changes in the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 4) Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

	Exploratory outcomes： 1) Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180. 2) Changes in fatigue severity (Fatigue Severity Scale, FSS) at on day 15, day 29, day 90, and day 180. 3) Changes in muscle strength on day 15, day 29, day 90, and day 180. 4) Changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180. 5) Changes in 7T MRI imaging features on day 29, day 90, and day 180. 6) Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180. 7) Changes in modified Norris scale scores on day 15, day 29, day 90, and day 180. 8) Changes in quality of life (including ALSAQ-40、EQ-5D-5L、HAMA and HAMD) on day 15, day 29, day 90, and day 180. 9) Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180 .
Statistical Analysis	• Statistical analysis will be performed with SAS software. • Demographic, medical, histories and baseline characteristics information will be listed and summarized. • The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after

	treatment. Safety evaluation will be analyzed using safety data set. The number of people of events should be recorded in detail and showing the event rate in summary statement. The event rate will be calculated as the number patients experiencing the event / the number of patients enrolled. • Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 180-day follow-up assessments for all participants, the data were analyzed.
Study Duration	2 years

1. Study Background

Amyotrophic lateral sclerosis (ALS) is a chronic, progressive neurodegenerative disease characterized by prominent damage to both upper and lower motor neurons, manifesting in symptoms such as muscle weakness, muscle atrophy, bulbar paralysis, and pyramidal signs. ALS is a rare disease, with a global standardized annual incidence rate of only 1.68 cases per 100,000 person-years[1]. The exact incidence rate of ALS in China still not well-established, but it is estimated that there are at least 100,000 patients [2]. The peak age of onset for ALS is around 50 years, with a growing trend toward earlier onset ages[3]. ALS is incurable, with a typical survival period of 3-5 years, and only approximately 10% of patients survive for more than 10 years[4]. As a result, it is listed by the World Health Organization as one of the "five major incurable diseases in the world" alongside cancer, acquired immunodeficiency syndrome, leukemia and rheumatoid arthritis.

Currently, the pathogenesis of ALS remains unclear, and available treatments are limited, primarily focusing on symptomatic relief rather than a cure. Since 1980, more than 80 randomized controlled trials (RCTs) have been conducted globally. Only three drugs for the treatment of ALS have been approved by the U.S. Food and Drug Administration (FDA): the neuroprotective drug riluzole, which inhibits excessive neurotransmission; the antioxidant edaravone; and Relyvrio (sodium phenylbutyrate and taurursodiol)[5-7]. Drug interventions for ALS include anti-apoptosis, anti-inflammation, anti-excitotoxicity, anti-oxidation, anti-aggregation, neurotrophic growth factors and neuroprotection, among others. In 2017, riluzole, the primary drug for ALS, became the first FDA-approved drug treatment for the disease. However, it cannot halt the progression of ALS, let alone reverse it, and only extends patient survival by 2 to 3 months. The riluzole costs about 1,800~4,000 RMB per month. However, it is only covered by medical insurance in certain provinces and cities. Edaravone is indicated for cerebral infarction and was approved for the treatment of ALS in the United States in 2019. It was approved for use in China in September 2020, specifically for patients with acceptable respiratory function in the early stage (within 2 years) of the disease. In September 2022, the FDA approved Relyvrio, a

combination drug composed of sodium phenylbutrate and taurursodiol, for the treatment of adult patients with ALS. The approval process for Relyvrio drug at the FDA has been marked by numerous challenges. At the first Peripheral and Central Nervous System Drug Advisory Committee (PCNSDAC) review meeting organized by the FDA in March 2022, the expert panel expressed concerns regarding issues such as the small scale of the Relyvri clinical trialo, data deficiencies, and the applicability of statistical methods, with a voting result of 6:4. In response to this, Amylyx reanalyzed the survival data of the CENTAUR trial of Relyvrio and submitted it, along with the biomarker data from another phase II clinical trial for Alzheimer's disease to the FDA for re-evaluation. At the second PCNSDAC meeting in September, the new drug application of Relyvrio was approved with a voting result of 7:2, and it was subsequently approved by the FDA. Currently, this drug has not been approved in China. As a result, existing therapies for ALS remain limited, and the demand for effective ALS treatments remains unmet.

Currently, the pathogenesis of ALS remains unclear, and available treatment are limited, mainly focusing on symptomatic relief rather than a cure. ALS can be divided into sporadic ALS (sALS) and familial ALS (fALS), with sALS accounting for the majority of cases (approximately 90%) and fALS accounting for 5-10%[8]. fALS can result from mutations in multiple genes, with the most common being a mutation in the *superoxide dismutase 1* (*SOD1*), which occurs in 2% to 7% of cases[9]. Approximately 20% of fALS and 5% of sALS cases are associated with the *SOD1* gene. Asia has the highest number of ALS, with Asian patients typically exhibiting a younger age of onset, a lower proportion of bulbar-onset ALS, and more pronounced cognitive dysfunction[10]. In Asia, fALS is mainly caused by *SOD1* gene mutations, whereas in Europe, mutations in the *C9ORF72* gene are more common. The frequency of the *SOD1* gene mutations is highest among ALS patient in central and southern China[11]. SOD1 is a potent antioxidant enzyme that, when combined with copper and zinc ions, helps convert superoxide into oxygen molecules and hydrogen peroxide, thereby protecting cells from damage[12-14]. Mutations in the *SOD1* gene in ALS patients lead to misfolding and accumulation of the SOD1 protein within

spinal motor neurons. While several mutations in SOD1 have been identified, not all of them result in ALS. Harmful mutations alter the functional activity of SOD1, with loss-of-function mutations impairing degradation of reactive oxygen species (ROS), which damages motor neurons, and gain-of-function mutations increasing oxidative activity, resulting in excessive production of hydrogen peroxide and enhancing protein-protein interactions[15-17]. These effects disrupt SOD1 protein level and activity, inhibit axonal transport, and cause mitochondrial dysfunction[15], ultimately leading to neuronal degeneration and death. Therefore, reducing the level of toxic SOD1 protein may offer an effective therapeutic approach for ALS caused by *SOD1* mutations. Researches have indicated that knockdown or knockout of normal SOD1 expression does not result in significant side effects[18-19].

Currently, several drugs under investigation have entered clinical studies, including small molecule drugs, antibody drugs, antisense oligonucleotides (ASO) drugs and cell therapy. Among these, except for small nucleic acid drugs (antisense oligonucleotides, small interfering RNA) which directly target pathogenic genes, the others are non-disease-modifying therapies. Tofersen, an antisense oligonucleotide drug developed by Biogen for the treatment of *SOD1*-ALS, binds to the mRNA encoding SOD1 and is degraded by ribonuclease H (RNase-H), thereby reducing the production of mutant SOD1 protein[20]. Tofersen is currently undergoing a New Drug Application (NDA) review by the U.S. FDA. The NDA submission for Tofersen is based on data from a Phase 1 study in healthy volunteers, a dose-escalation Phase 1/2 clinical study, a Phase 3 VALOR clinical study, and an open-label extension (OLE) study. While the Phase III of Tofersen for the treatment of did not meet the primary endpoint, Biogen has observed indications of reduced disease progression in several secondary and exploratory endpoints. Biogen's integration of 12-month data shows that earlier use of Tofersen, compared to delayed initiation, significantly reduces the neurofilament levels and slows the decline of various efficacy endpoints. In terms of adverse events, the most common side effects in patients were headache, falls, back pain and limb pain. About 6.7% of patients had serious neurological-related events, including myelitis, radiculitis, aseptic meningitis and optic disc edema[21-22].

RNA interference (RNAi) is a biopharmaceutical technology that utilizes double-stranded RNA molecules, known as small interfering RNA (siRNA), to target and silence specific genes at the mRNA level. Based on this mechanism, five drugs have been approved by the U.S. FDA, with nearly a hundred others currently in clinical development. RAG-17 is a 20 kDa siRNA targeting the *SOD1* gene. Through the RNAi mechanism, RAG-17 binds to *SOD1* mRNA, leading to the degradation of the target mRNA by the RNA-induced silencing complex (RISC). By recognizing and mediating the degradation of the target mRNA, RAG-17 silences the *SOD1* gene and downregulates the expression of the SOD-1 protein. RAG-17 is administered via intrathecal injection and is intended for the treatment of ALS patients with *SOD1* mutations. Compared to single-stranded self-delivering ASO drugs, siRNA offers a cascade amplification effect on the target gene and provides a higher gene silencing efficiency. This drug leverages a unique delivery technology for small nucleic acid drugs, enabling efficient siRNA delivery to the central nervous system and offering the potential for treatment of neurological diseases. The schematic diagram of the mechanism of RAG-17 in the treatment of ALS is shown below.

The *in vitro* pharmacodynamic results indicate that RAG-17 shows a concentration-dependent knockdown of *hSOD1* mRNA, with a half-maximal inhibitory concentration (IC50) of 0.011 nM. Compared to the control drug Tofersen, the IC50 of RAG-17 is 100 times lower, and the maximum inhibition rate reaches more than 90%. Cell apoptosis assays show that RAG-17 has no toxic response of cell apoptosis. Off-target effect analysis results show that RAG-17 dose not significantly affect the mRNA expression levels of non-target genes, demonstrating its specificity for *SOD1* gene knockdown.

The h*SOD1*^{G93A} transgenic mice exhibit progressive motor neuron degeneration, which closely resembles human ALS and is a widely recognized animal model for studying ALS. It has been extensively used in ALS pathogenesis research and drug screening [23-25]. To evaluated the inhibitory activity of RAG-17 on the target gene *in vivo*, a single-dose proof of concept (POC) experiment was conducted using the h*SOD1*^{G93A} ALS disease model mice. At 60 days of age, 200 μg of the siRNA drug

RAG-17 (~10 nmol) and the control drug Tofersen (28 nmol) were injected into the lateral ventricle. Tissue RNA was extracted 14 days post-administration, and RT-PCR was performed to assess the target gene, human *SOD1*. The results indicated that the mRNA expression of *SOD1* in both brain and spinal cord tissues was reduced by approximately 80%, with RAG-17 demonstrating superior gene down-regulation compared to the control drug.

Subsequently, a multi-dose pharmacodynamics (PD) experiment was conducted. Doses of 50 μg, 100 μg, 200 μg, and 400 μg of the siRNA drug RAG-17 were injected into the lateral ventricle. The PD indicators (relative expression levels of human *SOD1* mRNA) in the target tissues were assessed at 14 days and 56 days post-injection to determine the pharmacodynamic activity, dose response and the duration of effect at the respective doses. The results showed that gene knockdown was dose-dependent in three different regions of the brain (prefrontal cortex, cerebellum, and other brain tissues) and the spinal cord. Notably, only the high-dose group (400 μg) maintained 50% gene knockdown (KD) activity 56 days after injection, although this activity was weaker compared to 14 days post-injection. Compared to small molecule drugs, the significant advantage of small nucleic acid drugs is the extended duration of their therapeutic effect. Further testing at an extended time point (8 weeks) revealed that RAG-17 sustained the pharmacological activity with gene knockdown for at least 8 weeks in the model mice, providing valuable data to support the potential frequency of clinical administration.

To further verify the correlation between drug exposure in tissues and drug efficacy, the stem-loop PCR method was applied to detect the siRNA concentration in tissues at 14 days and 56 days post-administration. The results demonstrated a clear correlation between the drug concentration in tissues and PD effects.

To further explore the pharmacodynamics of RAG-17, human *SOD1*^{G93A} transgenic ALS disease model mice were used. Both male and female ALS disease model mice were administered intrathecally twice on the 68th and 100th days of life, with doses of the RAG-17 (RD-12500) at 75 μg, 150 μg, and 300 μg. Two negative control groups were included: a vehicle group and an inactvice (scrambled siRNA) group. The

scrambled siRNA served as a control for the target siRNA, being non-functional, and used to assess the potential inhibitory effects of the target siRNA. Additionally, 150 ug of Tofersen (RD-10748) was included as the positive control group. This study conducted pharmacodynamic evaluations based on multiple indicators, including animal body weight, motor function (grip strength in the fatigue rotating rod test, rotarod test, and open field test), disease onset time, lifespan, and neurological function score (neuroscore).

The results of animal weight changes indicated that both the vehicle group and the inactive group began to show a significant decrease in body weight around 130 days, with a more pronounced decrease in male mice. This suggests that the onset of h*SOD1*^{G93A} ALS disease model occurs earlier in males than in females. Among the groups, the positive group (RD-10748) showed improvements in body weight, with more significant weight maintenance in male mice. The body weight data from the drug treatment groups demonstrated a dose-dependent alleviation of weight loss, especially in female mice. As the disease progresses in ALS mice, their body weight gradually decreases. Compared to the vehicle control group, all dose groups of RD-12500 (RAG-17) and ASO (RD-10748) demonstrated a delayed onset of disease.

After two intrathecal administrations in the ALS disease model mice, all treatment groups of both male and female mice showed an extension in the median survival period. The low-dose group of of 75 μg RAG-17 and the medium-dose of 150 μg ASO (RD-10748) demonstrated comparable efficacy. The survival periods for male mice were 207 days (75 μg RAG-17) and 191 days (150 μg ASO), respectively. The survival periods of the female group were 198.5 days (75 μg RAG-17) and 189 days (150 μg ASO), respectively. In the female high-dose group (300 μg), the survival period was significantly extended to 227 days, representing a 54-day increase compared to the vehicle control group (173 days). However, the high-dose group (300 μg) in the male mice did not show a marked superiority. Female animals exhibited greater sensitive to drug treatment, and the results aligned with the trend of weight change.

The typical pathological feature of h*SOD1*^{G93A} ALS disease model mice is the

progressive loss of motor function. The Rotarod test for motor function fatigue and the Grip strength test were used to evaluate the therapeutic effect of the drug on the ALS model. The results showed that, in the Rotarod test, the drug-treated group demonstrated a significant increase in the time ALS mice were able to maintain their position on the accelerating rotating rod at 0-30 rpm, compared to both the vehicle and the inactive control groups. The Grip strength test revealed that the drug treatment group significantly improved the hindlimb muscle function of the disease model mice. Additionally, the open field test, which evaluates autonomous behavior, exploratory behavior and anxiety in a novel environment, showed that the drug-treatment group exhibited improved autonomous motor ability, with a dose-dependence effect.

Before performing the Rotarod and hindlimb grip strength behavioral tests, the neurological function of ALS mice was scored on a scale of 0 to 4 points. This scoring system objectively evaluates the common hindlimb dysfunction in h*SOD1*^{G93A} transgenic mice. The results showed that mice in the vehicle (aCSF) and inactive control groups exhibited abnormal tilting (NS2 symptoms) at PND130 days, with the severity progressively worsening until hindlimb paralysis (i.e., ≥ NS3). In contrast, at all time points during the study, the average score of all RAG-17 treatment doses never exceeded NS2. These results indicated that RAG-17 treatment, regardless of the dose, improved motor function in the animals compared with the control group.

In conclusion, RAG-17 treatment can effectively inhibit the gene expression of h*SOD1* in h*SOD1*^{G93A} transgenic mice, significantly delay the disease onset, extend the survival time of affected mice, enhance their grip strength , and improve the motor function.

Currently, the non-clinical safety evaluation of RAG-17 is ongoing. The safety dose exploration experiments have been completed in both rats (following two intrathecal administrations, a non-relevant species) and cynomolgus monkeys (a relevant species). In the rat safety dose exploration experiment, the main findings included transient neurological functions such as tremors, abnormal gait, and hindlimb weakness, in some animals of the medium-high dose group (2.5mg/dose) and high dose group (4.5mg/dose) after intrathecal drug injection. In the safety dose exploration

experiment with cynomolgus monkeys, one male monkey (3001) died after administration of the high dose of 200mg (equivalent to 2g in human dose), while another female monkey (3501) showed severe toxic reactions but gradually recovered over days 17-18. The two animals in the low-dose group of 30mg showed no abnormalities after the first administration, but tremors occurred after the second administration. As a result, animals in the vehicle group were used as alternatives and received the medium-dose experiment of 50mg. The 50mg dose group (equivalent to 500mg in human dose) showed transient tremors, body stiffness, and reduced activity after administration, but these symptoms resolved within approximately 2 to 4 hours.

In terms of histopathology, axonal degeneration of the sciatic nerve, unrelated to the dose, was observed in both species. In the cynomolgus monkey study (H59-0025-TX), mild to mild multifocal axonal degeneration was observed in both bilateral and unilateral sciatic nerves of the male in the 50 mg/dose and 30 mg/dose groups. Additionally, mild to mild multifocal axonal degeneration was observed in the female monkeys across all dose groups. This alteration is characterized by a "bubbly" appearance of multiple affected fibers, axonal rupture (aggregating into eosinophilic masses), and some cellular debris surrounding the vacuoles. Although there was no control group in the cynomolgus monkey study, the changes observed in the sciatic nerve were still considered related to the test drug, consistent with the findings from the rat study. In each dose group, mild multifocal axonal degeneration was observed at the injection site in the female monkeys. The characteristics of this alteration are similar to those observed in the sciatic nerve and may be due to the administration procedure. In addition, mild to mild diffuse vacuolation of macrophages in the medulla of mesenteric lymph nodes was observed in all dose groups. Since these adverse reactions were not dose-dependent, their correlation with the test drug cannot be determined at this time.

Based on the results of the safety dose exploration experiments, formal GLP toxicology experiments are currently underway. These formal GLP toxicology tests have reduced the administration doses for rats and cynomolgus monkeys, as well as optimized the intrathecal injection procedure. The preliminary safety dose exploration

experiment results of rats and cynomolgus monkeys can also provide insight into the potential clinical risks of this test drug.

This project conducts a single-center, dose-escalation clinical study, aiming to evaluate the safety, tolerance and pharmacokinetics of RAG-17 in adult ALS patients carrying the *SOD1* mutation, in preparation for subsequent efficacy clinical trial.

2. Study Purposes

This study primarily aims to evaluate the safety, tolerance and pharmacokinetics of RAG-17 in adult ALS patients with *SOD1* gene mutation.

2.1 Primary Purposes：

(1) To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17.

(2) To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

2.2 Second Purposes：

(1) To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(2) To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(3) To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(4) To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

2.3 Exploratory Purposes：

(1) To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180.

(2) To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180.

(3) To evaluate the changes in muscle strength on day 15, day 29, day 90, and day 180.

(4) To evaluate the changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180.

(5) To evaluate the changes in 7T MRI imaging features on day 29, day 90, and day 180.

(6) To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180.

(7) To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, and day 180.

(8) To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, and day 180.

(9) To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.

3. Study Design Type, Principles and Experimental Procedures

3.1 Study Design

The study is an ongoing open-label, single-center, first-in-human, and dose escalation clinical trial.

Adult patients with a confirmed or probable familial or sporadic of ALS with confirmed *SOD1* gene mutations (forced vital capacity ≥ 80% of predicted value) will be included according to the revised El Escorial criteria.

1) **Administration：** A total of 7 doses will be administered on Day 1, Day 15, Day 29, Day 60, Day 120, Day 180, and Day 240. The drug will be administered via

intrathecal injection of RAG-17, with doses of 60 mg per administration, 90 mg per administration, 120 mg per administration, 120 mg per administration, 150/180 mg (no more than 180 mg) per administration, with the last two doses being the optimal dose. The treatment period of this study is 8 months. Various indicators, adverse events, and concomitant medications will be collected on Day 1, Day 15, Day 29, Day 60, Day 90, and Day 180 post-treatment. The study is expected to be conducted at a single center, Beijing Tiantan Hospital, with the recruitment of 6 participants, aiming to complete the study within 2 years.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	120 mg/dose	Intrathecal injection	6
5th Administration	D120	150/180 (no more than 180) mg/dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

2) **Dose Escalation Criteria:** If no AEs or SAEs occur within 14 days after the first administration, the subject proceed with a dose escalation of 30 mg every 14 days during the observation period. It is planned to achieve DLT through 3 to 4 dose escalations, followed by continuous treatment with the optimal dose for a 6-month treatment period. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD.

3) **Dose Escalation Stopping Criteria：** 'Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0' will be used as the toxicity evaluation standard for this protocol.

If a dose meets any of the following criteria, dose escalation will be considered for stopping or the trial will be continued at an additional lower dose level, unless it can be clearly confirmed that the adverse event is unrelated to the drug (e.g., skin abrasions, hospitalization or death caused from a car accident).

① At the same dose level (within the same administration group), if ≥ 1 subject experiences an AE of grade ≥ 2;

② Other situations that may increase the risk of subjects, which will be discussed by the investigator to decide whether to terminate dose escalation.

4) **Individual Drug Withdrawal Criteria:** A subject will no longer participate in the subsequent administration, if they meet any of the following criteria:

① During the trial, the subject experiences a SAE or an AE of grade ≥ 3 (including laboratory abnormalities) , regardless of whether it is related to the drug;

② The subject experiences an event that, in the investigator's assessment, may significantly increase the risk to the subject.

3.2 Sample Size

This is an exploratory, hypothesis-generating study and a dose escalation clinical study to explore the maximum tolerated dose, so a total of 6 cases are required for sample size estimation.

3.3 Study Period

Work Progress	Timeline
Study Design, Study Approval Review, Ethical Review, Contract Signing, Human Genetic Resources Approval, Study Registration	2022.12-2023.05
Project Official Launch, Subject Enrollment and Follow-up	2023.06-2024.06
Data Cleaning and Analysis, Manuscript Writing	2024.06-2024.12
Total Duration	2 Years

3.4 Subject Selection

During the entire study period, it is planned to enroll 6 subjects. Ethical approval must be obtained before patient recruitment. The ethics committee will have access to all study documents and training materials.

- **Inclusion Criteria**

(1) Patients permitted by medical personnel who could complete the clinical trial.

(2) Age 18–75 years, gender unlimited.

(3) Clearly identify ALS patients with SOD1 gene mutations (known *SOD1* mutation sites and reported disease progression).

(4) Forced vital capacity during screening period ≥ 50% of expected lung capacity.

(5) Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology.

(6) The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form.

(7) The subjects (including male subjects) have no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum.

- **Exclusion Criteria**

(1) The *SOD1* mutation site occurs in patients with nucleotides 44-66 (calculated from the translation of SOD1 protein) and P.F21C mutations.

(2) Patients who have previously received or are currently receiving Tofersen treatment.
(3) HIV test positive or positive test history.
(4) Hepatitis C virus antibody positive or positive test history.
(5) Active hepatitis B infection (hepatitis B surface antigen positive and/or hepatitis B core antibody positive).
(6) Other experimental drugs used within one month or within five drug half-lives.
(7) Lumbar diseases and deformities.
(8) Suffering from other diseases known to be related to motor neuron dysfunction, which may confuse or blur the diagnosis of ALS.
(9) Other psychiatric disorders diagnosed according to DSM-V diagnostic criteria, or obvious suicidal intentions.
(10) Combined with severe liver dysfunction, renal dysfunction, or severe heart dysfunction (severe liver dysfunction refers to ALT value ≥ 2.0 times the upper limit of normal value or AST value ≥ 2.0 times the upper limit of normal value; severe renal dysfunction refers to CRE ≥ 1.5 times the upper limit of normal value or eGFR < 40mL/min/1.73m^2; severe heart dysfunction refers to NYHA 3-4 levels).
(11) Permanent and sustained dependence on ventilator assisted ventilation.
(12) History of combined alcohol and drug abuse.
(13) Pregnant, lactating, or potentially pregnant patients, as well as those planning to conceive.
(14) Patients participating in other clinical trials or applying other biologics, drugs, or devices under study.
(15) Patients who have received any vaccination within 28 days.
(16) Contraindications for MRI examination (ie. claustrophobia).
(17) Those who can't complete follow-up due to other reasons.

4. Study Method

(1) Drug Dosage Form

The aseptic aqueous solution of the RAG-17 preparation (sodium salt) used in the

study is stored at -20℃±5℃, with a specification of 75 mg (2.5 mL).

(2) Intervention Measures

1) Basic Treatment

All enrolled subjects will maintain basic medication treatment, and the dosage and type of medications will remain unchanged during the trial period.

Subjects who have already received riluzole treatment before enrollment may continue using it during the study period, maintaining a stable dosage (50mg per dose, twice daily). Throughout the study, discontinuation of riluzole for reasons other than medical necessity, as determined by the investigator, is not recommended.

The concomitant medications situation of the patients should be recorded in detail in the CRF form, including the name of the medication, the dosage, and frequency. If during the study, a change in the existing medication is deemed necessary by the investigator due to the patient's condition, it will be considered a protocol deviation. An adverse event record report and a concomitant medication form should be completed. The investigator will then assess whether the subject can continue participating in the study. If not, the subject will be treated as a withdrawn case.

2) **RAG-17 Treatment**

The administration begins with an initial intrathecal injection dose of 60 mg. Within the first 14 days following the initial dose, if no AEs or SAEs are observed, the dose may be escalated by 30mg at 14-day intervals. After reaching the maximum tolerated dose (MTD), an optimal dose between the safe dose and the MTD will be selected as the dose for continued treatment. Continuous treatment will be administered every 2 months, with a total treatment duration of 8 months.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	120 mg/dose	Intrathecal injection	6

5th Administration	D120	150/180 (no more than 180) mg/dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

5. Concomitant Medications

(1) Prohibited Concomitant Medications

Subjects must contact their investigators before taking any new medications, including over-the-counter drugs and herbal preparations.

• The use of any antiplatelet or anticoagulant drugs (e.g. aspirin, clopidogrel) is prohibited within 7 days prior to each lumbar puncture and until 48 hours after the procedure.

• The use of DPS, copper (II) (diacetylbis (N4-methylaminothiourea)), or pyrimethamine is prohibited during the study.

• The use of gamma globulin is not recommended during the study.

(2) Permitted Concomitant Medications

If subjects are taking concomitant vitamins, minerals and supplements, they should avoid exceeding the recommended daily dose within 14 days prior to the first administration of the RAG-17 and during the study period (e.g. no more than 5 grams of creatine per day and no more than 1000 IU of vitamin E per day). These daily limits include the total dose obtained from the combined use of daily multivitamins and supplements.

Medications not listed in the prohibited concomitant medications section above are considered permitted concomitant medications. Concomitant medications for symptom management during the study are determined by the investigator. All enrolled cases must maintain basic medication treatment, with no changes to the dosage or type of medications during the trial. If subjects have questions regarding permitted concomitant treatments, they should seek medical guidance from the investigator. The concomitant medications of the patients must be documented in

detail in the CRF form, including the name of the medication, the dosage, and frequency. If a change or adjustment to the existing medications become necessary due to the subject's condition during the study, and the investigator deems it essential, this will be considered a protocol deviation. The adverse event record form and the concomitant medication form should be promptly completed. The investigator will assess whether the subject can continue participating in the study. If continuation is not feasible, the case will be treated as an excluded case. In special circumstances (e.g. the subjects suffers from other diseases during the study), when additional treatments are necessary, these must be explained in the CRF form, including the disease diagnosis, medications, dosage, and usage details.

6. Observation Indicators and Examination Timeline

6.1 General Demographic Indicators and Clinical Data

(1) Collection time point: baseline

(2) Collection content: Basic demographic characteristics include gender, age, ethnicity, height, weight, education level, marital status; vital signs include body temperature, pulse, respiration rate, blood pressure; 12-lead electrocardiogram (ECG); waist circumference, physical examination; clinical data include relevant medical history, past medical history, medication used prior to enrollment, smoking and drinking history, and family medical history.

6.2 Daily Life Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo assessments using the ALSFRS-R and Modified Norris Scale before and after treatment. The results after treatment intervention will be compared with baseline data to evaluate the effect of RAG-17 on improving daily living function of the subjects.

(3) Observation indicators:

1) ALSFRS-R: This is a rapidly administered ordinal rating scale (grade 0-4) used to assess the subjects' ability and independence in 12 functional activities. ALSFRS-R measures 4 functional domains, including respiratory, bulbar, gross motor, and fine motor functions. The scale includes 12 items, each scored from 0 to 4, with a

total score of 48. Higher scores indicate better function, while lower scores reflect more severe neurological impairment.

2) Modified Norris Scale: This assessment tool for ALS consists of two parts: the Limb Norris Scale (comprises 21 items to evaluate limb function) and the Norris Bulbar Scale (includes 13 items to assess bulbar function). Lower scores indicate more severe neurological impairment.

6.3 Respiratory Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo forced vital capacity (FVC) testing before and after treatment. The results post-treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving the respiratory function.

(3) Contents of observation indicators:

FVC: The subjects are measured in an upright position. Measured as the maximum volume of air that can be forcefully exhaled as quickly as possible after a maximal inhalation.

6.4 Fatigue Severity Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements with the Fatigue Severity Scale (FSS) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving the severity of fatigue in subjects.

(3) Observation indicators:

Fatigue severity: The FSS consists of 9 items, designed to measure the degree of fatigue and its impact on a person's activities and lifestyle across various diseases. The evaluation covers areas such as daily activity ability, participation, and sleep. Each question is scored on a 7-point scale: 1 = strongly disagree and 7 = strongly agree. The minimum possible score = 9 and the maximum possible score = 63. Higher scores indicate the greater severity of fatigue.

6.5 Muscle Strength Test

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurement using the Medical Research Council (MRC) Scale (sternocleidomastoid muscle, deltoid muscle, biceps brachii, triceps brachii, common digital extensor muscle, abductor pollicis brevis muscle, abductor digiti minimi muscle, first dorsal interosseous muscle, and iliopsoas muscle, quadriceps femoris, biceps femoris, tibialis anterior, gastrocnemius muscle, flexor muscle, extensor muscle) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving muscle strength.

(3) Observation indicators:

MRC scale: The MRC muscle strength grading method, is an enhanced version of the Lovett grading method, further subdivided by the range of motion and the level of resistance applied. If the measured muscle strength is slightly stronger than a certain level, a "+" is added to the upper right corner of this level. If it is slightly weaker, a "-" is added to the upper right corner. The refinement addresses the limitation of Lovett grading method, providing a more precise assessment of muscle strength.

In this study, the MRC grading is used to measure the muscle strength of the abductor pollicis brevis muscle, abductor digiti minimi muscle and tibialis anterior muscle.

6.6 Electromyography Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements of compound muscle action potential (CMAP), motor unit number index (MUNIX), and motor unit size index (MUSIX) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the electromyographic parameters of the subjects.

(3) Observation indicators:

CMAP: CAMP represents the summated action potentials of muscle fibers that are almost simultaneously activated when a certain nerve is directly or indirectly

stimulated. The amplitude from baseline to peak, duration, latency of the negative phase of the potential, as well as the details of the stimulation method and recording method should be recorded.

MUNIX: A motor unit is a functional unit located in the anterior horn of the spinal cord, consisting of an alpha motor neuron and all the muscle fibers it innervates. It is the basic unit of motor function, with the minimum unit of normal muscle contraction being the contraction of one motor unit. MUNIX is a sensitive biomarker for monitoring the nerve innervation status of patients with neurodegenerative diseases and is a highly sensitive and effective marker for monitoring the loss of motor units in ALS. The method uses CMAP and the surface electromyographic interference pattern (SIP) to evaluate the number and size of motor units (MUs).

MUSIX: The size of a motor unit is determined by the number of nerve endings and branches of the neuron axon. Generally, larger muscles have larger motor unit. Each muscle contains a certain number of motor units. The calculation method is to divide the amplitude of the compound muscle action potential (CMAP) by the MUNIX value.

6.7 Imaging Indicators

(1) Collection time points: baseline, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurement with 7T MRI imaging before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the imaging indicators of the subjects.

(3) Observation indicators:

7T MRI imaging indicators: 7T ultra-high field magnetic resonance is an advanced magnetic resonance imaging system currently available for human body scanning. Compared with the traditional 1.5T and 3T magnetic resonances, 7T magnetic resonance can provide a doubled signal-to-noise ratio, finer spatial resolution, and more excellent tissue contrast. It is more suitable for observing the fine structure of tissues and physiological changes in functional metabolism and other information, thereby revealing physiological and pathological mechanisms that are

difficult to be discovered by traditional platforms.

6.8 Gait Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo the following assessments: three-dimensional gait analysis under multitasks, Short Physical Performance Battery (SPPB), item 1 (Tandem Walking) of the Scale for the Assessment and Rating of Ataxia (SARA), and 6-meter walking test before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the gait function of the subjects.

(3) Observation indicators: Semi-quantitative scales include the Short Physical Performance Battery (SPPB) and item 1 (Tandem Walking) of the Scale for the Assessment and Rating of Ataxia (SARA); Quantitative gait assessment includes 6-meter walking test.

SPPB: The SPPB is a widely used method for evaluating muscle function. It consists of 3 items: balance test, 4-meter timed walking test, and timed sit-to-stand test. Each individual test can score a maximum of 4 points, giving a total score of 12 points. To improve the accuracy of the test, each test is usually repeated 2 to 3 times, with the shortest time recorded for scoring.

6-meter normal walking test: It is conducted on an 8-meter walkway with a timing taken over a 6-meter distance. Landmark markers are placed at the start of the walkway, at the 1-meter, the 7-meter, and the 8-meter points, for a total of 4 markers. Timing starts at the 1-meter and stops at the 7-meter mark to avoid the influence of markers on the subject's behavior, such as intentional changes in walking pace or nervousness. The test is performed 4 times; the first trial serves as a practice and is not recorded. The times from the subsequent 3 trials are recorded, and the average of these times is used for analysis.

Item 1: SARA is a clinical scale used for the semi-quantitative assessment of cerebellar ataxia severity. It can also serve as a rehabilitation indicator for gait and independence in daily activities for ataxic stroke patients. In this study, the gait part (Item 1) is selected. It consists of 8 levels corresponding to scores ranging from 1 to 8.

Grade 1 is a mild abnormality corresponding to 1 point, up to grade 8 is unable to walk even with assistance corresponding to 8 points.

6.9 Quality of Life Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo measurements with the Amyotrophic Lateral Sclerosis Self-Assessment Questionnaire (ALSAQ-40), EQ-5D-5L scale, and HAMA and HAMD-17 scales before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the quality of life of the subjects.

(3) Observation indicators:

ALSAQ-40: ALSAQ-40 is a commonly used quality-of-life scale for ALS. The sections on social interaction and eating ability provide subjective insights into articulation and swallowing functions. ALSAQ-40 consists of 40 items involving the patient's condition, requiring a 5-point scale rating. The questions cover five health status dimensions affected by the disease: physical activity (including 10 items), daily living activities and independence (10 items), eating (3 items), communication (7 items), and emotional function (10 items). Each dimension is scored on a scale from 0 to 100: 0 represents the possible best health status and 100 represents the possible worst health status.

EQ-5D-5L: EQ-5D-5L is a standardized scale for measuring health status, that is an updated version of the EQ-5D scale. The scale consists of two parts: the EQ-5D-5L descriptive system and the visual analogue scale (EQ-VAS). The EQ-5D-5L descriptive system describes five dimensions: Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression. Each dimension contains five levels: 1=No difficulty, 2=Slight difficulty, 3=Moderate difficulty, 4=Severe difficulty, 5=Unable to perform/very severe difficulty. The responses of the five dimensions can generate a five-digit code to describe the health status of the respondent. The health status 1,1,1,1,1 indicates no problems in all five dimensions. EQ-VAS records the self-rated health status of the respondent on a vertical visual analogue scale, with the ends of the scale marked as "The best health status you can imagine" and "The worst health status

you can imagine".

6.10 Clinical Staging Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180

(2) Collection content: All subjects will undergo assessment with the London ALS staging and the Milan-Turin staging (ALS-MITOS) before and after treatment. The results after treatment intervention will be compared with the baseline data to assess the disease progression of ALS patients and evaluate the effect of RAG-17 on the condition of the subjects.

(3) Observation indicators:

London ALS staging: According to the most recently affected region in the course of the disease, ALS is divided into 5 stages: Stage 1 = symptom onset (involvement of the first area); Stage 2A = confirmed diagnosis, and Stage 2B = involvement of the second area; Stage 3 = involvement of the third area; Stage 4A = the patient requires percutaneous endoscopic gastrostomy (PEG), and Stage 4B = the patient requires non-invasive ventilation; Stage 5 = death or mechanical ventilation. The average survival time and 5-year survival rate vary across stages.

Milan-Turin staging: The patient's functions are divided into 4 domains by assessing 4 key points (including mobility, swallowing, communication, and respiration). Each domain is scored as either 0 (below the threshold) or 1 (above the threshold). The total score across these domains determines the stage: Stage 0 = no functional loss; Stage 1 = loss of one functional domain; Stage 2 = loss of two functional domains; Stage 3 = loss of three functional domains; Stage 4 = loss of four functional domains; Stage 5 = death or mechanical ventilation.

6.11 Laboratory Examination Data Collection

(1) Collection time points: baseline, day 1, day 15, day 29, day 60, day 120, day 180, day 240

(2) Collection content: All subjects will undergo laboratory tests including blood routine, urine routine, liver function, kidney function, and coagulation tests before and after treatment respectively. The results after treatment intervention will be compared with the baseline data to evaluate the impact of RAG-17 on the general physical

condition of the subjects and assist in determining the occurrence of adverse events and other related events.

All subjects will undergo the detection of SOD1 protein in cerebrospinal fluid, plasma neurofilament light chain protein (NFL), and the pharmacokinetics of RAG-17 in plasma before and after treatment.

6.12 Other Information Collection

(1) Collection time points: baseline, day 1, day 15, day 29, day 60, day 120, day 180, day 240

(2) Collection content: During the study period, all the medications taken by the subjects will be recorded, including the reasons for use, drug names, dosages, and duration of use. All AEs, adverse reactions and their handling processes will also be documented; The timing of tracheostomy, invasive mechanical ventilation, death, and the duration of invasive mechanical ventilation will be recorded; Subjects' medication adherence, cases of withdrawal or exclusion from the study will be recorded.

7. Follow-up Time

Follow-up plan: For all subjects, face-to-face visits will be conducted at baseline, day 1, day 15, day 29, day 60, day 120, day 180, and day 240. They will undergo tests including daily living function, respiratory function, muscle strength, quality of life, gait function, and clinical staging, as well as the collection of adverse events and concomitant medications use. The study is expected to be conducted at Beijing Tiantan Hospital, with a target recruitment of 6 participants, and is planned to be completed within 2 years.

Follow-up content: To evaluate the differences in clinical manifestations of the participants after receiving the RAG-17 intervention at baseline, day 1, day 15, day 29, day 60, day 120, and day 180, including:

- Laboratory examinations: tests including blood routine, urine routine, liver function, kidney function, coagulation, SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma will be conducted at baseline, day 1, day 15, day 29, day 60, day 120, day 180, and day 240.
- Daily living functions: ALSFRS-R scoring and Modified Norris Scale scoring

will be conducted at baseline, day 15, day 29, day 90, and day 180.

- Respiratory function: FVC evaluations will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Fatigue severity: FSS assessment will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Muscle strength test: MRC assessment will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Electromyogram indicators (abductor pollicis brevis, abductor digiti minimi, and anterior tibial muscle): CMAP, MUNIX, and MUSIX will be collected at baseline, day 15, day 29, day 90, and day 180.
- Imaging examinations: 7T magnetic resonance imaging will be conducted at baseline, day 29, day 90, and day 180.
- Gait function: SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Quality of life: assessments of ALSAQ-40, EQ-5D-5L, HAMA, and HAMD-17 will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Clinical staging: London ALS staging and Milan-Turin staging assessments will be conducted at baseline, day 15, day 29, day 90, and day 180.
- Other indicators: information such as vital signs, abnormalities in heart/lung/abdomen examination, neurological examination, electrocardiogram, adverse events, concomitant medications, the time to tracheostomy, invasive mechanical ventilation, death, and the duration of invasive mechanical ventilation of the patients will be collected at baseline, day 1, day 15, day 29, day 60, day 90, day 120, day 180, and day 240.

8. Study Process

8.1 Information Collection and Informed Consent (Visit 1)

Patients who meet the inclusion criteria but do not meet the exclusion criteria will be introduced to the study protocol and purposes by investigator. The patient or their legal representatives will be provided with complete information about the study,

accompanied by corresponding written materials, and a written informed consent form will be signed.

- Collection of medical history: including current medical history, past medical history, history of concomitant medication, history of smoking and alcohol consumption, family history;
- Physical examination: including vital signs, heart/lung/abdomen examination, neurological examination, 12-lead electrocardiogram;
- Laboratory examinations: including blood routine, urine routine, liver function, kidney function, coagulation function, BNP; Blood sample collection for verification of *SOD1* gene mutation sites, plasma neurofilament light chain protein (NFL);
- 7T magnetic resonance imaging;
- Assessment of respiratory function (including FVC)
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Assessment of daily living functions: ALSFRS-R score, Modified Norris Scale score;
- Assessment of fatigue severity: FSS scale score;
- Assessment of muscle strength and electromyogram indicators: MRC assessment (sternocleidomastoid muscle, deltoid muscle, biceps brachii, triceps brachii, common digital extensor muscle, abductor pollicis brevis, abductor digiti minimi, first dorsal interosseous muscle and iliopsoas muscle, quadriceps femoris, biceps femoris, anterior tibial muscle, gastrocnemius muscle, flexor muscle, extensor muscle grading); CMAP, MUNIX, MUSIX;
- Assessment of gait function: SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking;
- Assessment of quality of life: ALSAQ-40 and EQ-5D-5L scale scores;
- Assessment of emotional scale: HAMA and HAMD-17 scores.

8.2 Days 1-4 Post-Enrollment (First Treatment) (Visit 2)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These

samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;

- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications:
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 15, face-to-face follow-up.

8.3 Days 15-18 Post-Enrollment (Second Treatment) (Visit 3)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);

- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- Arrange the follow-up appointment for day 29, face-to-face follow-up.

8.4 Days 29-32 Post-Enrollment (Third Treatment) (Visit 4)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;

- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 60, face-to-face follow-up.

8.5 Days 60-63 Post-Enrollment (Fourth Treatment) (Visit 5)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 90, face-to-face follow-up.

8.6 Days 90-93 Post-Enrollment (Visit 6)

- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;

- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 120, face-to-face follow-up.

8.7 Days 120-123 after enrollment (Fifth treatment) (Visit 7)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 180, face-to-face follow-up.

8.8 Days 180-183 Post-Enrollment (Sixth Treatment) (Visit 8)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological

specialist examination, and electrocardiogram examination;

- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 240, face-to-face follow-up.

8.9 Days 240-243 after enrollment (Seventh treatment) (Visit 9)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.

8.10 Study Flowchart

Examination / Treatment	Screening / Baseline	Treatment and Follow-up							
		1st Administration	2nd Administration	3rd Administration	4th Administration		5th Administration	6th Administration	6th Administration
		Day 1-4	Day 15-18	Day 29-32	Day 60-63	Day 90-93	Day 120-123	Day 180-183	Day 240-243
Verification of inclusion / exclusion criteria	√								
Sign the informed consent form	√								
Demographic data	√								
Medical history / Personal history / Family history	√								
Height / Weight / Waist circumference / Physical examination	√	√	√	√	√	√	√	√	√
Concomitant medication	√	√	√	√	√	√	√	√	√
Blood pressure / Respiration / Heart rate / Body temperature	√	√	√	√	√	√	√	√	√
Neurological examination	√	√	√	√	√	√	√	√	√
12-lead electrocardiogram	√	√	√	√	√	√	√	√	√
Laboratory examination[1]	√	√	√	√	√		√	√	√
ALSFRS-R score	√		√	√		√		√	
Modified Norris Scale	√		√	√		√		√	
Forced vital capacity	√		√	√		√		√	
Fatigue Severity Scale	√		√	√		√		√	
Muscle strength / Electromyogram	√		√	√		√		√	
7T MRI	√			√		√		√	
SPPB	√		√	√		√		√	
Tandem Walking	√		√	√		√		√	
6-meter walking	√		√	√		√		√	
Three-dimensional gait analysis under multitasking	√		√	√		√		√	
ALSAQ-40	√		√	√		√		√	

EQ-5D-5L	√		√	√		√		√	
Anxiety Scale (HAMA)	√		√	√		√		√	
Depression Scale (HAMD-17)	√		√	√		√		√	
London ALS staging	√		√	√		√		√	
Milan-Turin staging (AKS-MITOS)	√		√	√		√		√	
Intrathecal administration of RAG-17	√	√	√	√	√		√	√	√
Blood sample collection [2]	√	√	√	√	√		√	√	√
Cerebrospinal fluid sample collection [3]	√	√	√	√	√		√	√	√
Adverse events / Severe adverse events	√	√	√	√	√	√	√	√	√
Study drug adherence	√	√	√	√	√		√	√	√

[1] Laboratory examinations: blood routine, urine routine, liver functions, renal function, coagulation function, BNP (BNP is only examined at baseline). Laboratory tests during treatments 1-7: Conduct laboratory tests before each treatment and 48 hours after each treatment.
[2] Samples are collected at baseline; during the treatments 1 to 7 , samples are collected at 4 hours before each treatment, and 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, and 48 hours after each treatment.
[3] During treatments 1 to 7, samples are collected 15 minutes before each treatment (Collection before drug administration via lumbar puncture, not restricted to 15 minutes).

9. Clinical Evaluation

9.1 Primary Outcomes：

1) Incidence of AEs and SAEs within 180 days after the first injection of RAG-17.
2) Changes in laboratory assessments, vital signs, ALSFRS-R scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

9.2 Second Outcomes：

1) Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
2) Changes in plasma NFL levels from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
3) Changes in the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29,

day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

4) Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

9.3 Exploratory outcomes：

1) Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180.
2) Changes in fatigue severity (Fatigue Severity Scale, FSS) at on day 15, day 29, day 90, and day 180.
3) Changes in muscle strength on day 15, day 29, day 90, and day 180.
4) Changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, and day 180.
5) Changes in 7T MRI imaging features on day 29, day 90, and day 180.
6) Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180.
7) Changes in modified Norris scale scores on day 15, day 29, day 90, and day 180.
8) Changes in quality of life (including ALSAQ-40、EQ-5D-5L、HAMA and HAMD) on day 15, day 29, day 90, and day 180.
9) Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.

10. Known and Potential Risks and Benefits, and Risk Management Plan

10.1 Adverse Event Monitoring

All information related to adverse events, whether reported by the participants, identified by the investigators, or detected through physical examinations, laboratory tests, or other methods, should be recorded on the adverse event page of the case report form. These events will be managed and reported in accordance with the relevant regulations. A comprehensive adverse events report will include in the final trial report.

10.2 Adverse Event Definition

10.2.1 Adverse Event (AE)

An adverse event refers to any unfavorable medical occurrence or worsening of a pre-existing condition that arises in a participant during the use of the investigational drug, regardless of whether it has a causal relationship with the investigational drug treatment. Adverse medical occurrences can manifest as clinical symptoms/signs, diseases, or abnormal laboratory findings. AEs include any unfavorable medical occurrences that occur at any stage of the clinical study, including the lead-in or washout periods, even before the investigational drug has been administered to the participant. AEs include both serious and non-serious adverse events.

10.2.2 Serious Adverse Event

An adverse event during the study will be classified as serious, if it meets one or more of the following criteria:

- Results in death
- Immediately life-threatening

Note: Life-threatening refers to an event that poses an immediate risk to the patient's life at the time it occurs, not an event that could result in death if it were to worsen.

- Requires hospitalization or prolongs the current hospitalization
- Results in permanent or severe disability/incapacity, or loss of essential abilities required for normal living
- Leads to congenital anomaly, birth defects, or reproductive issues
- Constitutes a significant medical event that may endanger the subject or necessitate medical intervention to prevent the outcomes listed above.

10.3 Adverse Events Documentation

Non-serious AE: Non-serious AEs of interest occurring during the study period from the start until the end of the study (e.g. renal function impairment/increased serum creatinine, elevated liver function test indicators, upper gastrointestinal discomfort, muscle/bone pain, hypocalcemia, ocular inflammation, arrhythmia, fractures, etc.) and information about drug discontinuation due to SAEs must be

documented. Documentation of other non-serious AEs is at the discretion of the investigator .

Serious AE: All SAEs must be documented.

10.4 Assessment of the Relationship Between AEs and the Investigational Drug

The relationship between AEs and the investigational drug will be assessed using a 5-level standard: ①Definitely related; ②Highly likely related; ③Possibly related; ④Unlikely related; ⑤Definitely unrelated; ⑥Unable to determine.

①Definitely related: Matches the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or disappears after dose reduction or drug withdrawal. Reaction reappears upon re-administration.

②Highly likely related: Matches to the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or disappears after dose reduction or drug withdrawal. However, the clinical status of the participants or other reasons may also cause the reaction.

③Possibly related: Matches the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or is not obvious after dose reduction or drug withdrawal. However, the clinical status of the subject or other reasons can equally explain the reaction.

④Unlikely related: Does not match the known reaction type of the suspected drug. Dose not align with the reasonable time frame after administration. The clinical status of the subject or other reasons may also cause the reaction.

⑤Definitely unrelated: Does not match the known reaction type of the suspected drug. Dose not align with the reasonable time frame after administration. The clinical status of the subject or other reasons can also explain the reaction. Reaction decreases or disappears when clinical symptoms or other causes are ruled out..

⑥Unable to determine.

The incidence of AEs will be calculated and summarized for classifications ① + ② + ③ from each case report form.

10.5 Unexpected Adverse Event

An unexpected adverse event refers to any event not mentioned in the investigator's brochure, or differs in nature and severity from what is described in the investigator's brochure. In the absence of an investigator's brochure, the event is inconsistent with the risk information described in the study protocol or application materials.

10.6 Reporting of Serious Adverse Events

Notify the designated representative of the sponsor immediately. Send the signed and dated relevant pages of the case report form to the sponsor’s representative, along with copies of all examination results and their completion dates. At the same time, ensure the patient privacy is protected by removing all identifiable information from the clinical source document copies provided to the sponsor. Laboratory results should include normal range values.

All SAEs must be reported within 24 hours of occurrence. Record the SAE in the designed section of the CRF. After signing, report it preferably via fax to the monitoring center. The name, address and fax number of the monitoring center will be specified in the clinical trial protocol.

10.7 Risk Control and Management

In this study, the investigational drug RAG-17 may cause adverse reactions such as tremors, abnormal gait, and hind limb weakness. If such actions occur and are deemed related to the drug, it is recommended to stop the administration, monitor vital signs, conduct auxiliary examinations such as blood routine, coagulation profile, muscle enzymes, liver function, kidney function, blood gas analysis, ultrasound, CT scans. Provide timely symptomatic and supportive treatment based on clinical findings. If necessary, consult with relevant specialists to manage the adverse events effectively. For serious adverse events, it is necessary to ensure airway patency, autonomous respiration, stable blood pressure and heart rate. If necessary, treatment should be carried out in the intensive care unit.

For all adverse events, the investigators should continue to follow up with patients and track any related outcomes (clinical symptoms, signs, laboratory test results) until the related clinical symptoms are completely resolved, the test results return to normal or the condition is stable. This follow-up should persist even if the patient withdraws

from the clinical trial. Additionally, the monitoring team may request further investigations as needed. After the termination of the study, the investigators should still pay attention to any serious adverse events that may occur.

10.8 Subjects Benefits

Participation in this clinical study may help alleviate the subject's condition, but it is also possible that the investigational drug will not provide the expected therapeutic effect. In other words, subjects may not gain any direct benefits from participating in this study. However, this study may contribute to the development of new treatment options for patients with similar conditions, offering more choices to others. In addition, subjects may gain valuable information about their conditions through the physical examinations and medical tests conducted during the study.

11. Quality Control and Quality Assurance of the Study

Representatives of the sponsor or collaborators will regularly monitor the clinical study. The scope and nature of supervision will be determined based on considerations such as the clinical study's objectives, purposes, design, complexity, scale, and endpoints.

11.1 Protocol Adherence Management

- Careful selection of participants: When selecting subjects, investigators should confirm accurate diagnoses, understand common factors leading to dropout, conduct a comprehensive assessment of participants, and perform a pre-evaluation of adherence. Participants with high compliance potential should be carefully screened and selectively included.
- Informed consent process: Before signing the informed consent form, investigators should provide detailed and clear explanations to the subjects and their families in an easy-to-understand manner. This ensure subjects fully understand the investigational drug and the treatment process. Investigators must thoroughly address participants' questions, respect their choices, and encourage confidence in completing the study, which fosters better adherence.
- Optimizing the protocol: The study protocol should be designed to minimize complexity, favoring simple and practical approaches that align with participants'

daily lives. A well-designed, scientifically sound, and highly operable protocol will enhance adherence.

- Managing adverse events: If participants experience adverse reactions, adverse events, or serious adverse events during the clinical study, regardless of whether they are related to the investigational drug, researchers must promptly take appropriate treatment measures. Ensuring timely medical intervention safeguards participants' safety, builds trust in the study, and improves adherence.
- Follow-up and reminders: A contact information sheet and visit schedule should be established to remind participants of follow-up visits. For those who miss scheduled visits, researchers should promptly follow up via phone. These efforts help ensure participants remain engaged and adhere to the study protocol.

11.2 Study Monitoring

The primary responsibility of the monitoring team of the collaborators is to assist investigators and the sponsor or collaborators in ensuring the clinical study adheres to the highest standards of ethics, scientific rigor, professionalism, and regulatory compliance. According to the Good Clinical Practice (GCP) principle, the monitoring team should verify the CRF against the original source documents.

The monitoring team will regularly assess the progress of the study, through on-site visits, letters, or telephone communication. This includes evaluating adherence to the clinical protocol by researchers and participants and addressing urgent issues. Medical monitors will also periodically review participant safety. During these monitoring visits, the monitors will collaborate with the investigators to focus on the following key points (not exhaustive): informed consent of subjects, recruitment and follow-up of subjects, documentation and reporting of serious adverse event, concomitant treatment and data quality.

11.3 Data Verification

The following measures will be taken to ensure the accuracy, consistency, completeness and reliability of the data:

- Regular on-site monitoring of the study;
- CRF summary based on source documents;

- Data management quality control checks;
- Medical review by the sponsor.

The sponsor or designated personnel (such as CRO) will be responsible for the data management in this clinical study. The responsible party will design and distribute CRFs and oversee their monitoring. If the data are incomplete or inconsistent, the responsible party will issue queries for site staff to address and make necessary corrections.

11.4 Personnel Training

The investigators shall ensure adherence to the clinical trial protocol, compliance with the Good Clinical Practice (GCP) guidelines outlined in the ICH, and conformity with applicable laws and regulations during the conduct of the clinical trial.

The investigators should ensure adherence to all study procedures outlined in the clinical trial protocol (including safety principles). The investigator should accurately and clearly provide reliable data and all information required by the clinical trial protocol (e.g., drug overdose, pregnancy) through appropriate methods, such as CRFs, deviation resolution forms (DRFs), and others, in accordance with relevant requirements. Additionally, the investigator must ensure that the monitoring team has direct access to the source documents.

The investigators may appoint individuals deemed suitable to assist in conducting the study. These assistants will help manage the clinical study in accordance with the study protocol. All assistants will be appointed and documented in a timely manner. They will work under the supervision and management of the investigator, who will provide them with a copy of the clinical study protocol and all necessary information. The sponsor or collaborator of the clinical study are accountable to the health authority for ensuring the proper management of the clinical study by employing all appropriate means, such as ethics, compliance with the clinical study protocol, completeness and validity of the data recorded in the CRF.

11.5 Data Management

During the clinical study, the data of the subjects should be collected anonymously in the CRF, with subjects identified only by their number and the initials of their name. In cases where participant identity must be disclosed due to safety or administrative reasons, both the investigator and any involved parties share the responsibility for maintaining confidentiality. The informed consent form allows the subject to agree to authorized personnel from the sponsor or collaborators, ethics committees, and regulatory authorities directly accessing relevant source documents associated with the CRF (e.g., medical records, appointment logs, original laboratory records). These individuals must adhere to professional confidentiality standards and ensure the privacy of all participant personal or medical information.

The medical information obtained in the study should remain confidential and may only be disclosed to third parties with the permission of the informed consent form and the subject's signed authorization form (or separate authorization for the use and disclosure of personal health information) or when permitted or required by law.

For treatment purposes, medical information may be shared with the participant's personal physician or other appropriate healthcare professionals responsible for the participant's well-being.

11.6 Handling of Biological Samples

The biological samples, such as blood and cerebrospinal fluid collected in this study, will be processed (e.g. centrifugation and aliquoting) at Beijing Tiantan Hospital, Capital Medical University. Samples designed for evaluation will be analyzed at a centralized clinical laboratory with recognized accredited certification. All biological including blood and cerebrospinal fluid will be stored at Beijing Tiantan Hospital, Capital Medical University for 10 years.

12. Data Retention

The reviewed CRF and imaging data will be carefully entered and processed by the data management center and properly stored. Once data entry is completed, the CRF will be stored by the research center.

The study monitor will perform ongoing source data verification to ensure that the

key study protocol data (i.e., source data) transcribed on the CRF by authorized on-site personnel are accurate and complete, and that they can be verified against the original source documents.

Source documents are the original records where subject data is first recorded and stored. These include, but are not limited to hospital records, clinical and office charts, laboratory records, memos, subject diaries or assessment checklists, pharmacy dispensing records, data from automated instrument records, verified and certified as accurate and complete copies of audio transcriptions, microfilms, photographic negatives, microfiche or magnetic media, computed tomography, subject files, and records kept in pharmacies, laboratories and medical technology departments related to clinical studies.

The source documents required to verify the validity and completeness of the data transcribed on the CRF must never be deleted or destroyed.

To facilitate source data verification, the investigator and the institution must allow the sponsor to access applicable source documents and reports for direct inspection. This is to support monitoring, sponsor audits, clinical research reviews, and summaries by the management committees or ethics committees. Additionally, appropriate regulatory agencies must be allowed to inspect the study site.

13. Data Safety Monitoring

The clinical study will develop a data safety monitoring plan based on the level of risk involved. All adverse events will be thoroughly documented, appropriately managed, and followed up until resolved or the condition stabilizes. Serious adverse events and unexpected events will be promptly reported to the ethics committee, regulatory authorities, the sponsor, and the drug regulatory agency in accordance with relevant regulations. The principal investigator will regularly conduct cumulative reviews of all adverse events and, when necessary, convene investigator meetings to assess the risks and benefits of the study. For studies with risks exceeding the minimal threshold, independent data monitors will be assigned to oversee the study data. For high-risk studies, an independent data safety monitoring board will be established to review the accumulated safety and efficacy data and provide recommendations on

whether the study should continue.

During the clinical trial, participant data will be collected anonymously in the CRF, with participants identified only by their participant number and the initials of their name. In cases where participant identity must be disclosed due to safety or administrative reasons, both the investigator and the involved parties share the responsibility for maintaining confidentiality. The informed consent form allows participants to consent to the authorized sponsor, ethics committee, and regulatory authorities directly accessing relevant source documents on the CRFs (e.g., medical records, appointment logs, original laboratory records). These individuals must adhere to professional confidentiality standards and maintain the privacy of all participant personal or medical information.

14. Statistical Analysis

- Statistical analysis will be performed with SAS software.
- Demographic, medical, histories and baseline characteristics information will be listed and summarized.
- The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after treatment. Safety evaluation will be analyzed using safety data set. The number of people of events should be recorded in detail and showing the event rate in summary statement. The event rate will be calculated as the number patients experiencing the event / the number of patients enrolled.
- Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 180-day follow-up assessments for all participants, the data were analyzed.

15. Ethical Considerations

The clinical study will adhere to the World Medical Association's Declaration of Helsinki and other relevant regulations. Before the study begins, the trial protocol will be approved by the ethics committee. Before enrollment, the investigator is responsible for providing a complete and comprehensive explanation of the study's purpose, procedures, and potential risks to the participant or their representative. A written informed consent form must be signed, ensuring that participants are aware of their right to withdraw from the study at any time. The informed consent form will be retained as part of the clinical study documentation for future reference. Throughout the study, the personal privacy and data confidentiality of participants will be safeguarded.

Screening and informed consent: The investigator (or designated personnel authorized and responsible by the investigator, in compliance with relevant regulations) will recruit participants in an independent consultation room at Beijing Tiantan Hospital, Capital Medical University, ensuring the privacy and confidentiality of the participants. Participants and their legal representatives should be fully informed about all relevant aspects of the clinical trial, including written information approved by the ethics committee. The investigator should use language or terms that the participant can understand, providing as much information as possible about the study, and allow sufficient time for the participant or their legal representative to consider whether to participate. Before participating in the clinical trial, the patient or their legal representative must discuss the informed consent with the investigator and sign the informed consent form (including the participant's name and the date). The investigator should provide the participant with a copy of the signed informed consent form.

16. Data Confidentiality

Throughout the entire research and data analysis process, participant information will be strictly protected. The executive committee will disclose the trial results in accordance with management regulations and procedures. Once the database is locked, the trial results will be published as soon as possible. Biostatistical experts involved in

the study have the right to access the dataset but will not be able to identify any individual participants. In line with the GCP principles outlined in the ICH guidelines, the monitoring team will verify the CRFs against the original source documents. The informed consent form will include a statement that allows participants to consent to authorized personnel from the sponsor, ethics committee, and regulatory authorities directly accessing relevant source documents on the CRFs (e.g., medical records, appointment logs, original laboratory records). Researchers must adhere to professional confidentiality standards and ensure the confidentiality of all personal and medical information of the participants.

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Safety and Tolerance of RAG-17 in Amyotrophic Lateral Sclerosis Patients With *SOD1* Gene Mutation (CREATION)

Study Protocol

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(Protocol Number HX-A-2023004)

Protocol Version: 2.1

Oct. 12th, 2023

Summary of the Trial Protocol

Study Title	Safety and tolerance of RAG-17 in amyotrophic lateral sclerosis patients with *SOD1* gene mutation
Study Purposes	To evaluate the safety, tolerance and pharmacokinetics (PK) of RAG-17 in adult ALS subjects with *SOD1* gene mutation. **Primary purposes：** 1. To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 240 days after the first injection of RAG-17. 2. To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 240 days after the first injection of RAG-17. **Secondary purposes：** 1. To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2. To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3. To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 4. To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

	Exploratory purposes： 1. To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, day 180, and day 240. 2. To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, day 180, and day 240. 3. To evaluate the changes in muscle strength on day 15, day 29, day 90, day 180, and day 240. 4. To evaluate the changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, day 180, and day 240. 5. To evaluate the changes in 7T MRI imaging features on day 29, day 90, day 180, and day 240. 6. To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, day 180, and day 240. 7. To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, day 180, and day 240. 8. To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, day 180, and day 240. 9. To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, day 180, and day 240.
Study	Ongoing open-label, single-center, first-in-human and dose escalation

Design	clinical study
Total number of cases	Six ALS patients with *SOD1* gene mutations
Subjects Selection	**Inclusion Criteria** (1) Patients permitted by medical personnel who could complete the clinical trial. (2) Age 18~75 years, gender unlimited. (3) Clearly identify ALS patients with *SOD1* gene mutations (known *SOD1* mutation sites and reported disease progression). (4) Forced vital capacity during screening period ≥ 50% of expected lung capacity. (5) Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology. (6) The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form. (7) The subjects (including male subjects) have no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum. **Exclusion Criteria** (1) The *SOD1* mutation site occurs in patients with nucleotides 44-66 (calculated from the translation of SOD1 protein) and P.F21C mutations. (2) Patients who have previously received or are currently receiving Tofersen treatment.

	(3) HIV test positive or positive test history. (4) Hepatitis C virus antibody positive or positive test history. (5) Active hepatitis B infection (hepatitis B surface antigen positive and/or hepatitis B core antibody positive). (6) Other experimental drugs used within one month or within five drug half-lives. (7) Lumbar diseases and deformities. (8) Suffering from other diseases known to be related to motor neuron dysfunction, which may confuse or blur the diagnosis of ALS. (9) Other psychiatric disorders diagnosed according to DSM-V diagnostic criteria, or obvious suicidal intentions. (10) Combined with severe liver dysfunction, renal dysfunction, or severe heart dysfunction (severe liver dysfunction refers to ALT value ≥ 2.0 times the upper limit of normal value or AST value ≥ 2.0 times the upper limit of normal value; severe renal dysfunction refers to CRE ≥ 1.5 times the upper limit of normal value or eGFR < 40mL/min/1.73m^2; severe heart dysfunction refers to NYHA 3-4 levels). (11) Permanent and sustained dependence on ventilator assisted ventilation. (12) History of combined alcohol and drug abuse. (13) Pregnant, lactating, or potentially pregnant patients, as well as those planning to conceive. (14) Patients participating in other clinical trials or applying other biologics, drugs, or devices under study. (15) Patients who have received any vaccination within 28 days. (16) Contraindications for MRI examination (ie. claustrophobia). (17) Those who can't complete follow-up due to other reasons.

Therapeutic Regimen

Study drug：Aseptic aqueous solution of the RAG-17 formulation (sodium salt)

Storage condition：2~8 °C, stored in refrigeration

Specification：75 mg (2.5 mL)

Route, dosage and schedule of administration: Intrathecal administration. The safety, tolerance, and pharmacokinetics of the RAG-17 drug will be evaluated by a dose-escalation approach. The dose of RAG-17 will range from a minimum of 60 mg to the maximum tolerated dose (MTD). The first three participants begin at a dose of 60 mg, while the last three participants start at 90 mg, for dose escalation. Once the tolerated dose is reached, a fixed dose of the drug will be administered every two months for continued treatment, with a total treatment duration of 8 months. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD. For the subsequent maintenance treatment phase, the optimal dose between the safe dose and the MTD will be selected. The dose for subsequent maintenance treatment should be ≤ MTD.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	150 mg/dose	Intrathecal injection	6
5th Administration	D120	150/180 (no more than 180) mg/dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

	Dose escalation criteria: If no AEs or SAEs occur within 14 days after the first administration, the subject proceed with a dose escalation of 30 mg every 14 days during the observation period. It is planned to achieve DLT through 3 to 4 dose escalations, followed by continuous treatment with the optimal dose for a 6-month treatment period. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD.
Study Outcomes	**Primary outcomes** 1) Incidence of AEs and SAEs within 240 days after the first injection RAG-17. 2) Changes in laboratory assessments, vital signs, ALSFRS-R scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 240 days after the first injection RAG-17. **Second outcomes：** 1) Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2) Changes in plasma NFL levels from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3) Changes in the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 4) Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

	Exploratory outcomes： 1) Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, day 180, and day 240. 2) Changes in fatigue severity (Fatigue Severity Scale, FSS) at on day 15, day 29, day 90, day 180, and day 240. 3) Changes in muscle strength on day 15, day 29, day 90, day 180, and day 240. 4) Changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, day 180, and day 240. 5) Changes in 7T MRI imaging features on day 29, day 90, day 180, and day 240. 6) Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, day 180, and day 240. 7) Changes in modified Norris scale scores on day 15, day 29, day 90, day 180, and day 240. 8) Changes in quality of life (including ALSAQ-40、EQ-5D-5L、HAMA and HAMD) on day 15, day 29, day 90, day 180, and day 240. 9) Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, day 180, and day 240.

Statistical Analysis	• Statistical analysis will be performed with SAS software. • Demographic, medical, histories and baseline characteristics information will be listed and summarized. • The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after treatment. Safety evaluation will be analyzed using safety data set. The number of people of events should be recorded in detail and showing the event rate in summary statement. The event rate will be calculated as the number patients experiencing the event / the number of patients enrolled. • Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 240-day follow-up assessments for all participants, the data were analyzed.
Study Duration	2 years

1. Study Background

Amyotrophic lateral sclerosis (ALS) is a chronic, progressive neurodegenerative disease characterized by prominent damage to both upper and lower motor neurons, manifesting in symptoms such as muscle weakness, muscle atrophy, bulbar paralysis, and pyramidal signs. ALS is a rare disease, with a global standardized annual incidence rate of only 1.68 cases per 100,000 person-years[1]. The exact incidence rate of ALS in China still not well-established, but it is estimated that there are at least 100,000 patients [2]. The peak age of onset for ALS is around 50 years, with a growing trend toward earlier onset ages[3]. ALS is incurable, with a typical survival period of 3-5 years, and only approximately 10% of patients survive for more than 10 years[4]. As a result, it is listed by the World Health Organization as one of the "five major incurable diseases in the world" alongside cancer, acquired immunodeficiency syndrome, leukemia and rheumatoid arthritis.

Currently, the pathogenesis of ALS remains unclear, and available treatments are limited, primarily focusing on symptomatic relief rather than a cure. Since 1980, more than 80 randomized controlled trials (RCTs) have been conducted globally. Only three drugs for the treatment of ALS have been approved by the U.S. Food and Drug Administration (FDA): the neuroprotective drug riluzole, which inhibits excessive neurotransmission; the antioxidant edaravone; and Relyvrio (sodium phenylbutyrate and taurursodiol)[5-7]. Drug interventions for ALS include anti-apoptosis, anti-inflammation, anti-excitotoxicity, anti-oxidation, anti-aggregation, neurotrophic growth factors and neuroprotection, among others. In 2017, riluzole, the primary drug for ALS, became the first FDA-approved drug treatment for the disease. However, it cannot halt the progression of ALS, let alone reverse it, and only extends patient survival by 2 to 3 months. The riluzole costs about 1,800~4,000 RMB per month. However, it is only covered by medical insurance in certain provinces and cities. Edaravone is indicated for cerebral infarction and was approved for the treatment of ALS in the United States in 2019. It was approved for use in China in September 2020, specifically for patients with acceptable respiratory function in the early stage (within 2 years) of the disease. In September 2022, the FDA approved Relyvrio, a

combination drug composed of sodium phenylbutrate and taurursodiol, for the treatment of adult patients with ALS. The approval process for Relyvrio drug at the FDA has been marked by numerous challenges. At the first Peripheral and Central Nervous System Drug Advisory Committee (PCNSDAC) review meeting organized by the FDA in March 2022, the expert panel expressed concerns regarding issues such as the small scale of the Relyvri clinical trialo, data deficiencies, and the applicability of statistical methods, with a voting result of 6:4. In response to this, Amylyx reanalyzed the survival data of the CENTAUR trial of Relyvrio and submitted it, along with the biomarker data from another phase II clinical trial for Alzheimer's disease to the FDA for re-evaluation. At the second PCNSDAC meeting in September, the new drug application of Relyvrio was approved with a voting result of 7:2, and it was subsequently approved by the FDA. Currently, this drug has not been approved in China. As a result, existing therapies for ALS remain limited, and the demand for effective ALS treatments remains unmet.

Currently, the pathogenesis of ALS remains unclear, and available treatment are limited, mainly focusing on symptomatic relief rather than a cure. ALS can be divided into sporadic ALS (sALS) and familial ALS (fALS), with sALS accounting for the majority of cases (approximately 90%) and fALS accounting for 5-10%[8]. fALS can result from mutations in multiple genes, with the most common being a mutation in the *superoxide dismutase 1* (*SOD1*), which occurs in 2% to 7% of cases[9]. Approximately 20% of fALS and 5% of sALS cases are associated with the *SOD1* gene. Asia has the highest number of ALS, with Asian patients typically exhibiting a younger age of onset, a lower proportion of bulbar-onset ALS, and more pronounced cognitive dysfunction[10]. In Asia, fALS is mainly caused by *SOD1* gene mutations, whereas in Europe, mutations in the *C9ORF72* gene are more common. The frequency of the *SOD1* gene mutations is highest among ALS patient in central and southern China[11]. SOD1 is a potent antioxidant enzyme that, when combined with copper and zinc ions, helps convert superoxide into oxygen molecules and hydrogen peroxide, thereby protecting cells from damage[12-14]. Mutations in the *SOD1* gene in ALS patients lead to misfolding and accumulation of the SOD1 protein within

spinal motor neurons. While several mutations in SOD1 have been identified, not all of them result in ALS. Harmful mutations alter the functional activity of SOD1, with loss-of-function mutations impairing degradation of reactive oxygen species (ROS), which damages motor neurons, and gain-of-function mutations increasing oxidative activity, resulting in excessive production of hydrogen peroxide and enhancing protein-protein interactions[15-17]. These effects disrupt SOD1 protein level and activity, inhibit axonal transport, and cause mitochondrial dysfunction[15], ultimately leading to neuronal degeneration and death. Therefore, reducing the level of toxic SOD1 protein may offer an effective therapeutic approach for ALS caused by *SOD1* mutations. Researches have indicated that knockdown or knockout of normal SOD1 expression does not result in significant side effects[18-19].

Currently, several drugs under investigation have entered clinical studies, including small molecule drugs, antibody drugs, antisense oligonucleotides (ASO) drugs and cell therapy. Among these, except for small nucleic acid drugs (antisense oligonucleotides, small interfering RNA) which directly target pathogenic genes, the others are non-disease-modifying therapies. Tofersen, an antisense oligonucleotide drug developed by Biogen for the treatment of *SOD1*-ALS, binds to the mRNA encoding SOD1 and is degraded by ribonuclease H (RNase-H), thereby reducing the production of mutant SOD1 protein[20]. Tofersen is currently undergoing a New Drug Application (NDA) review by the U.S. FDA. The NDA submission for Tofersen is based on data from a Phase 1 study in healthy volunteers, a dose-escalation Phase 1/2 clinical study, a Phase 3 VALOR clinical study, and an open-label extension (OLE) study. While the Phase III of Tofersen for the treatment of did not meet the primary endpoint, Biogen has observed indications of reduced disease progression in several secondary and exploratory endpoints. Biogen's integration of 12-month data shows that earlier use of Tofersen, compared to delayed initiation, significantly reduces the neurofilament levels and slows the decline of various efficacy endpoints. In terms of adverse events, the most common side effects in patients were headache, falls, back pain and limb pain. About 6.7% of patients had serious neurological-related events, including myelitis, radiculitis, aseptic meningitis and optic disc edema[21-22].

RNA interference (RNAi) is a biopharmaceutical technology that utilizes double-stranded RNA molecules, known as small interfering RNA (siRNA), to target and silence specific genes at the mRNA level. Based on this mechanism, five drugs have been approved by the U.S. FDA, with nearly a hundred others currently in clinical development. RAG-17 is a 20 kDa siRNA targeting the *SOD1* gene. Through the RNAi mechanism, RAG-17 binds to *SOD1* mRNA, leading to the degradation of the target mRNA by the RNA-induced silencing complex (RISC). By recognizing and mediating the degradation of the target mRNA, RAG-17 silences the *SOD1* gene and downregulates the expression of the SOD-1 protein. RAG-17 is administered via intrathecal injection and is intended for the treatment of ALS patients with *SOD1* mutations. Compared to single-stranded self-delivering ASO drugs, siRNA offers a cascade amplification effect on the target gene and provides a higher gene silencing efficiency. This drug leverages a unique delivery technology for small nucleic acid drugs, enabling efficient siRNA delivery to the central nervous system and offering the potential for treatment of neurological diseases. The schematic diagram of the mechanism of RAG-17 in the treatment of ALS is shown below.

The *in vitro* pharmacodynamic results indicate that RAG-17 shows a concentration-dependent knockdown of *hSOD1* mRNA, with a half-maximal inhibitory concentration (IC50) of 0.011 nM. Compared to the control drug Tofersen, the IC50 of RAG-17 is 100 times lower, and the maximum inhibition rate reaches more than 90%. Cell apoptosis assays show that RAG-17 has no toxic response of cell apoptosis. Off-target effect analysis results show that RAG-17 dose not significantly affect the mRNA expression levels of non-target genes, demonstrating its specificity for *SOD1* gene knockdown.

The h*SOD1*^{G93A} transgenic mice exhibit progressive motor neuron degeneration, which closely resembles human ALS and is a widely recognized animal model for studying ALS. It has been extensively used in ALS pathogenesis research and drug screening [23-25]. To evaluated the inhibitory activity of RAG-17 on the target gene *in vivo*, a single-dose proof of concept (POC) experiment was conducted using the h*SOD1*^{G93A} ALS disease model mice. At 60 days of age, 200 μg of the siRNA drug

RAG-17 (~10 nmol) and the control drug Tofersen (28 nmol) were injected into the lateral ventricle. Tissue RNA was extracted 14 days post-administration, and RT-PCR was performed to assess the target gene, human *SOD1*. The results indicated that the mRNA expression of *SOD1* in both brain and spinal cord tissues was reduced by approximately 80%, with RAG-17 demonstrating superior gene down-regulation compared to the control drug.

Subsequently, a multi-dose pharmacodynamics (PD) experiment was conducted. Doses of 50 μg, 100 μg, 200 μg, and 400 μg of the siRNA drug RAG-17 were injected into the lateral ventricle. The PD indicators (relative expression levels of human *SOD1* mRNA) in the target tissues were assessed at 14 days and 56 days post-injection to determine the pharmacodynamic activity, dose response and the duration of effect at the respective doses. The results showed that gene knockdown was dose-dependent in three different regions of the brain (prefrontal cortex, cerebellum, and other brain tissues) and the spinal cord. Notably, only the high-dose group (400 μg) maintained 50% gene knockdown (KD) activity 56 days after injection, although this activity was weaker compared to 14 days post-injection. Compared to small molecule drugs, the significant advantage of small nucleic acid drugs is the extended duration of their therapeutic effect. Further testing at an extended time point (8 weeks) revealed that RAG-17 sustained the pharmacological activity with gene knockdown for at least 8 weeks in the model mice, providing valuable data to support the potential frequency of clinical administration.

To further verify the correlation between drug exposure in tissues and drug efficacy, the stem-loop PCR method was applied to detect the siRNA concentration in tissues at 14 days and 56 days post-administration. The results demonstrated a clear correlation between the drug concentration in tissues and PD effects.

To further explore the pharmacodynamics of RAG-17, human *SOD1*^{G93A} transgenic ALS disease model mice were used. Both male and female ALS disease model mice were administered intrathecally twice on the 68th and 100th days of life, with doses of the RAG-17 (RD-12500) at 75 μg, 150 μg, and 300 μg. Two negative control groups were included: a vehicle group and an inactvice (scrambled siRNA) group. The

scrambled siRNA served as a control for the target siRNA, being non-functional, and used to assess the potential inhibitory effects of the target siRNA. Additionally, 150 ug of Tofersen (RD-10748) was included as the positive control group. This study conducted pharmacodynamic evaluations based on multiple indicators, including animal body weight, motor function (grip strength in the fatigue rotating rod test, rotarod test, and open field test), disease onset time, lifespan, and neurological function score (neuroscore).

The results of animal weight changes indicated that both the vehicle group and the inactive group began to show a significant decrease in body weight around 130 days, with a more pronounced decrease in male mice. This suggests that the onset of h*SOD1*^{G93A} ALS disease model occurs earlier in males than in females. Among the groups, the positive group (RD-10748) showed improvements in body weight, with more significant weight maintenance in male mice. The body weight data from the drug treatment groups demonstrated a dose-dependent alleviation of weight loss, especially in female mice. As the disease progresses in ALS mice, their body weight gradually decreases. Compared to the vehicle control group, all dose groups of RD-12500 (RAG-17) and ASO (RD-10748) demonstrated a delayed onset of disease.

After two intrathecal administrations in the ALS disease model mice, all treatment groups of both male and female mice showed an extension in the median survival period. The low-dose group of of 75 μg RAG-17 and the medium-dose of 150 μg ASO (RD-10748) demonstrated comparable efficacy. The survival periods for male mice were 207 days (75 μg RAG-17) and 191 days (150 μg ASO), respectively. The survival periods of the female group were 198.5 days (75 μg RAG-17) and 189 days (150 μg ASO), respectively. In the female high-dose group (300 μg), the survival period was significantly extended to 227 days, representing a 54-day increase compared to the vehicle control group (173 days). However, the high-dose group (300 μg) in the male mice did not show a marked superiority. Female animals exhibited greater sensitive to drug treatment, and the results aligned with the trend of weight change.

The typical pathological feature of h*SOD1*^{G93A} ALS disease model mice is the

progressive loss of motor function. The Rotarod test for motor function fatigue and the Grip strength test were used to evaluate the therapeutic effect of the drug on the ALS model. The results showed that, in the Rotarod test, the drug-treated group demonstrated a significant increase in the time ALS mice were able to maintain their position on the accelerating rotating rod at 0-30 rpm, compared to both the vehicle and the inactive control groups. The Grip strength test revealed that the drug treatment group significantly improved the hindlimb muscle function of the disease model mice. Additionally, the open field test, which evaluates autonomous behavior, exploratory behavior and anxiety in a novel environment, showed that the drug-treatment group exhibited improved autonomous motor ability, with a dose-dependence effect.

Before performing the Rotarod and hindlimb grip strength behavioral tests, the neurological function of ALS mice was scored on a scale of 0 to 4 points. This scoring system objectively evaluates the common hindlimb dysfunction in h*SOD1*^{G93A} transgenic mice. The results showed that mice in the vehicle (aCSF) and inactive control groups exhibited abnormal tilting (NS2 symptoms) at PND130 days, with the severity progressively worsening until hindlimb paralysis (i.e., ≥ NS3). In contrast, at all time points during the study, the average score of all RAG-17 treatment doses never exceeded NS2. These results indicated that RAG-17 treatment, regardless of the dose, improved motor function in the animals compared with the control group.

In conclusion, RAG-17 treatment can effectively inhibit the gene expression of h*SOD1* in h*SOD1*^{G93A} transgenic mice, significantly delay the disease onset, extend the survival time of affected mice, enhance their grip strength , and improve the motor function.

Currently, the non-clinical safety evaluation of RAG-17 is ongoing. The safety dose exploration experiments have been completed in both rats (following two intrathecal administrations, a non-relevant species) and cynomolgus monkeys (a relevant species). In the rat safety dose exploration experiment, the main findings included transient neurological functions such as tremors, abnormal gait, and hindlimb weakness, in some animals of the medium-high dose group (2.5mg/dose) and high dose group (4.5mg/dose) after intrathecal drug injection. In the safety dose exploration

experiment with cynomolgus monkeys, one male monkey (3001) died after administration of the high dose of 200mg (equivalent to 2g in human dose), while another female monkey (3501) showed severe toxic reactions but gradually recovered over days 17-18. The two animals in the low-dose group of 30mg showed no abnormalities after the first administration, but tremors occurred after the second administration. As a result, animals in the vehicle group were used as alternatives and received the medium-dose experiment of 50mg. The 50mg dose group (equivalent to 500mg in human dose) showed transient tremors, body stiffness, and reduced activity after administration, but these symptoms resolved within approximately 2 to 4 hours.

In terms of histopathology, axonal degeneration of the sciatic nerve, unrelated to the dose, was observed in both species. In the cynomolgus monkey study (H59-0025-TX), mild to mild multifocal axonal degeneration was observed in both bilateral and unilateral sciatic nerves of the male in the 50 mg/dose and 30 mg/dose groups. Additionally, mild to mild multifocal axonal degeneration was observed in the female monkeys across all dose groups. This alteration is characterized by a "bubbly" appearance of multiple affected fibers, axonal rupture (aggregating into eosinophilic masses), and some cellular debris surrounding the vacuoles. Although there was no control group in the cynomolgus monkey study, the changes observed in the sciatic nerve were still considered related to the test drug, consistent with the findings from the rat study. In each dose group, mild multifocal axonal degeneration was observed at the injection site in the female monkeys. The characteristics of this alteration are similar to those observed in the sciatic nerve and may be due to the administration procedure. In addition, mild to mild diffuse vacuolation of macrophages in the medulla of mesenteric lymph nodes was observed in all dose groups. Since these adverse reactions were not dose-dependent, their correlation with the test drug cannot be determined at this time.

Based on the results of the safety dose exploration experiments, formal GLP toxicology experiments are currently underway. These formal GLP toxicology tests have reduced the administration doses for rats and cynomolgus monkeys, as well as optimized the intrathecal injection procedure. The preliminary safety dose exploration

experiment results of rats and cynomolgus monkeys can also provide insight into the potential clinical risks of this test drug.

This project conducts a single-center, dose-escalation clinical study, aiming to evaluate the safety, tolerance and pharmacokinetics of RAG-17 in adult ALS patients carrying the *SOD1* mutation, in preparation for subsequent efficacy clinical trial.

2. Study Purposes

This study primarily aims to evaluate the safety, tolerance and pharmacokinetics of RAG-17 in adult ALS patients with *SOD1* gene mutation.

2.1 Primary Purposes：

(1) To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 240 days after the first injection of RAG-17.

(2) To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 240 days after the first injection of RAG-17.

2.2 Second Purposes：

(1) To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(2) To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(3) To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.

(4) To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

2.3 Exploratory Purposes：

(1) To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, day 180, and day 240.

(2) To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, day 180, and day 240.

(3) To evaluate the changes in muscle strength on day 15, day 29, day 90, day 180, and day 240.

(4) To evaluate the changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, day 180, and day 240.

(5) To evaluate the changes in 7T MRI imaging features on day 29, day 90, day 180, and day 240.

(6) To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, day 180, and day 240.

(7) To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, day 180, and day 240.

(8) To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, day 180, and day 240.

(9) To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, day 180, and day 240.

3. Study Design Type, Principles and Experimental Procedures

3.1 Study Design

The study is an ongoing open-label, single-center, first-in-human, and dose escalation clinical trial.

Adult patients with a confirmed or probable familial or sporadic of ALS with confirmed *SOD1* gene mutations (forced vital capacity ≥ 80% of predicted value) will be included according to the revised El Escorial criteria.

1) **Administration：** A total of 7 doses will be administered on Day 1, Day 15, Day 29, Day 60, Day 120, Day 180, and Day 240. The drug will be administered via

intrathecal injection of RAG-17, with doses of 60 mg per administration, 90 mg per administration, 120 mg per administration, 150 mg per administration, 150/180 mg (no more than 180 mg) per administration, with the last two doses being the optimal dose. The treatment period of this study is 8 months. Various indicators, adverse events, and concomitant medications will be collected on Day 1, Day 15, Day 29, Day 60, Day 90, and Day 180 post-treatment. The study is expected to be conducted at a single center, Beijing Tiantan Hospital, with the recruitment of 6 participants, aiming to complete the study within 2 years.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	150 mg/dose	Intrathecal injection	6
5th Administration	D120	150/180 (no more than 180) mg/dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

Dose Escalation Criteria: If no AEs or SAEs occur within 14 days after the first administration, the subject proceed with a dose escalation of 30 mg every 14 days during the observation period. It is planned to achieve DLT through 3 to 4 dose escalations, followed by continuous treatment with the optimal dose for a 6-month treatment period. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD. The dose for subsequent maintenance treatment should be ≤ MTD.

2) **Dose Escalation Stopping Criteria：** 'Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0' will be used as the toxicity evaluation standard

for this protocol.

If a dose meets any of the following criteria, dose escalation will be considered for stopping or the trial will be continued at an additional lower dose level, unless it can be clearly confirmed that the adverse event is unrelated to the drug (e.g., skin abrasions, hospitalization or death caused from a car accident).

① At the same dose level (within the same administration group), if ≥ 1 subject experiences an AE of grade ≥ 2;

② Other situations that may increase the risk of subjects, which will be discussed by the investigator to decide whether to terminate dose escalation.

3) **Individual Drug Withdrawal Criteria:** A subject will no longer participate in the subsequent administration, if they meet any of the following criteria:

① During the trial, the subject experiences a SAE or an AE of grade ≥ 3 (including laboratory abnormalities) , regardless of whether it is related to the drug;

② The subject experiences an event that, in the investigator's assessment, may significantly increase the risk to the subject.

3.2 Sample Size

This is an exploratory, hypothesis-generating study and a dose escalation clinical study to explore the maximum tolerated dose, so a total of 6 cases are required for sample size estimation.

3.3 Study Period

Work Progress	Timeline
Study Design, Study Approval Review, Ethical Review, Contract Signing, Human Genetic Resources Approval, Study Registration	2022.12-2023.05
Project Official Launch, Subject Enrollment and Follow-up	2023.06-2024.06
Data Cleaning and Analysis, Manuscript Writing	2024.06-2024.12
Total Duration	2 Years

3.4 Subject Selection

During the entire study period, it is planned to enroll 6 subjects. Ethical approval must be obtained before patient recruitment. The ethics committee will have access to all study documents and training materials.

- **Inclusion Criteria**

(1) Patients permitted by medical personnel who could complete the clinical trial.

(2) Age 18–75 years, gender unlimited.

(3) Clearly identify ALS patients with SOD1 gene mutations (known *SOD1* mutation sites and reported disease progression).

(4) Forced vital capacity during screening period ≥ 50% of expected lung capacity.

(5) Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology.

(6) The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form.

(7) The subjects (including male subjects) have no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum.

- **Exclusion Criteria**

(1) The *SOD1* mutation site occurs in patients with nucleotides 44-66 (calculated from the translation of SOD1 protein) and P.F21C mutations.

(2) Patients who have previously received or are currently receiving Tofersen treatment.
(3) HIV test positive or positive test history.
(4) Hepatitis C virus antibody positive or positive test history.
(5) Active hepatitis B infection (hepatitis B surface antigen positive and/or hepatitis B core antibody positive).
(6) Other experimental drugs used within one month or within five drug half-lives.
(7) Lumbar diseases and deformities.
(8) Suffering from other diseases known to be related to motor neuron dysfunction, which may confuse or blur the diagnosis of ALS.
(9) Other psychiatric disorders diagnosed according to DSM-V diagnostic criteria, or obvious suicidal intentions.
(10) Combined with severe liver dysfunction, renal dysfunction, or severe heart dysfunction (severe liver dysfunction refers to ALT value ≥ 2.0 times the upper limit of normal value or AST value ≥ 2.0 times the upper limit of normal value; severe renal dysfunction refers to CRE ≥ 1.5 times the upper limit of normal value or eGFR < 40mL/min/1.73m^2; severe heart dysfunction refers to NYHA 3-4 levels).
(11) Permanent and sustained dependence on ventilator assisted ventilation.
(12) History of combined alcohol and drug abuse.
(13) Pregnant, lactating, or potentially pregnant patients, as well as those planning to conceive.
(14) Patients participating in other clinical trials or applying other biologics, drugs, or devices under study.
(15) Patients who have received any vaccination within 28 days.
(16) Contraindications for MRI examination (ie. claustrophobia).
(17) Those who can't complete follow-up due to other reasons.

4. Study Method

(1) Drug Dosage Form

The aseptic aqueous solution of the RAG-17 preparation (sodium salt) used in the

study is stored at -20℃±5℃, with a specification of 75 mg (2.5 mL).

(2) Intervention Measures

1) Basic Treatment

All enrolled subjects will maintain basic medication treatment, and the dosage and type of medications will remain unchanged during the trial period.

Subjects who have already received riluzole treatment before enrollment may continue using it during the study period, maintaining a stable dosage (50mg per dose, twice daily). Throughout the study, discontinuation of riluzole for reasons other than medical necessity, as determined by the investigator, is not recommended.

The concomitant medications situation of the patients should be recorded in detail in the CRF form, including the name of the medication, the dosage, and frequency. If during the study, a change in the existing medication is deemed necessary by the investigator due to the patient's condition, it will be considered a protocol deviation. An adverse event record report and a concomitant medication form should be completed. The investigator will then assess whether the subject can continue participating in the study. If not, the subject will be treated as a withdrawn case.

2) **RAG-17 Treatment**

The first three participants receive intrathecal injection begin at a dose of 60 mg, while the last three participants start at 90 mg. Within the first 14 days following the initial dose, if no AEs or SAEs are observed, the dose may be escalated by 30mg at 14-day intervals. After reaching the maximum tolerated dose (MTD), an optimal dose between the safe dose and the MTD will be selected as the dose for continued treatment. Continuous treatment will be administered every 2 months, with a total treatment duration of 8 months.

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6

4th Administration	D60	150 mg/dose	Intrathecal injection	6
5th Administration	D120	150/180 (no more than 180) mg/dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

5. Concomitant Medications

(1) Prohibited Concomitant Medications

Subjects must contact their investigators before taking any new medications, including over-the-counter drugs and herbal preparations.

• The use of any antiplatelet or anticoagulant drugs (e.g. aspirin, clopidogrel) is prohibited within 7 days prior to each lumbar puncture and until 48 hours after the procedure.

• The use of DPS, copper (II) (diacetylbis (N4-methylaminothiourea)), or pyrimethamine is prohibited during the study.

• The use of gamma globulin is not recommended during the study.

(2) Permitted Concomitant Medications

If subjects are taking concomitant vitamins, minerals and supplements, they should avoid exceeding the recommended daily dose within 14 days prior to the first administration of the RAG-17 and during the study period (e.g. no more than 5 grams of creatine per day and no more than 1000 IU of vitamin E per day). These daily limits include the total dose obtained from the combined use of daily multivitamins and supplements.

Medications not listed in the prohibited concomitant medications section above are considered permitted concomitant medications. Concomitant medications for symptom management during the study are determined by the investigator. All enrolled cases must maintain basic medication treatment, with no changes to the dosage or type of medications during the trial. If subjects have questions regarding permitted concomitant treatments, they should seek medical guidance from the

investigator. The concomitant medications of the patients must be documented in detail in the CRF form, including the name of the medication, the dosage, and frequency. If a change or adjustment to the existing medications become necessary due to the subject's condition during the study, and the investigator deems it essential, this will be considered a protocol deviation. The adverse event record form and the concomitant medication form should be promptly completed. The investigator will assess whether the subject can continue participating in the study. If continuation is not feasible, the case will be treated as an excluded case. In special circumstances (e.g. the subjects suffers from other diseases during the study), when additional treatments are necessary, these must be explained in the CRF form, including the disease diagnosis, medications, dosage, and usage details.

6. Observation Indicators and Examination Timeline

6.1 General Demographic Indicators and Clinical Data

(1) Collection time point: baseline

(2) Collection content: Basic demographic characteristics include gender, age, ethnicity, height, weight, education level, marital status; vital signs include body temperature, pulse, respiration rate, blood pressure; 12-lead electrocardiogram (ECG); waist circumference, physical examination; clinical data include relevant medical history, past medical history, medication used prior to enrollment, smoking and drinking history, and family medical history.

6.2 Daily Life Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180, day 240

(2) Collection content: All subjects will undergo assessments using the ALSFRS-R and Modified Norris Scale before and after treatment. The results after treatment intervention will be compared with baseline data to evaluate the effect of RAG-17 on improving daily living function of the subjects.

(3) Observation indicators:

1) ALSFRS-R: This is a rapidly administered ordinal rating scale (grade 0-4) used to assess the subjects' ability and independence in 12 functional activities. ALSFRS-R measures 4 functional domains, including respiratory, bulbar, gross motor,

and fine motor functions. The scale includes 12 items, each scored from 0 to 4, with a total score of 48. Higher scores indicate better function, while lower scores reflect more severe neurological impairment.

2) Modified Norris Scale: This assessment tool for ALS consists of two parts: the Limb Norris Scale (comprises 21 items to evaluate limb function) and the Norris Bulbar Scale (includes 13 items to assess bulbar function). Lower scores indicate more severe neurological impairment.

6.3 Respiratory Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180, day 240

(2) Collection content: All subjects will undergo forced vital capacity (FVC) testing before and after treatment. The results post-treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving the respiratory function.

(3) Contents of observation indicators:

FVC: The subjects are measured in an upright position. Measured as the maximum volume of air that can be forcefully exhaled as quickly as possible after a maximal inhalation.

6.4 Fatigue Severity Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180, day 240

(2) Collection content: All subjects will undergo measurements with the Fatigue Severity Scale (FSS) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving the severity of fatigue in subjects.

(3) Observation indicators:

Fatigue severity: The FSS consists of 9 items, designed to measure the degree of fatigue and its impact on a person's activities and lifestyle across various diseases. The evaluation covers areas such as daily activity ability, participation, and sleep. Each question is scored on a 7-point scale: 1 = strongly disagree and 7 = strongly agree. The minimum possible score = 9 and the maximum possible score = 63. Higher scores indicate the greater severity of fatigue.

6.5 Muscle Strength Test

(1) Collection time points: baseline, day 15, day 29, day 90, day 180, day 240

(2) Collection content: All subjects will undergo measurement using the Medical Research Council (MRC) Scale (sternocleidomastoid muscle, deltoid muscle, biceps brachii, triceps brachii, common digital extensor muscle, abductor pollicis brevis muscle, abductor digiti minimi muscle, first dorsal interosseous muscle, and iliopsoas muscle, quadriceps femoris, biceps femoris, tibialis anterior, gastrocnemius muscle, flexor muscle, extensor muscle) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on improving muscle strength.

(3) Observation indicators:

MRC scale: The MRC muscle strength grading method, is an enhanced version of the Lovett grading method, further subdivided by the range of motion and the level of resistance applied. If the measured muscle strength is slightly stronger than a certain level, a "+" is added to the upper right corner of this level. If it is slightly weaker, a "-" is added to the upper right corner. The refinement addresses the limitation of Lovett grading method, providing a more precise assessment of muscle strength.

In this study, the MRC grading is used to measure the muscle strength of the abductor pollicis brevis muscle, abductor digiti minimi muscle and tibialis anterior muscle.

6.6 Electromyography Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180, day 240

(2) Collection content: All subjects will undergo measurements of compound muscle action potential (CMAP), motor unit number index (MUNIX), and motor unit size index (MUSIX) before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the electromyographic parameters of the subjects.

(3) Observation indicators:

CMAP: CAMP represents the summated action potentials of muscle fibers that

are almost simultaneously activated when a certain nerve is directly or indirectly stimulated. The amplitude from baseline to peak, duration, latency of the negative phase of the potential, as well as the details of the stimulation method and recording method should be recorded.

MUNIX: A motor unit is a functional unit located in the anterior horn of the spinal cord, consisting of an alpha motor neuron and all the muscle fibers it innervates. It is the basic unit of motor function, with the minimum unit of normal muscle contraction being the contraction of one motor unit. MUNIX is a sensitive biomarker for monitoring the nerve innervation status of patients with neurodegenerative diseases and is a highly sensitive and effective marker for monitoring the loss of motor units in ALS. The method uses CMAP and the surface electromyographic interference pattern (SIP) to evaluate the number and size of motor units (MUs).

MUSIX: The size of a motor unit is determined by the number of nerve endings and branches of the neuron axon. Generally, larger muscles have larger motor unit. Each muscle contains a certain number of motor units. The calculation method is to divide the amplitude of the compound muscle action potential (CMAP) by the MUNIX value.

6.7 Imaging Indicators

(1) Collection time points: baseline, day 29, day 90, day 180, day 240

(2) Collection content: All subjects will undergo measurement with 7T MRI imaging before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the imaging indicators of the subjects.

(3) Observation indicators:

7T MRI imaging indicators: 7T ultra-high field magnetic resonance is an advanced magnetic resonance imaging system currently available for human body scanning. Compared with the traditional 1.5T and 3T magnetic resonances, 7T magnetic resonance can provide a doubled signal-to-noise ratio, finer spatial resolution, and more excellent tissue contrast. It is more suitable for observing the fine structure of tissues and physiological changes in functional metabolism and other

information, thereby revealing physiological and pathological mechanisms that are difficult to be discovered by traditional platforms.

6.8 Gait Function Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180, day 240

(2) Collection content: All subjects will undergo the following assessments: three-dimensional gait analysis under multitasks, Short Physical Performance Battery (SPPB), item 1 (Tandem Walking) of the Scale for the Assessment and Rating of Ataxia (SARA), and 6-meter walking test before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the gait function of the subjects.

(3) Observation indicators: Semi-quantitative scales include the Short Physical Performance Battery (SPPB) and item 1 (Tandem Walking) of the Scale for the Assessment and Rating of Ataxia (SARA); Quantitative gait assessment includes 6-meter walking test.

SPPB: The SPPB is a widely used method for evaluating muscle function. It consists of 3 items: balance test, 4-meter timed walking test, and timed sit-to-stand test. Each individual test can score a maximum of 4 points, giving a total score of 12 points. To improve the accuracy of the test, each test is usually repeated 2 to 3 times, with the shortest time recorded for scoring.

6-meter normal walking test: It is conducted on an 8-meter walkway with a timing taken over a 6-meter distance. Landmark markers are placed at the start of the walkway, at the 1-meter, the 7-meter, and the 8-meter points, for a total of 4 markers. Timing starts at the 1-meter and stops at the 7-meter mark to avoid the influence of markers on the subject's behavior, such as intentional changes in walking pace or nervousness. The test is performed 4 times; the first trial serves as a practice and is not recorded. The times from the subsequent 3 trials are recorded, and the average of these times is used for analysis.

Item 1: SARA is a clinical scale used for the semi-quantitative assessment of cerebellar ataxia severity. It can also serve as a rehabilitation indicator for gait and independence in daily activities for ataxic stroke patients. In this study, the gait part

(Item 1) is selected. It consists of 8 levels corresponding to scores ranging from 1 to 8. Grade 1 is a mild abnormality corresponding to 1 point, up to grade 8 is unable to walk even with assistance corresponding to 8 points.

6.9 Quality of Life Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180, day 240

(2) Collection content: All subjects will undergo measurements with the Amyotrophic Lateral Sclerosis Self-Assessment Questionnaire (ALSAQ-40), EQ-5D-5L scale, and HAMA and HAMD-17 scales before and after treatment. The results after treatment intervention will be compared with the baseline data to evaluate the effect of RAG-17 on the quality of life of the subjects.

(3) Observation indicators:

ALSAQ-40: ALSAQ-40 is a commonly used quality-of-life scale for ALS. The sections on social interaction and eating ability provide subjective insights into articulation and swallowing functions. ALSAQ-40 consists of 40 items involving the patient's condition, requiring a 5-point scale rating. The questions cover five health status dimensions affected by the disease: physical activity (including 10 items), daily living activities and independence (10 items), eating (3 items), communication (7 items), and emotional function (10 items). Each dimension is scored on a scale from 0 to 100: 0 represents the possible best health status and 100 represents the possible worst health status.

EQ-5D-5L: EQ-5D-5L is a standardized scale for measuring health status, that is an updated version of the EQ-5D scale. The scale consists of two parts: the EQ-5D-5L descriptive system and the visual analogue scale (EQ-VAS). The EQ-5D-5L descriptive system describes five dimensions: Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression. Each dimension contains five levels: 1=No difficulty, 2=Slight difficulty, 3=Moderate difficulty, 4=Severe difficulty, 5=Unable to perform/very severe difficulty. The responses of the five dimensions can generate a five-digit code to describe the health status of the respondent. The health status 1,1,1,1,1 indicates no problems in all five dimensions. EQ-VAS records the self-rated health status of the respondent on a vertical visual analogue scale, with the ends of the

scale marked as "The best health status you can imagine" and "The worst health status you can imagine".

6.10 Clinical Staging Evaluation Indicators

(1) Collection time points: baseline, day 15, day 29, day 90, day 180, day 240

(2) Collection content: All subjects will undergo assessment with the London ALS staging and the Milan-Turin staging (ALS-MITOS) before and after treatment. The results after treatment intervention will be compared with the baseline data to assess the disease progression of ALS patients and evaluate the effect of RAG-17 on the condition of the subjects.

(3) Observation indicators:

London ALS staging: According to the most recently affected region in the course of the disease, ALS is divided into 5 stages: Stage 1 = symptom onset (involvement of the first area); Stage 2A = confirmed diagnosis, and Stage 2B = involvement of the second area; Stage 3 = involvement of the third area; Stage 4A = the patient requires percutaneous endoscopic gastrostomy (PEG), and Stage 4B = the patient requires non-invasive ventilation; Stage 5 = death or mechanical ventilation. The average survival time and 5-year survival rate vary across stages.

Milan-Turin staging: The patient's functions are divided into 4 domains by assessing 4 key points (including mobility, swallowing, communication, and respiration). Each domain is scored as either 0 (below the threshold) or 1 (above the threshold). The total score across these domains determines the stage: Stage 0 = no functional loss; Stage 1 = loss of one functional domain; Stage 2 = loss of two functional domains; Stage 3 = loss of three functional domains; Stage 4 = loss of four functional domains; Stage 5 = death or mechanical ventilation.

6.11 Laboratory Examination Data Collection

(1) Collection time points: baseline, day 1, day 15, day 29, day 60, day 120, day 180, day 240

(2) Collection content: All subjects will undergo laboratory tests including blood routine, urine routine, liver function, kidney function, and coagulation tests before and after treatment respectively. The results after treatment intervention will be compared

with the baseline data to evaluate the impact of RAG-17 on the general physical condition of the subjects and assist in determining the occurrence of adverse events and other related events.

All subjects will undergo the detection of SOD1 protein in cerebrospinal fluid, plasma neurofilament light chain protein (NFL), and the pharmacokinetics of RAG-17 in plasma before and after treatment.

6.12 Other Information Collection

(1) Collection time points: baseline, day 1, day 15, day 29, day 60, day 120, day 180, day 240

(2) Collection content: During the study period, all the medications taken by the subjects will be recorded, including the reasons for use, drug names, dosages, and duration of use. All AEs, adverse reactions and their handling processes will also be documented; The timing of tracheostomy, invasive mechanical ventilation, death, and the duration of invasive mechanical ventilation will be recorded; Subjects' medication adherence, cases of withdrawal or exclusion from the study will be recorded.

7. Follow-up Time

Follow-up plan: For all subjects, face-to-face visits will be conducted at baseline, day 1, day 15, day 29, day 60, day 120, day 180, and day 240. They will undergo tests including daily living function, respiratory function, muscle strength, quality of life, gait function, and clinical staging, as well as the collection of adverse events and concomitant medications use. The study is expected to be conducted at Beijing Tiantan Hospital, with a target recruitment of 6 participants, and is planned to be completed within 2 years.

Follow-up content: To evaluate the differences in clinical manifestations of the participants after receiving the RAG-17 intervention at baseline, day 1, day 15, day 29, day 60, day 120, day 180, and day 240 including:

- Laboratory examinations: tests including blood routine, urine routine, liver function, kidney function, coagulation, SOD1 protein in CSF, plasma NFL, and pharmacokinetic of RAG-17 in plasma will be conducted at baseline, day 1, day 15, day 29, day 60, day 120, day 180, and day 240.

- Daily living functions: ALSFRS-R scoring and Modified Norris Scale scoring will be conducted at baseline, day 15, day 29, day 90, day 180, and day 240.
- Respiratory function: FVC evaluations will be conducted at baseline, day 15, day 29, day 90, day 180, and day 240.
- Fatigue severity: FSS assessment will be conducted at baseline, day 15, day 29, day 90, day 180, and day 240.
- Muscle strength test: MRC assessment will be conducted at baseline, day 15, day 29, day 90, day 180, and day 240.
- Electromyogram indicators (abductor pollicis brevis, abductor digiti minimi, and anterior tibial muscle): CMAP, MUNIX, and MUSIX will be collected at baseline, day 15, day 29, day 90, day 180, and day 240.
- Imaging examinations: 7T magnetic resonance imaging will be conducted at baseline, day 29, day 90, day 180, and day 240.
- Gait function: SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking will be conducted at baseline, day 15, day 29, day 90, day 180, and day 240.
- Quality of life: assessments of ALSAQ-40, EQ-5D-5L, HAMA, and HAMD-17 will be conducted at baseline, day 15, day 29, day 90, day 180, and day 240.
- Clinical staging: London ALS staging and Milan-Turin staging assessments will be conducted at baseline, day 15, day 29, day 90, day 180, and day 240.
- Other indicators: information such as vital signs, abnormalities in heart/lung/abdomen examination, neurological examination, electrocardiogram, adverse events, concomitant medications, the time to tracheostomy, invasive mechanical ventilation, death, and the duration of invasive mechanical ventilation of the patients will be collected at baseline, day 1, day 15, day 29, day 60, day 90, day 120, day 180, and day 240.

8. Study Process

8.1 Information Collection and Informed Consent (Visit 1)

Patients who meet the inclusion criteria but do not meet the exclusion criteria will be introduced to the study protocol and purposes by investigator. The patient or their

legal representatives will be provided with complete information about the study, accompanied by corresponding written materials, and a written informed consent form will be signed.

- Collection of medical history: including current medical history, past medical history, history of concomitant medication, history of smoking and alcohol consumption, family history;
- Physical examination: including vital signs, heart/lung/abdomen examination, neurological examination, 12-lead electrocardiogram;
- Laboratory examinations: including blood routine, urine routine, liver function, kidney function, coagulation function, BNP; Blood sample collection for verification of *SOD1* gene mutation sites, plasma neurofilament light chain protein (NFL);
- 7T magnetic resonance imaging;
- Assessment of respiratory function (including FVC)
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Assessment of daily living functions: ALSFRS-R score, Modified Norris Scale score;
- Assessment of fatigue severity: FSS scale score;
- Assessment of muscle strength and electromyogram indicators: MRC assessment (sternocleidomastoid muscle, deltoid muscle, biceps brachii, triceps brachii, common digital extensor muscle, abductor pollicis brevis, abductor digiti minimi, first dorsal interosseous muscle and iliopsoas muscle, quadriceps femoris, biceps femoris, anterior tibial muscle, gastrocnemius muscle, flexor muscle, extensor muscle grading); CMAP, MUNIX, MUSIX;
- Assessment of gait function: SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking;
- Assessment of quality of life: ALSAQ-40 and EQ-5D-5L scale scores;
- Assessment of emotional scale: HAMA and HAMD-17 scores.

8.2 Days 1-4 Post-Enrollment (First Treatment) (Visit 2)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal

injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;

- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the pharmacokinetic analysis of RAG-17 in plasma;
- Record adverse events and concomitant medications:
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 15, face-to-face follow-up.

8.3 Days 15-18 Post-Enrollment (Second Treatment) (Visit 3)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the pharmacokinetic analysis of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP,

MUNIX, MUSIX);

- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- Arrange the follow-up appointment for day 29, face-to-face follow-up.

8.4 Days 29-32 Post-Enrollment (Third Treatment) (Visit 4)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the pharmacokinetic analysis of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver

function, kidney function, and coagulation function;

- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 60, face-to-face follow-up.

8.5 Days 60-63 Post-Enrollment (Fourth Treatment) (Visit 5)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the pharmacokinetic analysis of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 90, face-to-face follow-up.

8.6 Days 90-93 Post-Enrollment (Visit 6)

- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);

- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 120, face-to-face follow-up.

8.7 Days 120-123 after enrollment (Fifth treatment) (Visit 7)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the pharmacokinetic analysis of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.
- Arrange the follow-up appointment for day 180, face-to-face follow-up.

8.8 Days 180-183 Post-Enrollment (Sixth Treatment) (Visit 8)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the pharmacokinetic analysis of RAG-17 in plasma;
- Record adverse events and concomitant medications;

- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;
- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- 7T magnetic resonance imaging;
- Arrange the follow-up appointment for day 240, face-to-face follow-up.

8.9 Days 240-243 after enrollment (Seventh treatment) (Visit 9)

- Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis, including but not limited to NFL, RAG-17, and SOD1;
- Administration: Intrathecally injection of RAG-17 according to the treatment protocol;
- Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the pharmacokinetic analysis of RAG-17 in plasma;
- Record adverse events and concomitant medications;
- Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale

(FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale;

- Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX);
- Assessment of respiratory function (FVC);
- Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking);
- Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging;
- Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function;
- 7T magnetic resonance imaging.

8.10 Study Flowchart

Examination / Treatment	**Screening / Baseline**	Treatment and Follow-up							
		1st Administration	2nd Administration	3rd Administration	4th Administration		5th Administration	6th Administration	6th Administration
		Day 1-4	Day 15-18	Day 29-32	Day 60-63	Day 90-93	Day 120-123	Day 180-183	Day 240-243
Verification of inclusion / exclusion criteria	√								
Sign the informed consent form	√								
Demographic data	√								
Medical history / Personal history / Family history	√								
Height / Weight / Waist circumference / Physical examination	√	√	√	√	√	√	√	√	√
Concomitant medication	√	√	√	√	√	√	√	√	√
Blood pressure / Respiration / Heart rate / Body temperature	√	√	√	√	√	√	√	√	√
Neurological examination	√	√	√	√	√	√	√	√	√
12-lead electrocardiogram	√	√	√	√	√	√	√	√	√
Laboratory examination[1]	√	√	√	√	√		√	√	√
ALSFRS-R score	√		√	√		√		√	√
Modified Norris Scale	√		√	√		√		√	√

Forced vital capacity	√		√	√		√		√	√
Fatigue Severity Scale	√		√	√		√		√	√
Muscle strength / Electromyogram	√		√	√		√		√	√
7T MRI	√			√		√		√	√
SPPB	√		√	√		√		√	√
Tandem Walking	√		√	√		√		√	√
6-meter walking	√		√	√		√		√	√
Three-dimensional gait analysis under multitasking	√		√	√		√		√	√
ALSAQ-40	√		√	√		√		√	√
EQ-5D-5L	√		√	√		√		√	√
Anxiety Scale (HAMA)	√		√	√		√		√	√
Depression Scale (HAMD-17)	√		√	√		√		√	√
London ALS staging	√		√	√		√		√	√
Milan-Turin staging (AKS-MITOS)	√		√	√		√		√	√
Intrathecal administration of RAG-17	√	√	√	√	√		√	√	√
Blood sample collection [2]	√	√	√	√	√		√	√	√
Cerebrospinal fluid sample collection [3]	√	√	√	√	√		√	√	√
Adverse events / Severe adverse events	√	√	√	√	√	√	√	√	√
Study drug adherence	√	√	√	√	√		√	√	√

[1] Laboratory examinations: blood routine, urine routine, liver functions, renal function, coagulation function, BNP (BNP is only examined at baseline). Laboratory tests during treatments 1-7: Conduct laboratory tests before each treatment and 48 hours after each treatment.

[2] Samples are collected at baseline; during the treatments 1 to 7 , samples are collected at 4 hours before each treatment, and 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, and 48 hours after each treatment.

[3] During treatments 1 to 7, samples are collected 15 minutes before each treatment (Collection before drug administration via lumbar puncture, not restricted to 15 minutes).

9. Clinical Evaluation

9.1 Primary Outcomes：

1) Incidence of AEs and SAEs within 240 days after the first injection of RAG-17.
2) Changes in laboratory assessments, vital signs, ALSFRS-R scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological

examination, as well as electrocardiogram within 240 days after the first injection of RAG-17.

9.2 Second Outcomes：

1) Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
2) Changes in plasma NFL levels from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
3) Changes in the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
4) Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

9.3 Exploratory outcomes：

1) Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, day 180, and day 240.
2) Changes in fatigue severity (Fatigue Severity Scale, FSS) at on day 15, day 29, day 90, day 180, and day 240.
3) Changes in muscle strength on day 15, day 29, day 90, day 180, and day 240.
4) Changes in features of electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day90, day 180, and day 240.
5) Changes in 7T MRI imaging features on day 29, day 90, day 180, and day 240.
6) Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, day 180, and day 240.
7) Changes in modified Norris scale scores on day 15, day 29, day 90, day 180, and day 240.

8) Changes in quality of life (including ALSAQ-40、EQ-5D-5L、HAMA and HAMD) on day 15, day 29, day 90, day 180, and day 240.
9) Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, day 180, and day 240.

10. Known and Potential Risks and Benefits, and Risk Management Plan

10.1 Adverse Event Monitoring

All information related to adverse events, whether reported by the participants, identified by the investigators, or detected through physical examinations, laboratory tests, or other methods, should be recorded on the adverse event page of the case report form. These events will be managed and reported in accordance with the relevant regulations. A comprehensive adverse events report will include in the final trial report.

10.2 Adverse Event Definition

10.2.1 Adverse Event (AE)

An adverse event refers to any unfavorable medical occurrence or worsening of a pre-existing condition that arises in a participant during the use of the investigational drug, regardless of whether it has a causal relationship with the investigational drug treatment. Adverse medical occurrences can manifest as clinical symptoms/signs, diseases, or abnormal laboratory findings. AEs include any unfavorable medical occurrences that occur at any stage of the clinical study, including the lead-in or washout periods, even before the investigational drug has been administered to the participant. AEs include both serious and non-serious adverse events.

10.2.2 Serious Adverse Event

An adverse event during the study will be classified as serious, if it meets one or more of the following criteria:

- Results in death
- Immediately life-threatening

Note: Life-threatening refers to an event that poses an immediate risk to the patient's life at the time it occurs, not an event that could result in death if it were to worsen.

• Requires hospitalization or prolongs the current hospitalization

• Results in permanent or severe disability/incapacity, or loss of essential abilities required for normal living

• Leads to congenital anomaly, birth defects, or reproductive issues

• Constitutes a significant medical event that may endanger the subject or necessitate medical intervention to prevent the outcomes listed above.

10.3 Adverse Events Documentation

Non-serious AE: Non-serious AEs of interest occurring during the study period from the start until the end of the study (e.g. renal function impairment/increased serum creatinine, elevated liver function test indicators, upper gastrointestinal discomfort, muscle/bone pain, hypocalcemia, ocular inflammation, arrhythmia, fractures, etc.) and information about drug discontinuation due to SAEs must be documented. Documentation of other non-serious AEs is at the discretion of the investigator .

Serious AE: All SAEs must be documented.

10.4 Assessment of the Relationship Between AEs and the Investigational Drug

The relationship between AEs and the investigational drug will be assessed using a 5-level standard: ①Definitely related; ②Highly likely related; ③Possibly related; ④Unlikely related; ⑤Definitely unrelated; ⑥Unable to determine.

①Definitely related: Matches the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or disappears after dose reduction or drug withdrawal. Reaction reappears upon re-administration.

②Highly likely related: Matches to the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or disappears after dose reduction or drug withdrawal. However, the clinical status of the participants or other reasons may also cause the reaction.

③Possibly related: Matches the known reaction type of the suspected drug. Occurs within a reasonable time frame after administration. Adverse reaction reduces or is not obvious after dose reduction or drug withdrawal. However, the clinical status of the

subject or other reasons can equally explain the reaction.

④Unlikely related: Does not match the known reaction type of the suspected drug. Dose not align with the reasonable time frame after administration. The clinical status of the subject or other reasons may also cause the reaction.

⑤Definitely unrelated: Does not match the known reaction type of the suspected drug. Dose not align with the reasonable time frame after administration. The clinical status of the subject or other reasons can also explain the reaction. Reaction decreases or disappears when clinical symptoms or other causes are ruled out..

⑥Unable to determine.

The incidence of AEs will be calculated and summarized for classifications ① + ② + ③ from each case report form.

10.5 Unexpected Adverse Event

An unexpected adverse event refers to any event not mentioned in the investigator's brochure, or differs in nature and severity from what is described in the investigator's brochure. In the absence of an investigator's brochure, the event is inconsistent with the risk information described in the study protocol or application materials.

10.6 Reporting of Serious Adverse Events

Notify the designated representative of the sponsor immediately. Send the signed and dated relevant pages of the case report form to the sponsor’s representative, along with copies of all examination results and their completion dates. At the same time, ensure the patient privacy is protected by removing all identifiable information from the clinical source document copies provided to the sponsor. Laboratory results should include normal range values.

All SAEs must be reported within 24 hours of occurrence. Record the SAE in the designed section of the CRF. After signing, report it preferably via fax to the monitoring center. The name, address and fax number of the monitoring center will be specified in the clinical trial protocol.

10.7 Risk Control and Management

In this study, the investigational drug RAG-17 may cause adverse reactions such as tremors, abnormal gait, and hind limb weakness. If such actions occur and are deemed

related to the drug, it is recommended to stop the administration, monitor vital signs, conduct auxiliary examinations such as blood routine, coagulation profile, muscle enzymes, liver function, kidney function, blood gas analysis, ultrasound, CT scans. Provide timely symptomatic and supportive treatment based on clinical findings. If necessary, consult with relevant specialists to manage the adverse events effectively. For serious adverse events, it is necessary to ensure airway patency, autonomous respiration, stable blood pressure and heart rate. If necessary, treatment should be carried out in the intensive care unit.

For all adverse events, the investigators should continue to follow up with patients and track any related outcomes (clinical symptoms, signs, laboratory test results) until the related clinical symptoms are completely resolved, the test results return to normal or the condition is stable. This follow-up should persist even if the patient withdraws from the clinical trial. Additionally, the monitoring team may request further investigations as needed. After the termination of the study, the investigators should still pay attention to any serious adverse events that may occur.

10.8 Subjects Benefits

Participation in this clinical study may help alleviate the subject's condition, but it is also possible that the investigational drug will not provide the expected therapeutic effect. In other words, subjects may not gain any direct benefits from participating in this study. However, this study may contribute to the development of new treatment options for patients with similar conditions, offering more choices to others. In addition, subjects may gain valuable information about their conditions through the physical examinations and medical tests conducted during the study.

11. Quality Control and Quality Assurance of the Study

Representatives of the sponsor or collaborators will regularly monitor the clinical study. The scope and nature of supervision will be determined based on considerations such as the clinical study's objectives, purposes, design, complexity, scale, and endpoints.

11.1 Protocol Adherence Management

- Careful selection of participants: When selecting subjects, investigators should

confirm accurate diagnoses, understand common factors leading to dropout, conduct a comprehensive assessment of participants, and perform a pre-evaluation of adherence. Participants with high compliance potential should be carefully screened and selectively included.

- Informed consent process: Before signing the informed consent form, investigators should provide detailed and clear explanations to the subjects and their families in an easy-to-understand manner. This ensure subjects fully understand the investigational drug and the treatment process. Investigators must thoroughly address participants' questions, respect their choices, and encourage confidence in completing the study, which fosters better adherence.
- Optimizing the protocol: The study protocol should be designed to minimize complexity, favoring simple and practical approaches that align with participants' daily lives. A well-designed, scientifically sound, and highly operable protocol will enhance adherence.
- Managing adverse events: If participants experience adverse reactions, adverse events, or serious adverse events during the clinical study, regardless of whether they are related to the investigational drug, researchers must promptly take appropriate treatment measures. Ensuring timely medical intervention safeguards participants' safety, builds trust in the study, and improves adherence.
- Follow-up and reminders: A contact information sheet and visit schedule should be established to remind participants of follow-up visits. For those who miss scheduled visits, researchers should promptly follow up via phone. These efforts help ensure participants remain engaged and adhere to the study protocol.

11.2 Study Monitoring

The primary responsibility of the monitoring team of the collaborators is to assist investigators and the sponsor or collaborators in ensuring the clinical study adheres to the highest standards of ethics, scientific rigor, professionalism, and regulatory compliance. According to the Good Clinical Practice (GCP) principle, the monitoring team should verify the CRF against the original source documents.

The monitoring team will regularly assess the progress of the study, through on-site

visits, letters, or telephone communication. This includes evaluating adherence to the clinical protocol by researchers and participants and addressing urgent issues. Medical monitors will also periodically review participant safety. During these monitoring visits, the monitors will collaborate with the investigators to focus on the following key points (not exhaustive): informed consent of subjects, recruitment and follow-up of subjects, documentation and reporting of serious adverse event, concomitant treatment and data quality.

11.3 Data Verification

The following measures will be taken to ensure the accuracy, consistency, completeness and reliability of the data:

- Regular on-site monitoring of the study;
- CRF summary based on source documents;
- Data management quality control checks;
- Medical review by the sponsor.

The sponsor or designated personnel (such as CRO) will be responsible for the data management in this clinical study. The responsible party will design and distribute CRFs and oversee their monitoring. If the data are incomplete or inconsistent, the responsible party will issue queries for site staff to address and make necessary corrections.

11.4 Personnel Training

The investigators shall ensure adherence to the clinical trial protocol, compliance with the Good Clinical Practice (GCP) guidelines outlined in the ICH, and conformity with applicable laws and regulations during the conduct of the clinical trial.

The investigators should ensure adherence to all study procedures outlined in the clinical trial protocol (including safety principles). The investigator should accurately and clearly provide reliable data and all information required by the clinical trial protocol (e.g., drug overdose, pregnancy) through appropriate methods, such as CRFs, deviation resolution forms (DRFs), and others, in accordance with relevant requirements. Additionally, the investigator must ensure that the monitoring team has direct access to the source documents.

The investigators may appoint individuals deemed suitable to assist in conducting the study. These assistants will help manage the clinical study in accordance with the study protocol. All assistants will be appointed and documented in a timely manner. They will work under the supervision and management of the investigator, who will provide them with a copy of the clinical study protocol and all necessary information. The sponsor or collaborator of the clinical study are accountable to the health authority for ensuring the proper management of the clinical study by employing all appropriate means, such as ethics, compliance with the clinical study protocol, completeness and validity of the data recorded in the CRF.

11.5 Data Management

During the clinical study, the data of the subjects should be collected anonymously in the CRF, with subjects identified only by their number and the initials of their name. In cases where participant identity must be disclosed due to safety or administrative reasons, both the investigator and any involved parties share the responsibility for maintaining confidentiality. The informed consent form allows the subject to agree to authorized personnel from the sponsor or collaborators, ethics committees, and regulatory authorities directly accessing relevant source documents associated with the CRF (e.g., medical records, appointment logs, original laboratory records). These individuals must adhere to professional confidentiality standards and ensure the privacy of all participant personal or medical information.

The medical information obtained in the study should remain confidential and may only be disclosed to third parties with the permission of the informed consent form and the subject's signed authorization form (or separate authorization for the use and disclosure of personal health information) or when permitted or required by law.

For treatment purposes, medical information may be shared with the participant's personal physician or other appropriate healthcare professionals responsible for the participant's well-being.

11.6 Handling of Biological Samples

The biological samples, such as blood and cerebrospinal fluid collected in this study, will be processed (e.g. centrifugation and aliquoting) at Beijing Tiantan Hospital,

Capital Medical University. Samples designed for evaluation will be analyzed at a centralized clinical laboratory with recognized accredited certification. All biological including blood and cerebrospinal fluid will be stored at Beijing Tiantan Hospital, Capital Medical University for 10 years.

12. Data Retention

The reviewed CRF and imaging data will be carefully entered and processed by the data management center and properly stored. Once data entry is completed, the CRF will be stored by the research center.

The study monitor will perform ongoing source data verification to ensure that the key study protocol data (i.e., source data) transcribed on the CRF by authorized on-site personnel are accurate and complete, and that they can be verified against the original source documents.

Source documents are the original records where subject data is first recorded and stored. These include, but are not limited to hospital records, clinical and office charts, laboratory records, memos, subject diaries or assessment checklists, pharmacy dispensing records, data from automated instrument records, verified and certified as accurate and complete copies of audio transcriptions, microfilms, photographic negatives, microfiche or magnetic media, computed tomography, subject files, and records kept in pharmacies, laboratories and medical technology departments related to clinical studies.

The source documents required to verify the validity and completeness of the data transcribed on the CRF must never be deleted or destroyed.

To facilitate source data verification, the investigator and the institution must allow the sponsor to access applicable source documents and reports for direct inspection. This is to support monitoring, sponsor audits, clinical research reviews, and summaries by the management committees or ethics committees. Additionally, appropriate regulatory agencies must be allowed to inspect the study site.

13. Data Safety Monitoring

The clinical study will develop a data safety monitoring plan based on the level of risk involved. All adverse events will be thoroughly documented, appropriately

managed, and followed up until resolved or the condition stabilizes. Serious adverse events and unexpected events will be promptly reported to the ethics committee, regulatory authorities, the sponsor, and the drug regulatory agency in accordance with relevant regulations. The principal investigator will regularly conduct cumulative reviews of all adverse events and, when necessary, convene investigator meetings to assess the risks and benefits of the study. For studies with risks exceeding the minimal threshold, independent data monitors will be assigned to oversee the study data. For high-risk studies, an independent data safety monitoring board will be established to review the accumulated safety and efficacy data and provide recommendations on whether the study should continue.

During the clinical trial, participant data will be collected anonymously in the CRF, with participants identified only by their participant number and the initials of their name. In cases where participant identity must be disclosed due to safety or administrative reasons, both the investigator and the involved parties share the responsibility for maintaining confidentiality. The informed consent form allows participants to consent to the authorized sponsor, ethics committee, and regulatory authorities directly accessing relevant source documents on the CRFs (e.g., medical records, appointment logs, original laboratory records). These individuals must adhere to professional confidentiality standards and maintain the privacy of all participant personal or medical information.

14. Statistical Analysis

- Statistical analysis will be performed with SAS software.
- Demographic, medical, histories and baseline characteristics information will be listed and summarized.
- The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after treatment. Safety evaluation will be analyzed using safety data set. The number of people of events should be recorded in detail and showing the event rate in summary statement. The event rate will be calculated as

the number of patients experiencing the event/ the number of patients enrolled.

- Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 240-day follow-up assessments for all participants, the data were analyzed.

15. Ethical Considerations

The clinical study will adhere to the World Medical Association's Declaration of Helsinki and other relevant regulations. Before the study begins, the trial protocol will be approved by the ethics committee. Before enrollment, the investigator is responsible for providing a complete and comprehensive explanation of the study's purpose, procedures, and potential risks to the participant or their representative. A written informed consent form must be signed, ensuring that participants are aware of their right to withdraw from the study at any time. The informed consent form will be retained as part of the clinical study documentation for future reference. Throughout the study, the personal privacy and data confidentiality of participants will be safeguarded.

Screening and informed consent: The investigator (or designated personnel authorized and responsible by the investigator, in compliance with relevant regulations) will recruit participants in an independent consultation room at Beijing Tiantan Hospital, Capital Medical University, ensuring the privacy and confidentiality of the participants. Participants and their legal representatives should be fully informed about all relevant aspects of the clinical trial, including written information approved by the ethics committee. The investigator should use language or terms that the participant can understand, providing as much information as possible about the study, and allow sufficient time for the participant or their legal representative to consider whether to participate. Before participating in the clinical trial, the patient or their legal representative must discuss the informed consent with the investigator and

sign the informed consent form (including the participant's name and the date). The investigator should provide the participant with a copy of the signed informed consent form.

16. Data Confidentiality

Throughout the entire research and data analysis process, participant information will be strictly protected. The executive committee will disclose the trial results in accordance with management regulations and procedures. Once the database is locked, the trial results will be published as soon as possible. Biostatistical experts involved in the study have the right to access the dataset but will not be able to identify any individual participants. In line with the GCP principles outlined in the ICH guidelines, the monitoring team will verify the CRFs against the original source documents. The informed consent form will include a statement that allows participants to consent to authorized personnel from the sponsor, ethics committee, and regulatory authorities directly accessing relevant source documents on the CRFs (e.g., medical records, appointment logs, original laboratory records). Researchers must adhere to professional confidentiality standards and ensure the confidentiality of all personal and medical information of the participants.

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Summary of protocol amendments

Protocol changes version 1.1 (Feb. 14th, 2023) to version 1.2 (Apr. 26th, 2023)

Protocol V1.1	Protocol V1.2
Secondary purposes 1)To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2)To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3)To evaluate the pharmacokinetics of RAG-17 in plasma and cerebrospinal fluid on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients. 4)To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.	**Secondary purposes** 1)To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2)To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3)To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients. 4)To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.
Secondary outcomes •Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. •Changes in NFL levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. •Pharmacokinetics of RAG-17 in plasma and cerebrospinal fluid on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients. Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS	**Secondary outcomes** •Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. •Changes in NFL levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. •Pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients. Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS

patients.	patients.
Storage condition Stored at room temperature	**Storage condition：** -20°C ± 5°C, stored in a frozen state

Protocol changes version 1.2 (Apr. 26th, 2023) to version 1.3 (Jun. 1st, 2023)

Protocol V1.2	Protocol V1.3
Days 1-4 Post-Enrollment (First Treatment) (Visit 2) Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;	**Days 1-4 Post-Enrollment (First Treatment) (Visit 2)** Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
Days 15-18 Post-Enrollment (Second Treatment) (Visit 3) Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;	**Days 15-18 Post-Enrollment (Second Treatment) (Visit 3)** Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
Days 29-32 Post-Enrollment (Third Treatment) (Visit 4) Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;	**Days 29-32 Post-Enrollment (Third Treatment) (Visit 4)** Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
Days 60-63 Post-Enrollment (Fourth Treatment) (Visit 5) Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;	**Days 60-63 Post-Enrollment (Fourth Treatment) (Visit 5)** Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
Days 120-123 after enrollment (Fifth treatment) (Visit 7) Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4	**Days 120-123 after enrollment (Fifth treatment) (Visit 7)** Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12

days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;	hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
Days 180-183 Post-Enrollment (Sixth Treatment) (Visit 8) Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;	**Days 180-183 Post-Enrollment (Sixth Treatment) (Visit 8)** Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;
Days 240-243 after enrollment (Seventh treatment) (Visit 9) Post-Treatment Sampling: Collect 5 mL of whole blood 15 minutes, 4 hours, 48 hours, and 4 days post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;	**Days 240-243 after enrollment (Seventh treatment) (Visit 9)** Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma;

Protocol changes version 1.3 (Jun. 1st, 2023) to version 2.0 (Aug. 9th, 2023)

Protocol V1.3	Protocol V2.0
Inclusion Criteria (1)Patients permitted by medical personnel who could complete the clinical trial. (2)Age 18~75 years, gender unlimited. (3)Clearly identify ALS patients with SOD1 gene mutations (known SOD1 mutation sites and reported disease progression). (4)Forced vital capacity during screening period ≥ 80% of expected lung capacity. (5)Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology. (6)The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form. (7)The subjects (including male subjects) have	**Inclusion Criteria** (1)Patients permitted by medical personnel who could complete the clinical trial. (2)Age 18~75 years, gender unlimited. (3)Clearly identify ALS patients with SOD1 gene mutations (known SOD1 mutation sites and reported disease progression). (4)Forced vital capacity during screening period ≥ 50% of expected lung capacity. (5)Confirmed or probable diagnosis of familial or sporadic ALS, in accordance with the revised EI Escorial criteria for amyotrophic lateral sclerosis as defined by the World Federation of Neurology. (6)The patient or their legal representative clearly understands and voluntarily agrees to participate, and signs an informed consent form. (7)The subjects (including male subjects) have

<table>
<tr><td>no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum</td><td>no family planning throughout the entire study and within 3 months after, voluntarily take effective contraceptive measures and without any plans to donate sperm or ovum</td></tr>
<tr><td>Route, dosage and schedule of administration
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Protocol changes version 2.0 (Aug. 9th, 2023) to version 2.1 (Oct 12th, 2023)

Protocol V2.0	Protocol V2.1
Primary purposes (1) To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17; (2) To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.	**Primary purposes** (1) To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 240 days after the first injection of RAG-17; ***(2)*** To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 240 days after the first injection of RAG-17.
Exploratory purposes 1)To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90 and day 180. 2)To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180. 3)To evaluate the changes in muscle strength on day 15, day 29, day 90 and day 180. 4)To evaluate the changes in electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>,	**Exploratory purposes** 1)To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, day 180 and day 240. 2)To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, day 180 and day 240. 3)To evaluate the changes in muscle strength on day 15, day 29, day 90, day 180 and day 240. 4)To evaluate the changes in electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>,

and motor unit size index <MUSIX>) on day 29, day 90 and day 180. 5)To evaluate the changes in 7T MRI imaging features on day 29, day 90 and day 180. 6)To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90 and day 180. 7)To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90 and day 180. 8)To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90 and day 180. 9)To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90 and day 180.	and motor unit size index <MUSIX>) on day 29, day 90, day 180 and day 240. 5)To evaluate the changes in 7T MRI imaging features on day 29, day 90, day 180 and day 240. 6)To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, day 180 and day 240. 7)To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, day 180 and day 240. 8)To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, day 180 and day 240. 9)To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, day 180 and day 240.
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Exploratory outcomes •Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90,	**Exploratory outcomes** •Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, day 180

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Storage condition -20°C ± 5°C, stored in a frozen state	**Storage condition：** 2~8°C, stored in refrigeration
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Days 240-243 after enrollment (Seventh treatment) (Visit 9) •Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers	**Days 240-243 after enrollment (Seventh treatment) (Visit 9)** •Pre-Treatment Sampling: Collect whole blood 4 hours before the intrathecal injection of RAG-17, and 5 mL of CSF 15 minutes prior to treatment. These samples are for biomarkers analysis,

analysis, including but not limited to NFL, RAG-17, and SOD1; •Administration: Intrathecally injection of RAG-17 according to the treatment protocol; •Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the analysis of plasma NFL, and pharmacokinetic of RAG-17 in plasma; •Record adverse events and concomitant medications; •Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination; Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function.	including but not limited to NFL, RAG-17, and SOD1; •Administration: Intrathecally injection of RAG-17 according to the treatment protocol; •Post-Treatment Sampling: Collect 5 mL of whole blood 1 hour, 2hours, 4 hours, 6 hours, 12 hours, and 48 hours post-treatment for the pharmacokinetic analysis of RAG-17 in plasma; •Record adverse events and concomitant medications; •Record vital signs, conduct heart/lung/abdomen examination, neurological specialist examination, and electrocardiogram examination; •Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function; •Assessment of scale : ALSFRS-R, Modified Norris Scale, Fatigue Severity Scale (FSS), ALSAQ-40, EQ-5D-5L, HAMD-17, Hamilton Anxiety Scale; •Assessment of muscle strength and electromyogram (MRC grading, CMAP, MUNIX, MUSIX); •Assessment of respiratory function (FVC); •Assessment of gait function (SPPB, SARA Item 1 (Tandem Walking), 6-meter walking test, and three-dimensional gait analysis under multitasking); •Assessment of ALS clinical staging: London ALS staging, Milan-Turin staging; •Conduct laboratory examinations, including blood routine, urine routine, liver function, kidney function, and coagulation function; •7T magnetic resonance imaging.

Supplemental Information_SAP

This statistical analysis plan has been provided by the authors to give readers additional information about the work.

This supplement contains the following items:

No.	Item	Version	Date	Page No.
1	Original statistical analysis plan	V1.0	Feb. 14th, 2023	2 to 10
2	First amendment analysis plan	V1.1	Apr. 26th, 2023	11 to 19
3	Second amendment analysis plan	V1.2	Jun. 1st, 2023	20 to 28
4	Third amendment analysis plan	V2.0	Aug. 9th, 2023	29 to 37
5	Final statistical analysis plan	V2.1	Oct 12th, 2023	38 to 46
6	Summary of statistical analysis plan amendments	-	-	47 to 52

Safety and Tolerance of RAG-17 in Amyotrophic Lateral Sclerosis Patients With *SOD1* Gene Mutation (CREATION)

Statistical Analysis Plan

Principal Investigator

Yilong Wang

Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Prepared by

Yuesong Pan, PhD

Xuan Wang, MM

Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Version 1.0

Feb. 14th, 2023

1. Introduction

This statistical analysis plan (SAP) documents the planned statistical analyses for the CREATION study and is based on the protocol, together with any subsequent amendments.

This SAP is intended for the use of project team members and should be read in conjunction with the aforementioned protocol.

2. Study Purposes

The primary purposes of the study are:

1) To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17;
2) To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

The secondary purposes of the study are:

1) To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
2) To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
3) To evaluate the pharmacokinetics of RAG-17 in plasma and cerebrospinal fluid on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients.
4) To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

The exploratory purposes of the study are:

1) To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180.
2) To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180.
3) To evaluate the changes in muscle strength on day 15, day 29, day 90, and day 180.
4) To evaluate the changes in electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day 90, and day 180.
5) To evaluate the changes in 7T MRI imaging features on day 29, day 90, and day 180.
6) To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180.
7) To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, and day 180.
8) To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, and day 180.
9) To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.

3. Study Outcomes

Primary outcome:

- Incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17.

- Changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

Secondary outcomes:

- Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
- Changes in NFL levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
- Pharmacokinetics of RAG-17 in plasma and cerebrospinal fluid on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients.
- Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

Exploratory outcomes:

- Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180.
- Changes in fatigue severity (FSS) on day 15, day 29, day 90, and day 180.
- Changes in muscle strength on day 15, day 29, day 90, and day 180.
- Changes in electromyography (including CMAP, MUNIX and MUSIX) on day 29, day 90, and day 180.
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- Changes in quality of life (including ALSAQ-40, EQ-5D-5L, HAMA and HAMD) on day 15, day 29, day 90, and day 180.
- Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.

4. Statistical Hypotheses

This was a phase 1 exploratory safety, tolerability, pharmacokinetics (PK), and preliminary efficacy study. The primary outcome for this study includes 1) the incidence of AEs and SAEs within 180 days after the first injection of RAG-17, 2) the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

This is an exploratory, hypothesis-generating study, and the target enrollment was not based on formal statistical power calculations, so there is no test hypothesis.

5. Design

Study design

- An ongoing open-label, single-center, first-in-human, and dose escalation clinical trial
- The trial is intended to enroll 6 participants and complete follow-up of all subjects within 240 days.
- A total of 7 doses will be administered on Day 1, Day 15, Day 29, Day 60, Day 120, Day 180, and Day 240. The drug will be administered via intrathecal injection of RAG-17, with doses of 60 mg per administration, 90 mg per administration, 120 mg per administration, DLT per administration, with the last three doses being the optimal dose. The treatment period of this study is 8 months. Various indicators, adverse events, and concomitant medications will be collected on Day 1, Day 15, Day 29, Day 60, Day 90, and Day 180 post-treatment. .

Dosing regimen of CREATION trial:

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	DLT/dose	Intrathecal injection	6
5th Administration	D120	Optimal dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

The dose of RAG-17 will range from a minimum of 60 mg to the maximum tolerated dose (MTD). Administration will be conducted once every two weeks, starting at 60 mg, for dose escalation. Once the tolerated dose is reached, a fixed dose of the drug will be administered every two months for continued treatment, with a total treatment duration of 8 months. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD. For the subsequent maintenance treatment phase, the optimal dose between the safe dose and the MTD will be selected as the dose for continued treatment.

Follow-up plan

The first 3 patients will receive a face-to-face visit at baseline, day 1~4, day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243, while the last 3 patients will be seen at day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243. Venous blood samples will be collected at 4 hours before, and 15 minutes, 4 hours, 48 hours and 4 days after each injection of RAG-17 for assessment of pharmacokinetics. Cerebrospinal fluid will be collected from all subjects 15 minutes before and 15 minutes after each injection of RAG-17.

Planned Analyses

The analyses that are detailed in this SAP will be performed only when the database

has been locked, all protocol violators identified. Membership of the Full Analysis and Per Protocol populations will be determined using the rules set out in this SAP. At a date to be agreed within the project team, a data look will be performed. This will involve production of all data displays on a subset of the data using dummy treatment codes. These are produced purely as an aide to the pre-programming of the study.

Interim Analyses

No interim analyses are planned for this study. However, a Data and Safety Monitoring Board (DSMB) is in place to ensure the safety of patients in the study. An independent statistician will prepare summary tables of SAEs, selected demographic data and patients exposure data and these will be examined by the DSMB. These tables will be provided to the DSMB at regular intervals. If the tables give rise to safety concerns for any treatment, the DSMB may recommend that the trial should be modified or stopped prematurely. The Steering Committee will, in conjunction with the Sponsor, decide whether to act on this recommendation. Further discussion of these safety tabulations is provided in a specific study protocol.

6. Sample size

This is an exploratory, hypothesis-generating study and a dose escalation clinical study to explore the maximum tolerated dose, so total of 6 cases are required for sample size estimation.

7. General considerations for data analyses

All programming will be performed using SAS Version 9.4.

8. Data handling conventions

Premature Withdrawal and Missing Data

1) Loss to follow-up

Investigators should try their best to keep contact with every patient, making sure the reason of loss to follow-up and their health situation. All CRFs of missed patients should be recorded until the last follow-up visit.

2) Quit from study

Those who quit the study should not be included again. Investigators should confirm the withdrawal along with the monitoring committee. Subjects who had one or more doses recorded were required to complete the calendar visits according to the protocol.

Event Rates

The number of people of events should be recorded in detail and showing the event rate in 180 days in summary statement.

The event rate will be calculated as: the sum of number of event for all the patients / the number of patients enrolled.

9. Study Population

Disposition of Patients

The number of patients in each analysis population will be presented, patients to be excluded from the Per Protocol population will be listed, and the total number of patients attending each clinic visit will also be summarized by treatment group.

The number of patients enrolled, completed and prematurely withdrawn from the study will be presented for each treatment group. The primary reasons for withdrawal will also be presented.

A data display listing and summary of deviations from the inclusion/exclusion criteria will be presented for all patients who were either entered into the trial.

Protocol Deviations

Patient data will be examined for evidence of protocol violators in order to assess how well the protocol was followed. Inclusion and exclusion criteria are detailed in the study protocol.

Patients who commit protocol violations will be included in the full analysis set (FAS) Population but excluded from the Per Protocol Population. These protocol violations will be shown in a listing. Patients can either be full or partial protocol

violators. A full protocol violator is completely excluded from the Per Protocol Population. A partial protocol violator has only some data excluded. For patients who violated the protocol during the treatment period due to unpermitted changes in the medication or prohibited concurrent medication, the analysis will only use data recorded prior to the violation. For all violations which reference the treatment period, the treatment start date will be used as the reference date.

A listing of all possible protocol violators will be produced for clinical review. The final list of patients who are protocol violators and are therefore excluded from the Per-Protocol population will be agreed by the study team.

Demographic and Baseline Characteristics

Analysis was conducted on an intention-to-treat population, which was defined as all subjects in whom was treated. Demographic, medical, histories and baseline characteristics information will be listed and summarized.

10. Safety and Efficacy Analyses

The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after treatment. Safety evaluation will be analyzed using safety data set.

Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). Following the completion of the 180-day follow-up assessments for all participants, the data were analyzed.

Safety and Tolerance of RAG-17 in Amyotrophic Lateral Sclerosis Patients With *SOD1* Gene Mutation (CREATION)

Statistical Analysis Plan

Principal Investigator

Yilong Wang

Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Prepared by

Yuesong Pan, PhD

Xuan Wang, MM

Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Version 1.1

Apr. 26th, 2023

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This statistical analysis plan (SAP) documents the planned statistical analyses for the CREATION study and is based on the protocol, together with any subsequent amendments.

This SAP is intended for the use of project team members and should be read in conjunction with the aforementioned protocol.

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9) To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, and day 180.

3. Study Outcomes

Primary outcome:

- Incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17.

- Changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

Secondary outcomes:

- Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
- Changes in NFL levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
- Pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients.
- Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

Exploratory outcomes:

- Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180.
- Changes in fatigue severity (FSS) on day 15, day 29, day 90, and day 180.
- Changes in muscle strength on day 15, day 29, day 90, and day 180.
- Changes in electromyography (including CMAP, MUNIX and MUSIX) on day 29, day 90, and day 180.
- Changes in 7T MRI imaging features on day 29, day 90, and day 180.
- Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180.
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4. Statistical Hypotheses

This was a phase 1 exploratory safety, tolerability, pharmacokinetics (PK), and preliminary efficacy study. The primary outcome for this study includes 1) the incidence of AEs and SAEs within 180 days after the first injection of RAG-17, 2) the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

This is an exploratory, hypothesis-generating study, and the target enrollment was not based on formal statistical power calculations, so there is no test hypothesis.

5. Design

Study design

- An ongoing open-label, single-center, first-in-human, and dose escalation clinical trial
- The trial is intended to enroll 6 participants and complete follow-up of all subjects within 240 days.
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Dosing regimen of CREATION trial:

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
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The dose of RAG-17 will range from a minimum of 60 mg to the maximum tolerated dose (MTD). Administration will be conducted once every two weeks, starting at 60 mg, for dose escalation. Once the tolerated dose is reached, a fixed dose of the drug will be administered every two months for continued treatment, with a total treatment duration of 8 months. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD. For the subsequent maintenance treatment phase, the optimal dose between the safe dose and the MTD will be selected as the dose for continued treatment.

Follow-up plan

The first 3 patients will receive a face-to-face visit at baseline, day 1~4, day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243, while the last 3 patients will be seen at day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243. Venous blood samples will be collected at 4 hours before, and 15 minutes, 4 hours, 48 hours and 4 days after each injection of RAG-17 for assessment of pharmacokinetics. Cerebrospinal fluid will be collected from all subjects 15 minutes before each injection of RAG-17.

Planned Analyses

The analyses that are detailed in this SAP will be performed only when the database

has been locked, all protocol violators identified. Membership of the Full Analysis and Per Protocol populations will be determined using the rules set out in this SAP. At a date to be agreed within the project team, a data look will be performed. This will involve production of all data displays on a subset of the data using dummy treatment codes. These are produced purely as an aide to the pre-programming of the study.

Interim Analyses

No interim analyses are planned for this study. However, a Data and Safety Monitoring Board (DSMB) is in place to ensure the safety of patients in the study. An independent statistician will prepare summary tables of SAEs, selected demographic data and patients exposure data and these will be examined by the DSMB. These tables will be provided to the DSMB at regular intervals. If the tables give rise to safety concerns for any treatment, the DSMB may recommend that the trial should be modified or stopped prematurely. The Steering Committee will, in conjunction with the Sponsor, decide whether to act on this recommendation. Further discussion of these safety tabulations is provided in a specific study protocol.

6. Sample size

This is an exploratory, hypothesis-generating study and a dose escalation clinical study to explore the maximum tolerated dose, so total of 6 cases are required for sample size estimation.

7. General considerations for data analyses

All programming will be performed using SAS Version 9.4.

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Premature Withdrawal and Missing Data

1) Loss to follow-up

Investigators should try their best to keep contact with every patient, making sure the reason of loss to follow-up and their health situation. All CRFs of missed patients should be recorded until the last follow-up visit.

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Those who quit the study should not be included again. Investigators should confirm the withdrawal along with the monitoring committee. Subjects who had one or more doses recorded were required to complete the calendar visits according to the protocol.

Event Rates

The number of people of events should be recorded in detail and showing the event rate in 180 days in summary statement.

The event rate will be calculated as: the sum of number of event for all the patients / the number of patients enrolled.

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Disposition of Patients

The number of patients in each analysis population will be presented, patients to be excluded from the Per Protocol population will be listed, and the total number of patients attending each clinic visit will also be summarized by treatment group.

The number of patients enrolled, completed and prematurely withdrawn from the study will be presented for each treatment group. The primary reasons for withdrawal will also be presented.

A data display listing and summary of deviations from the inclusion/exclusion criteria will be presented for all patients who were either entered into the trial.

Protocol Deviations

Patient data will be examined for evidence of protocol violators in order to assess how well the protocol was followed. Inclusion and exclusion criteria are detailed in the study protocol.

Patients who commit protocol violations will be included in the full analysis set (FAS) Population but excluded from the Per Protocol Population. These protocol violations will be shown in a listing. Patients can either be full or partial protocol

violators. A full protocol violator is completely excluded from the Per Protocol Population. A partial protocol violator has only some data excluded. For patients who violated the protocol during the treatment period due to unpermitted changes in the medication or prohibited concurrent medication, the analysis will only use data recorded prior to the violation. For all violations which reference the treatment period, the treatment start date will be used as the reference date.

A listing of all possible protocol violators will be produced for clinical review. The final list of patients who are protocol violators and are therefore excluded from the Per-Protocol population will be agreed by the study team.

Demographic and Baseline Characteristics

Analysis was conducted on an intention-to-treat population, which was defined as all subjects in whom was treated. Demographic, medical, histories and baseline characteristics information will be listed and summarized.

10. Safety and Efficacy Analyses

The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after treatment. Safety evaluation will be analyzed using safety data set.

Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 180-day follow-up assessments for all participants, the data were analyzed.

Safety and Tolerance of RAG-17 in Amyotrophic Lateral Sclerosis Patients With *SOD1* Gene Mutation (CREATION)

Statistical Analysis Plan

Principal Investigator

Yilong Wang

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Version 1.2

Jue. 1st, 2023

1. Introduction

This statistical analysis plan (SAP) documents the planned statistical analyses for the CREATION study and is based on the protocol, together with any subsequent amendments.

This SAP is intended for the use of project team members and should be read in conjunction with the aforementioned protocol.

2. Study Purposes

The primary purposes of the study are:

1) To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17;
2) To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.

The secondary purposes of the study are:

1) To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
2) To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
3) To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients.
4) To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

The exploratory purposes of the study are:

1) To evaluate the changes in pulmonary function (including forced vital capacity) on

day 15, day 29, day 90, and day 180.

2) To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180.
3) To evaluate the changes in muscle strength on day 15, day 29, day 90, and day 180.
4) To evaluate the changes in electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day 90, and day 180.
5) To evaluate the changes in 7T MRI imaging features on day 29, day 90, and day 180.
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7) To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, and day 180.
8) To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, and day 180.
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Statistical Analysis Plan

Principal Investigator

Yilong Wang

Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Prepared by

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Xuan Wang, MM

Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Version 2.0

Aug. 9th, 2023

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This statistical analysis plan (SAP) documents the planned statistical analyses for the CREATION study and is based on the protocol, together with any subsequent amendments.

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The primary purposes of the study are:

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The secondary purposes of the study are:

1) To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
2) To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
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4th Administration	D60	120 mg/dose	Intrathecal injection	6
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6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

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Follow-up plan

The first 3 patients will receive a face-to-face visit at baseline, day 1~4, day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243, while the last 3 patients will be seen at day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243. Venous blood samples will be collected at 4 hours before, and 1 hours, 2 hours, 4 hours, 6 hours, 12 hours and 48 hours after each injection of RAG-17 for assessment of pharmacokinetics. Cerebrospinal fluid will be collected from all subjects 15 minutes before each injection of RAG-17.

Planned Analyses

The analyses that are detailed in this SAP will be performed only when the database has been locked, all protocol violators identified. Membership of the Full Analysis and Per Protocol populations will be determined using the rules set out in this SAP. At a date to be agreed within the project team, a data look will be performed. This will involve production of all data displays on a subset of the data using dummy treatment codes. These are produced purely as an aide to the pre-programming of the study.

Interim Analyses

No interim analyses are planned for this study. However, a Data and Safety Monitoring Board (DSMB) is in place to ensure the safety of patients in the study. An independent statistician will prepare summary tables of SAEs, selected demographic data and patients exposure data and these will be examined by the DSMB. These tables will be provided to the DSMB at regular intervals. If the tables give rise to safety concerns for any treatment, the DSMB may recommend that the trial should be modified or stopped prematurely. The Steering Committee will, in conjunction with the Sponsor, decide whether to act on this recommendation. Further discussion of these safety tabulations is provided in a specific study protocol.

6. Sample size

This is an exploratory, hypothesis-generating study and a dose escalation clinical study to explore the maximum tolerated dose, so total of 6 cases are required for sample size estimation.

7. General considerations for data analyses

All programming will be performed using SAS Version 9.4.

8. Data handling conventions

Premature Withdrawal and Missing Data

1) Loss to follow-up

Investigators should try their best to keep contact with every patient, making sure the reason of loss to follow-up and their health situation. All CRFs of missed patients should be recorded until the last follow-up visit.

2) Quit from study

Those who quit the study should not be included again. Investigators should confirm the withdrawal along with the monitoring committee. Subjects who had one or more doses recorded were required to complete the calendar visits according to the protocol.

Event Rates

The number of people of events should be recorded in detail and showing the event rate in 180 days in summary statement.

The event rate will be calculated as: the sum of number of event for all the patients / the number of patients enrolled.

9. Study Population

Disposition of Patients

The number of patients in each analysis population will be presented, patients to be excluded from the Per Protocol population will be listed, and the total number of patients attending each clinic visit will also be summarized by treatment group.

The number of patients enrolled, completed and prematurely withdrawn from the study will be presented for each treatment group. The primary reasons for withdrawal will also be presented.

A data display listing and summary of deviations from the inclusion/exclusion criteria will be presented for all patients who were either entered into the trial.

Protocol Deviations

Patient data will be examined for evidence of protocol violators in order to assess how well the protocol was followed. Inclusion and exclusion criteria are detailed in the study protocol.

Patients who commit protocol violations will be included in the full analysis set (FAS) Population but excluded from the Per Protocol Population. These protocol violations will be shown in a listing. Patients can either be full or partial protocol violators. A full protocol violator is completely excluded from the Per Protocol

Population. A partial protocol violator has only some data excluded. For patients who violated the protocol during the treatment period due to unpermitted changes in the medication or prohibited concurrent medication, the analysis will only use data recorded prior to the violation. For all violations which reference the treatment period, the treatment start date will be used as the reference date.

A listing of all possible protocol violators will be produced for clinical review. The final list of patients who are protocol violators and are therefore excluded from the Per-Protocol population will be agreed by the study team.

Demographic and Baseline Characteristics

Analysis was conducted on an intention-to-treat population, which was defined as all subjects in whom was treated. Demographic, medical, histories and baseline characteristics information will be listed and summarized.

10. Safety and Efficacy Analyses

The overall of safety assessment is all used test medications and the safety follow-up case should be recorded at least once. The safety evaluation data includes adverse reactions observed during the trial and changes in laboratory data before and after treatment. Safety evaluation will be analyzed using safety data set.

Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 180-day follow-up assessments for all participants, the data were analyzed.

Safety and Tolerance of RAG-17 in Amyotrophic Lateral Sclerosis Patients With *SOD1* Gene Mutation (CREATION)

Statistical Analysis Plan

Principal Investigator

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Prepared by

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Version 2.1

Oct. 12th, 2023

1. Introduction

This statistical analysis plan (SAP) documents the planned statistical analyses for the CREATION study and is based on the protocol, together with any subsequent amendments.

This SAP is intended for the use of project team members and should be read in conjunction with the aforementioned protocol.

2. Study Purposes

The primary purposes of the study are:

1) To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 240 days after the first injection of RAG-17;
2) To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 240 days after the first injection of RAG-17.

The secondary purposes of the study are:

1) To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
2) To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
3) To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients.
4) To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

The exploratory purposes of the study are:

1) To evaluate the changes in pulmonary function (including forced vital capacity) on

day 15, day 29, day 90, day 180 and day 240.

2) To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, day 180 and day 240.
3) To evaluate the changes in muscle strength on day 15, day 29, day 90, day 180 and day 240.
4) To evaluate the changes in electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day 90, day 180 and day 240.
5) To evaluate the changes in 7T MRI imaging features on day 29, day 90, day 180 and day 240.
6) To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, day 180 and day 240.
7) To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, day 180 and day 240.
8) To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety Scale <HAMA> and Hamilton Depression Scale <HAMD>) on day 15, day 29, day 90, day 180 and day 240.
9) To evaluate the changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, day 180 and day 240.

3. Study Outcomes

Primary outcome:

- Incidence of adverse events (AEs) and serious adverse events (SAEs) within 240 days after the first injection of RAG-17.
- Changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and

abdomen examinations, neurological examination, as well as electrocardiogram within 240 days after the first injection of RAG-17.

Secondary outcomes:

- Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
- Changes in NFL levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients.
- Pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients.
- Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.

Exploratory outcomes:

- Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, day 180 and day 240.
- Changes in fatigue severity (FSS) on day 15, day 29, day 90, day 180 and day 240.
- Changes in muscle strength on day 15, day 29, day 90, day 180 and day 240.
- Changes in electromyography (including CMAP, MUNIX and MUSIX) on day 29, day 90, day 180 and day 240.
- Changes in 7T MRI imaging features on day 29, day 90, day 180 and day 240.
- Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, day 180 and day 240.
- Changes in modified Norris scale scores on day 15, day 29, day 90, day 180 and day 240.
- Changes in quality of life (including ALSAQ-40, EQ-5D-5L, HAMA and HAMD)

on day 15, day 29, day 90, day 180 and day 240.

- Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, day 180 and day 240.

4. Statistical Hypotheses

This was a phase 1 exploratory safety, tolerability, pharmacokinetics (PK), and preliminary efficacy study. The primary outcome for this study includes 1) the incidence of AEs and SAEs within 240 days after the first injection of RAG-17, 2) the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 240 days after the first injection of RAG-17.

This is an exploratory, hypothesis-generating study, and the target enrollment was not based on formal statistical power calculations, so there is no test hypothesis.

5. Design

Study design

- An ongoing open-label, single-center, first-in-human, and dose escalation clinical trial
- The trial is intended to enroll 6 participants and complete follow-up of all subjects within 240 days.
- A total of 7 doses will be administered on Day 1, Day 15, Day 29, Day 60, Day 120, Day 180, and Day 240. The drug will be administered via intrathecal injection of RAG-17, with doses of 60 mg per administration, 90 mg per administration, 120 mg per administration, 150 mg per administration, 150 or 180 (no more than 180) mg per administration, with the last two doses being the optimal dose. The treatment period of this study is 8 months. Various indicators, adverse events, and concomitant medications will be collected on Day 1, Day 15, Day 29, Day 60, Day 90, and Day 180 post-treatment.

Dosing regimen of CREATION trial:

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	150 mg/dose	Intrathecal injection	6
5th Administration	D120	150/180 (no more than 180) mg/dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

The dose of RAG-17 will range from a minimum of 60 mg to the maximum tolerated dose (MTD). The first three participants begin at a dose of 60 mg, while the last three participants start at 90 mg, for dose escalation. Once the tolerated dose is reached, a fixed dose of the drug will be administered every two months for continued treatment, with a total treatment duration of 8 months. The MTD in this study will be determined based on the safety and efficacy (pharmacodynamic indicators) data from the currently explored doses, after discussion between the investigators and the sponsor, with the lower dose that results in DLT selected as the MTD. For the subsequent maintenance treatment phase, the optimal dose between the safe dose and the MTD will be selected. The dose for subsequent maintenance treatment should be ≤ MTD.

Follow-up plan

The first 3 patients will receive a face-to-face visit at baseline, day 1~4, day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243, while the last 3 patients will be seen at day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243. Venous blood samples will be collected at 4 hours before, and 1 hours, 2 hours, 4 hours, 6 hours, 12 hours and 48 hours after each injection of RAG-17 for assessment of pharmacokinetics. Cerebrospinal fluid will be collected from all subjects 15 minutes before each injection of RAG-17.

Planned Analyses

The analyses that are detailed in this SAP will be performed only when the database has been locked, all protocol violators identified. Membership of the Full Analysis and Per Protocol populations will be determined using the rules set out in this SAP. At a date to be agreed within the project team, a data look will be performed. This will involve production of all data displays on a subset of the data using dummy treatment codes. These are produced purely as an aide to the pre-programming of the study.

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Investigators should try their best to keep contact with every patient, making sure the reason of loss to follow-up and their health situation. All CRFs of missed patients

should be recorded until the last follow-up visit.

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Those who quit the study should not be included again. Investigators should confirm the withdrawal along with the monitoring committee. Subjects who had one or more doses recorded were required to complete the calendar visits according to the protocol.

Event Rates

The number of people of events should be recorded in detail and showing the event rate in 180 days in summary statement.

The event rate will be calculated as: the sum of number of event for all the patients / the number of patients enrolled.

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Disposition of Patients

The number of patients in each analysis population will be presented, patients to be excluded from the Per Protocol population will be listed, and the total number of patients attending each clinic visit will also be summarized by treatment group.

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Analysis was conducted on an intention-to-treat population, which was defined as all subjects in whom was treated. Demographic, medical, histories and baseline characteristics information will be listed and summarized.

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Given the exploratory design and limited sample size of this research, all safety and efficacy analyses were conducted in a descriptive manner. For categorical variables, data are reported as frequencies and corresponding percentages. Continuous variables are summarized as mean ± SD/SE or median (25th-75th percentiles). No missing data imputation techniques were applied in the analysis. Following the completion of the 240-day follow-up assessments for all participants, the data were analyzed.

Summary of statistical analysis plan amendments

SAP changes version 1.0 (Feb 14th, 2023) to version 1.1 (Apr. 26th, 2023)

SAP V1.0	SAP V1.1
The secondary purposes of the study 1)To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2)To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3)To evaluate the pharmacokinetics of RAG-17 in plasma and cerebrospinal fluid on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients. 4)To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.	***The secondary purposes of the study*** 1)To evaluate the changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 2)To evaluate the changes in neurofilament light chain (NFL) levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. 3)To evaluate the pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients. 4)To evaluate the time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.
Secondary outcomes • Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. • Changes in NFL levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. • Pharmacokinetics of RAG-17 in plasma and cerebrospinal fluid on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients.	***Secondary outcomes*** • Changes in SOD1 protein levels in cerebrospinal fluid from baseline, on day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. • Changes in NFL levels in plasma from baseline, on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the first injection of RAG-17 in adult ALS patients. • Pharmacokinetics of RAG-17 in plasma on day 1, day 15, day 29, day 60, day 120, day 180, and day 240 after the injection of RAG-17 in adult ALS patients.

Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.	Time to invasive mechanical ventilation or death after the first injection of RAG-17 in ALS patients.
Follow-up plan The first 3 patients will receive a face-to-face visit at baseline, day 1~4, day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243, while the last 3 patients will be seen at day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243. Venous blood samples will be collected at 4 hours before, and 15 minutes, 4 hours, 48 hours and 4 days after each injection of RAG-17 for assessment of pharmacokinetics. Cerebrospinal fluid will be collected from all subjects 15 minutes before and 15 minutes after each injection of RAG-17.	***Follow-up plan*** The first 3 patients will receive a face-to-face visit at baseline, day 1~4, day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243, while the last 3 patients will be seen at day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243. Venous blood samples will be collected at 4 hours before, and 15 minutes, 4 hours, 48 hours and 4 days after each injection of RAG-17 for assessment of pharmacokinetics. Cerebrospinal fluid will be collected from all subjects 15 minutes before each injection of RAG-17.

SAP changes version 1.1 (Apr. 26th, 2023) to version 1.2 (Jun. 1st, 2023)

SAP V1.1	SAP V1.2
Follow-up plan The first 3 patients will receive a face-to-face visit at baseline, day 1~4, day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243, while the last 3 patients will be seen at day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243. Venous blood samples will be collected at 4 hours before, and 15 minutes, 4 hours, 48 hours and 4 days after each injection of RAG-17 for assessment of pharmacokinetics. Cerebrospinal fluid will be collected from all subjects 15 minutes before each injection of RAG-17.	***Follow-up plan*** The first 3 patients will receive a face-to-face visit at baseline, day 1~4, day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243, while the last 3 patients will be seen at day 15~18, day 29~32, day 60~63, day 90~93, day 120~123, day 180~183 and day 240~243. Venous blood samples will be collected at 4 hours before, and 1 hours, 2 hours, 4 hours, 6 hours, 12 hours and 48 hours after each injection of RAG-17 for assessment of pharmacokinetics. Cerebrospinal fluid will be collected from all subjects 15 minutes before each injection of RAG-17.

SAP changes version 1.2 (Apr. 26th, 2023) to version 2.0 (Aug. 9th, 2023)

SAP V1.2

Dosing regimen of CREATION trial

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	DLT/dose	Intrathecal injection	6
5th Administration	D120	Optimal dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

SAP V2.0

Dosing regimen of CREATION trial

Treatment	Administration Day	Dose Level	Administration Route	Number of Subjects
1st Administration	D1	60 mg/dose	Intrathecal injection	3
2nd Administration	D15	90 mg/dose	Intrathecal injection	6
3rd Administration	D29	120 mg/dose	Intrathecal injection	6
4th Administration	D60	120 mg/dose	Intrathecal injection	6
5th Administration	D120	150/180(no more than 180) mg/dose	Intrathecal injection	6
6th Administration	D180	Optimal dose	Intrathecal injection	6
7th Administration	D240	Optimal dose	Intrathecal injection	6

SAP changes version 2.0 (Aug. 9th, 2023) to version 2.1 (Oct 12th, 2023)

SAP V2.0	SAP V2.1
The primary purposes of the study 1)To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17;	***The primary purposes of the study*** 1)To evaluate the incidence of adverse events (AEs) and serious adverse events (SAEs) within 240 days after the first injection of RAG-17; To evaluate the changes in laboratory

To evaluate the changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.	assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 240 days after the first injection of RAG-17.
The exploratory purposes of the study 1)To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90 and day 180. 2)To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, and day 180. 3)To evaluate the changes in muscle strength on day 15, day 29, day 90 and day 180. 4)To evaluate the changes in electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day 90 and day 180. 5)To evaluate the changes in 7T MRI imaging features on day 29, day 90 and day 180. 6)To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90 and day 180. 7)To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90 and day 180. 8)To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions Questionnaire <EQ-5D-5L>, Hamilton Anxiety	***The exploratory purposes of the study*** 1)To evaluate the changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, day 180 and day 240. 2)To evaluate the changes in fatigue severity (Fatigue Severity Scale, FSS) on day 15, day 29, day 90, day 180 and day 240. 3)To evaluate the changes in muscle strength on day 15, day 29, day 90, day 180 and day 240. 4)To evaluate the changes in electromyography (including composite muscle action potential <CMAP>, motor unit number index <MUNIX>, and motor unit size index <MUSIX>) on day 29, day 90, day 180 and day 240. 5)To evaluate the changes in 7T MRI imaging features on day 29, day 90, day 180 and day 240. 6)To evaluate the changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, day 180 and day 240. 7)To evaluate the changes in modified Norris scale scores on day 15, day 29, day 90, day 180 and day 240. 8)To evaluate the changes in quality of life (including Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 <ALSAQ-40>, Five-level version of EuroQol Five Dimensions

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Primary outcome • Incidence of adverse events (AEs) and serious adverse events (SAEs) within 180 days after the first injection of RAG-17. • Changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 180 days after the first injection of RAG-17.	***Primary outcome*** • Incidence of adverse events (AEs) and serious adverse events (SAEs) within 240 days after the first injection of RAG-17. • Changes in laboratory assessments, vital signs, ALS Functional Rating Scale-Revised (ALSFRS-R) scores, incidence of abnormalities in heart, lung and abdomen examinations, neurological examination, as well as electrocardiogram within 240 days after the first injection of RAG-17.
Exploratory outcomes • Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, and day 180. • Changes in fatigue severity (FSS) on day 15, day 29, day 90, and day 180. • Changes in muscle strength on day 15, day 29, day 90, and day 180. • Changes in electromyography (including CMAP, MUNIX and MUSIX) on day 29, day 90, and day 180. • Changes in 7T MRI imaging features on day 29, day 90, and day 180. • Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, and day 180. • Changes in modified Norris scale scores on	***Exploratory outcomes*** • Changes in pulmonary function (including forced vital capacity) on day 15, day 29, day 90, day 180 and day 240. • Changes in fatigue severity (FSS) on day 15, day 29, day 90, day 180 and day 240. • Changes in muscle strength on day 15, day 29, day 90, day 180 and day 240. • Changes in electromyography (including CMAP, MUNIX and MUSIX) on day 29, day 90, day 180 and day 240. • Changes in 7T MRI imaging features on day 29, day 90, day 180 and day 240. • Changes in gait function (including 3D gait analysis, short physical performance battery, tandem walking, and 6-meter walk test) on day 15, day 29, day 90, day 180 and day 240. • Changes in modified Norris scale scores on

<table>
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<td>day 15, day 29, day 90, day 180 and day 240.
• Changes in quality of life (including ALSAQ-40, EQ-5D-5L, HAMA and HAMD) on day 15, day 29, day 90, day 180 and day 240.
•Changes in clinical staging (including London ALS Stage and Milano-Torino staging) on day 15, day 29, day 90, day 180 and day 240.</td>
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<tr>
<td>Dosing regimen of CREATION trial
<table>
<tr><th>Treatment</th><th>Administration Day</th><th>Dose Level</th><th>Administration Route</th><th>Number of Subjects</th></tr>
<tr><td>1stAdministration</td><td>D1</td><td>60 mg/dose</td><td>Intrathecal injection</td><td>3</td></tr>
<tr><td>2ndAdministration</td><td>D15</td><td>90 mg/dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>3rdAdministration</td><td>D29</td><td>120 mg/dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>4thAdministration</td><td>D60</td><td>120 mg/dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>5thAdministration</td><td>D120</td><td>150/180(no more than 180) mg/dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>6thAdministration</td><td>D180</td><td>Optimal dose</td><td>Intrathecal injection</td><td>6</td></tr>
<tr><td>7thAdministration</td><td>D240</td><td>Optimal dose</td><td>Intrathecal injection</td><td>6</td></tr>
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Supplement information_Sequence for primers

Gene name	Target Species	Sequence (5′→3′)
SOD1 detection primer F	Human	5′-AAGCATTAAAGGACTGACTGAAGG-3′
SOD1 detection primer R	Human	5′-CAAGTCTCCAACATGCCTCTC-3′
CYTH2 detection primer F	Human	5′-TACTTTGAGTACACCACGGACAA-3′
CYTH2 detection primer R	Human	5′-CCTCTCGGATGCTCAGATTCTC-3′
NDUFC2 detection primer F	Human	5′-CCTTACGGTTTETGCCGGAT-3′
NDUFC2 detection primer R	Human	5′-CTGGCGATGCAAACCAGC-3′
DISP2 detection primer F	Human	5′-CTGCCCACTTCACCTATCCC-3′
DISP2 detection primer R	Human	5′-GGCTCGTTCCTGCCTGAC-3′
KNOP1 detection primer F	Human	5′-CGCACATAGACCAGGTGAGG-3′
KNOP1 detection primer R	Human	5′-AGCTTCCGTTTTGCCTGACT-3′
UBAP2L detection primer F	Human	5′-GTCGCCTGGATCTTCAGACA-3′
UBAP2L detection primer R	Human	5′-ACAGCCAGAGCAGGAATCTTA-3′
TBP detection primer F	Human	5′-TGCTCACCCACCAACAATTTAG-3′
TBP detection primer R	Human	5′-TCTGCTCTGACTTTAGCACCTG-3′
SOD1 detection primer F	*Macaca fascicularis*	5′-AAGGTGTGGGGAAGCATTAC-3′
SOD1 detection primer R	*Macaca fascicularis*	5′-CCACCGTGTTGTCTGGATAG-3′
GAPDH detection primer F	*Macaca fascicularis*	5′-ATCACCATCTTCCAGGAGCGA-3′
GAPDH detection primer R	*Macaca fascicularis*	5′-TTCTCCGTGGTGGTGAAGACG-3′