CLINICAL STUDY PROTOCOL

A multi-center, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of tetramethylpyrazine nitrone (TBN) tablets for treatment of amyotrophic lateral sclerosis (ALS)

Protocol Number: MP-2019-004

Investigational Product: TBN

Version: 1.1

Date: Jul 29, 2020

Study phase: Phase 2

Sponsor: Guangzhou Magpie Pharmaceuticals Co., Ltd.

17 SYNOPSIS

Sponsor	Guangzhou Magpie Pharmaceuticals Co., Ltd.
Investigational Product	TBN Tablets
Name of Active Ingredient	TBN
Study Title	A multi-center, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of tetramethylpyrazine nitrone (TBN) tablets for treatment of amyotrophic lateral sclerosis (ALS)
Study Objectives	To evaluate the efficacy and safety of TBN tablets in patients with ALS, and to explore the optimal effective dose.
Clinical Phase	Phase 2
Study Duration	Treatment period: 180 days
Study Design	This study is a randomized, double-blind, placebo-controlled parallel study. Patients with ALS will be enrolled. After screening for inclusion and exclusion criteria, subjects are to be randomized to receive either TBN or placebo. **Treatment group 1:** TBN tablets 600 mg group (TBN 300 mg × 2 tablets + placebo 300 mg × 2 tablets are taken orally twice daily, TBN 100 mg × 6 tablets + placebo 100 mg × 6 tablets instead if the subjects are not able to take 300 mg tablets because of disease progression). **Treatment group 2:** TBN tablets 1200 mg group (TBN 300 mg × 4 tablets are taken orally twice daily, TBN 100 mg × 12 tablets instead if the subjects are not able to take 300 mg tablets because of disease progression). **Placebo group:** placebo group (placebo 300 mg × 4 tablets are taken orally twice daily, placebo 100 mg × 12 tablets instead if the subjects are not able to take 300 mg tablets because of disease progression).

Visit Plan	Screening period: 3 months. The eligible subjects will be randomized, with no administration on the day of randomization and the first administration on the next day (Day 1). Clinic visits will occur at the end of Month 1, Month 3, and Month 6 (or withdrawal) for safety- and efficacy-related examinations. Phone calls will be conducted at Day 7, the end of Month 2, Month 4, and Month 5, and the 2nd week after the last dosing.
Planned Enrollment Number of Patients	**Number of patients to be enrolled:** 150, all of whom are to be randomized to 2 treatment groups and 1 placebo group, 50 patients per group, respectively.
Study Population	Patients with mild to moderate ALS
Inclusion Criteria	Subjects will be enrolled in this trial only if they meet all the following criteria: 1) Male or female, aged 45 to 70 years old (including 45 and 70 years old); 2) Subjects diagnosed with clinically definite or clinically probable ALS according to revised El Escorial criteria-1998 by the World Federation of Neurology; 3) Subjects of ≤ 2 years after the onset of ALS before randomization; 4) The ALSFRS-R scores are all ≥ 2 points, and the respiratory function is 4 points before randomization; 5) ALSFRS-R has a decrease of 1-4 points during a 3-month screening period; 6) Forced Vital Capacity (%FVC) ≥ 80% before randomization; 7) Understand and comply with the study rules, voluntarily participate, and sign an informed consent form (informed consent form is voluntarily signed by the subject himself/herself or his/her legal representative).
Exclusion Criteria	Subjects who meet one of the following conditions will not be eligible for this trial: 1) Familial ALS (judge by family history); 2) Those with obvious cognitive impairment (MMSE scale: illiterate group ≤ 19 points, primary school group ≤ 22 points, junior high

	school and above group (≥ 8 years of education) ≤ 26 points; 3) Obvious dysphagia; 4) Severe renal insufficiency: creatinine clearance < 30 mL / min (Cockcroft-Gault formula), or other known severe renal insufficiency disease; 5) Severe liver damage: ALT, AST > 3 times the upper limit of normal level, or other known liver diseases such as acute and chronic hepatitis, cirrhosis, etc.; 6) Acute myocardial infarction or interventional therapy in the past 6 months, heart failure patients (in accordance with NYHA classification III-IV patients) at the screening period; 7) Combined with malignant tumors and serious diseases of blood, digestion or the other systems; 8) Allergic to Investigational Product (IMP) or tetramethylpyrazine; 9) Female patients during pregnancy and lactation; 10) Participate in other clinical studies within 30 days before screening, or is participating in other clinical studies; 11) Other circumstances that the investigator considered unsuitable for participation in the study.
Investigational medicinal product	TBN tablets, strengths: 100 mg / tablet and 300 mg / tablet, provided by Guangzhou Magpie Pharmaceuticals Co., Ltd. Placebo tablets, strengths: 100 mg / tablet and 300 mg / tablet, provided by Guangzhou Magpie Pharmaceuticals Co., Ltd.
Efficacy Endpoints	**Primary Endpoint:** ✓ Difference between the groups in the ALSFRS-R scores change from baseline at Day 180; **Secondary Endpoints:** ✓ Difference between the groups in the proportion of events such as death, tracheotomy, invasive ventilation, or continuous non-invasive ventilation (≥ 22 h per day for ≥10 days);

	✓ Difference between the groups in gripe strength change from baseline at Day 180; ✓ Difference between the groups in respiratory function (%FVC) change from baseline at day 180; ✓ Difference between the groups in ALSAQ-40 scores change from baseline at Day 180.
Safety Assessment Indicators	Safety evaluation including: adverse events (AEs), serious adverse events (SAEs), clinical laboratory tests (hematology, urinalysis, blood biochemistry, thyroid function), vital signs (blood pressure, respiratory rate, pulse and body temperature), 12-lead electrocardiogram (ECG) examination, physical examination. **Primary safety indicators:** Percentage of subjects with serious adverse reactions within 180 days; (Adverse reactions: AEs related to the IMP) **Secondary safety indicators:** Percentage of subjects with adverse reactions within 180 days; AEs, SAEs, and their incidence, the clinical manifestations, severity, time of occurrence, end time, duration, treatment measures and outcomes of these are to be recorded, and the correlation between AE and IMP are to be determined.
PK/PD and Biomarkers	The cerebrospinal fluid (Tmax after administration, optional) is to be taken (in part of subjects) at baseline period before administration and 180 days after administration, and the concentration of TBN and its metabolites in cerebrospinal fluid and plasm are to be measured for PK/PD analysis. Biomarkers, such as 3-NT, 8-OHdG, NFL levels in cerebrospinal fluid are to be determined.
Treatment Termination	Subjects need to terminate the treatment permanently if the following events occur: ✓ The subject is pregnant; ✓ The subject withdraws informed consent; ✓ The subject has a medical emergency that requires to terminate the

	treatment permanantly, such as death, tracheotomy, invasive ventilator assisted ventilation, or continuous non-invasive ventilator assisted ventilation (≥ 22 h per day for ≥10 days); ✓ The subject has a medical emergency requiring a unblinding of the treatment of the subject; ✓ Determinations based on medical reasons made by investigator.
Combined Medication and Treatment	✓ Except edaravone, all other drugs are not prohibited. ✓ Riluzole administration is permitted during the study, but is regulated under the guidance of the investigator. ✓ All concomitant medications other than the IMP within 180 days should be recorded and counted for the impact on the subject's outcome; ✓ All non-medicine therapies need to be recorded in the medical records.
Estimating Sample Size	This trial is an exploratory study in safety and efficacy. No statistical assumptions will be made, and statistics will not calculate the sample size.

18 TRIAL SCHEDULE

Items \ Time		Screening period*		Baseline# Day 0	Day 7[T]	Day 30 ± 3	Day 60 ±3[T]	Day 90 ± 7	Day 120 ± 3[T]	Day 150 ±3[T]	Day 180 ± 7	End-of-study visit[T]	Withdrawal[&]
		First	Second										
Informed consent		X											
Review of inclusion and exclusion criteria		X		X									
Subject characteristics	Medical history/allergic history	X											
	Demographics	X											
	Height	X											
	Weight	X		X		X	X[¥]	X	X[¥]	X[¥]	X		X
Efficacy evaluation	ALSFRS-R	X	X	X		X		X			X		X
	ALSAQ-40			X							X		X
	Grip strength test [1]	X		X		X		X			X		X
	Respiratory Function (%FVC)	X		X		X		X			X		X
Safety evaluation	Vital signs (blood pressure, respiratory rate, pulse, temperature)	X		X		X	X[¥]	X	X[¥]	X[¥]	X		X
	Physical examination	X		X		X	X[¥]	X	X[¥]	X[¥]	X		X

	12-lead ECG	X		X		X		X			X		X
	Hematology, urinalysis, chemistry[2]	X		X		X		X			X		X
	Thyroid function[3]			X		X		X			X		X
MMSE scale[4]		X											
Urine pregnancy test for women of childbearing age		X		X		X		X			X		X
Cerebrospinal fluid sampling [5]				X							X		
Blood sampling for drug concentration[6]											X		
Assigning randomization numbers				X									
Dispensing IMP				X		X	X	X	X	X			
Drug accountability/return, diary card return						X	X	X	X	X	X		X
Drug administration					X	X	X	X	X	X	X		
Concomitant treatment (medication & non- medication)		X	X	X	X	X	X	X	X	X	X	X	X
Safety evaluation	Monitoring and recording AEs		X	X	X	X	X	X	X	X	X	X	X
	Monitoring and recording SAEs		X	X	X	X	X	X	X	X	X	X	X

Note:* Screening period: Perform ALSFRS-R examinations twice to confirm subjects meeting the inclusion criteria; the two ALSFRS-R examinations will be performed with 90 ± 3 days interval. For subjects having an ALSFRS-R scores within the previous 3 months with appropriate documentation (such as medical record), the results of the previous examination can be used as the first ALSFRS-R result for the screening period, at the discretion of the investigator.

Baseline: Subjects enter baseline period after screening successfully. The examination cannot be repeated if the same examinations are performed at

baseline and screening with the results within 7 days. Randomization is performed after eligibility for the baseline assessment, with no administration on the day of randomization and the first administration on the next day (Day 1).

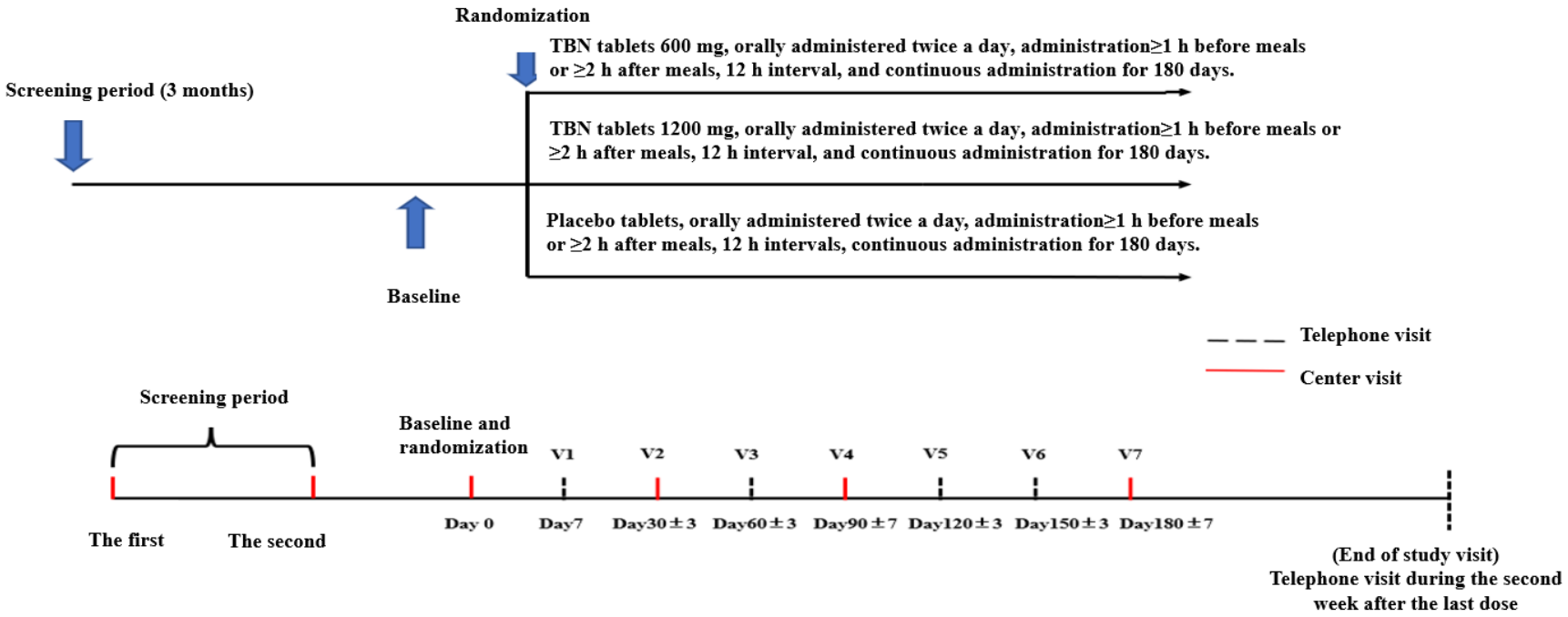
& Withdrawal: Only for subjects dropping out of the study, withdrawal visits from 7 days to 14 days after the last dose.

T Visiting by phone: Subjects are encouraged to visit the hospital for follow-up at Day 60, Day 120, and Day 150; If the subject cannot visit due to medical reasons, phone visits can be accepted for the subject, but the family member need to visit the hospital at the specified visit to get the new medication and diary card, as well as to retrieve the left-over medication and the diary card recording the medication taken; the end-of-study visit is at the 2nd week after the end of the study.

￥ Perform when the subject arrives at the hospital to collect the new medication and diary card in person, but not if the family member delegating visit.

1. Grip strength measurement: Measure the subject's maximum grip strength (using an electronic grip strength meter).
2. Hematology, urinalysis, chemistry: On the day of testing, subjects attend the study site fasting for blood collection and testing; Hematology includes white blood cell count, red blood cell count, platelet count, hemoglobin, neutrophil percentage, and lymphocyte percentage. Chemistry includes ALT、AST、TBIL (Total bilirubin), Cr (Serum creatinine), BUN (Blood Urea Nitrogen), CK (Creatine Kinase);
3. Thyroid function includes free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH);
4. Subjects with significant cognitive impairment (MMSE score: illiterate group ≤ 19, primary school group ≤ 22, middle school group and above (>8 years of schooling) ≤ 26);
5. Cerebrospinal fluid (CSF) collection: For subjects agreeing cerebrospinal fluid collection for drug concentration and biomarker levels, 4 mL of CSF is collected at baseline and 180 ± 3 days after treatment (3 h ± 30 min after the first dose on the same day), and the levels of TBN and its metabolite, 5-OH-TBN, are determined in the CSF of less than 10 subjects in each group at 180 ± 3 days after treatment; The levels of 3-NT, 8-OHdG, and NFL (Neurofilament light chain) in the CSF of all subjects agreeing CSF collection at baseline and at 180 ± 3 days after treatment are determined. The sample is not collected at 180 ± 3 days after treatment for the subject without baseline sampling.
6. Blood sampling for drug concentration: Blood sample is collected before the first dose and at 3h±30 min after the first dose on 180±3 day (the same day) after administration, 4 mL each time, and plasma levels of TBN and its metabolite 5-OH-TBN are determined.

SCHEMA


Randomization
TBN tablets 600 mg, orally administered twice a day, administration≥1 h before meals or ≥2 h after meals, 12 h interval, and continuous administration for 180 days.
Screening period (3 months)
TBN tablets 1200 mg, orally administered twice a day, administration≥1 h before meals or ≥2 h after meals, 12 h interval, and continuous administration for 180 days.
Placebo tablets, orally administered twice a day, administration≥1 h before meals or ≥2 h after meals, 12 h intervals, continuous administration for 180 days.
Baseline
Telephone visit
Center visit
Screening period
Baseline and randomization
V1
V2
V3
V4
V5
V6
V7
The first
The second
Day 0
Day7
Day30±3
Day60±3
Day90±7
Day120±3
Day150±3
Day180±7
(End of study visit)
Telephone visit during the second week after the last dose

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1. INTRODUCTION

1.1. Scientific Background

Amyotrophic Lateral Sclerosis (ALS) is an insidious, fatal neurodegenerative disease that mainly involves the cerebral cortex, brainstem, and spinal motor neuron. The onset of ALS is heterogeneous, and it usually starts at a single site and then develops throughout the body, with initial clinical symptoms varying from one site to another. Typical clinical symptoms are progressive skeletal muscle weakness, atrophy, muscle fasciculations, bulbar palsy and pyramidal signs. In the later stage of the disease, most patients eventually progress to respiratory muscle weakness causing respiratory failure and death. Recent studies have found that there are also some atypical clinical manifestations in ALS patients such as cognitive dysfunction, sensory deficits and autonomic dysfunction. The anatomical pathological features are mainly characterized by degenerative lesions of brain and spinal cord motor neurons.

The incidence of ALS is estimated to be 1.5-3/100,000/year, with a prevalence of 3-6/100,000 [1]. At May 11, 2018, ALS was included in the list of the National Catalogue of Rare Diseases. Professor Fan Dongsheng from the Third Affiliated Hospital of Peking University etc. [2] summarized the epidemiological characteristics of ALS in China by summarizing the epidemiological data of ALS in China, Hong Kong, and Taiwan. There ae no data on the incidence rate of the disease in China. Relevant studies in Taiwan (0.51/100,000/year) and Hong Kong (0.31-0.6/100,000/year) shown that the incidence of ALS in China is lower than Japan (2.2 per 100,000/year), Europe (2.16 per 100,000/year) and Australia (2.74 per 100,000/year). The mean age of onset of ALS in China is 55.5-58.76 years, which is lower than Japan (62.1 years) and Europe (62.1-66.3 years). In addition, the percentage of familial ALS in China is 1.2%-2.7%, which is similar to Southern Europe but lower than the rest of the world (10%).

The cause of ALS is not yet clear, and studies have shown that 5 to 15 percent of patients are familial, and is called familial Amyotrophic Lateral Sclerosis(fALS). The main genes involved are TDP-43, sod1, C90RF72 and FUS [3]. The remaining patients are sporadic Amyotrophic Lateral Sclerosis (sALS) whose pathogenic factors are unclear and multiple factors may be involved.

There is lack of effective treatment and high mortality for ALS. Relevant domestic and overseas guidelines all emphasize that ALS should be treated at an early stage to prolong survival as much as possible, including comprehensive management such as nutritional management and respiratory support, in addition to the use of drugs that slow down the progression of the disease. Currently, the two drugs for ALS have been approved, including Riluzole and Edaravone. Riluzole is a glutamate antagonist, which reduces damage to motor neurons by decreasing glutamate release and decreasing neuronal excitability [4]; the mechanism of edaravone in the treatment of ALS has not been clarified, and it may work by protecting neurons.

TBN, an innovative chemical drug with independent intellectual property rights, is developed by Guangzhou Magpie Pharmaceuticals Co., Ltd. TBN is designed by combing tetramethylpyrazine (TMP), a main active ingredient of Chinese herb medicine Ligusticum (Chuanxiong) and nitrone with strong free radical scavenging property. TBN acts through multiple mechanisms: Firstly, TBN has strong free radical scavenging activity which can protect motor neurons from free radical damage. In addition, TBN can activate the AMPK/PGC-1α/Nrf2/HO-1 signaling pathway to improve mitochondrial function of motor neurons.

1.2. Introduction of Investigational Product (IMP)

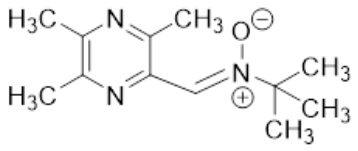
1.2.1. Brief Introduction of IMP

Common Name: TBN

Chemical Name: (Z)-2-Methyl-N-((3,5,6-trimethylpyrazin-2-yl)methylene)propan-2-amine oxide

CAS No: 1083171-75-8

Chemical Structure:



Molecular Formula: $C_{12}H_{19}N_3O$

Molecular Weight: 221.30

Physicochemical Property: Physicochemical property of TBN is shown in Table 1.

Table 1. Physicochemical property of TBN

Item	Description
Appearance	Off-white to pale yellow crystalline powder, odorless.
Melting point	72-76 ^{o}C
Solubility	Freely soluble in methanol, ethanol, acetonitrile and 0.1 mol/L hydrochloric acid and 0.1 mol/L sodium hydroxide, and soluble in water.
PH	25 mg/ml, pH 5.0 ~ 7.0.
Hygroscopicity	No hygroscopicity.
Polymorph	XRD shows that TBN is a monocrystalline powder and with no polycrystalline phenomenon.

1.2.2. Mechanism of Action

The possible mechanisms of action of TBN fighting against ALS is primarily through scavenging free radicals of motor neuronal cells and improving mitochondrial function. In addition, TBN can activate the AMPK/PGC-1α/Nrf2/HO-1 signaling pathway to improve mitochondrial function of motor neurons.

1.2.3. Nonclinical Studies

1.2.3.1. Nonclinical pharmacodynamics studies

The mutant hSOD1*G93A mice is the most used animal model for ALS. This mice model spontaneously manifest clinical symptoms of ALS, including muscle weakness, muscle atrophy,

fasciculation, and pyramidal tract signs, on top of selective loss of motor neurons in the anterior horn of spinal cord. After TBN treatment, the behavioral function was improved compared with the control group. The results of pole climbing test, suspension test and limb grasping test showed that TBN treatment reduced the pole climbing time, improved the motor retardation symptoms, prolonged the suspension time and enhanced grip strength of the mice.

The gastrocnemius muscle of SOD1 mice was taken and its atrophy degree was measured. After treatment with TBN, muscle atrophy was significantly decreased and fiber atrophy within the gastrocnemius muscle was alleviated. Compared with the control group, the protection rates of gastrocnemius muscle in SOD1 mice treated with TBN (10, 30, 60 mg/kg) were 0.43%, 19.77% and 6.19%, respectively, among which the 30 mg/kg dose presented the best therapeutic effect ($P<0.05$). In 30 and 60 mg/kg TBN groups, the muscle fibers of the gastrocnemius muscle in SOD1 mice retained a good appearance of integrity. The number of muscle fibers within a cross-sectional area of 800-1200 μm^2 was used as the main evaluating indicator, and the results showed that TBN significantly reduced the atrophy of the gastrocnemius muscle fibers. The results of Masson staining showed that TBN decreased the fibrosis of SOD1 mice.

The primary pathological feature of ALS is the loss of motor neurons in the anterior horn of the spinal cord, which results in disordered motor behaviors and muscle atrophy. In the study, Nissl staining was used to observe the survival of motor neurons in the anterior horn of the spinal cord. After treatment with 10, 30 and 60 mg/kg of TBN, the protection rate of motor neurons was 25.52%, 37.91, and 32.34%, respectively, among which 30 and 60mg/kg groups were significantly higher than that of the control group ($P<0.01$ and $P<0.05$).

Another pathological feature of ALS is the high activation of glial cells in the nervous system. In this study, GFAP (a marker of neurotrophic astrocyte activation) and Iba-1 (a marker of neuronal microglial activation) were used for IHC staining to observe changes in the number of activated astrocytes in the spinal cords of SOD1 mice after drug administration. Results showed that the number of activated astrocytes and microglial in the SOD1 mice at the period of onset was abnormally increased when compared with wild-type mice. After treatment with different dose of TBN, the number of activated astrocytes and microglial in the SOD1 mice was significantly decreased.

Protein aggregation due to misfolding of SOD1 may be responsible for SOD1-mediated ALS cytotoxicity. Therefore, the level of human SOD1 in the spinal cord is a key indicator for evaluating the efficacy of ALS treatments. The level of hSOD1 in the spinal cords of SOD1 mice was significantly higher than that of the wild-type mice at the time of onset. After treatment with 30 and 60 mg/kg of TBN, the level of hSOD1 in the spinal cords of the SOD1 mice was significantly reduced.

1.2.3.2. Nonclinical Safety Pharmacology Study

No significant CNS toxicity was observed in SD rats following a single intravenous injection of 20, 60, and 120 mg/kg of TBN, and the no observed adverse effect level (NOAEL) was 120 mg/kg.

Single intravenous injection of TBN at doses of 12, 36, and 120 mg/kg had no effect on the cardiovascular system of conscious unrestrained Beagle dogs. The NOAEL was 120 mg/kg.

Single intravenous injection of TBN at doses of 20, 60, and 120 mg/kg had no effect on the respiratory system of conscious unrestrained SD rats. The NOAEL was 120 mg/kg.

The results of study on effects of TBN on gastrointestinal irritation in ICR Mice/SD rats following single oral gavage administration showed that TBN had no significant effects on gastrointestinal propulsion function in the both female and male ICR mice after a single oral gavage administration at doses of 25 and 75mg/kg. At dosage of 150 mg/kg, TBN had no significant effects on the gastrointestinal propulsion function in the female mice, but reduced the charcoal advancement rate in male mice after a single oral gavage administration, TBN had no significant effects on the bile secretion in SD rats after a single oral gavage administration at dosage of 25, 75 and 150 mg/kg and TBN had no significant effects on the gastric secretion in SD rats after a single oral gavage administration at dosage of 25, 75 and 150 mg/kg.

1.2.3.3. Nonclinical Toxicology Studies

Single-dose toxicity: The single-dose toxicology studies on rats and beagles showed that TBN was well tolerated, with the maximum tolerated dose (MTD) via intravenous injection both in rats and beagles of 625 mg/kg (about 62.5-fold of the effective dose). The main toxic target organs are the central nervous system and gastrointestinal system. The toxic reactions were

reversible and could be quickly recovered after the administration stopped.

Repeat-dose toxicity: The study was conducted on SD rats and Beagle dogs. After the consecutive 4-week of TBN for injection treatment, the main adverse reactions in SD rats were weight growth slowing down, food consumption decreasing, animal activity decreasing, the emergence of tachypnea and spasm; while the main adverse reactions in beagles were food consumption decreasing, muscle tone increasing, unstable gait, motor activity decreasing, prone and motionless, curled up and less movement, loose stools, drooling and the weight of thymus losing. The toxicity of continuous administration was mainly reflected in the nervous system and digestive system. Except for body-weight growth slowing down and food intake decreasing, the other toxic effects, were short-lived. The NOAEL in SD rat and beagle were 75 mg/kg and 60 mg/kg, respectively. No clinical abnormalities and accumulation of TBN were observed in the recovery period.

Long-term toxicity: SD rats were orally administrated with different doses of TBN (50, 100, 200 mg/kg) once daily, for 26 consecutive weeks, and then animals were observed for 4 weeks in the recovery period. The maximum tolerated dose was no less than 200 mg/kg. During the study period, there was no obvious drug-related organic pathological changes found in clinical observation, body-weight, food consumption, eye examination, hematological test, coagulation test, serum biochemical test, urinary examination and bone marrow cell examination. The results of histopathological examination showed that at the study doses of TBN (50 to 200 mg/kg), male rats presented drug-related mild or moderate diffuse follicular atrophy of the thyroid gland. Compared with at the end period of the administration, at the end of recovery period, the incidence of drug-related changes in thyroid gland was decreased or alleviated, indicating that the lesions were recovering. For the female rats in 50 mg/kg TBN group didn’t show any drug-related changes in thyroid gland during the study. In 100 mg/kg TBN group, female rats didn’t show changes in thyroid gland on day 92. On day 183 two female rats presented follicular atrophy of the thyroid gland (one was mild and the other one was moderate) and on day 210 the above changes recovered completely.

Genotoxicity: The results of Ames test, CHL chromosome aberration test and mouse micronucleus test were all negative.

Reproductive Toxicity: The NOAEL of TBN for injection in the male and female SD rats was 150 mg/kg (TBN doses of 150 mg/kg/day caused decreased motor activity and transient convulsions after dosing). The NOAEL of TBN for injection of fertility and early embryo development in male and female SD rats was 150 mg/kg. The NOAEL was 170 mg/kg/day in pregnant maternal SD rats, which were intravenously injected with TBN at doses of 0, 75, 170 and 400 mg/kg/day on the Day 6 to Day 15 after pregnancy, respectively, and no obvious adverse effect was observed. The NOAEL for embryos and fetuses was 400 mg/kg/day.

1.2.3.4. Nonclinical pharmacokinetic study

The animal species/lines, administration route, and dosage formulations of non-clinical pharmacokinetic studies of TBN were consistent with its pharmacological studies. After administration of different doses of TBN in SD rats, the peak concentration was observed at 0.32 h to 0.38 h after oral administration, and the dose-related linearity was higher than that in the dose range from 10 to 90 $mg \cdot kg^{-1}$. After the administration of different doses TBN tablets in Beagles, the peak concentration was observed at 1.25 h to 2.50 h, and it was also higher than the dose-related linear increase in the range of 100 mg to 900 mg per dog. No drug accumulation and sex differences were observed in rats or beagles after administration of TBN. TBN was rapidly distributed to various tissues after oral administration, in which the kidney and brain tissues were observed with higher TBN distribution rate than that in other tissues. TBN was cleared from various tissues 4 h after administration, and no drug accumulation was observed. In vitro studies, plasma protein binding rate of TBN was low and no species differences were observed.

Blood-brain barrier permeability study: Healthy cynomolgus monkeys were given 30 mg/kg of TBN via intravenous injection twice, and the second administration was 6 h after the first dose. The concentration of TBN in cerebrospinal fluid was 176 μM 10 min after the second dose and in plasma was 195 μM. The blood-brain barrier permeability of TBN in cynomolgus monkeys is about 90%. According to the above results, TBN has good blood-brain barrier permeability.

The primary metabolic pathway of TBN in vivo was shown to form 5-hydroxymethyl-TBN through oxidation and then to be excreted by urine. TBN administrated with clinical doses showed good stability in liver microsomes. The CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4 subtypes of CYP were not observed to contribute significantly to the metabolism of TBN. In

addition, TBN showed no inhibitory effect on the enzyme subtypes like CYP3A4, 1A2, 2C9, 2D6, 2C19, 2C8 and 2B6, and had no inducing activity against CYP3A4, 1A2 and 2B6. In the drug interaction study, the transporters OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2K were not shown transport effect on TBN, while TBN was not displayed inhibitory activity in transporters OATP1B1, OAT1, OAT3, OCT2, MATE1 and MATE2K. The inhibitory IC_{50} of TBN on transporter OATP1B3, however, was 90.2 μM. OATP1B3 is an ingestion transporter primarily distributed in hepatocytes. TBN is mainly excreted by kidney (urine discharge in 0-168 h accounted for 84.5% of the dose administered in SD rats), while liver and bile excretion only accounted for a very small proportion (fecal discharge in 0-168 h accounted for 3.8% of the dose administered in SD rats). Thus, when TBN is administered in combination with the substrate or inhibitor of OATP1B3 transporter in humans, the risk of increasing the substrate plasma exposure is very low.

TBN had low binding rate of plasma protein, of which the different species was observed to be between 12.1% and 25.1%, and no significant differences of during concentrations or species were observed after TBN administration. So, the risk of competitive protein binding of TBN with other drugs could be low.

1.2.4. Clinical study

Clinical pharmacokinetics

The single-ascending-dose (SAD) study and multiple-ascending-dose (MAD) study of TBN tablets in healthy Chinese volunteers have been completed. The efficacy study of TBN tablets in patients has not been carried out. The details of the clinical pharmacokinetic information see the *Part 5.2, Pharmacokinetic Study of TBN in Human, Investigator's Brochure.*

Pharmacokinetics

Absorption: The oral bioavailability of 400 mg TBN tablets (1 tablet of 300 mg + 1 tablet of 100 mg) was 95.31%. The oral bioavailability of 1800 mg TBN tablets (6 tablets of 300 mg) was 120.01%. In a dose range from 400-1800 mg, TBN reached peak concentration at 2.48-3.70 h, and 5-OH-TBN reached peaked concentration at 3.23-5.11 h. TBN half-life was 1.28-2.10 h, and 5-OH-TBN half-life was 2.91-3.67 h. In addition, pharmacokinetic data results show that the

absorption of TBN tablets was affected by food. Specifically, fed subjects exhibited decreases in $AUC_{0\text{-}24h}$ of about 29% when compared to fasted subjects; similarly, C_{max} decreased by about 45%, and T_{max} was extended from 3.34 h to 5.77 h.

Distribution: The V_Z/F of each dose group of healthy subjects given TBN tablets ranged from 60302.84 mL to 81875.92 mL. TBN tablets taken twice a day for 6.5 days was not observed to cause drug accumulation within the body. The accumulation index of TBN was 1.00-1.04, whereas that of 5-OH-TBN was 1.08-1.12. The plasma protein binding rate of TBN in humans was low. In vitro experiments showed that, at a concentration of 0.1-10 μM, the binding rate of TBN to human plasma protein was around 15.9-20.9%.

Metabolism: In humans, TBN undergoes primarily oxidative metabolism, and the main metabolite of orally administered TBN tablets is 5-OH-TBN. At doses of 400 mg to 1800 mg after a single-dose administration, the plasma exposure of 5-OH-TBN was 2.28-4.10 times that of TBN. The level of another metabolite of TBN, TMP-CHO, was very low in the human body. The C_{max} of TMP-CHO was 67.65-533.94 ng/mL after a single dose of 600-1800 mg. Therefore, TBN is unlikely to cause genotoxicity under clinical dose.

Excretion: In rats, TBN is excreted mainly in the form of metabolites. In study ICP-I-2016-07, a single-ascending-dose study in healthy human subjects treated with TBN for Injection, after a single-dose administration of 700 mg TBN for Injection, the amount of parent TBN and 5-OH-TBN found in the urine was 4.7% of the administered dose. The amount of parent TBN and 5-OH-TBN found excreted through feces was 0.0% of the dose. The total cumulative excretion rate of these two forms of TBN through both urine and feces was thus 4.7%. Combining these results with the non-clinical radiolabeled pharmacokinetics, metabolite, and animal studies, we speculate that *tert*-butylamine is the main metabolite through which TBN is excreted in humans.

Safety and Efficacy

TBN is a new chemical drug, whose single-ascending-doses study (SAD) and multiple-ascending-doses study (MAD) has been completed. The summary table of adverse reaction information can be found in section 5 of the investigator's brochure.

In the SAD and MAD study, TBN was well tolerated in all subjects without SAE observed, and no AEs leading to early withdrawal from the trial and AEs leading to drug or non-drug intervention in this study.

During the study, 52 subjects were enrolled and all of the subjects were capable of compliance the protocol. A total of 3 drug-related AEs occurred in 2 subjects (3.85%), among which 1 subject in the 1200 mg single dose group had a decrease in the percentage of neutrophils (1.9%), and this subject had a drug eruption after multiple administration of 1200 mg (1.9%). One subject in the 1200 mg food-affected group was found elevated alanine aminotransferase (1.9%). The above adverse reactions were of grade 1 in CTCAE and recovered to normal without any medical intervention. No adverse reactions were observed in 400 mg, 600 mg and 1800 mg groups.

In this study, a total of 23 AEs occurred in 18 subjects, which were determined by the investigator to be unrelated to the IMP. Among the above AEs, the incidence of elevated white blood cell count was 7.7% (4 cases), increased oral ulcer was 5.8% (3 cases), elevated neutrophilic granulocyte count was 3.8% (2 cases), and increased platelet count, positive urine white blood cell count, and elevated creatinine phosphokinase in blood was 1.9% (1 case), respectively. All of the above AEs were of CTCAE grade 1 severity and disappeared spontaneously without any treatment.

Other safety indicators, including vital signs, physical examination and electrocardiogram examination, no abnormal results of clinical significance at baseline and each visit were observed.

TBN for injection is used for the treatment of acute ischemic stroke. The phase I clinical trial has been completed and the phase II clinical trial is ongoing. The safety information of the phase I clinical study of TBN for injection is as follows:

In the single-dose safety, tolerability and pharmacokinetics study of TBN for Injection, a total of 68 subjects were enrolled, 52 subjects in the TBN for Injection group and 16 subjects in the placebo group. No AEs, SAEs and toxic reactions were observed. There were 10 adverse reactions in 3 subjects (37.5%) in the 400 mg group, 3 adverse reactions in 3 subjects (37.5%) in

the 700 mg group, 6 adverse reactions in 2 subjects (25.0%) in the 1000 mg group, and there were no adverse reactions of the other groups. The highest incidence of adverse reactions was decreased white blood cell count and decreased neutrophil count, both in 3 cases (4.4%), followed by decreased haemoglobin, increased blood lactate dehydrogenase, and decreased neutrophil percentage, both in 2 cases (2.9%). The overall severity of adverse reactions was grade 1 in 7 subjects (10.3%), grade 2 in 3 subjects (4.4%) with decreased neutrophil counts (400 and 1000 mg groups), and grade 3 in 1 subject (1.5%) with decreased neutrophil counts (1000 mg group). Safety analyses including laboratory parameters, vital signs, physical examination, electrocardiogram, neurological and behavioral tests, did not change significantly from baseline at each post-baseline visit for each dose group.

In the multiple-dose safety, tolerability and pharmacokinetics study of TBN for Injection, a total of 24 subjects were enrolled. Among the 24 subjects, 3 (12.5%) were observed to have 6 AEs, all of which were treatment emergent adverse events (TEAE). The incidences of TEAEs in each group were as follows: 1 subject (8.3%) in the 700 mg group had 2 AEs, and 2 subjects (16.7%) in the 1400 mg group had 4 AEs. No SAEs, AEs leading to withdrawal from the study, or AEs leading to death or dose-limiting toxicity occurred during the study. No significant effects on the safety indicators, such as laboratory tests, vital signs, physical examination, electrocardiogram data, and nervous system and behavioral examination, were observed. Studies showed that TBN and 5-OH-TBN had the best protective effect on cerebral cortical neurons at the concentrations of 30 μM~300 μM ($P < 0.01$, compared with the control group) in vitro, followed by 10 μM ($P < 0.05$, compared with the control group). After 7 days of continuous administration of TBN tablets, the C_{max} of TBN in 600 mg and 1200 mg groups were 43 μM and 114 μM, respectively; the C_{max} of 5-OH-TBN was 51 μM and 111 μM, respectively. The concentration of TBN and 5-OH in the two dose groups can reach the range of effective drug protection concentrations in vitro. In 600 mg and 1200 mg groups, the prototype of TBN and 5-OH-TBN are both higher than 30 μM, which can reach the minimum effective protective concentration of drug in vitro and should display a good neuroprotective effect.

1.3. Summarized Risk-benefit Assessment

1.3.1. Known Potential Risks

In a non-clinical study in SD rats, after 4 consecutive weeks of treatment with TBN for injection, the main adverse reactions observed were slow weight gain, decreased food consumption, decreased animal activity, and the emergence of tachypnea and spasm. The main adverse reactions in beagles, by contrast, were increased muscle tone, decreased food consumption, unstable gait, decreased motor activity, prone and motionless stance, curling up and decreased movement in behavior, loose stools, drooling and decreased thymic weight. Symptoms of toxicity were apparent primarily in the nervous system in the form of altered behavioral activities. With the exception of slow weight gain and decreased food intake, the other toxic effects, such as decreased activity, tachypnea and twitching, were short-lived. No clinical abnormalities were observed during the recovery period. In rats administered for a prolonged period of 26 weeks, a decrease in the weight of the thyroid and parathyroid glands was observed in the ≥50 mg/kg dose group, and diffuse follicular atrophy of the thyroid gland was observed in the ≥50 mg/kg dose group. In beagle dogs administered for a prolonged period of 39 weeks, an increase in alkaline phosphatase was observed in the blood of the males in the 100 mg/kg and 200 mg/kg dose groups.

A total of 128 healthy subjects in completed clinical trials received TBN, including TBN tablets and TBN for injection. The safety and tolerability evaluation included vital signs, physical examination, ECG, and laboratory test. All AEs were recorded. The TBN was safe, with no SAEs, AEs leading to withdrawal from the study, or AEs leading to death or dose-limiting toxicity occurred during the study. Summarized safety for TBN administered orally and intravenously in healthy subjects, drug-related safety information is as follows: The adverse reactions in the single-dose, multiple-dose and food effect study in healthy Chinese human subjects receiving TBN tablets orally included a decreased percentage of neutrophils (1.9%), drug eruption (1.9%), and increased alanine aminotransferase (1.9%). In the single-ascending-dose study of healthy subjects receiving TBN for injection intravenously, the adverse reactions included decreased white blood cell count (4.4%), decreased neutrophil count (4.4%), decreased hemoglobin (2.9%), increased blood lactate dehydrogenase (2.9%), decreased percentage of neutrophils (2.9%), decreased hematocrit (1.5%), positive Coombs test (1.5%), decreased mean cellular hemoglobin (1.5%), decreased mean cellular hemoglobin concentration (1.5%). In the multiple-ascending-dose study of healthy subjects receiving TBN for Injection

intravenously, adverse reactions included increased gamma-glutamyl transferase (4.2%), decreased erythrocyte count (4.2%), decreased hemoglobin (4.2%), and decreased erythrocyte pressure volume (4.2%).

1.3.2. Known potential benefits

The benefits of TBN for ALS treatment include three aspects:

1) Nonclinical studies have shown that TBN was effective in the treatment of ALS. 10, 30, and 60 mg/kg of TBN can improve motor behavioral deficits caused by loss of spinal cord motor neurons in a mice model of ALS, markedly reduce the expression of SOD1 in the spinal cord, and significantly reduce the atrophy of muscle fibers of the gastrocnemius muscle, as well as improve the survival of motor neurons in the spinal cord and attenuating astrocyte activation.

2) TBN has good blood-brain barrier permeability, which is crucial for diseases in central nervous system. TBN has strong free radical scavenging ability, which can attenuate mitochondrial damage caused by oxidative stress. TBN can inhibit calcium inward flow. TBN also has anti-inflammatory activity. TBN can activate the AMPK/PGC1 pathway/Nrf2/HO-1 pathway, which improves the mitochondrial function, and thus attenuates damage in neuronal cell. These properties suggest that TBN is a potential ALS treatment.

3) The combination of TBN and riluzole is expected to be safe. The two drugs have different mechanisms of action (riluzole acts by inhibiting excitatory neuronal activation and TBN acts mainly by scavenging free radicals), and it is expected there may be a synergistic effect in efficacy. In vitro findings suggesting that the combination of riluzole with CYP1A2 inhibitors or inducers may increase or decrease the blood concentration of riluzole, thereby increasing the adverse effects of riluzole or affecting its efficacy. The results of nonclinical studies have demonstrated that TBN has no inhibitory or inducing activity on CYP1A2. Therefore, drug-drug interactions between TBN and riluzole are unlikely to occur.

1.3.3. Potential risk-benefit assessment

Completed studies suggest that TBN may be safe and effective in the treatment for ALS. The investigator will monitor the side effects of TBN. If any side effects or discomfort occur during the study, the investigator may use other medications to control it. If the investigator verify that

the subject cannot tolerate these side effects, TBN administration may be stopped and the subject may withdraw from this study.

2. STUDY OBJECTIVES

To evaluate the efficacy and safety of TBN tablets in patients with ALS, and to explore the optimal effective dose.

3. STUDY DESIGN

3.1. Overall study design

The study is designed to be a multi-center, randomized, double-blind, placebo-controlled study of efficacy and safety of TBN tablets for the treatment of ALS.

3.1.1 Design Rationale

The design basis of this study is as follows:

(1) General Considerations for Clinical Trials, NMPA, Jan 18, 2017

(2) *Safety Data Rapid Reporting Standards and Procedures During Drug Clinical Trials, NMPA, Apr 27,2018*

(3) Chinese Guidelines for Diagnosis and Treatment of Amyotrophic Lateral Sclerosis, Jul, 2012

(4) Amyotrophic Lateral Sclerosis Developing Drugs for Treatment Guidance for Industry, Feb, 2018

As for the screening of subjects, the sponsor requires that incapacitated subjects should obtain the consent of their legal guardians to subjects in the test, and vulnerable groups such as children, pregnant women, mentally retarded people, mental patients and prisoners should be given special protection according to the *Good Clinical Practice (GCP)* and *Methods for ethical review of biomedical research involving human beings* promulgated by the National Medical Products Administration (NMPA).

In addition, during the design of the protocol, we fully refer to the NDA clinical trial scheme and

results applied by Edaravone, a drug with similar mechanism of action, for the treatment of ALS. ALSFRS-R was the main endpoint index. Patients with a short duration of ALS (within 2 years from onset) and rapid progression (ALSFRS-R functional score decreased by -1 to -4 points within 3 months of screening period) and mild or moderate ALS (The ALSFRS-R scores are all ≥ 2 points when enrolled and the respiratory function is 4 points) and well respiratory function (%FCV ≥ 80%) are our target subjects. Compared to the control group, the decline of ALSFRS-R scores in Edaravone group were reduced by 2.5 points after a 6 months treatment period, therefore, the treatment period of TBN is designed as 6 months. We anticipate that TBN tablets can benefit patients with early mild ALS by improving motor dysfunction and delaying disease progression.

3.1.2. Dose selection rationale

According to the results of efficacy of TBN in the animal model of ALS, PK results of animals and phase I clinical trial results of TBN tablets, the doses are designed to be 600 or 1200 mg per subject, twice a day.

In the SOD1 mutant ALS mice model, the pharmacodynamic study showed that the efficacy of TBN was demonstrated at 10 mg/kg, and the efficacy of the 30 and 60 mg/kg groups was significant, and the efficacy of the 30 mg/kg group was comparable with that of the 60 mg/kg group. The Cmax of each dose in the rats were 18 μM, 52 μM, and 105 μM, respectively. In vitro, TBN at 30~300 μM had excellent free radical scavenging and neuronal protection activities. TBN tablets 1200 mg and 1800 mg were well tolerated in single administration safety, tolerance and pharmacokinetic tests in healthy subjects. 1200 mg and 1800 mg were also well tolerated in multiple administration tests. The pharmacokinetic parameters of TBN tablets in healthy subjects showed that the Cmax of TBN were 23.85 μM (5277.57 ng/mL), 43.31 μM (9583.79 ng/mL), and 114.19 μM (25,271.09 ng/mL) for 400 mg, 600 mg, and 1,200 mg of TBN tablets respectively. The Cmax of 5-OH-TBN (in vitro study showed that its activity was comparable to that of TBN) were 37.58 μM, 50.8 μM, and 110.3 μM. The dose of TBN administered was linearly correlated with the Cmax range and the Cmax (43.31 μM and 114.19 μM) were calculated for 600 mg and 1200 mg of TBN corresponding to 30 mg/kg, 60 mg/kg TBN Cmax

(52 μM, 105 μM) in animals. Therefore the 600 mg and 1200 mg TBN were chosen as low-dose and high-dose in this study.

3.1.3. Definition of the End of Study

When a subject finish with the last visit in the study schedule or the last study procedure, he/she is considered to have completed the study.

The end of the last visit of the last subject is considered to be the end of the clinical study.

3.1.4. Study Method

This study complies the *Guidelines for the Good Clinical Practices (GCP)* and is designed as a multi-center, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of TBN tablets in patients with amyotrophic lateral sclerosis (ALS). The planed enrolled number of subjects in this study are 150. Subjects will be randomly assigned according to the ratio: low-dose TBN group: high-dose TBN group: placebo group = 1:1:1. The administration time for each patient in this study is about 180 days. This study is an exploratory study, aiming to explore the safety and initial efficacy of IMP for the treatment ALS and provide support for phase 3 clinical trial.

3.2. Selection of Study Population

3.2.1 Inclusion Criteria

Subjects will be enrolled into this trial only if they meet all the following criteria:

(1) Male or female, aged 45 to 70 years old (including 45 and 70 years old).

(2) Subjects diagnosed with clinically definite or clinically probable ALS according to revised El Escorial criteria-1998 by the World Federation of Neurology.

(3) Subjects of ≤ 2 years after the onset of ALS before randomization.

(4) The ALSFRS-R scores all ≥ 2 points, and the respiratory function is 4 points before randomization.

(5) ALSFRS-R has a decrease of 1-4 points during a 3-month screening period.

(6) Forced Vital Capacity (%FVC) ≥ 80% before randomization.

(7) Understand and comply with to the study protocol, voluntarily participate, and sign an informed consent form (informed consent form is voluntarily signed by the subject himself/herself or his/her legal representative).

3.2.2 Exclusion Criteria

Subjects who meet one of the following conditions will not be eligible for this trial:

(1) Familial ALS (judged by family history).

(2) Those with obvious cognitive impairment (MMSE scale: illiterate group ≤ 19 points, primary school group ≤ 22 points, junior high school and above group (more than 8 years of education) ≤ 26 points.

(3) Obvious dysphagia.

(4) Severe renal insufficiency: creatinine clearance < 30 mL / min (Cockcroft-Gault formula), or other known severe renal insufficiency disease.

(5) Severe liver damage: ALT, AST > 3 times the upper limit of normal level, or other known liver diseases such as acute and chronic hepatitis, cirrhosis, etc.

(6) Acute myocardial infarction or interventional therapy in the past 6 months, heart failure patients (in accordance with NYHA classification III-IV patients) at the screening period.

(7) Combined with malignant tumors and serious diseases of blood, digestion or the other systems;

(8) Allergic to IMP or tetramethylpyrazine.

(9) Female patients during pregnancy and lactation.

(10) Participate in other clinical studies within 30 days before screening, or is participating in other clinical studies.

(11) Other circumstances that the investigator considered unsuitable for participation in the study.

3.3 Study Endpoints and Schedule

3.3.1 Efficacy Endpoints

- ✓ Difference between the groups in the ALSFRS-R scores change from baseline at Day 180.
- ✓ Difference between the groups in the proportion of events such as death, tracheotomy, invasive ventilator assisted ventilation, or continuous non-invasive ventilator assisted ventilation (≥ 22 h per day for ≥10 days).
- ✓ Difference between the groups in grip strength change from baseline at Day 180.
- ✓ Difference between the groups in respiratory function (%FVC) change from baseline at day 180.
- ✓ Difference between the groups in ALSAQ-40 scores change from baseline at Day 180.

3.3.2 Safety Assessment

The endpoints consist of treatment-related adverse events (TRAEs), serious adverse events (SAEs), clinical laboratory tests (hematology, urinalysis, blood biochemistry, thyroid function), vital signs (blood pressure, respiration, pulse and body temperature), 12-lead electrocardiogram (ECG) examination, and physical examination.

(1) Percentage of participants with serious adverse reactions within 180 days;

(2) Percentage of participants with adverse reactions within 180 days;

Record the clinical manifestations, severity, time of occurrence, end time, duration, treatment measures and outcomes of AEs and SAEs during clinical study and determine the correlation between them and the IMP.

3.4 Concomitant Treatments

3.4.1 Treatment Compliance

IMP and other drug combinations must be documented in the appropriate section of eCRF.

3.4.2 Concomitant Illnesses

The diseases that existed prior to screening are considered as concomitant diseases and should be recorded in eCRF, the following items have to be documented specifically:

(1) Smoking habits within the last 12 months before screening.

(2) Alcohol consumption within the last 12 months before screening.

Concomitant diseases are listed according to the latest edition of the Medical Dictionary for Regulatory Activities (MedDRA) in terms of "System Organ Class (SOC)" and "Preferred Term (PT)".

3.4.3 Prohibited Medication

During the trial, there will be no restrictions on other concomitant medication except edaravone.

3.4.4 Permitted medications and non-medications

Riluzole will be a permitted medication during the trial, but it must be used under the guidance of the investigator.

When an adverse event occurs during the trial, such as an abnormal laboratory test such as a decreased white blood cell count, it should be closely observed, and according to the Common Terminology Criteria for AEs (CTCAE) 5.0, severity grade 1 AEs are usually not treated symptomatically in order to observe the extent and reversibility. However, when a grade 2 or higher adverse event occurs, it should be treated according to the investigator's judgement, and if treated, information such as the drug name, administration route, dosage, and start and end times should be clarified and documented on the original record and eCRF. It is recommended to note that the concomitant medication is for the treatment of a specific AE/SAE. If the concomitant medication is required for other conditions, such as clinical routine diagnosis and treatment assistance, the use is recorded as “for clinical routine use” to distinguish from the treatment for AEs.

Concomitant medication/treatment during the trial should be mandatory for the subject and the dose should be as stable as possible.

Coding of concomitant medications will base on WHO (World Health Organization)

Pharmacopeial Dictionary terminology. The concomitant medications are summarized according to the frequency of WHO coded terms.

3.5 Study Schedule

3.5.1 Screening Period

After signing the informed consent form, inclusion and exclusion criteria will be reviewed and demographic, medical and allergy history inquiries will be recorded. Height, weight, physical examination, vital signs, hematology, urinalysis, blood biochemistry, 12-lead electrocardiogram, respiratory function (%FVC), grip strength, MMSE, and ALSFRS-R scale will be performed. Women of child-bearing age will undergo a urine pregnancy test.

During the screening period, twice ALSFRS-R score examinations are performed to confirm that the subject meets the inclusion criteria, and the twice ALSFRS-R examinations should be 90 ±3 days apart. If the subject has an ALSFRS-R score examination within 3 months prior to this trial, the results can be used as the first ALSFRS-R score during the Screening Period, upon presentation of appropriate documentation (e.g., medical records). During the screening period, concomitant treatments (medications & non-medications) need to be documented, and AEs and SAEs need to be monitored and documented.

3.5.2 Baseline and randomization

Screened subjects complete a baseline program that includes: weight, physical examination, vital signs, hematology, urinalysis, blood biochemistry, thyroid function, 12-lead electrocardiogram, respiratory function (%FVC), grip strength, ALSFRS-R scale, ALSAQ-40 scale, and cerebrospinal fluid collection (if consented). Women of child-bearing potential undergo a urine pregnancy test. Repeat testing is not required if the same items are checked at baseline and screening and the results within 7 days.

Once inclusion and exclusion criteria have been verified, randomization number will be requested for distribution of the drug, and subjects will be instructed on how to take the medication at the study center. The first dose of medication will be taken on the day after randomization (Day 1). Diary cards will be distributed and subjects will be instructed to record concomitant medication and AEs.

3.5.3 Clinic Visit

3.5.3.1 Time of Clinic Visit

Subjects will be followed up at the study center at day 30 ± 3 (V2), day 90 ± 7 (V4), and day 180 ± 7 (V7) after randomization. During the course of the trial, the investigator may increase the visit depending on the patient's condition, and record in the medical record form.

3.5.3.2 Items of Clinic Visit

Each clinic visit should evaluate and record the occurrence of AEs, concomitant medication, dispense IMP and return the unused IMP, and dispense and return of diary cards.

V2, V4: weight, ALSFRS-R score, grip strength, respiratory function (%FVC), vital signs, physical examination, electrocardiogram, hematology, urinalysis, blood biochemistry, urine pregnancy, and thyroid function will be test.

V7: Weight, physical examination, vital signs, hematology, urinalysis, blood biochemistry, thyroid function, 12-lead electrocardiogram, respiratory function (%FVC), grip strength, ALSFRS-R scale, ALSAQ-40 scale, centralized blood collection (if consent), cerebrospinal fluid collection (if consent), and urine pregnancy in women of childbearing age.

3.5.4 Phone Visit

3.5.4.1 Time of phone Visit

After signing the informed consent form, subjects will be visited by phone at the Screening Period, Day 7 (V1), Day 60 ± 3 (V3), Day 120 ± 3 (V5), Day 150 ± 3 (V6), and the 2nd weeks after the end of the trial.

3.5.4.2 Items of phone Visit

The visits (V1, V3, V5 and V6) will be to inquire and teach about treatment compliance in addition to documenting AEs and concomitant treatments (medication & non-medication). On days 60 ± 3 (V3), 120 ± 3 (V5), and 150 ± 3 (V6), subjects will be encouraged to follow up in the hospital, where vital signs, physical examination, and weight will be recorded. If the subject unable to the hospital, family will be required to the hospital at the scheduled visit to collect new

medication and diary cards. The post-study visit will take within 2 weeks of the end of the trial.

3.5.4.3 Post-study Visit

Subjects will be followed up by phone within the 2nd week after the last dosing, and will be asked if they are experiencing any discomfort such as dizziness, headache, vomiting, etc. The eCRF for the phone visit will complete.

3.5.5 Visit for Subjects Who Withdraw Early"

If subject is randomized and treated with the IMP and withdraws early for reasons attributable to the subject or at the discretion of the investigator, the subject should come to the study site to complete the withdrawal visit: weight, physical examination, vital signs, hematology, urinalysis, blood biochemistry, thyroid function, 12-lead electrocardiogram, respiratory function (%FVC), grip strength, ALSFRS-R scale, ALSAQ-40 scale, urine pregnancy test in women of childbearing age. Documentation of concomitant treatments (medication & non-medication), monitoring and recording of AEs and SAEs up to 14 days after the last dosing.

3.6 Visit Description

3.6.1 Screening Visit

The following twice procedures will be performed at a clinic visit to determine the subject's eligibility for the study.

First screening

(1) Signing informed consent;

(2) Obtaining a detailed medical history, allergy history, medication history, smoking history, alcohol history, and assessment of demographic information;

(3) Height and weight;

(4) Routine physical examination and vital signs;

(5) Laboratory tests: hematology, urinalysis, blood biochemistry, 12-lead electrocardiogram, and urinary pregnancy test for women of childbearing potential;

(6) Respiratory function test (% FVC);

(7) Grip strength test;

(8) ALSFRS-R scale;

(9) MMSE scale;

(10) Verification of inclusion and exclusion criteria;

(11) Reminding subjects to visit the center for next time for screening tests.

Second Screening (90 ± 3 days apart from the first**)**

(1) ALSFRS-R scale;

(2) Recording of concomitant medications;

(3) Monitoring and recording of AEs and SAEs.

NOTE: Twice ALSFRS-R scores are performed to confirm that the subject meets the inclusion criteria, and should be performed 90 ± 3 days apart between the twice. If the subject has ALSFRS-R score examination within 3 months prior to this trial, the results may be used as the first ALSFRS-R score during the Screening Period, upon presentation of appropriate documentation (e.g., medical records).

3.6.2 Baseline Period

(1) Routine physical examination, vital signs, weight;

(2) Laboratory tests: hematology, urinalysis, blood biochemistry, thyroid function, 12-lead electrocardiogram, urine pregnancy test for women of childbearing age;

(3) Respiratory function (%FVC);

(4) Grip strength test;

(5) ALSFRS-R scale;

(6) ALSAQ-40 scale;

(7) Verification of inclusion and exclusion criteria;

(8) Cerebrospinal fluid collection (if agreed, then sent to the central laboratory);

(9) Recording of concomitant medications;

(10) Monitoring and recording of AEs and SAEs.

Note: After passing the screening, subject can enter the baseline period. If the same items are examined during the baseline and screening periods and the results within 7 days, repeat testing is not required.

3.6.3 Randomization (Day 0)

(1) Assign randomization numbers in a randomized system;

(2) Dispense IMP and confirm that is enough for use until the next visit;

(3) Instruct subjects in the use of the IMP and have subjects use the study drug as prescribed by the investigator;

(4) Instruct females of childbearing potential to use contraception throughout the course of the study;

(5) Distribute and instruct in the use of the subject diary;

(6) Record the concomitant medications;

(7) Remind subjects that a phone visit on Day 7 of dosing.

Note: The first dosing of IMP started the day after randomization is (Day 1).

3.6.4 Visit 1 (Day 7, phone visit)

(1) Inquire and record the occurrence of AEs and concomitant medication;

(2) Inquire treatment compliance;

(3) Remind the subject or his/her family to collect the next month's drug and diary card from the study center and return the unused medication and diary card at the same time.

3.6.5 Visit 2 (Day 30 ± 3)

(1) Routine physical examination, vital signs, weight;

(2) Respiratory function (%FVC);

(3) Grip strength test;

(4) ALSFRS-R scale;

(5) Laboratory tests: hematology, urinalysis, blood biochemistry, thyroid function, urine pregnancy test for women of childbearing age;

(6) 12-lead electrocardiogram;

(7) Verify and return all unused IMP and used and unused packages;

(8) Record the AEs and concomitant medications;

(9) Verify, return and distribute diary cards to subjects;

(10) Dispense IMP and confirm that the drug will be enough until the next visit;

(11) Make an appointment with the subject for the next visit.

3.6.6 Visit 3 (Day 60 ± 3, phone visit)

(1) Weight;

(2) Physical examination;

(3) Vital signs;

(4) Inquire and record AEs, concomitant medications;

(5) Inquire treatment compliance;

(6) Remind the subject or his/her family members to come to the center to get the next month's medications and diary card and return any unused medication and diary card at the same time.

3.6.7 Visit 4 (Day 90 ± 7)

(1) Routine physical examination, vital signs, weight;

(2) Respiratory function (%FVC);

(3) Grip strength test;

(4) ALSFRS-R scale;

(5) Laboratory tests: hematology, urinalysis, blood biochemistry, thyroid function, urine pregnancy test for women of childbearing age;

(6) 12-lead electrocardiogram;

(7) Verify and return all used IMP and used and unused packages;

(8) Record the AEs and concomitant medications;

(9) Verify, return and distribute diary cards to subjects;

(10) Dispense IMP and confirm that the drug will be enough until the next visit;

(11) Make an appointment with the subject for the next visit.

3.6.8 Visit 5 (Day 120 ± 3)

(1) Weight;

(2) Physical examination;

(3) Vital signs;

(4) Inquire and record AEs, concomitant medications;

(5) Inquire treatment compliance;

(6) Remind the subject or his/her family members to come to the center to get the next month's medication and diary card and return any unused medication and diary card at the same time.

3.6.9 Visit 6 (Day 150 ± 3)

(1) Weight;

(2) Physical examination;

(3) Vital signs;

(4) Inquire and record AEs, concomitant medications;

(5) Inquire treatment compliance;

(6) Remind the subject or his/her family members to come to the center to get the next month's medication and diary card and return any unused medication and diary card at the same time.

3.6.10 Visit 7 (Day 180 ± 7)

(1) Routine physical examination, vital signs, weight;

(2) Laboratory tests: hematology, urinalysis, blood biochemistry, thyroid function, urine pregnancy test for women of childbearing age;

(3) 12-lead electrocardiogram;

(4) Respiratory function (%FVC);

(5) Grip strength test;

(6) ALSFRS-R scale;

(7) ALSAQ-40 scale;

(8) Cerebrospinal fluid collection (if agreed, then sent to the central laboratory)

(9) Blood Collection for Drug Concentration Studies (sent to the central laboratory)

(10) Verify and return all used IMP and used and unused packages;

(11) Verify and return diary cards;

(12) Record the AEs and concomitant medications.

3.6.11 Safety follow-up after discharge / withdrawal

Follow-up phone call within 2 weeks of discharged / withdrawal to inquire about symptoms such as dizziness, headache, and vomiting, and complete an eCRF for the visit.

3.7 Plasma and cerebrospinal fluid drug concentration studies, Measurement of

Biomarker Levels in Cerebrospinal Fluid

Collection of plasma and cerebrospinal fluid from subjects, and determinate of TBN and its metabolite 5-OH-TBN in plasma and cerebrospinal fluid and biomarker in cerebrospinal fluid.

Collection of plasma and cerebrospinal fluid requires the following steps:

Screening Period

Signed informed consent for collection of blood and cerebrospinal fluid for drug concentration studies.

Baseline period and Visit 7 (Day 180 ± 7)

Subjects who sign the informed consent form will have their cerebrospinal fluid collected at any time of baseline period. Blood sample for the drug concentration study will be collected prior to the first dose of medication on the day of Visit 7 (Day 180 ± 7), and then a blood and cerebrospinal fluid sample will be collected 3±0.5 hours after the first dosing of drug on the same day (based on the T_{max} time for the PK of the TBN Tablets Phase I clinical trial). 4 mL of blood samples and 4 mL of cerebrospinal fluid will be collected each time. The collection, dispensation, preservation, transportation, and test are in strict accordance with the Central Laboratory Service Manual. No more than 10 subjects per group shall be tested for blood samples and cerebrospinal fluid for drug concentration study. Cerebrospinal fluid biomarker testing will be performed on all subjects who consent as possible.

4 THE MANAGEMENT OF IMP

4.1 Investigational medicinal product (IMP)

4.1.1 Treatment Groups

(1) TBN tablets: strengths: 100 mg/tablet and 300 mg/tablet, provided by Guangzhou Magpie Pharmaceuticals Co., Ltd.

(2) Placebo: Excipient tablets without TBN, strengths: 100 mg/tablet and 300 mg/tablet, provided by Guangzhou Magpie Pharmaceuticals Co., Ltd.

4.1.2 Mode of Administration

Treatment Group 1: TBN tablets 600 mg group (TBN 300 mg × 2 tablets + placebo 300 mg × 2 tablets are taken orally twice daily, TBN 100 mg × 6 tablets + placebo 100 mg × 6 tablets instead if the subjects are not able to take 300 mg tablets because of disease progression).

Treatment Group 2: TBN tablets 1200 mg group (TBN 300 mg× 4 tablets are taken orally twice daily, TBN 100 mg × 12 tablets instead if the subjects are not able to take 300 mg tablets because of disease progression).

Control Group: placebo group (placebo 300 mg × 4 tablets are taken orally twice daily, placebo 100 mg × 12 tablets instead if the subjects are not able to take 300 mg tablets because of disease progression).

Dosage and Administration: orally administered, twice a day, once in the morning and evening (or the interval ≥ 8 hours), at least 1 hour before meals (≥ 1 hour before meals) or at least 2 hours after meals (≥ 2 hours after meals), for 180 ± 3 days.

Note: If the subjects are not able to take 300 mg tablets of TBN or placebo because of disease progression, the tables may be exchanged for the 100 mg/tablet, with no change in the dose.

During the treatment, subjects should record the time and dose of the drug in the subject's diary card. Any adverse reactions should be promptly treated and recorded in the original medical records.

4.2 Preparation/Package/Storage/Responsibility

4.2.1 Packaging and Labeling of IMP

Packaging specification: the packaging material is 60 ml oral solid medical high-density polyethylene (HDPE) bottle with child protection cover, containing silica gel desiccant and deoxidizer, 25 pills/bottle.

The labels of 100 mg TBN tablets/placebo and 300 mg TBN tablets/placebo are as follows:

Investigational Product for Clinical Studies of TBN tablet (Phase II, Protocol No.: MP-2019-004)

Clinical Study Approval Letter No：CXHL2000168 / CXHL2000169

Drug No：

Indication: Amyotrophic lateral sclerosis (ALS)

Dosage and Administration: oral (see the protocol for specific dosing regimen)

Dosage Form: Tablet

Strength：100 mg/tablet or 300 mg/tablet

Storage：Away from light, sealed, and under room temperature (15 ^{o}C-30 ^{o}C)

Batch No：See the product label for details.

Expiry Date：See the product label for details.

Sponsor: Guangzhou Magpie Pharmaceuticals Co., Ltd.

Note：For clinical study only. Consult your doctor if you have any questions.

4.2.2 Dispensing of IMP Numbers

In this study, the central random system will be used to generate a random unique number for subjects having passed the screening and match a corresponding code of IMP. The drug is then administered according to the randomization. The use and return of each bottle of drug should be documented on a specified record sheet.

4.2.3 Storage and Dispensing of IMP

The dispensing and management of IMP should be carried out in accordance with the protocol. According to the registration regulations, only the subjects who enrolled the study are able to obtain the IMP; only the delegated person at each study site could dispense and store the IMP. No IMP should be provided to any third parties or any person other than the clinical study participants. The IMP should be stored properly and only limited number of staffs have the access to where IMP are stored. The monitor is responsible to ensure that the amount of IMP is consistent with the amount on the IMP dispensing records.

The IMP should be away from light, sealed and stored under room temperature (15 to 30 ℃).

The study site should designate a dedicated person to be responsible for the storage and dispensing of IMP and document on a specified sheet.

Notice

- Drugs should be kept in their original packaging;
- Check the packaging before first use. If the package seal is damaged or the drug is lost, it should not be used;
- Save all remaining IMP and packaging.

4.2.4 Inventory of IMP

It is the responsibility of the investigator to ensure that the quantity of IMP is in line with the documented quantity, and to save the record of the drug registration. As required by regulations, the investigator or delegated person must maintain the records of the use and return of IMP throughout the study, including the amount of the IMP receipted, the amount of the IMP stored and/or dispensing, and the amount of the IMP returned to the study site.

When the IMP is delivered to each study site, the investigator or the delegated person must sign the drug receipt form. The list includes: the package number, quantity of IMP and the date of receipt. The investigator or the delegated person should record all IMP on a specified record form. At the end of the study, the amount of IMP on the sheets should be consistent with the amount of drugs stored in the center, the amount of drugs delivered to the subjects, and the amount of drugs that the subjects later returned, and there should be a reasonable explanation for any discrepancies. All containers and unused IMP should be returned and signed on the return form. In most cases, the returned drugs are collected centrally in Guangzhou Magpie Pharmaceuticals Co., Ltd. for subsequent destruction; in rare cases, the returned IMP are destructed at each research center. Destruction of the drug is carried out by delegated person. The process of destruction will be documented and archived.

4.2.5 Transportation of IMP

All IMP used in the trial are provided by Guangzhou Magpie Pharmaceuticals Co., Ltd. and are transported by the courier company delegated by Guangzhou Magpie Pharmaceuticals Co., Ltd.

During the transportation, the temperature should be kept within a specified temperature range, and a thermometer with real-time temperature monitoring should be used to monitor the drug transportation. After receiving the drug, the drug clinical trial institution should present the receipt certificate and accurately record all the IMP received and the date of receipt.

5 METHODS FOR REDUCING BIAS: RANDOMIZATION AND BLINDING

5.1 Randomization

This study uses Interactive Web Response System (IWRS) to dispense random numbers and corresponding IMP. Subjects' random numbers are generated by independent statisticians unrelated to the study using SAS 9.4 software or higher version. The subject-drug randomization table is in duplicate, sealed and handed over to the main study unit and the sponsor.

After a 90 day observation period, eligible subjects are to be randomly assigned to high or low dose of the IMP group or placebo group (1:1:1). The investigator log into the IWRS system, where they are to be given a random number and a drug code.

In order to ensure that the number of patients in each trial group is similar and the distribution of important prognostic factors is balanced, this study uses the minimization method in dynamic randomization. The stratification factors are: the diagnostic grade of ALS (clinical diagnosis vs clinical diagnosis and laboratory support - The diagnostic criteria for clinical diagnosis), the change in ALSFRS-R scores during observation period (-1 or -2 vs -3 or -4) and age (<65 years vs >65 years).

Once having been randomly assigned in the study, subjects withdrawing from the clinical trials for any reason, regardless of whether or not having been administered with the IMP, will retain their random number and the subject will not be allowed to subjects in the trial again.

5.2 Blinding

The study is designed as a double-blind, which means that participants, investigators, supervisors and data analyzers are unaware of the assignment of subjects. The statistical analysts randomly assign subjects to the treatment or control groups, and keep the distribution information in the IWRS system. The sponsor or its delegated unit shall, in accordance with the relevant provisions

of the GCP on the administration of IMP, uniformly pack and label the IMP and label them for clinical trials, and pack them by relevant personnel. Each drug shall be packaged in a separate package. The whole process should be checked and recorded in detail by delegated person. After the IMP are transported to study sites, the study sites shall set up a special place (drug depository) for safekeeping by delegated person.

The blinding of drug is conducted by statisticians and staffs from sponsor who are irrelevant with the study. The TBN and placebo are labeled based on the randomization table. The process of blinding should be documented and signed by relevant person.

5.2.1 Emergency Unblinding

Investigators cannot open the unblinding envelope unless there is a medical reason for it. On occurrence of a SAE or emergency rescue required by the subject, the responsible investigator of the site should notify the principal investigator to decide whether to open the emergency unblinding envelope for identification of the drug administered before providing prompt rescue or taking appropriate measures. Once the emergency unblinding envelope is opened, the subject will be considered a drop-out. Meanwhile, the investigator should record the information relevant to the unblinding in detail, including the unblinding time, the reason, the study treatment, and the outcome of the rescue. The principal investigator should notify the sponsor in written format as soon as possible and describe the details and necessity of the unblinding.

5.2.2 Unblinding Procedure

After verification under blinded status, the data will be locked and unblinded. The statistician will be informed of the drug names corresponding to the drug codes to perform statistical analysis for all data. The unblinding documents will be signed jointly by the principal investigators, sponsors, CROs, and statisticians.

6 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

6.1 Adverse Events

6.1.1 Definition of Adverse Events

Adverse Events refer to all adverse medical events that occur after subject receives the IMP, which can be manifested as symptom signs, diseases or abnormal laboratory tests, but are not necessarily causally related to the IMP. In this trial, safety information should be collected from the time the subject signs informed consent until the last follow-up visit. Clinical AEs occurring after the signing of informed consent and prior to the first dose of drug are recorded in the original medical record as medical history/concomitant illnesses and are not recorded as AEs, but injuries/damages resulting from clinical trial investigations are required to be recorded as AEs. Any clinical abnormality that occurs during the time period starting from the first dosing and the last clinic follow-up visit that is judged by the clinician to be clinically significant, regardless of whether it is causally related to the trial drug or not, is judged to be an adverse event. Laboratory test abnormalities without clinically significant are not documented as AEs or SAEs.

AEs include but not limited to the following:

A. Newly diagnosed disease;

B. Abnormal laboratory test result with clinical significance, and/or associated with concomitant symptoms;

C. Clinically significant symptoms and signs;

D. Clinically significant findings (e.g., physical examination, ECG, etc.);

E. Exacerbation of pre-existing disease, symptoms, signs, or laboratory abnormalities;

F. Allergy.

6.1.2 Access to Adverse Events

The investigator should report all AEs directly observed or spontaneously reported by the subjects. In addition, investigators are required to regularly inquire about AEs from the participants after the initiation of this trial.

6.1.3 Recording of adverse events

Adverse event record forms should be completed faithfully during the trial period (after the first dosing to the last follow-up visit), including the time of occurrence, severity, duration, measures

taken and outcome of the adverse event. AEs should be recorded in the designated adverse event form in the original medical record and case report form.

The adverse event should be named in medical terminology, and prefer using medical diagnosis. That is, multiple signs, symptoms, and laboratory abnormalities can be called or attributed to a disease or impairment, then this is treated as an adverse event. Symptoms/signs will be used if the diagnosis cannot be made definitively. When the diagnosis is clear at a later stage, the record should be updated to replace the previous symptoms/signs with the diagnosis.

When determining the names of AEs, ensure that each adverse event name consists of a single event. One diagnosis, sign/symptom is one adverse event. Therefore, when a subject presents with symptoms of "vomiting and diarrhea," the adverse event name should be recorded as two AEs, e.g., (1) diarrhea and (2) vomiting, rather than one "diarrhea and vomiting" for both symptoms. If the laboratory test abnormality is part of a syndrome, record the syndrome or diagnosis (e.g., anemia) rather than the laboratory test result (i.e., decreased hemoglobin).

AEs are recorded according to the latest edition of the International Medical Dictionary for Regulatory Activities (MedDRA) as "System Organ Class (SOC)" and "Preferred Term (PT)"."Preferred Term (PT). Describe the number of cases, number of occurrences and incidence of all AEs, all TEAEs, TEAEs related to IMP, serious TEAEs, serious TEAEs related to IMP, TEAEs leading to withdrawal from the trial, TEAE-related deaths, etc., according to treatment group.

Duration of Adverse Event Collection

In this trial, safety information should be collected from the time the subject signs informed consent until 14 days after the last dosing.

6.1.4 Follow-up of unresolved adverse events

All AEs and serious AEs for each subject should be actively followed up throughout the study. All events should be followed up until resolved, or the subject’s conditions are stable, or the cause of the event has other explanations, or the subject is lost to follow-up, even if the event persists after discontinuation of treatment or termination of the study. As long as there is a medical indication, the investigator should follow up any AEs that were not resolved at the time

of the last visit, but the follow-up not need to be recorded in the eCRF.

The sponsor reserves the right to request more information (if necessary) from any subject regarding the ongoing AEs/serious AEs after the end of the study.

Post-study event

After the end of the last visit or permanent suspension of the study, the investigator is no longer obliged to inform the sponsor of new AEs or serious AEs occurred in subjects after the safety follow-up. However, the investigator should inform the sponsor's pharmacovigilance team or representative if the investigator is informed of any serious adverse event (including death) that has occurred after the subject has permanently withdrawn from the study and there is reasonable reason to believe that the event may be related to the investigational product.

Variable

The following variables will be collected for each adverse event (AE):

(1) AEs (written records);

(2) The start and end dates of AEs;

(3) The severity grade according to CTCAE 5.0;

(4) Whether it is a serious adverse event (SAE);

(5) The causal relationship between the AEs and the IMP assessed by the investigator;

(6) Measures taken on IMP;

(7) Treatment for the AE;

(8) Outcome of the AE.

In addition, the following variables will be collected from each serious adverse event:

(1) The date of the adverse event meets the criteria for the SAE;

(2) The date of the investigator is informed of the SAE;

(3) The cause of the SAE;

(4) Date of discharge;

(5) Possible causes of death;

(6) The date of death;

(7) Autopsy (if any);

(8) Assessment of causality between SAE and research operations;

(9) Assessment of causality between SAE and other medications;

(10) Description of the SAE.

6.2 Serious Adverse Events

6.2.1 Definition of Serious Adverse Events

According to the ICH, a SAE refers to events that have caused one of the following damages after the use of IMP:

(1) Death;

(2) Life-threatening (Subjects face the risk of death when an event occurs. It does not refer to events that theoretically may lead to death if exacerbated);

(3) Permanent or severe disability or loss of function;

(4) Hospitalization or prolongation of existing hospitalization;

(5) Congenital anomalies or birth defects;

(6) Other important medical event.

Other important medical events: Medical and scientific judgment should be exercised in deciding whether to speed up reporting on other situations. Important medical events may not immediately endanger life, lead to death or hospitalization, but if medical measures are needed to prevent one of the above situations, it is usually considered to be serious. Example of such events include important treatment in the emergency room or allergic bronchospasm or convulsions occurring at home or resulting in drug dependence or abuse, etc.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

SUSAR means the suspected and unexpected serious adverse reaction whose clinical presentation is of a nature and severity that exceeds the information available in the investigator's brochure for the IMP. The definition of predictability of AEs will be judged based on whether they are listed in the investigator's brochure.

Hospitalization

AEs during the study leading to hospitalization or prolongation of existing hospitalization are attributed to SAEs (the reason for this event should be due to AEs, not due to selective surgery, non-medical reasons, etc.). Any new medical facility hospitalization (even if the time of hospitalization is less than 24 hours) meets this standard. Hospitalization also includes in-hospital transport to emergency/intensive care unit (e.g. from department of internal medicine transport to coronary care unit, from neurology department transport to tuberculosis ward, etc.). Hospitalization does not include the following situations:

(1) Rehabilitation institutions;

(2) Hospice care institutions;

(3) Short-term care institutions (e.g. nursing care);

(4) Professional nursing institutions;

(5) Nursing home;

(6) Admission to the regular emergency department;

(7) Day surgery (outpatient/day surgery/daytime operation).

Hospitalization or prolonged hospitalization due to the following reasons is not a SAE:

(1) Hospitalization due to progression of pre-existing disease that unrelated to new AEs (for example, an examination of an abnormal laboratory test that has been performed prior to treatment);

(2) Hospitalization for non-medical reasons (e.g. the subject is homeless);

(3) Transactional hospitalization (e.g. annual medical examination);

(4) Hospitalization as planned by the clinical trial protocol (e.g., for the required operation of the clinical trial protocol);

(5) Voluntary hospitalization that does not cause clinical AEs (e.g. for elective plastic surgery);

(6) Pre-planned treatment or surgery for the entire clinical trial protocol and/or subject should be recorded in the baseline document;

(7) Specially admitted to hospital for the use of IMP.

6.2.2 Severity assessment

SAEs from severe adverse events need to be identified. An adverse event that is severe in severity is not necessarily a serious adverse event. For example, vomiting that lasts for several hours may be considered severe but is not a serious adverse event. On the other hand, a stroke that results in only minor functional impairment may be considered a mild stroke but is a serious adverse event.

The investigator should assess the severity of each adverse event and serious adverse event reported during the study according to NCI-CTCAE v5.0.

AEs not included in the standard are referred to the following criteria:

Grade I: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade II: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living, which refers to preparing meals, shopping for groceries, using the phone, managing money, etc.

Grade III: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living, which refers to: bathing, dressing and undressing, feeding self, using the toilet, taking medications, etc., not bedridden.

Grade IV: Life-threatening consequences; urgent intervention indicated.

Grade V: Death related to adverse events.

6.2.3 Causality relation assessment

The analysis of the correlation between AEs and the IMP requires comprehensive consideration of the following factors:

(1) Whether there is a reasonable temporal relation between the AEs and the action time of the IMP;

(2) Whether the AE disappears or resolves after the IMP is discontinued or the dose reduced;

(3) Whether the clinical or pathological manifestations of the AEs are consistent with the known pharmacological and toxicological characteristics of the IMP;

(4) Whether the adverse event can be explained by the subject's own clinical status, psychological factors, or other environmental factors.

The investigator should evaluate the possible relationship among the AEs, the IMP, and concomitant medications according to the following five-level classification criteria:

1) Definitely related: Occurrence of the reaction is in line temporally with the IMP administration, and the reaction is consistent with the type of reaction known for the IMP; the AE may have improved after discontinuing IMP, and recurred after resuming dosing.

2) Probably related: Occurrence of the reaction is in line temporally with the treatment, the reaction is consistent with the known principles of the treatment, and cannot be explained by the subject's clinical status, psychological factors, or other environmental factors; the adverse event is resolved after the treatment is discontinued.

3) Possibly related: Occurrence of the reaction is in line temporally with the IMP, the reaction is consistent with the type of reaction known for the IMP, and the subject's clinical status, psychological factors, or other environmental factors also could have caused the reaction.

4) Unlikely related: Occurrence of the reaction is not in line temporally with the IMP, the reaction is not quite consistent with the type of reaction known for the IMP, and the subject's clinical status, psychological factors, or other environmental factors also could have caused

the reaction.

5) Not related: Occurrence of the reaction is not in line temporally with the IMP, the reaction is not consistent with the type of reaction known for the IMP, and the subject's clinical status, psychological factors, or other environmental factors also could have caused; the reaction resolved when the external conditions were improved, or when the treatment is discontinued.

Adverse Events Related to the Investigational Product

	Definitely related	Probably related	Possibly related	Unlikely related	Not related
Reasonable time sequence with investigational product	+	+	+	+	-
Known types of drug reaction	+	+	+	-	-
Decrease or disappearance of the reaction after stopping the drug	+	+	±	±	-
Repeated reactions after re-administration	+	?	?	?	-
Cannot be explained by subject's disease	+	+	-	±	-

Note: + denotes affirmation, - denotes negation, ± denotes difficulty in affirmation or negation, ? indicates that the situation is unknown. Definitely related, probably related, possibly related, these three levels can be considered as adverse reactions caused by the drug.

6.2.4 Management of SAEs

Any SAE must be treated immediately with appropriate therapeutic measures.

6.2.5 Follow-up of SAEs

When a SAE occurs, subjects must be followed up until complete clinical recovery and normalization of laboratory test results, or until their condition is stabilized. Follow-up information must be recorded on the SAE follow-up form and reported to the regulatory authority, ethics committee and sponsor.

If at any time after discontinuation of the IMP there is a SAE that comes to the attention of the investigator, and the investigator believes that there is a reasonable probability that the SAE is caused by the IMP, it should be reported to the sponsor and the ethics committee.

6.2.6 Disease progression

Progression of the disease refers to the deterioration of the subject's condition by the disease being studied. Progression of the disease includes an increase in the severity of the disease being studied and/or an increase in the symptoms of the disease. In this trial, events clearly attributed to disease progression should not be reported as AEs.

6.3 Report of AEs/ SAEs /Suspected Unexpected Serious Adverse Reaction (SUSAR)

Each AE should be assessed to see if it meets the criteria for a SAE. If a SAE occurs, it should be reported in accordance with relevant regulations.

6.3.1 Report of Serious Adverse Events

Except for SAEs that are not required to be reported immediately as specified in the trial protocol or other documents (e.g., investigator's brochure), the investigator should immediately report all SAEs in writing to the sponsor, and should subsequently provide a detailed, written follow-up report in a timely manner. Serious adverse event reports and follow-up reports should indicate the subject's identification code for the clinical trial, rather than the subject's real name, civil identification number, and residential address and other identifying information. The AEs important for safety evaluation and laboratory outliers, which are specified in the trial protocol, should be reported to the sponsor in accordance with the requirements and timeframe of the trial protocol.

For reports involving death events, the investigator should provide other required data, such as autopsy reports and final medical reports, to the sponsor and the ethics committee.

The investigator should sign and read the relevant safety information of the clinical trial provided by the sponsor in a timely manner upon receipt and consider the treatment of the subjects, whether to adjust accordingly, and communicate with the subjects at the earliest possible time if necessary, and the SUSAR provided by the sponsor should be reported to the ethics committee.

6.3.2 Suspected Unexpected Serious Adverse Reaction Reporting Requirements

The sponsor should timely report to the Center for Drug Evaluation information of SUSAR and other potential serious safety risks that occurs during the clinical trial in accordance with the relevant requirements. According to the severity of safety risks, the sponsor can be required to take measures to adjust the drug clinical trial protocol, informed consent form, investigator's brochure and other measures to strengthen risk control. If necessary, the sponsor can be required to suspend or terminate the drug clinical trial.

The sponsor should report adverse drug reactions in accordance with the requirements and timelines. Upon receipt of safety-related information from any source, the sponsor should immediately analyze and assess it, including the severity, relevance to the IMP and whether it is an expected event, etc. The sponsor should expeditiously report SUSAR to all investigators participating in the clinical trial as well as to the sites and the ethics committees; the sponsor should report SUSAR to the National Medical Products Administration and the health authorities.

Expedited reporting to the Center for Drug Evaluation according to the nature (category) of the SAE in accordance with the following timeframes:

(i) For fatal or life-threatening unexpected serious adverse reactions, the applicant should report as soon as first informed, but not more than 7 days. And report and complete the follow-up information within the following 8 days.

Note: The day the applicant is first informed is day 0.

(ii) For non-fatal or life-threatening unexpected serious adverse reactions, the applicant should report as soon as possible after first informed, but not more than 15 days.

Expedited reporting of other potentially serious safety risk information can be emailed to:

lcqjywjj@cde.org.cn

SAE and Other Safety Information Reporting Requirements

	SAE	SUSAR	Other potentially serious safety risk information	Periodic Safety Update Report
Reporter	Investigator	The sponsor	The sponsor	The sponsor
Report Recipient	Sponsor Ethics (Death event only)	The site/ Ethics Committee/The Center for Drug Evaluation	Investigator Ethics Committee The Center for Drug Evaluation	Investigator Ethics Committee The Center for Drug Evaluation
Time limit	Immediate (no specific time limit)	CDE corresponds to a expedited reporting time limit of 7/15 days. Not specified for the other department.	Expedited reporting by mail is available. lcqjywjj@cde.org.cn	Once a year or as specified by CDE, not specified by other departments

6.3.3 Death

All deaths that occur during the study period or during the follow-up period following the IMP administration as specified in the clinical trial protocol must be reported as follows:

- When it’s confirmed that the death is caused by disease progression, it should be reported to the study monitor during the next monitor visit and should be recorded in the eCRF, but not as a SAE.
- Unexplained deaths should always be reported as SAEs. An autopsy may help assess the cause of death. If an autopsy is performed, a copy of the autopsy report should be forwarded to the sponsor's pharmacovigilance team or its representative within reasonable time frame.

6.3.4 Non-serious adverse event reporting requirements

All AEs must be recorded under the corresponding item on the AEs page /eCRF. Please note that the adverse event information collection form and AEs CRF are two different forms. When collecting same information, the forms must be filled in consistently. For example, the same adverse event term should be used on both tables. AEs should be reported in concise medical terms on both the CRF and serious adverse event information collection form.

6.3.5 Pregnancy

To ensure the safety of the subject, each pregnancy and pregnancy outcome of the subject treated with the trial must be reported to the sponsor using a corresponding pregnancy report form within 24 hours after being informed. Abnormal pregnancy outcomes should be considered as SAEs and reported using a table of SAEs.

Pregnancy is not considered as an adverse event unless it is suspected that the IMP may affect the effectiveness of the contraceptive. Congenital malformations/ birth defects and spontaneous abortion should be reported and treated as SAEs. Selective abortion without complications will not be treated as an adverse event. All pregnancy outcomes (spontaneous abortion, elective abortion, ectopic pregnancy, normal production, or congenital anomalies) should be followed up and recorded, even if the subject has withdrawn from the study.

If any pregnancy event occurs during the study, the investigator or other person in the site should report the incident to the appropriate sponsor representative within 24 hours of the event being known.

Access to pregnancy outcome information should also follow the same time requirements.

The following CRF modules should be used for reporting: use the pregnancy reporting template in the CRF to report pregnancy events and use the pregnancy outcome reporting template to report pregnancy outcomes.

Pregnancy events that occur within 6 months of the end of the study should also be reported to the sponsor.

7 STUDY TERMINATION AND SUBJECT WITHDRAWAL

7.1 Study Termination

The termination of the study means that the clinical study has not been completed according to the protocol, and all studies are stopped in the middle. The primary purpose of the study termination is to protect the rights of the subjects, or to ensure the quality of the study, or to avoid unnecessary economic losses. Early termination of clinical trials should be promptly notified to the investigating parties. The termination of the study includes the following:

(1) Serious safety problems are found during the study, and the study needs to be terminated after the drug safety committee and the investigator jointly evaluate;

(2) The IMP is found to have no clinical value during the study;

(3) During the study, it is found that there are major errors in the design of the protocol, it is difficult to evaluate the drug, or there is a major deviation in the implementation of the protocol, which affects the final evaluation of the drug, and the study should be suspended;

(4) The sponsor requests to stop the study (such as funding reasons, management reasons, etc.);

(5) The drug regulatory authority orders the study have to stop for some reason.

7.2 Subject Withdrawal

7.2.1 Withdrawal Determined by Investigator

The investigator decides to withdraw subjects from the study, including following situations:

(1) There are some complications or special physiological changes in the subjects during the study, which are not suitable for further study;

(2) Poor compliance of subjects, which affect safety and efficacy evaluation;

(3) Subjects who have SAEs and are not suitable to continue the study;

(4) Other circumstances that the investigator determines require withdrawal from the study.

The proposed indication of TBN is ALS, which is a progressive disease with the possibility of disability and death. The withdrawal criteria for ALS patients with significant deterioration of the condition are set, and the rescue treatment measures are improved as follows:

1) The patient cannot swallow or come to the hospital for visits, affecting administration and routine follow-up, the investigator decides whether the subject withdraws from the trial, and follows up regularly until the end of the study.

2) The patient has respiratory failure and relies on a ventilator to maintain ventilation (≥ 22 h per day for ≥10 days), which may endanger their life, continuing the trial may affect the patients' life safety, and the investigator decides whether the subject withdraws from the trial.

3) To protect the subjects, when they have respiratory failure or other emergency situations, the department should have the ability to deploy emergency treatment personnel and equipment, and use ventilators and other rescue equipment in time, and open the green treatment channel of the department when needed.

4) The patient has a serious adverse event or the condition worsens to the point of endangering their life. To protect the subject and respect their wishes, the investigator can consider letting them withdraw and receive more treatment after assessing their survival status, and follow up until the end of the study.

7.2.2 Withdrawal Decided by Subject

According to the provisions of the informed consent form, subjects have the right to withdraw from the study halfway, or not receive the IMP or test, or be lost to the visit (also referred to as withdrawal, or drop-out). The reason for withdrawal should be known and recorded. If subjects withdraw from the study due to AEs or abnormal laboratory test results, this important and special event and its test results should be recorded on the electronic case report form (eCRF).

For a subject who drops out of the study or drops out of follow-up, the investigator should take active measures to complete the last examination as much as possible, so as to analyze the efficacy and safety of the patients. The cause of drop-out should be documented in detail, safety and efficacy data for the subject should be available to the extent possible, and all end-of-study evaluations should be conducted with subject consent and compliance. All case data should be kept integrally for reference. Subjects who sign informed consent forms and have been randomly assigned in groups cannot be replaced.

8 STATISTICAL ANALYSIS

Statistical analysis will be carried out under the authorization of the sponsor. The following sections describe the statistical methods used for the efficacy and safety analysis of this study. A detailed description will be provided in the Statistical Analysis Plan (SAP).

All statistical analyses will be programmed using SAS 9.4 or higher version software. All statistical tests will be performed on a two-sided test. $P \leq 0.05$ will be considered statistically significant, and the confidence interval is 95% confidence.

8.1 Sample Size Estimation

The trial is an exploratory study of safety and efficacy, no statistical assumptions will be made and no sample calculations.

This study is expected to enroll a total of 150 subjects, with 50 in each group.

8.2 Analysis Population

This study used three data sets: Full Analysis Set, Per-Protocol analysis set, and Safety analysis set.

Full Analysis Set (FAS): A collection of all randomized subjects with at least one study administration and at least one valid post-dose efficacy evaluation data. FAS is the primary analysis set. The FAS will be used to analyze the subject's distribution, demographic data, and baseline characteristics and efficacy. Subjects will be analyzed according to their randomized groups.

Per-Protocol Set (PPS): It included FAS subjects who meet the main inclusion criteria and do not meet the main exclusion criteria, complete the treatment without any major protocol deviations. The PPS is mainly used for the sensitivity analysis of efficacy.

Safety analysis set (SS): A collection of subjects with at least one administration and actual safety data of the post study administration. Missing safety values are generally not carried forward. SS will be used for safety analysis. Subjects will be analyzed according to randomized groups.

8.3 Statistical Method

8.3.1 Enrollment Analysis

List the number of enrolled and completed subjects in total and for each site. Identify three analysis data sets (FAS, PPS, SS). List the reasons for excluding subjects from the PPS."

The disposition of missing values data is detailed in the statistical analysis plan.

8.3.2 Demographic Data and Baseline Analysis

Descriptive statistical demographic data and other baseline characteristics:

- Continuous variables: Calculate the number of subjects, mean, standard deviation (SD), median (Q1, Q2), minimum, and maximum;
- Count data and categorical data to calculate frequency and composition ratio;
- Perform a difference test between groups, with P values listed as exploratory results.

8.3.3 Primary Efficacy Endpoint Evaluation

The primary endpoint is difference between the groups in the ALSFRS-R score change from baseline at Day 180.

ALSFRS-R data of each visit in different treatment groups are statistically described by mean ± standard deviation and median (minimum and maximum). Comparing with the baseline, the paired T test is to be used to compare the within-group before-after differences. Analysis of variance (ANOVA) is used for comparing changes before and after treatment in each group, and the linear model will include three random stratification factors. The Tukey method-corrected 95% confidence interval for each group's least squares means (LS Means) and Least Squares Means Difference is to be calculated according to the model's least squares means (LS Means) to compare the statistical differences between the two groups. The model is to be used to further investigate the interaction between groups and three random stratification factors. If there is a qualitative interaction, a subgroup analysis of the relevant random stratification factors will be performed.

8.3.4 Analysis of Secondary Efficacy Endpoint

The following four secondary efficacy endpoints, the continuous data of each visit in different groups will be described using mean ± standard deviation and median (minimum, maximum). Comparing with the baseline data, the paired T test is to be used to compare the within-group before-after differences. Analysis of variance (ANOVA) is to be used before and after treatment between groups. For the first secondary endpoint (endpoint event and disease exacerbation event), Kaplan-Meier plots will be used to describe the survival rate of patients without endpoint event, and Log-Rank test or non-parametric statistical method is to be used to compare the indicators of each group.

(1) Proportion of endpoint events, including death, tracheotomy, invasive ventilator-assisted ventilation, or continuous non-invasive ventilator-assisted ventilation (≥ 22 h per day for ≥ 10 days);

(2) Difference between the groups in grip strength change from baseline at Day 180;

(3) Difference between the groups in respiratory function (%FVC) change from baseline at Day 180;

(4) Difference between the groups in ALSAQ-40 scores change from baseline at Day 180.

8.3.5 Safety Assessment

Descriptive statistical analysis is mainly to be used to list the AE and SAE, drug-related AE and SAE that occurs in this trial, and between-group comparisons are conducted using the X^2 test or Fisher's test.

Descriptive statistics are to be used to summarize laboratory tests, vital signs, and electrocardiograms, etc. Shift tables are to be used to describe changes in normal/abnormal results before and after administration, such as laboratory tests, electrocardiograms, and physical examinations.

8.3.6 Interim Analysis

Not Applicable

9 QUALITY CONTROL, QUALITY ASSURANCE AND DATA

MANAGEMENT

According to the GCP Guidelines, the sponsor is responsible for implementing and maintaining the quality assurance and quality control system and should have written standard operating procedures (SOPs).

Quality control applies to every stage of data processing. The following steps will be taken to ensure the accuracy, consistency, completeness and reliability of the data:

- Conference of investigator;
- Site initiation visit;
- The early stage site visit after enrollment;
- Site routine monitoring;
- Continuous site communication and training;
- Data management quality control check;
- Continuous data collection and organization;
- Internal data review;
- Quality control of final clinical study report.

In addition, the sponsor and/or CRO Clinical Quality Assurance department may conduct periodic audits of the study process, including but not limited to site visits, suppliers, clinical databases, and final clinical study reports.

All study-related documents, including medical history and combined medication records, authorized by the sponsor's authorized representatives and regulatory authorities, must be audited.

9.1 Clinical Monitoring

In accordance with the relevant regulations, GCP and sponsor procedures, the sponsor conducts all monitoring activities for this clinical trial. The CRA will work in accordance with the SOPs

and monitoring plan set by the sponsor. The CRA will establish and maintain regular contact between the investigator and the sponsor. When contacting, the CRA will:

- Check the progress of the study;
- Review the collected data;
- Verify source data;
- Identify and resolve issues.

Its purpose is to confirm:

- The data is true, accurate and intact;
- Whether the safety and rights of subject are protected;
- The study is conducted in accordance with the currently approved clinical trial protocol (and any supplements), GCP and all relevant regulatory requirements.

Investigators agreed to allow the CRA to view all relevant documents directly, arrange the time of the individual and staff, discuss the findings and all related issues with the CRA.

9.2 Quality Assurance Audit/Inspection

The sponsor's authorized representative, regulatory authority or ethics committee may conduct audits or inspections at the site, including source data checks. The purpose of the audit or inspection is to systematically and independently review all study-related activities and records to determine that whether the records, analysis, and reports of these activities and data meet the requirements of the clinical trial protocol, GCP, ICH guidelines, and any relevant regulations. Such audits/inspections may be conducted at any time during the study or at the end of the study. If an audit or inspection occurs, the investigator and agency should agree to allow the auditor/monitor to view all relevant documents, arrange the time and discuss the findings and all related issues with the auditor/monitor.

9.3 Data Management

The clinical trial data management process of this study should meet the requirements of the

National Medical Products Administration Good Clinical Practice and corresponding regulations, and comply with the Standard Operating Procedures (SOP) of the data management department to ensure the authenticity, accuracy, integrity, reliability and traceability of clinical trial data. The details of clinical trial data management will be specified in the data management plan.

9.3.1 Data Management Collection and Entry

In this study, the data is to be entered directly into the Electronic Data Collection (EDC). The investigator or CRC will fill in the subject information through the EDC system accurately, timely, integrally and systematically based on the source data of the subject.

The investigator's primary responsibility is to ensure that the data reported in an electronic case report form or other form is accurate, intact, and timely, and that the data on the electronic case report form is from the subject's source data and must account for any differences.

The CRA should monitor whether the clinical trial follows the protocol and conduct a Source Document Verification (SDV) to confirm that all electronic case report forms (eCRFs) are in accordance with the source data. In case of errors or discrepancies, the investigator should be notified, and the corresponding questions should be recorded based on the errors or differences found, to ensure that all data is recorded and reported correctly and completely.

9.3.2 Data Verification and Query Management

The data manager performs data verification according to the data verification plan, which mainly includes manual verification and logical verification of computer system. Queries raised from the verification are answered by the data entry personnel. If the questions are resolved by the answer, the data management will shut down the queries, and if the queries are not resolved, it will be raised again. This process continues until the data is “clean”.

9.3.3 Data Modification and Review

Data entry personnel or investigator can modify the data after verifying the data, and the modified data need to fill in the reason for modification on the eCRF. The investigator has the authority to review all final data.

9.3.4 Electronic signature

After the data in the database have been verified and cleared beyond doubt by the CRA and the data manager, the data will be frozen by the data manager, at which point the data can no longer be modified, and the principal investigator will confirm the authenticity and integrity of the frozen data and electronically sign it.

9.3.5 Database Locking

According to the database locking process, once all the previous steps are completed, the locking of database should be approved in written. The data editing permission of the database should be withdrawn to lock the database. If problems are discovered after the locking of database and need to be modified, the data should be modified in strict accordance with the process of unlocking and re-locking. All data are eventually exported from the EDC database by the data manager to the statistician for analysis.

9.3.6 Archiving of Data

- After the trial is over, the relevant data will be archived and saved;
- The project data management document is kept for at least 2 years after the locking as required by the regulations;
- The data should be kept for a longer period of time if required by current regulations or agreements with sponsors.

9.4 Independent data monitoring (Data and Safety Monitoring Board)

During the course of the study, the sponsor establishes a data and safety monitoring committee that periodically evaluates the progress of the clinical trial, safety data, and important effectiveness endpoints, and recommends to the sponsor whether to continue, adjust, or discontinue the trial in order to identify drug inefficacies or safety problems as early as possible, and to minimize the risk to the subjects.

10 ETHICS AND REGULATORY REQUIREMENTS

10.1 Ethical conduct and Ethical Approval

This study will be conducted followed the Helsinki Declaration (2013 Edition), GCP and related

regulations.

The investigator (or sponsor, if applicable) is responsible for obtaining the review and approval of clinical trial protocol, of informed consent form providing to the site and of any other information providing to potential subjects (such as advertising or information supporting or supplement of the informed consent) from the ethics committee. Investigators agree that the ethics committee has direct access to all relevant documents. The composition of the ethics committee must comply with relevant regulatory requirements. The sponsor will provide the investigator with the relevant documents/information required by the ethics committee to review and approve the study.

If the clinical trial protocol, informed consent form, or any other information that the ethics committee has approved to provide to potential subjects is revised during the study, investigators are responsible for ensuring that the Ethics committee approves the revised documents (if applicable). Investigator must follow all relevant regulatory requirements for the use of the revised informed consent, including obtaining approval from the ethics committee for revised informed consent form before the new subject agrees to participate in the study using the new form. A copy of the Ethics committee's approval of the informed consent form / other information and approval of the revised informed consent form /other information must be sent to the sponsor in a timely manner.

10.2 Informed Consent Procedure

The principal investigator in each site will:

- Ensure that each subject or his/her legal representative is provided with a comprehensive and sufficient oral or written information about the nature, purpose, risks and benefits of the study; Ensure that each subject or his/her legal representative knows that participation in this clinical study is completely voluntary, that the subjects can withdraw from the study unconditionally at any time during the study;
- Ensure that each subject or his/her legal representative has the opportunity to ask questions and be given sufficient time to consider the information provided;
- Ensure that each subject or his/her legal representative provides an informed consent form

(ICF) with signature and date prior to any study procedures;

- Ensure that the signed ICF original is kept in the investigator site file;
- Ensure that a copy of signed ICF is provided to the subject;
- Ensure that any benefit to the subject of the study and any potential harm to the subject is stated in the ICF approved by the Ethics committee.

10.3 Study Protocol and Informed Consent Form Revised Procedures

The study process may be modified only after consultation between the principal investigator and the sponsor.

If there are any significant changes to the clinical study protocol, these changes must be documented in the protocol amendment of the latest clinical trial protocol (if needed). The amendment should be approved by ethics committee and national regulatory authority (if applicable) before implementation. The sponsor will release the latest version of the protocol and any amendments to each principal investigator.

If the clinical trial protocol revision requires changes to the ICF of the site, the sponsor and the ethics committee of the site will approve the ICF amendment before it can be used. Any management changes should be notified and approved by each ethics committee.

10.4 Site Close-out

At the end of the study, the CRA will conduct the following activities with the investigator or site staff (if applicable):

- Send all study data back to the sponsor;
- Data query;
- Counting, verification and disposal of unused IMP;
- Check the integrity of the study record;
- Send all treatment numbers back to the sponsor.

In addition, the sponsor reserves the right to suspend or permanently terminate one site or all site at any time for reasons including but are not limited to safety, ethical issues or serious non-compliance. If the sponsor decides to take this action, the sponsor will discuss with the investigator (including the reasons for the action). If feasible, the sponsor will notify the investigator in advance before taking action.

If the study needs to be suspended or terminated for safety reasons, the sponsor will promptly notify all other investigators and/or sites participating in the study, and will also inform the regulatory authorities of the suspension or termination of the study and the reasons for such action. The investigator must promptly notify the ethics committee and provide reasons for suspension or termination.

If the study is suspended early, all research data must be sent back to the sponsor. In addition, all unused IMP will be disposed of according to the sponsor procedures.

The economic compensation will be given to the investigator and/or the site according to the agreement between the investigator and the sponsor.

10.5 Record Retention

Research materials are saved as follows:

- The investigator must maintain all research materials;
- The sponsor may establish a computer database of all or part of the subjects if necessary;
- The sponsor and the clinical study leader shall have the right to access or review the source medical records of all subjects upon completion of certain procedures if necessary;
- The retention period is 5 years after the completion of the trial.
- All data of this clinical trial belong to Guangzhou Magpie Pharmaceutical Co., LTD., and the investigator shall not provide any information in any form to any third party without written consent of the sponsor, unless required by the regulatory authorities.

10.6 Confidentiality and Privacy

The informed consent form may contain information that complies with relevant data protection

and privacy rights. In some cases, this content will be recorded in a separate companion file.

The privacy of the subjects will be strictly confidential to the investigator, the research staff and the sponsor. The clinical study protocol, records, data and all other information generated will be kept strictly confidential. Research information or data will not be disclosed to any unauthorized third party without the written permission of the sponsor.

All study activities should be conducted in as private a place as possible.

The CRA, the sponsor's authorized representative, the ethics committee representative, the regulatory agency, or the pharmaceutical company that supplies the IMP may request that all documents and records kept by the investigator, including but not limited to the medical record of the study subjects (clinic, outpatient or hospital) and drug records. The site will allow these individuals to obtain these records.

Each site will strictly maintain the contact information of the subjects for internal use during the study period. At the end of the study, all records will continually to be kept in a safe place and will be determined according to the requirements of the Ethics Committee, the site system or the sponsor.

10.7 Publishing privacy

All data provided by Guangzhou Magpie Pharmaceuticals Co., Ltd. and all data generated during the study (except medical records of the subjects) should be kept confidential by the investigator and other staff of the site. The investigator or other staff members of the site may not use the material, data or records for any purpose other than for this study. These restrictions do not apply to:

(1) The data have been disclosed not due to errors by the investigator or the staff of the site;

(2) Information that must be made public in order to acquire trust from the academic committee or the ethics committee to evaluate the study;

(3) Information that must be made public in order to provide appropriate medical care to subjects in the study.

After the sponsor and the principal investigator of the site review, the site can summarize, submit and publish.

REFERENCE

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[3] Renton A E, Chio A, Traynor B J. State of play in amyotrophic lateral sclerosis genetics [J]. Nat Neurosci, 2014, 17(1): 17-23.

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LIST OF ABBREVIATIONS

Abbreviation/Term	Full Name
AE	Adverse event
ALS	Amyotrophic Lateral Sclerosis
ALSAQ-40	The amyotrophic lateral sclerosis assessment questionnaire-40
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale-revised
ALT	Alanine transaminase
AST	Aspartate aminotransferase
CFDA	China food and drug administration
CRC	Clinical research coordinator
CRF	Clinical report form
CRO	Contract research organization
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data and safety monitoring board
ECG	Electrocardiogram
eCRF	electronic case report form
EDC	Electronic data capture
FAS	Full analysis set

FDA	Food and drug administration
FVC	Forced vital capacity
GCP	Good clinical practice
ICF	Informed consent form
IMP	Investigational Product
MedDRA	Medical Dictionary for Drug Regulatory Activities
MRI	Magnetic resonance imaging
NOAEL	No observed adverse effect level
PPS	Per-protocol set
PT	Preferred term
SAE	Serious adverse event
SDV	Source data verification
SOC	System organ class
SOP	Standard operating procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TRAEs	Treatment-Related Adverse Events

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Appendix

Appendix 1. El Escorial World Federation of Neurology Criteria for the Diagnosis of ALS (Revised)

Diagnostic category	Diagnosis Criteria	
Clinically Definite ALS	UMN and LMN clinical signs in ≥ 3 body regions	**Without following situations:**
Clinically Probable ALS	UMN and LMN clinical signs in 2 body regions, and some UMN signs rostral to the LMN signs	1. Electrophysiological or pathologic examinations suggest that the signs of UMN and/or LMN may be caused by other diseases.
Clinically Probable ALS - Laboratory-supported	UMN and LMN clinical signs in 1 body region and EMG findings in 2 body regions	2. Neuroimaging suggests the presence of other diseases that may explain clinical signs or electrophysiological changes
Clinically Possible ALS	Clinical signs of UMN and LMN dysfunction together in only 1 region or UMN signs alone in 2 or more regions; or LMN signs rostral to UMN signs and the diagnosis of Clinically Probable ALS–Laboratory – supported cannot be proven by evidence on clinical grounds	
Clinically Suspected ALS*	**Clinically Suspected ALS** may be suspected in many settings, where the diagnosis of ALS could not be regarded as sufficiently certain to include the patient in a research study.	

* *This category is deleted from the revised EI Escorial Criteria for the Diagnosis of ALS.*

Appendix 2. The Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R)

Measure	Finding	Points
(1) Speech	Normal	4
	Detectable speech disturbance	3
	Intelligible with repeating	2
	Speech combined with non-vocal communications	1
	Loss of useful speech	0
(2) Salivation	Normal	4
	Slight but definite excess of saliva in mouth; may have nighttime drooling	3
	Moderately excessive saliva; may have minimal drooling	2
	Marked excess of saliva with some drooling	1
	Marked drooling; requires constant tissue or handkerchief	0
(3) Swallowing	Normal	4
	Early eating problems; occasional choking	3
	Dietary consistency changes	2
	Needs supplemental tube feedings	1
	Nothing by mouth (NPO); exclusively parenteral or enteral feeding	0
(4) Handwriting	Normal	4

(dominant hand before ALS)	Slow or sloppy; all words are legible	3
	Not all words are legible	2
	Able to grasp pen but unable to write	1
	Unable to grip pen	0
(5a) Cutting food and handling utensils (subjects without gastrostomy)	Normal	4
	Somewhat slow and clumsy but no help required	3
	Can cut most foods although clumsy and slow; some help needed	2
	Food must be cut by someone but can still feed slowly	1
	Needs to be fed	0
(5b) Cutting food and handling utensils (subjects with gastrostomy)	Normal	4
	Clumsy but able to perform with manipulations independently	3
	Some help needed with closures and fasteners	2
	Needs minimal assistance from a caregiver	1
	Unable to perform any aspect of task	0
(6) Dressing and hygiene	Normal	4
	Independent and complete self-care with effort or decreased efficiency	3
	Intermittent assistance or substitute methods	2
	Needs attendant for self-care	1

	Total dependence	0
(7) Turning in bed and adjusting bed clothes	Normal	4
	Somewhat slow and clumsy but no help needed	3
	Can turn alone or adjust sheets but with great difficulty	2
	Can initiate but not turn or adjust sheets alone	1
	Helpless	0
(8) Walking	Normal	4
	Early ambulation difficulties	3
	Walks with assistance (any auxiliaries, including AFOs)	2
	Non-ambulatory functional movement only	1
	No purposeful leg movement	0
(9) Climbing stairs	Normal	4
	Slow	3
	Mild unsteadiness or fatigue	2
	Needs assistance (including handrails)	1
	Cannot do	0
(10) Dyspnea	None	4
	Occurs while walking	3
	Occurs in one or more of the following scenarios: eating, bathing, and dressing	2

	Occurs at rest: when sitting or lying down	1
	Very difficult, considers mechanical respiratory supports	0
(11) Orthopnoea	None	4
	Difficult to sleep due to shortness of breath but no need for more than two pillows during daily sleep	3
	Need extra pillows to fall asleep (more than 2)	2
	Can only sleep sitting up	1
	Difficulty to sleep without mechanical support	0
(12) Respiratory insufficiency	None	4
	Intermittent use of ventilator	3
	Needs the ventilator every night	2
	Needs the ventilator all day and night	1
	Invasive ventilation: intubated or a tracheostomy has been performed	0
	Total	

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Appendix 3. The amyotrophic lateral sclerosis assessment questionnaire-40 (ALSAQ-40)

NAME: **Birth Day:** **Sex (Male/Female)**

Medical record number:

Assessment Date:

Please complete this questionnaire as soon as possible. If you have any difficulties filling in the questionnaire by yourself, please get someone else to help you with it. However, it is your responses that we are interested in.

The questionnaire consists of a number of statements about difficulties that you may have experienced **during the last 2 weeks**. There are no right or wrong answers: your first response is likely to be the most accurate for you. **Please tick the box which best describes your own experience or feelings.** For example, if you are unable to walk at all, please tick the box in Always or cannot do at all. Please tick the box for every question.

Please try to answer every question even though some may seem rather similar to others, or may not seem relevant to you.

	Never	**Occasionally**	**Rarely**	**Often**	**Always or cannot do at all**
Physical mobility, 10					
1. I have found it difficult to walk short distances, e.g around the house.	□	□	□	□	□
2. I have fallen over whist walking.	□	□	□	□	□
3. I have stumbled or tripped whilst walking.	□	□	□	□	□
4. I have Lost my balance whilst walking.	□	□	□	□	□
5. I have had to concentrate whilst walking.	□	□	□	□	□
6. Walking has tired me out.	□	□	□	□	□
7. I have had pains in my legs whilst walking.	□	□	□	□	□

8. I have found it difficult to go up and down the stairs.	□	□	□	□	□
9. I have found it difficult to stand up.	□	□	□	□	□
Activities of daily living/independence, 10					
10. I have found it difficult to get myself up out of chairs.	□	□	□	□	□
11. I have had difficulty using my arms and hands.	□	□	□	□	□
12. I have found turning and moving in bed difficult.	□	□	□	□	□
13. I have found picking things up difficult.	□	□	□	□	□
14. I have found holding books or newspapers, or turning pages, difficult.	□	□	□	□	□
15. I have had difficulty writing clearly.	□	□	□	□	□
16. I have found it difficult to do jobs around the house.	□	□	□	□	□
17 I have found it difficult to feed myself.	□	□	□	□	□
18. I have had difficulty combing my hair or cleaning my teeth.	□	□	□	□	□
19. I have had difficulty getting dressed.	□	□	□	□	□
20. I have had difficulty washing at the hand basin.	□	□	□	□	□
Eating and drinking, 3					

21. I have had difficulty swallowing.	□	□	□	□	□
22. I have had difficulty eating solid food.	□	□	□	□	□
23. I have found it difficult to drink liquids.	□	□	□	□	□
Communication, 7					
24. I have found it difficult to participate in conversations.	□	□	□	□	□
25. I have felt that my speech has not been easy to understand.	□	□	□	□	□
26. I have slurred or stuttered whilst speaking.	□	□	□	□	□
27. I have had to talk very slowly.	□	□	□	□	□
28. I have talked less than I used to do.	□	□	□	□	□
29. I have been frustrated by my speech.	□	□	□	□	□
30. I have felt self- conscious about my speech.	□	□	□	□	□
Emotional well-being, 10					
31. I have felt lonely.	□	□	□	□	□
32. I have been bored.	□	□	□	□	□
33. I have felt embarrassed in social situations.	□	□	□	□	□
34. I have felt hopeless about the future.	□	□	□	□	□
35. I have worried that I am a burden to other people.	□	□	□	□	□
36. I have wondered why I keep going.	□	□	□	□	□

37. I have felt angry because of the disease.	□	□	□	□	□
38. I have felt depressed.	□	□	□	□	□
39. I have worried about how the disease will affect me in the future.	□	□	□	□	□
40. I have felt as if I have no freedom.	□	□	□	□	□

Note:

The ALSAQ40 consists of 40 questions covering 5 domains of competence to assess the physical and mental health status of subject with ALS. The 5 domains are physical mobility, activities of daily living/independence, eating and drinking, communication, and emotional well-being, each of which constitutes a separate rating scale.

Scoring: Subjects choose appropriate descriptions for each question, and each description corresponds to a certain score (Never-0, Rarely-1, Sometimes-2, Often-3, Always or cannot do at all-4), with higher scores representing poorer ability. For each domain, the score is calculated by the following formula:

$$\text{Scale score} = \frac{\text{Total of raw scores of each scale item}}{\text{Maximum possible raw score of all scale items}} \times 100$$

Example: A score is now calculated for the subject's ability to eat and drink, and the subject describes the 3 questions as: difficulty swallowing-sometimes, difficulty eating solid food-sometimes, and difficult to drink liquids-rarely, the raw total score for this domain is 2+2+1=5. The theoretical maximum raw total score for this domain is 12 (3×4), and a score for this domain can be calculated as: 5/12 × 100 = 41.67 points.

Appendix 4. Mini-Mental State Examination (MMSE)

I'm going to ask you some questions to check your attention and memory. Most of them are easy.

(Examiner: Record the answers and circle the scores. Those who don't know are counted as wrong.)

		Right	Wrong
1.What is the year?	Year_____	1	5
2.What is the season?	Season_____	1	5
3.What is the day of the week?	Day_____	1	5
4.What is the date?	Date_____	1	5
5.What is the month?	Month_____	1	5
6. Can you please tell me where we are?			
For example, what province (city) are we in?	Province (City) _____	1	5
7.What district (county) is here?	District (county) _____	1	5
8.What street (township) is here?	Street (township) _____	1	5
9.What floor of the building are we on?	Floor _____	1	5
10. What is this place?	Address_____	1	5
	(Address or building name)		

11. I am going to name three objects. When I am finished, I want you to repeat them. Remember what they are because I am going to ask you to name them again in a few minutes. (Examiner: Say the following words slowly at one-second intervals):

"Ball" "Flag" "Tree"

Please repeat the three items for me. (Only score first attempt)	Right	Wrong	Refuse to answer

Ball	1	5	9
Flag	1	5	9
Tree	1	5	9

(Examiner: If the answer is wrong the first time, continue to repeat the three objects until the subject can repeat them correctly, up to six trials. Count trials and record.)

Trials _____

12. I would like you to count backward from 100 by 7. Tell me every answer until I say "stop".

(If the answer is wrong, but the following answer with the number of errors minus 7 is correct, then only the previous wrong answer is recorded.)

	Right	Wrong	Say can’t do it	Refuse to do it
93_____	1	5	7	9
86_____	1	5	7	9
79_____	1	5	7	9
72_____	1	5	7	9
65_____	1	5	7	9

Stop!

13. Now what were the three objects I asked you to remember?

Ball	Right	Wrong	Say can’t do it	Refuse to do it
Flag	1	5	7	9
Tree	1	5	7	9

14. (Examiner: show wristwatch) What is this called?

	Right	Wrong	Say can't do it	Refuse to do it
Wristwatch	1	5	7	9

14. (Examiner: show pencil) What is this called?

Pencil	1	5	7	9

15. Now I am going to say a phrase, and please repeat it clearly. The phrase is:"forty-four stone lions (in Mandarin Chinese)"

(Allow only one trial. Only correct and exact words count for one point.)

forty-four stone lions (in Mandarin Chinese)	1	5	7	9

16. (Examiner: Hand Card 1 to the subject) Please do what is written on this card.

	Right	Wrong	Say can't do it	Refuse to do it
Close your eyes	1	5	7	9

17. (Examiner: Say the following paragraph and give the subject a piece of paper; do not repeat the instructions or demonstrate) Please take this piece of paper in your right hand, then fold it in half with both hands and put it on your lap.

	Right	Wrong	Say can't do it	Refuse to do it
Take the paper in right hand.	1	5	7	9
Fold the paper in half	1	5	7	9
Put the paper on lap	1	5	7	9

18. Please say a complete and meaningful sentence. (Requirements: The sentence must have subject and verb, make sense, and record the sentence)

(Only score first attempt)	Meeting the requirements	Not meeting the requirements	Refuse to do it

Record_____	1	5	9

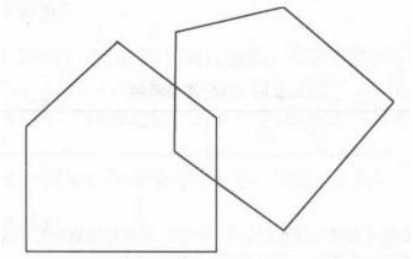
19. (Examiner: Hand Card 2 to subject) This is a picture, please copy it as it is.

(Requirements: The subject must draw a four-sided figure between two five-sided figures.)

	Right	Wrong	Say can’t do it	Refuse to do it
Record_____	1	5	7	9

Card 1 “Please close your eyes”

Card 2



Scoring Guidelines:

The scale consists of 19 questions with 30 subitems. Correct answers or operations are scored as "1", wrong answers are scored as "5" and refusals or say can’t do it are scored as "9" and "7". When calculating the score, the sum of all items (and subtests) marked "1", i.e., the number of items/subtests answered/operated correctly, is counted and is called the total MMSE score, which ranges from 0 to 30.