Registration classification: Class 2.2 chemical drug

A randomized, double-blind, placebo-controlled, multicenter phase III clinical trial to evaluate the efficacy and safety of TTYP01 tablets in the treatment of acute ischemic stroke

Protocol No.: TTYP01-III-AIS

Version: V2.0

Version Date: June 30, 2023

Sponsor:	Suzhou Auzone Biotechnology Co., Ltd.
Leading Site of Clinical Study:	Army Medical Center of PLA
Principal Investigator:	Prof. Yanjiang Wang
Data Management and Statistical Analysis Unit:	Beijing SSYP Data Technology Development Co., Ltd.
Contract Research Organization:	Guoxin Medical Technology (Beijing) Co., Ltd.

Statement and Signature Page of Sponsor

I have read and approved this protocol, Protocol No.: TTYP01-III-AIS, Version No.: V1.0 (dated 28 Oct 2022). I agree to the contents of this protocol and will conduct the study in strict accordance with this protocol. I will also strictly fulfill my obligations to the sponsor and other relevant requirement as required by the laws of China, Declaration of Helsinki, Good Clinical Practice (GCP) and this study protocol. I agree to comply with all SOPs of the hospital and Suzhou Auzone Biological Technology Co., Ltd. related to the conduct of this trial.

I will be responsible for initiating, applying for, organizing, funding, and monitoring this clinical trial in compliance with GCP regulations. I will establish a quality control and quality assurance system for the clinical trials and may conduct audits to ensure the quality of the study. I will cover the costs of treatment and provide appropriate economic compensation for subjects who experience trial-related injuries or death during the clinical trial. Additionally, I will offer legal and financial guarantees for the investigators involved.

At the same time, I will appoint a qualified CRO to manage and monitor the project, ensuring that the entire trial process is accurate, complete, and standardized.

Sponsor: Suzhou Auzone Biological Technology Co., Ltd.

Responsible Person: Xiaolong Hou

Signature: _____ __DD__MM__YYYY

Statement and Signature Page of Principal Investigator

I have read and approved this protocol, Protocol No.: TTYP01-III-AIS, Version No.: V1.0 (dated 28 Oct 2022). I agree to perform my duties in strict accordance with the laws of China, the Declaration of Helsinki, GCP, and this study protocol. I will provide copies of this protocol to all study teams involved in this trial and will discuss the protocol and related materials with them to ensure that they fully understand the study drug and how to conduct the trial prior to its initiation.

I will submit a CV to the Ethics Committee and, if necessary, to the Drug Administration before the start of the study. The study will only commence after receiving approval from the Ethics Committee. During the study, I will conduct the research in strict accordance with the requirements outlined in this protocol. Any modifications to the protocol will be implemented only after notifying the sponsor to obtain consent and securing re-approval or filing from the Ethics Committee, unless immediate measures are necessary to protect the safety, rights, and interests of the subjects.

My study team will make medical decisions related to the clinical trial to ensure that subjects receive timely treatment in the event of adverse events during the trial. I will ensure that all personnel are aware of the requirements for proper reporting of serious adverse events (SAEs) and will record and report these events as required.

I guarantee that the data will be accurately, completely, timely and legally recorded in the source documents and clinical observation record forms. I will accept monitoring or audits conducted by the monitor or auditor appointed by the sponsor, as well as audits and inspections by the drug regulatory authority, to ensure the quality of the clinical trial.

I agree that the research results may be used for drug registration and publicly published.

At the same time, I will keep this protocol and related content confidential.

Clinical Research Unit: Army Medical Center of PLA

Principal Investigator: Yanjiang Wang

Signature: _____	__DD__MM__YYYY

Statement and Signature Page of Subcenter Investigator

I have read and approved this protocol, Protocol No.: TTYP01-III-AIS, Version No.: V1.0 (dated 28 Oct 2022). I agree to perform my duties in strict accordance with the laws of China, the Declaration of Helsinki, GCP, and this study protocol. I will provide copies of this protocol to all study teams involved in this trial and will discuss the protocol and related materials with them to ensure that they fully understand the study drug and how to conduct the trial prior to its initiation.

I will submit a CV to the Ethics Committee and, if necessary, to the Drug Administration before the start of the study. The study will only commence after receiving approval from the Ethics Committee. During the study, I will conduct the research in strict accordance with the requirements outlined in this protocol. Any modifications to the protocol will be implemented only after notifying the sponsor to obtain consent and securing re-approval or filing from the Ethics Committee, unless immediate measures are necessary to protect the safety, rights, and interests of the subjects.

My study team will make medical decisions related to the clinical trial to ensure that subjects receive timely treatment in the event of adverse events during the trial. I will ensure that all personnel are aware of the requirements for proper reporting of serious adverse events (SAEs) and will record and report these events as required.

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I agree that the research results may be used for drug registration and publicly published.

At the same time, I will keep this protocol and related content confidential.

Clinical Research Unit:

Principal Investigator:

Signature: _____	__DD__MM__YYYY

Statement and Signature Page of Contract Research Organization

I have read and approved this protocol, Protocol No.: TTYP01-III-AIS, Version No.: V1.0 (dated 28 Oct 2022). I agree to this study protocol and perform my duties in strict accordance with the laws of China, the Declaration of Helsinki, GCP, and this study protocol. I will ensure that all personnel involved in this trial receive a copy of the study protocol. I will discuss the protocol and relevant materials with them to ensure that they fully understand the study drug and the procedures for conducting the trial prior to its initiation.

I will be responsible for medical and safety affairs, project management, clinical monitoring, and related tasks in accordance with GCP regulations and the requirements of the contract with the sponsor. I will establish a quality control and quality assurance system for the clinical trial and appoint qualified personnel to monitor and audit the entire process in line with the company's quality system, thereby ensuring that the trial process is accurate, complete, and standardized.

Contract Research Organization: Guoxin Medical Technology (Beijing) Co., Ltd.

Project Leader:

Signature: _____ __DD__MM__YYYY

Statement and Signature Page of Data Management and Statistical Analysis Unit

I have read and approved this protocol, Protocol No.: TTYP01-III-AIS, Version No.: V1.0 (dated 28 Oct 2022). I agree to this study protocol and perform my duties in strict accordance with the laws of China, the Declaration of Helsinki, GCP, and this study protocol. I will provide copies of this protocol to all relevant personnel involved in data management and statistical analysis of this trial within our company. I will discuss the protocol and relevant data with them to ensure that they fully understand the protocol and how to effectively perform data management and statistical analysis.

Data Management and Statistical Analysis Unit: Beijing SSYP Data Technology Development Co., Ltd.

Responsible Person: Jianmin Zhao

Signature: _____ __DD__MM__YYYY

Table of Contents

Abbreviations

Abbreviation	Definition
ADR	Adverse drug reaction
AE	Adverse event
AIS	Acute ischemic stroke
ALB	Albumin
A/G	Serum albumin to globulin ratio
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
ALS	Amyotrophic lateral sclerosis
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
AUC_{0-24h}	Area under the drug concentration-time curve from time 0 to 24 hours
AUC_{0-last}	Area under the concentration-time curve from 0 to the last measurable concentration
BASO	Basophils
BI	Barthel Index
BID	Twice daily
BUN	Blood urea nitrogen
CDE	Center for Drug Evaluation, NMPA
CFDA	China Food and Drug Administration
CI	Confidence interval
CK	Creatine kinase
CL	Clearance
C_{max}	Maximal concentration
Cr	Serum creatinine
CRE	Creatinine
CRO	Contract research organization
CT	Computed tomography
CTCAE	Common terminology criteria for adverse events
DIC	Disseminated intravascular coagulation
DMP	Data management plan
DSUR	Development safety update report
DVP	Data verification plan
eCRF	Electronic case report form
EDC	Electronic data capture
EOS	Eosinophils
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIB	Fibrinogen
GCP	Good Clinical Practice
GLU	Blood glucose
HCT	Haematocrit
HDRS	Hamilton Depression Scale-21
HGB	Hemoglobin
IC_{50}	Half maximal inhibitory concentration
ICF	Informed consent form
IDMC	Independent Data Monitoring Committee
INR	International normalized ratio
IWRS	Interactive Web Response System
LDH	Lactate dehydrogenase
LY	Lymphocytes
MCI	Mild cognitive impairment
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MONO	Monocyte

Abbreviation	Definition
MRI	Magnetic resonance imaging
mg	Milligram
ml	Milliliter
mRS	Modified Rankin Scale
MoCA	Montreal Cognitive Assessment
MTD	Maximum tolerated dose
NEUT	Neutrophils
NIHSS	National Institutes of Health Stroke Scale
NMPA	National Medical Products Administration
NOAEL	No-observed-adverse-effect level
PMDA	Pharmaceuticals and Medical Devices Agency
PLT	Platelets
PPS	Per-Protocol Analysis Set
PT	Prothrombin time
PT	Preferred term
QD	Quaque die
RBC	Red blood cell
RDW	Red blood cell distribution width
RET	Reticulocyte
SAE	Serious adverse event
SDV	Source data verification
SOC	System organ class
SS	Safety Set
SUSAR	Suspected unexpected serious adverse reaction
$t_{1/2}$	Elimination half-life
TBIL	Total bilirubin
TC	Total cholesterol
TEAE	Treatment emergent adverse event
TG	Triglycerides
T_{max}	Time to maximum
TP	Total protein
TT	Thrombin time
UA	Uric acid
UNL	Upper limit of normal
Urea	Urea
WBC	White blood cells
WHO	World Health Organization
γ-GTP	Gamma-glutamyltranspeptidase

Protocol Synopsis

Study Title	A randomized, double-blind, placebo-controlled, multi-center, phase III clinical trial to evaluate the efficacy and safety of TTYP01 tablets in the treatment of acute ischemic stroke
Registration Category	Class 2.2 chemical drug
Investigational Drug Name	TTYP01 tablets
Study Phase	III
Sponsor	Suzhou Auzone Biological Technology Co., Ltd.
Indication	Acute ischemic stroke
Study Objective	To evaluate the efficacy and safety of TTYP01 tablets in the treatment of acute ischemic stroke
Sample Size	The trial is expected to enroll 618 subjects, and all subjects are randomized (1:1) to the investigational drug group (TTYP01 group) and placebo control group (control group), with 309 subjects in each group.
Study Design	The study is designed as a multi-center, randomized, double-blind, parallel, placebo-controlled trial to evaluate the efficacy and safety of TTYP01 tablets in the treatment of AIS. **Study groups:** 309 subjects in the TTYP01 group and 309 subjects in the control group are randomly enrolled in parallel: • TTYP01 group: 2 tablets (edaravone, 60 mg) of TTYP01, twice daily, administered 30–60 minutes before breakfast or dinner; • Control group: 2 tablets (edaravone, 0 mg) of placebo, twice daily, administered 30–60 minutes before breakfast or dinner. **Course of treatment:** Subjects in the TTYP01 group or control group are orally administrated with two TTYP-01 tablets (edaravone 30 mg per tablet) or two placebo tablets (edaravone 0 mg per tablet) twice daily for consecutive 28 days (56 times in total), respectively. The first dose should be given within 1 hour after randomization and remaining doses should be administrated within 30–60 minutes before breakfast or dinner. In case of postprandial randomization, the second dose should not be earlier than 6 hours after the first dose. **Study periods:** Normally, the duration of the study is approximately 90 days. There are two periods in the study, with five visits in total: the Treatment period: Day 1–Day 28, with a minimum of 7 days of the inpatient treatment, including 3 visits (V1–V3); and the Follow-up period: Day 29–Day 90, including 2 visits (V4: Day 60 ± 5, telephone visit; V5: Day 90 ± 5, returning to hospital for the End-of-Study visit). Eligible subjects will be screened based on inclusion and exclusion criteria, treated with either the investigational drug or placebo, and evaluated for the efficacy and safety of the investigational drug. **Neurological and clinical symptom assessments:** - modified Rankin Scale (mRS) - National Institutes of Health Stroke Scale (NIHSS) - Barthel Index (BI) - Montreal Cognitive Assessment Scale (MoCA) - 21-item Hamilton Depression Rating Scale (HDRS)

<table>
<tr><td>Investigational drug</td><td>
<table>
<tr><th>Item</th><th>Investigational drug</th><th>Control drug</th></tr>
<tr><td>Name</td><td>TTYP01 tablets</td><td>Placebo
(Simulant TTYP01 Tablets)</td></tr>
<tr><td>Dosage formulation</td><td>Oral tablet</td><td>Oral tablet</td></tr>
<tr><td>Strength</td><td>30 mg</td><td>0 mg</td></tr>
<tr><td>Ingredients</td><td>Edaravone, polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus), sodium bisulfite,microcrystalline cellulose, polycrystalline</td><td>Microcrystalline cellulose,magnesium stearate</td></tr>
<tr><td>Storage conditions</td><td>Store at room temperature</td><td>Store at room temperature</td></tr>
<tr><td>Shelf life</td><td>18 months tentatively</td><td>18 months tentatively</td></tr>
<tr><td>Manufacturer</td><td>STA Pharmaceutical Co. Ltd.</td><td>STA Pharmaceutical Co. Ltd.</td></tr>
<tr><td>Supplier</td><td>Suzhou Auzone Biological Technology Co., Ltd.</td><td>Suzhou Auzone Biological Technology Co., Ltd.</td></tr>
</table>
</td></tr>
<tr><td>Dosing Regimen</td><td>Treatment group (TTYP01 tablets):
Subjects in the TTYP01 group are orally administrated with two TTYP01 tablets (edaravone 30 mg per tablet) twice daily for consecutive 28 days (56 times in total). The first dose should be given within 1 hour after randomization and remaining doses should be administrated within 30–60 minutes before breakfast or dinner. In case of postprandial randomization, the second dose should not be earlier than 6 hours after the first dose.
Control group (placebo tablets, simulant TTYP01 tablets):
Subjects in the control group are orally administrated with two placebo tablets (edaravone 0 mg per tablet) twice daily for consecutive 28 days (56 times in total). The first dose should be given within 1 hour after randomization and remaining doses should be administrated within 30–60 minutes before breakfast or dinner. In case of postprandial randomization, the second dose should not be earlier than 6 hours after the first dose.</td></tr>
<tr><td>Inclusion Criteria</td><td>Subjects eligible for enrollment in this trial must meet all of the following inclusion criteria:
1. Patient whose age is ≥ 18 and ≤ 80 years;
2. Patients diagnosed with AIS according to the Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association;
3. Patients who refuse thrombolysis or thrombectomy, or do not meet the indications for thrombolysis or thrombectomy;
4. Patients who are within ≤ 24 hours after the symptom onset. For patients with uncertain onset time due to wake-up stroke, aphasia, consciousness disorders, etc., the last known asymptomatic time is considered as the onset time;
5. Patients with a first stroke, or a recurrent stroke who recovered well from a previous stroke (mRS score ≤ 1);
6. Patients whose NIHSS score is ≥ 6 and ≤ 20, and the sum of upper limb and lower limb scores in item 5 and 6 is ≥ 2 at the initial diagnosis;
7. Patients or their legal guardians are informed of and voluntarily participate in this study, and have signed a written informed consent form.</td></tr>
</table>

Exclusion Criteria	Subjects who meet any of the following criteria will be excluded from the study: 1. Patients with any contraindications for CT or MRI examination such as metal implants, pacemakers, claustrophobia, etc.; 2. Patients with intracranial hemorrhage confirmed by CT or MRI including but not limited to cerebral hemorrhage, epidural hematoma, subdural hematoma, intracranial hematoma, ventricular hemorrhage, subarachnoid hemorrhage, traumatic cerebral hemorrhage, hemorrhagic transformation in this stroke, etc.; 3. Patients with massive cerebral infarction (i.e., the infarcted area > 1/3 of the cerebral hemisphere as indicated by CT or MRI); 4. Patients with consciousness disorders (Ia > 1 in NIHSS), posterior circulation infarction, or transient ischemic attack (TIA); 5. Patients who are unable or unwilling to cooperate with the study due to comorbidity such as severe mental disorders, depression, Alzheimer's disease, Parkinson's dementia, and Louis body dementia; 6. Patients who are scheduled for or have already received intravenous thrombolysis after the onset of this stroke, or those who plan to receive endovascular therapy urgently or within following 90 days; 7. Patients with a history of malignant tumors or parasitic diseases in the brain; 8. Patients with a history of severe traumatic brain injury or intracranial infection and with a mRS score > 1 before this stroke onset; 9. Patients with severe hypertension (systolic blood pressure ≥ 220 mmHg or diastolic blood pressure ≥ 120 mmHg) before the first administration, which cannot be controlled by treatment; 10. Patients with blood glucose < 2.7 mmol/L and being untreated, or blood glucose > 22.2 mmol/L at initial diagnosis; 11. Patients with dysphagia; 12. Patients with a history of rheumatic heart disease or atrial fibrillation, or with heart rate < 40 or > 120 beats/min, or with second- or third-degree atrioventricular block and being untreated, or with other kinds of malignant arrhythmia, or with acute myocardial infarction or heart failure (grade III–IV according to the New York Heart Association [NYHA]) or cardiac intervention within the past 6 months; 13. Patients with other serious systemic or organ diseases which, in the opinion of the investigator, may hinder the evaluation of efficacy or make it unlikely for the patient to complete the expected course of treatment and follow-up (e.g., malignancy with the life expectancy < 90 days); 14. Patients with severe liver or kidney diseases, or significant abnormalities in renal or hepatic function tests such as alanine aminotransferase [ALT] or aspartate aminotransferase [AST] ≥ 3.0 × upper limit of normal [ULN], or serum creatinine [Cr] > 2.0 × ULN; 15. Patients with bleeding tendencies (e.g., the platelet count [PLT] < 75.0×10^9/L), or severe bleeding within 90 days before this stroke onset; 16. Patients with contraindications for antiplatelet or statin therapy; 17. Patients who are allergic to edaravone or excipients; 18. Patients with a suspected or proven history of alcohol or drug abuse; 19. Female patients who are pregnant, lactating, having a recent pregnancy plan, or unwilling to use contraception; 20. Patients who have participated in other clinical trials within 90 days before this stroke onset or are participating in other clinical trials; 21. Patients who, in the opinion of the investigator, are not suitable to participate in the clinical trial.
Withdrawal Criteria	**Withdrawal as determined by the investigator** 1. Subjects who experience other comorbidities, complications, or special physiological changes during the study, such as recurrent stroke or symptomatic intracranial hemorrhage (sICH), classified as PH I or II according to ECASS criteria;

	2. Subjects with poor compliance, such as failure to take investigational drugs after enrollment, lack of records for follow-up visits, unauthorized self-dressing changes, or the use of prohibited drugs specified in the protocol, which may adversely affect the assessment of the efficacy and safety; 3. Subjects who are taking other concurrent treatments that compromised the evaluation in this trial; 4. Serious adverse events (SAEs) or significant adverse events (AEs) may occur, making it inappropriate to continue the trial; 5. The occurrence of intolerable toxicity related to the drug may include any of the following criteria: (1) serum ALT or AST levels > 8× ULN; (2) ALT or AST levels > 5 × ULN for two consecutive weeks; (3) ALT or AST levels > 3 × ULN with total bilirubin (TBIL) > 2 × ULN; (4) ALT or AST levels > 3 × ULN accompanied by progressively worsening fatigue and gastrointestinal symptoms, and/or eosinophilia (> 5%); (5) serum creatinine (Cr) levels reaching three times the baseline value or higher; 6. Subjects who do not meet the inclusion criteria or who meet any of the exclusion criteria are mistakenly included in this trial; 7. Subjects who participate in another intervention study during this trial; 8. Because of changes in the subject's medical condition unrelated to the investigational drug that occurred during treatment period, such as severe nosocomial infections, heart failure (graded III–IV according to NYHA), and epilepsy that was difficult to control with medication, the investigator determines that it is not in the subject's best interest to continue participation in this trial; 9. Other conditions that, in the opinion of the investigator, necessitate withdrawal from this trial. **Subjects' voluntary withdrawal from the trial** 1. Subjects who are unwilling or unlikely to continue in this trial may request withdrawal to the investigator; 2. Subjects may withdraw spontaneously for various reasons, including perceived poor efficacy, intolerance to certain adverse reactions, economic factors, or other unexplained reasons. The reason for withdrawal should be recognized as far as possible and documented; 3. Subjects who, although not explicitly withdrawing from the trial, no longer undergo visits and are lost to follow-up.
Study Procedure	This trial is divided into two periods, with a total of five visits. The treatment period: Day 1–Day 28 (the minimum length of in-hospital treatment is not less than 7 days), which includes 3 visits (V1 to V3); The follow-up period: Day 29–Day 90, including 2 visits (V4: Day 60 ± 5 for telephone visit; V5: Day 90 ± 5, returning to hospital for End-of-Study visit). **Treatment period (D1–D28; V1–V3)** **V1 (D1):** • Signing the informed consent form; • Assessment based on inclusion and exclusion criteria; • Demographic data collection, including initials, sex, date of birth, ethnicity, height, weight, education level, etc.; • Recording of the relevant medical history, including history of present illness, past history (including dementia/cognitive dysfunction, depression, etc.), medication history, allergy history, smoking history, history of alcohol consumption, etc.; • Vital signs, including body temperature, blood pressure, pulse, and respiratory rate; • Physical examination, including general condition, head, face, skin, lymph nodes, eyes, ears, nose, throat, mouth, respiratory system, cardiovascular system, abdomen, muscles, bones, and nervous system;

	• 12-lead electrocardiogram (ECG); • Laboratory tests: Blood routine, blood biochemistry, coagulation function, urine routine, etc.; • Head CT or MRI scanning (T1, T2, FLAIR, and DWI + ADC); • Blood or urine pregnancy test (for pre-menopausal women only); • Randomization; • Hospitalization; • Distribution of investigational drugs: Distribution of investigational drugs of day 1 to day 7; • mRS score; • NIHSS score; • BI score; • Recording concomitant therapies and drugs; • Collection and evaluation of adverse events. **V2 (D7):** • Vital sign examination; • Physical examination; • 12-lead ECG; • Laboratory tests: Blood routine, blood biochemistry, coagulation function, urine routine, etc.; • Distribution of investigational drugs: Distribution of investigational drug for the period until the next visit; • Distribution of subject diary cards: Distribution of subject diary cards required at the next visit; • mRS score; • NIHSS score; • BI score; • MoCA score: Completing baseline assessment within 7 days (including day 7) after enrollment; • HDRS score: Completing baseline assessment within 7 days (including day 7) after enrollment; • Record concomitant therapies and concomitant drugs; • Collection and evaluation of adverse events. **V3 (D28 + 3):** • Vital sign examination; • Physical examination; • 12-lead ECG; • Laboratory tests: Blood routine, blood biochemistry, coagulation function, urine routine, etc.; • Head MRI scanning: Selecting some eligible sites to perform post-stroke head MRI scanning (T1, T2, and FLAIR); • Return of investigational drugs: Subjects returning unused drugs and/or empty packages to investigators; • Collection and assessment of subject diary cards: Subjects submitting completed diary cards to investigators for assessment; • Distribution of subject diary cards: Distribution of the subject diary cards required for the next return visit; • mRS score; • NIHSS score; • BI score; • MoCA score; • HDRS score; • Recording concomitant therapies and drugs; • Collection and evaluation of adverse events. *Note:*

	1. V1 should be conducted during this episode prior to the first administration of the treatment. 2. If the subject has completed some protocol-required examinations (e.g., physical examination, 12-lead ECG, laboratory tests, and blood or urine pregnancy tests) at the site within 24 hours of admission, and the investigator deems these examinations sufficient to support the screening of the subject, they may not be repeated. This consideration is based on the potential health risks associated with conducting these examinations in a short time, which could also significantly impact the subject's treatment schedule. 3. Selected sites with specific medical conditions will conduct head MRI scans on Day 2 and Day 5 (including T1, T2, FLAIR, and DWI + ADC) and on Day 28 + 3 (T1, T2, and FLAIR). It is expected to collect MRI data from approximately 75% of all subjects and perform centralized reading to compare changes in MRI variables (infarct volume and area). If a participant undergoes an MRI scan on Day 1, the MRI scan on Day 2 can be skipped and only the remaining MRI tests are required. 4. Dosing regimen during the treatment period: Subjects in the TTYP01 group or control group are orally administrated with two TTYP01 tablets (edaravone 30 mg per tablet) or two placebo tablets (edaravone 0 mg per tablet) twice daily for consecutive 28 days (56 times in total), respectively. The first dose should be given within 1 hour after randomization and remaining doses should be administrated within 30–60 minutes before breakfast or dinner. In case of postprandial randomization, the second dose should not be earlier than 6 hours after the first dose. **Follow-up period (D29–D90; V4–V5)** **V4 (D60 ± 5, telephone visit):** • mRS score; • Recording concomitant therapies and concomitant drugs; • Collection and evaluation of adverse events. *Note:* The investigator will record the telephone visiting for the subject or his/her guardian on Day 60 ± 5. **V5 (D90 ± 5, returning to hospital for End-of-Study visit):** • Vital sign examination; • Physical examination; • 12-lead ECG; • Laboratory tests: Blood routine, blood biochemistry, coagulation function, urine routine, etc.; • Blood or urine pregnancy tests (pre-menopausal women only); • mRS score; • NIHSS score; • BI score; • MoCA score; • HDRS score; • Collection and assessment of subject diary cards: Subjects submitting completed diary cards to the investigator for the assessment; • Recording concomitant therapies and concomitant drugs; • Collection and evaluation of adverse events. *Note:* Efficacy and safety data for early withdrawal subjects are collected as much as possible by performing the same processes and examination items as V5.
Concomitant medications	**Permitted Concomitant Drugs During the Trial** **Basic medication** Basic drugs are administered according to the *Guideline for the Prevention and*

	Treatment of Stroke in China (Version 2021), including general treatment, antiplatelet therapy, etc. Drugs prohibited in this trial, such as neuroprotective agents, should be excluded. **Other permitted drugs during the trial** In case of adverse reactions, the investigator should decide whether the symptomatic treatment is needed, and should record the drug name, administration route, dosage, and starting and ending time of administration in the source records and electronic case report form (eCRF). All other treatments administered for the subject between the enrollment and the End-of-Study are considered concomitant therapies, and the drugs are recorded in the eCRF in the generic name. Concomitant drugs during the trial must be essential for the subject, and the investigator should ensure concomitant drugs do not interfere with the trial drug. The dose should be kept at the minimum level. **Non-pharmacological treatment** It's necessary to accurately and thoroughly document any permitted non-pharmacological treatments during the trial, such as rehabilitation therapy. **Prohibited drugs during the trial** The combination use of neuroprotective agents listed in the *Chinese Guideline for Diagnosis of Acute Ischemic Stroke 2018,* the *Guideline for the Prevention and Treatment of Stroke in China (Version 2021),* or the *Chinese Guideline for the Secondary Prevention of Ischemic Stroke and Transient Ischemic Attack (Version 2010)* is prohibited in this trial, such as commercial available edaravone, edaravone dexborneol, nimodipine, gangliosides, phosphatidylcholine, piracetam, olactam, butylphthalide, urinary kallindinogenase, cinepazide, nerve growth factor, cerebrolysin, brain protein hydrolysate, deproteinised calf serum injection, and deproteinisd calf blood extract injection, as well as herbs with neuroprotective effects stated in instructions, etc. Other drugs in trial or unmarketed are also prohibited in this trial.
Efficacy Evaluation Measures	**Primary outcome measure:** Proportion of subjects with mRS score ≤ 1 on Day 90 after stroke onset. **Secondary outcome measure:** 1. mRS score on day 90 after stroke onset; 2. Proportion of patients with a mRS score ≤ 2 on Day 90 after stroke onset; 3. Changes from baseline in mRS score on Day 7, 28, 60, and 90 after stroke onset; 4. Proportion of patients with an improvement of ≥ 4 in NIHSS score on Day 7, 28, and 90 after stroke onset; 5. Proportion of patients with a NIHSS score of 0–1 on Day 7, 28, and 90 after stroke onset; 6. Changes from baseline in NIHSS score on Day 7, 28, and 90 after stroke onset; 7. Proportion of patients with Barthel Index (BI) score ≥ 95 on Day 7, 28, and 90 after stroke onset; 8. Changes from baseline in BI score on Day 7, 28, and 90 after stroke onset; 9. MoCA score on Day 7, 28, and 90 after stroke onset ; 10. HDRS score on Day 7, 28, and 90 after stroke onset; 11. Changes from baseline in brain MRI variables (e.g., infarct volume and area) on Day 5 and 28 after stroke onset.
Safety Evaluation Measures	Safety endpoints include the occurrence of adverse events (AEs) from the start of study to the end of follow-up period, including treatment-emergent adverse events (TEAEs), adverse drug reactions (ADRs), serious adverse events (SAEs), and TEAEs leading to subject termination from the trial. 1. Proportion of all-cause deaths within 90 days after stroke onset; 2. Proportion of symptomatic intracranial hemorrhages within 90 days after stroke

	onset; 3. Proportion of recurrent ischemic strokes within 90 days after stroke onset; 4. Proportion of AEs within 90 days after stroke onset; 5. Proportion of SAEs within 90 days after stroke onset; 6. Discontinuation due to any AE within 90 days after stroke onset; 7. Discontinuation due to any other non-AE within 90 days after stroke onset.
Statistical Analyses	**Sample Size Determination:** A sample size of 274 subjects per each treatment group (total of 548 subjects) will provide at least 90% power to detect a difference of 15% (60% in the TTYP01 group versus 45% in the placebo group) for the primary endpoint the proportion of subjects who achieved mRS ≤1 on Day 90 with a one-sided α value of 0.025. Assuming drop-out rate of ~20%, 618 subjects (309 in each group) will be planned to be enrolled for the trial. **Analysis Sets:** 1. Full analysis set (FAS): FAS will included all the randomized subjects who receive at least one dose of investigational drug and who have at least one post-dose primary efficacy assessment (i.e., mRS). 2. Per-protocol set (PPS): As the subset of FAS, PPS will include all the subjects who have the primary efficacy endpoint available on D90, with treatment compliance of 80% to 120% and who have no major protocol deviations affecting the primary efficacy assessment (e.g. not meeting important inclusion criteria, use of prohibited medications impacting the primary efficacy outcomes during the trial). 3. Safety set (SS): SS will include all the subjects who receive at least one dose of investigational drug. **General Consideration:** All statistical analyses will be performed using SAS version 9.4 or above. Detailed statistical methods will be described in the SAP and finalized prior to database lock. In general, all study data will be pooled and summarized/analyzed by treatment group: descriptive statistics (n, mean, median, first quartile (Q1), third quartile (Q3), standard deviation, minimum and maximum values) will be used for continuous variables; categorical variables will be described using frequency tables (frequencies and percentages). All statistical hypothesis tests will use a 2-sided test at α = 0.05 and two-sided 95% confidence intervals (CIs) will be calculated, unless otherwise specified. **Demographics and Baseline Characteristics** Demographic data (Age and Age Group (<65, ≥65), Race, Gender, Height, Weight, Body Mass Index (BMI)) will be analyzed with descriptive statistics as appropriate, respectively. In addition, baseline disease characteristics including AIS history, time from onset of AIS to treatment, baseline NIHSS, concomitant illness with AIS (hypertension, diabetes, hyperlipidemia and cardiac disease), alcohol history, smoking history and etc. will be also summarized. **Efficacy Analyses:** The number and percentage of subjects achieving mRS≤1 on Day 90 and the corresponding 95% CI will be summarized (Clopper-Pearson interval) by treatment group. The treatment group difference between TTYP01 and placebo group will be tested using Chi-square test, and the 95% CI of the difference will be estimated. In addition, odds ratio (OR) with the 95% CI as well as p-value will be also reported using logistic regression. Multiple imputation will be used to account for missing mRS; Death will be scored as 6 in mRS and thus there will be no missing mRS values due to death. If the data are applicable, supplementary or sensitivity analyses will be performed to assess the robustness of the primary analysis results. Further more, subgroup analyses will be conducted based on the demographic and baseline characteristics of subjects as applicable.

	The shift in Day 90 mRS as a 6-category ordinal scale will be analysed using ordinal logistic regression, common OR, the associated 95% CI as well as the p-value will be reported. Similar statistical methods as the primary endpoint will be applied for binary secondary endpoints including mRS ≤2 on Day 90, NIHSS improvement ≥4 or NIHSS ≤1 on Day 7, 28 and 90 and etc. For the continuous secondary outcomes such as change in NIHSS from baseline, the least-square mean differences with 95% CI between treatment groups will be estimated by analysis of covariance (ANCOVA) or analysis of variance (ANOVA) as appropriate. **Safety Analyses:** AEs will be coded using Medical Dictionary for Regulatory Activities (MedDRA). AE analysis will be based on TEAE. Based on SS, the number and percentage of subjects in each treatment group with overall and various types of TEAEs including all TEAEs, all ADRs, CTCAE grade ≥3 TEAEs/ADRs, SAEs, serious ADRs, TEAEs/ADRs that leading to treatment discontinuation will be counted and also summarized by System Organ Class (SOC) and Preferred Term (PT), respectively. The proportions of all AEs, symptomatic intracranial hemorrhage, recurrent symptomatic stroke (cerebral infarction), all-cause mortality, serious adverse events, discontinuation due to any adverse event and etc. and the corresponding 95% CI will be summarized by treatment group. The relative risk of the treatment group compared to the control group and the associated 95% CI will be also reported. Cross-tabulation tables before and after treatment will be provided to analyze the post-treatment changes in the laboratory tests, 12-lead ECG, and other applicable safety parameters. Vital signs and the changes from baseline will be analyzed using descriptive statistics by treatment group. **Interim Analysis:** An interim analysis will be planned at the timing of 50% subjects complete D90 or early terminate, whichever occurs first, for the assurance of patient safety and trial integrity and monitoring the effectiveness data. To maintain an overall one-sided α of 0.025, the α-spending function to approximate O'Brien-Fleming will be implemented. The interim analysis will be monitored by Independent Data Monitoring Committee (IDMC). The purpose of IDMC, IDMC members and their responsibilities, meeting frequency and etc. will be described in the IDMC Charter.

Schedule of Study Activities

Trial Phase	Treatment Period			Follow-up Period	
Test Days	**D1**	**D7**	**D28 + 3**	**D60±5**	**D90±5**
Visit Number	**V1[a]**	**V2**	**V3**	**V4 Telephone visit[b]**	**V5/Early withdrawal[c]**
Signing the informed consent form	X				
Assessment based on inclusion and exclusion criteria	X				
Collection of demographic data	X				
Recording relevant medical history (present, past, etc.)	X				
Vital signs examination[1]	X	X	X		X
Physical examination[2]	X	X	X		X
12-lead ECG examination	X	X	X		X
Randomization	X				
Hospitalization[3]	----------				
Distribution of investigational drugs[4]	X	X			
Administration of investigational drugs	----------				
Distribution of the subject diary card[5]		X	X		
Return of investigational drugs[6]			X		
Collection and Assessment of the subject diary card[7]			X		X
Blood routine test[8]	X	X	X		X
Urine routine test[9]	X	X	X		X
Blood biochemistry[10]	X	X	X		X
Coagulation function[11]	X	X	X		X
Blood or urine pregnancy test[12]	X				X
Head computed tomography (CT) or magnetic resonance imaging (MRI)[13]	X		X		
Modified Rankin Scale (mRS) Score[14]	X	X	X	X	X
National Institutes of Health Stroke Scale (NIHSS) score[15]	X	X	X		X
Barthel Index (BI) score[16]	X	X	X		X
Montreal Cognitive Assessment (MoCA) score[17]		X	X		X
Hamilton Depression Scale (HDRS) score[18]		X	X		X
Collection and evaluation of adverse events (AEs)	X				
Concomitant Drugs	X				
Concomitant Therapies	X				

Note:

If the subject has completed all protocol-required examinations (e.g., physical examination, 12-lead ECG, laboratory tests, imaging tests) at the participating site within 24 hours of admission for this episode, and the investigator deems these examinations sufficient to support the diagnosis of the subject's condition, they may not be repeated. This consideration is based on the potential health risks associated with conducting these examinations in a short time frame, which could also significantly impact the subject's treatment schedule.

a. V1: V1 should be conducted during this episode prior to the first administration of the treatment.

b. V4 Telephone visit: The investigator shall conduct a telephone visit with the subject or their guardian on Day 60 ± 5, and document the conversation. At this visit, the investigator will complete the modified Rankin Scale (mRS) score assessment, record basic treatments, record any concomitant treatments and drug combinations,

and collect and evaluate any adverse events.

c. Early withdrawal: Efficacy and safety data should be collected from participants who withdraw early, following the same procedures and tests as those conducted at the V5 whenever possible.

1. Vital signs: Including body temperature, blood pressure, pulse, and respiratory rate.
2. Physical examination: Including general condition, head, face, skin, lymph nodes, eyes, ears, nose, throat, mouth, respiratory system, cardiovascular system, abdomen, muscles, bones, and nervous system.
3. Hospitalization: The length of hospitalization in this trail will be determined by the actual condition of the patients; however, the minimum duration will be set at no less than 7 days. This corresponds to the treatment period from Day 1 to Day 28, during which a minimum of 7 days of hospitalization is required.
4. Distributing investigational drugs: Investigational drugs from Day 1 to Day 7 will be distributed on Day 1, and investigational drugs required for the next visit will be distributed on Day 7.
5. Distribution of the subject diary card: Subject diary cards for the next return visit will be distributed at V2 and V3.
6. Return of investigational drugs: Subjects return unused drugs and/or empty packaging to investigators at V3.
7. Collection and assessment of subject diary cards: Subjects submit completed diary cards to investigators for assessment at V3 and V5.
8. Blood routine test: White blood cell (WBC), neutrophil (NEUT), eosinophil (EOS), basophil (BASO), lymphocyte (LY), monocyte (MONO), red blood cell (RBC), hemoglobin (HGB), platelet (PLT), and hematocrit (HCT). Assessments were performed on Day 1, Day 7, Day 28 + 3, Day 90 ± 5, or early withdrawal.
9. Urine routine test: Including pH value, urine specific gravity, urine protein (albumin), glucose, ketone body, red blood cells, white blood cells, and urine occult blood. Assessments were performed on Day 1, Day 7, Day 28 + 3, Day 90 ± 5, or early withdrawal. If results of the urine routine test are not available due to emergency, investigational drugs can be administered first. If the subject has no urine temporarily, the test results of first urine sample collected upon admission will serve as baseline data.
10. Blood biochemistry: Total bilirubin (TBIL), total protein (TP), albumin (ALB), serum albumin-to-globulin ratio (A/G), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRE), total cholesterol (TC), triglyceride (TG), urea (Urea), uric acid (UA), alkaline phosphatase (ALP), creatine kinase (CK), blood glucose (GLU), sodium, potassium, calcium, chloride and phosphorus or blood urea nitrogen (BUN). Assessments of high-density lipoprotein (HDL), low-density lipoprotein (LDL), and D-dimer will be performed on Day 1, Day 7, Day 28 + 3, Day 90 ± 5 or upon early withdrawal.
11. Coagulation function: Including international normalized ratio (INR), activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen (FIB). Assessments performed on D1, D7, D28+ 3, D90±5.
12. Blood or urine pregnancy test: For premenopausal women only. Conduct tests on Day 1 and Day 90 (± 5 days) during the follow-up period.
13. Head computed tomography (CT) or magnetic resonance imaging (MRI): Head CT or MRI will be performed on Day 1 (including T1, T2, FLAIR, and DWI + ADC). Selected sites with specific conditions will conduct head MRI scans on post-stroke Day 2 and Day 5 (including T1, T2, FLAIR, and DWI + ADC) and on Day 28 + 3 (including T1, T2, and FLAIR). The plan is to collect MRI data from approximately 75% of the subjects and perform centralized reading to compare changes in MRI findings (infarct volume and area). If a participant undergoes an MRI scan on Day 1, the MRI scan on Day 2 may be skipped, and only subsequent MRI scans are required.
14. Modified Rankin Scale (mRS) scores will be assessed on Day 1, Day 7, Day 28 + 3, Day 60 ± 5 by telephone visit, and Day 90 ± 5.
15. National Institutes of Health Stroke Scale (NIHSS) scores will be assessed on Day 1, Day 7, Day 28 + 3, and Day 90 ± 5.
16. Barthel Index (BI) scores will be assessed on Day 1, Day 7, Day 28 + 3, and Day 90 ± 5.
17. Montreal Cognitive Assessment (MoCA) scores will be assessed at baseline within 7 days (including Day 7) after enrollment, as well as on Day 28 + 3 and Day 90 ± 5.
18. Hamilton Depression Rating Scale (HDRS) scores will be assessed at baseline within 7 days (including Day 7) after enrollment, as well as on Day 28 + 3 and Day 90 ± 5.

1. Background Information

1.1 Rationale for this trial

Acute ischemic stroke (AIS) is the most common type of stroke, with localized softening or necrosis of brain tissue caused by cerebral blood supply disorder and hypoxia-ischemia. Stroke is the third leading cause of death worldwide, causing 5.7 million deaths annually and is one of the most common causes of death, long-term disability and hospitalization [1]. AIS is the most common type of stroke, accounting for 69.6% to 70.8% of stroke in China. The time division of acute phase is not uniform, which generally refers to within 2 weeks after onset, within 1 week for mild cases and within 1 month for severe cases. The mortality of inpatients with AIS in China is 2.3% to 3.2% within 1 month, 9% to 9.6% at 3 months, with a death/disability rate of 34.5% to 37.1%. The one-year mortality is 14.4% to 15.4% and the death/disability rate is 33.4% to 33.8%. Management of AIS includes early diagnosis and treatment, early prevention of recurrence (secondary prevention) and early rehabilitation [2]. The rising incidence of AIS has placed a great burden on China's health care system. AIS has high recurrence rate, complication rate, disability rate and case fatality rate, bringing great pain and economic burden to patients and families [3].

The acute phase of ischemic stroke is often accompanied by complications, including cerebral edema and intracranial hypertension, hemorrhagic transformation, epilepsy, dysphagia, pneumonia, dysuria and urinary tract infection, deep vein thrombosis, etc. Among them, cerebral edema and intracranial hypertension, hemorrhagic transformation and pneumonia are the main causes of death of stroke patients. Stroke in China has become a serious medical and public health problem. Its high morbidity, high disability rate, high mortality, high recurrence rate and high economic burden bring heavy burden to society and family. It is urgent to develop effective prevention and treatment strategies and drugs with clinical value to improve the current situation.

Currently, basic therapy with specific medication is still the major treatment of AIS. Commonly used drugs mainly include those to improve cerebral blood circulation (intravenous thrombolysis, antiplatelet therapy, anticoagulation, fibrin reduction, volume expansion, vasodilatation, etc.), statins, neuroprotective agents (calcium antagonists, free radical scavengers, cell membrane stabilizers, etc.) and traditional Chinese medicine preparations for activating blood circulation and dredging collaterals. Thrombolysis treatment in the ultra-early stage yields the best efficacy. But it has multiple limitations and is accompanied by the risk of bleeding transformation; Anti-platelet agents and cerebral protective agents are the most popular treatment for stroke, where neuroprotective agents have not yet demonstrated satisfactory clinical efficacy, and anti-platelet therapy may also result in reperfusion injury. Therefore, there is still large unmet clinical request in the treatment of ischemic stroke, where it is urgent to find new drugs with wide effect, multiple

mechanisms, which can really reduce the mortality, improve the neurological deficit, reduce the complications and sequelae, and with higher safety [2].

Edaravone is a novel radical scavenger that has been shown to inhibit lipid peroxidation and vascular endothelial cell injury and improve brain edema, tissue damage, and delayed neuronal death [4]. Currently, edaravone injection has been approved for the treatment of AIS in China and Japan, and for the treatment of amyotrophic lateral sclerosis (ALS) in Japan, South Korea, the United States, Canada, Switzerland, and China. However, for either AIS or ALS, the first course of treatment requires consecutive administration for 14 days, which is extremely inconvenient for clinical use and has poor patient compliance.

The TTYP01 tablet is an edaravone oral tablet independently developed by Suzhou Auzone Biological Technology Co., Ltd., which is derived from the modified form of edaravone injection. The non-clinical study results show that TTYP01 tablet has good safety and tolerability. Phase I clinical trials in Australia and China have demonstrated good safety and tolerability of TTYP01 tablets. Compared with edaravone injection, TTYP01 tablets can be administered by patients themselves without relying on professional medical personnel, which improves compliance, reduces medical costs, leading to significant clinical development potential. In this clinical trial, the efficacy and safety of TTYP01 tablets in the treatment of AIS will be studied to provide a more scientific and reasonable basis for the use of TTYP01 tablets in patients with AIS and to maximize the benefits to them.

1.2 Study Product Description

1.2.1 Basic Information

Generic name: Edaravone Tablets

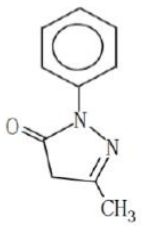
R&D code: TTYP01

Main ingredient: Edaravone

Chinese chemical name: 3-甲基-1-苯基-5吡唑啉酮

English chemical name: 3-Methyl-1-phenyl-5-pyrrazolone

Structure formula:



Molecular formula: $C_{10}H_{10}N_2O$

Molecular weight: 174.20

Strength: 30 mg

Excipients: Polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus), sodium bisulfite, microcrystalline cellulose, polyvinylpyrrolidone, magnesium stearate

Storage condition: Store at room temperature

Validity period: 18 months tentatively

1.2.2 Mechanism of Action

Edaravone inhibits membrane lipid peroxidation chain reaction by providing electrons to oxygen free radicals, thereby attenuating cytotoxicity caused by oxygen free radicals and inhibiting irreversible destruction of protein and nucleic acid mediated by oxygen free radicals [5].

1.3 Nonclinical Studies

1.3.1 Pharmacology Studies

According to the pharmacological review of FDA [6] and the review report of PMDA [7], edaravone, as a free radical scavenger, will be converted in tissues to 2-oxo-3-(phenylhydrazino)-butyric acid, which is its free radical reaction product, indicating its interaction with free radicals (IC_{50}=6.7 μM). In *in vitro* studies, edaravone has been demonstrated to reduce lipid peroxidation in rat brain homogenate (IC_{50}=15.3 μM) by preventing linoleic acid oxidation via hydrogen peroxide (IC_{25} = 33.8 μM), and to reduced oxidative damage to cultured endothelial cells and neuronal apoptosis [7,8]. The safety pharmacology of edaravone includes assessment of respiratory function, central nervous system and cardiovascular system. In vitro test results showed that edaravone does not inhibit the hERG channel. For the central nervous system, the associated symptoms include mainly increased lacrimation and ptosis and decreased spontaneous movement in male mice and rats following a single intravenous injection of 30 or 100 mg/kg of edaravone. A decrease in body temperature was observed in male mice following a single intravenous injection of 100 mg/kg edaravone. In beagle dogs, a single intravenous injection of 30 or 100 mg/kg edaravone caused a temporary decrease in blood pressure and an increase in carotid blood flow and heart rate. A single intravenous injection of edaravone up to 100 mg/kg in beagle dogs did not affect respiratory rate [6].

1.3.2 Toxicology Studies

As is demonstrated in the literature of the marketed edaravone injection Radicava®, the no observed adverse effect level (NOAEL) in rats is the lowest in toxicity studies, where rats are believed to be the most sensitive species, and are therefore selected for general toxicity studies of TTY01-ASD oral formulation, including single-dose and repeat-dose toxicity studies.

1.3.2.1 Single-dose toxicity

Under good laboratory practice of drug (GLP) conditions, TTY01-ASD was administered to Sprague dawley (SD) rats at a single dose of 0, 50, 250, or 500 mg/kg/day by oral gavage twice

daily at 8-hour intervals (administration doses of 0, 100, 500, and 1000 mg/kg/day) for 14 days, where the maximum tolerated dose (MTD) of the study product, the reversibility, persistence, or delayed effects of toxic effects were observed and assessed. No unscheduled deaths or test product-related changes in body weight, food consumption, hemagglutination, serum chemistry, urinalysis parameters, and macroscopic necropsy were reported during the study. Slight test product-related salivation was observed in both males and females in the ≥ 500 mg/kg/day dose groups, and episodic abnormal respiratory sounds and/or decreased activity was observed in both males and females in the 1000 mg/kg/day dose group. Clinical signs related to drug administration were only seen on the day of administration and were no longer evident 24 hours after administration. A slight decrease in the neutrophil count was observed in animals in the administration groups (-30.06% in males and -25.79% in females). Since this change is small and reversible, it is not considered a harmful change. The MTD is 1000 mg/kg (500 mg/kg, twice daily).

In addition, single-dose intravenous toxicity studies of edaravone have been conducted in mice, rats, and beagle dogs. The MTD of edaravone administered by intravenous bolus injection was less than 478 mg/kg in mice, 580 mg/kg in rats, and 600 mg/kg in beagle dogs. Edaravone was administered by intravenous drip over 4 hours and the MTD in beagle dogs was 500 mg/kg. Specific study results can be found in the pharmacologic review of Edaravone Radicava® published by the Food and Drug Administration (FDA) [6].

1.3.2.2 Repeat-dose toxicity

1.3.2.2.1 Relevant tests of TTY01-ASD

Under non-GLP conditions, TTY01-ASD was administered by oral gavage to SD rats twice daily for 14 consecutive days at doses of 40, 150, or 400 mg/kg/dose, and no animals were found to be moribund or dead during the test. Test product-related clinical signs included salivation (slight to moderate) in both sexes at 400 mg/kg/day and transient fecal abnormalities (red/brown) in females. No relevant histopathological changes were noted. Therefore, this change is considered harmless. At the end of the dosing period, a slight and dose-dependent decrease in body weight was observed in females in the low, medium and high treatment groups, compared to the control group (-3.27%, -3.45% and -6.76% respectively). These changes were due to decreased body weight gain and abnormal feces. Consistent with the observed decrease in body weight gain, decreased food consumption was observed in females in all test product-treated groups. These changes were considered test product-related but not harmful due to their small magnitude. Test product-related hematology changes were limited to an increase in monocytes in males of each treatment group (49.30%, 52.11%, and 73.24% in the low, mid, and high dose groups, respectively). Since no relevant histopathological changes were found, the changes were considered non-hazardous. There

were no test product-related changes in hemagglutination, serum chemistry, urine, organ weights, macroscopic and histopathological changes.

Therefore, oral gavage administration of TTY01-ASD to SD rats for 14 consecutive days at doses of 40, 150, or 400 mg/kg/day was tolerated, and clinical signs in both sexes at 400 mg/kg/day, decreases in body weight and food consumption in females in each treatment group, and hematological changes in males were considered harmless. The NOAEL was 400 mg/kg/day. At this dose, $AUC_{0\text{-}24h}$ and C_{max} were 96000 h*ng/mL and 96800 ng/mL in males and 110000h*ng/mL and 77000 ng/mL in females after the last dose.

Administration of TTY01-ASD at doses of 0, 20, 75, or 200 mg/kg/dose (0, 40, 150, or 400 mg/kg/day) by oral gavage to rats for 28 consecutive days under GLP conditions followed by a 28-day recovery period was well tolerated.

All animals survived to the scheduled dissection day. Test product-related clinical signs included salivation (slight to moderate) in both sexes in the medium and high dose groups, occasional fecal abnormalities in females and abnormal respiratory sounds in both sexes in the 400 mg/kg/day dose group. All of the above symptoms recovered during the recovery period and were considered harmless due to the mild severity of the symptoms. Test product-related changes in body weight and food consumption were also observed in animals in the medium and high dose groups. At the end of the dosing period, compared with the control group, a slight decrease in body weight was observed in both sexes of animals in the medium and high dose groups (Males: -2.23% and -10.23%; Females: -4.42% and -8.38%). These changes were dose-related. Decreased body weight was associated to declined food consumption (males: -2.6% and -10.0%; Females: -5.2% and -10.4%). These changes were considered harmless due to their small magnitude.

Test product-related clinical pathology changes were limited to changes in hematology parameters in females of each treatment group. At the end of the dosing period, dose-related decreases in RBC, HGB, and HCT, accompanied by increases in RET and RDW were observed in females in each treatment group. These changes were within historical reference values and associated histopathological changes were not available, they were considered test product-related but non-adverse. These changes were partially recovered at the end of the recovery period. No test product-related changes were observed in ophthalmology, hemagglutination, blood chemistry, urinalysis, macroscopic observations, organ weights, and histopathological examinations in any dose group.

For toxicokinetics, the time to maximum concentration in each dose group was observed at 0.1 hours after the first or second dose and 0.2 hours after the first dose. There were no apparent gender differences in any of the dose groups except for the 40 and 150 mg/kg/day dose groups, where

females had higher systemic exposure than males on day 1. Systemic exposure increased dose-proportionally on day 1 and day 28 in female rats as dose increased from 40 to 400 mg/kg/day, but increased more than dose-related proportion in male rats. No significant drug accumulation was observed in any of the dose groups except for the increase in systemic exposure in males in the 400 mg/kg/day dose group.

In conclusion, administration of TTY01-ASD to rats at doses of 0, 20, 75, or 200 mg/kg (0, 40, 150, 400 mg/kg/day) by oral gavage twice daily for 28 consecutive days followed by a 28-day recovery period was well tolerated. Oral administration of the test product did not cause death of the animals or result in any adverse change in associated clinical signs, body weight, food consumption, ophthalmological examination, clinical examination, or histopathology. NOAEL for this test was determined to be 400 mg/kg/day. At this dose, the $AUC_{0\text{-}24h}$ and C_{max} of edaravone were 72100 h*ng/mL and 32100 ng/mL in males and 99000h*ng/mL and 45100 ng/mL in females, respectively.

1.3.2.2.2 Studies on Edaravone Radicava®

Repeat-dose toxicity of edaravone was evaluated in rats, beagle dogs, and cynomolgus monkeys by intravenous bolus and 24-hour intravenous infusion. Results of the study are presented in the FDA published pharmacology review of Edaravone Radicava® [6]. A summary of the results are as follows:

1.3.2.2.2.1 Repeat-dose toxicity study in rats

Under GLP conditions, Radicava® was given to Wistar rats through intravenous bolus administration at 10, 30, 100, and 300 mg/kg/day for 28 consecutive days. The NOAEL for this study was 10 mg/kg/day. Major findings included incomplete lid closure and lacrimation at doses greater than 30 mg/kg in the intravenous bolus experiment. At 300 mg/kg, a decrease in righting reflex was observed immediately after injection, with a transition to unsteady gait after 5 minutes. All clinical symptoms disappeared within 24 hours. Decreased RBC, HCT and HGB, and elevated RET and MCV were observed in the high dose (300 mg/kg/day) group, which were associated with increases in bone marrow erythroid cells in the high dose group. All these changes were reversible during the recovery period. The NOAEL for this experiment was finally determined to be 10 mg/kg/day.

1.3.2.2.2.2 Repeat-dose toxicity study in Beagle dogs

A 28-day repeat-dose toxicity study of Edaravone (Radicava®) was conducted in Beagle dogs under GLP conditions by daily intravenous bolus injection (10, 30, 100, and 300 mg/kg/day). The NOAEL was 30 mg/kg/day.

In the daily intravenous bolus study, clinical signs observed at doses greater than 30 mg/kg/day included decreased activity, hindlimb weakness, convulsions, sneezing, and salivation. Examination

of the sample revealed signs of anemia at 300 mg/kg/day. Bone marrow smear results showed an approximately 50% decrease in the granulocyte/red blood cell ratio at this dose relative to the baseline value. Enlarged and dark spleen was observed at 100 and 300 mg/kg/day. The mean absolute liver and spleen weights were increased at 300 mg/kg/day. These findings were associated with increased congestion and hemosiderin deposition in the spleen, as well as the increase of hematopoietic cells and hemosiderin deposition in hepatic Kupffer cells.

1.3.2.3 Genotoxicity

Genotoxicity of edaravone has been fully assessed by the Ames test, the *in vitro* chromosome aberration test, and the *in vivo* micronucleus test in mice, all of which showed negative results. The results of the study can be found in the FDA published pharmacology review of Edaravone Radicava® [6].

Three impurities in TTY01-ASD tablets were genotoxic predicted by Leadscope software. The results indicated low genotoxic risk.

1.3.3 Non-clinical pharmacokinetics study

Pharmacokinetic studies evaluated the absorption of TTY01-ASD in SD rats and Beagle dogs following single or multiple oral administration. The metabolism of edaravone in rats and Beagle dogs was compared between intravenous injection and single oral administration, and the excretion of edaravone in rats after a single oral dose of TTY01-ASD was investigated. The results of tissue distribution and drug-drug interactions are primarily referenced from FDA data on marketed injections.

1.3.3.1 Absorption

The *in vivo* pharmacokinetics of edaravone and its preparation TTY01-ASD were studied in SD rats and Beagle dogs, including: (1) A study of single intravenous administration of edaravone; (2) Dose escalation study of intragastric administration of TTY01-ASD; and (3) A study of TTY01-ASD following the administration of once a day for 7 consecutive doses by gavage.

The test results of Caco-2 were referenced to the information of the marketed injections, where edaravone has good permeability at a concentration of 100 μM (32.0×10^{-6} cm/sec) [8].

After single gavage administration of TTY01-ASD at 20, 60, and 180 mg/kg to male and female SD rats, the mean bioavailability was 40.7%, 44.1%, and 58.8%, respectively, and the systemic exposure ($AUC_{0\text{-last}}$ and C_{max}) increased generally in proportion to the dose. There was a marked difference in $AUC_{0\text{-last}}$ of edaravone in male and female SD rats at 60 and 180 mg/kg by gavage. The corresponding C_{max} ratios (female/male) were 1.63, 1.75, and 1.36 and the $AUC_{0\text{-last}}$ ratios (female/male) were 1.86, 2.05, and 2.06 at doses of 20, 60, and 180 mg/kg, respectively. After administration of 60 mg/kg of TTY01-ASD by gavage once daily for 7 consecutive days, systemic

exposure to the drug was essentially unchanged with no apparent accumulation.

The bioavailability of TTY01-ASD was 29.3%, 58.2%, and 93.7% in male and female Beagle dogs after single gavage administration of 5, 15, and 50 mg/kg TTY01-ASD, respectively. As the gavage dose increased from 5 to 15 mg/kg, the systemic exposure to edaravone ($AUC_{0\text{-last}}$ and C_{max}) increased more than dose proportionally in male Beagle dogs, while C_{max} increased more than dose proportionally in female Beagle dogs, but $AUC_{0\text{-last}}$ increased generally in proportion to the dose. Systemic exposure ($AUC_{0\text{-last}}$ and C_{max}) to the drug in male and female Beagle dogs increased generally dose-proportionally as the gavage dose increased from 15 mg/kg to 50 mg/kg. There were no significant gender differences in the systemic exposure ($AUC_{0\text{-last}}$ and C_{max}) of edaravone in male and female Beagle dogs at each gavage dose. When TTY01-ASD was administered by gavage at 15 mg/kg once daily for 7 consecutive days, systemic exposure to the drug was essentially unchanged with no apparent accumulation in male and female Beagle dogs.

1.3.3.2 Distribution

Data on marketed injections [6,8] show that the protein binding of edaravone injection in human serum is between 91 and 92%, and the amount of edaravone in the brain is about 5% of the plasma content at 5 minutes of administration. ^{14}C-edaravone labeled experiments showed that tissues with radioactivity levels above plasma levels were the aorta and kidney.

1.3.3.3 Metabolism

The main metabolic pathway of edaravone is glucuronide conjugation, where multiple uridine diphosphate glucuronosyltransferases (UGT1A6, UGT1A9, UGT2B7, and UGT2B17) and sulfotransferase are involved. Edaravone is mainly metabolized to sulfate conjugates and glucuronide conjugates in human plasma [6].

Comparison of the metabolism of edaravone in rats and Beagle dogs by injection and oral administration showed that the major metabolic pathways of edaravone in rats and Beagle dogs plasma were sulfate binding and glucuronide binding, and the secondary metabolic pathway was oxidation. Compared with intravenous administration, no new metabolites were produced after oral administration of TTY01-ASD.

1.3.3.4 Excretion

Edaravone is excreted mainly in the urine as a sulfate conjugate and a glucuronide conjugate [6]. The ratio of the parent drug and its metabolites excreted in the urine after 24 h of intravenous administration is shown in Table 1 Proportions of parent drug and its metabolites excreted in the urine 24 h after intravenous administration:

Table 1 Proportions of parent drug and its metabolites excreted in the urine 24 h after intravenous

administration

Species	%Excretion in Urine			
	Parent drug	Sulfuric acid conjugate	Glucuronide Conjugate	Total Quantity
Rats	1.59	51.19	8.25	61.04
Beagle dogs	2.82	64.47	9.15	76.44
Cynomolgus monkeys	7.4	52.3	40.4	83
Human	0.68	6.58	83.17	90.43

After oral administration of TTY01-ASD to rats for 72 h, the recoveries were 57.0 ± 6.35% and 62.7 ± 8.50% in urine, 0.594 ± 0.361% and 0.280 ± 0.0826% in feces and 29.5 ± 10.9% and 22.4 ± 19.1% in bile in male and female rats, respectively. The total recovery from feces, urine and bile accounted for 87.0 ± 13.2% and 85.4 ± 11.6% of the administered dose in male and female SD rats, respectively, with urine being the major route of excretion.

Edaravone and 15 metabolites were detected in the urine of both male and female rats. Gluconate conjugate and sulfate conjugate were the two most abundant metabolites in both male and female rats. The relative abundance of the two substances was 18.42% and 68.91% for males and 36.79% and 51.46% for females, respectively. The relative abundance of other metabolites was less than 5%-6%. The relative abundance of edaravone in urine of male and female rats was 5.99% and 5.88%, respectively.

Edaravone and 11 metabolites were detected in the bile of both male and female rats. Gluconate conjugate and sulfate conjugate remained the two most abundant metabolites. The relative abundance of both substances was 82.16% and 12.10% for males and 84.34% and 11.06% for females, respectively, and the relative abundance of the other metabolites was less than 4%. The relative abundance of edaravone in bile of both male and female rats was ≤ 0.09%.

1.3.3.5 Drug-Drug Interactions

The effect of edaravone as an inhibitor or inducer on cytochrome enzymes P450 has been reported in the marketed injection dossier [8]. Edaravone and its sulfate conjugates did not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, UGT1A1 and UGT2B7 (IC_{50} > 50 μM). Edaravone and its sulfate conjugates induced CYP1A2, CYP3A4 and CYP2B6, where the induction effect on CYP1A2 > CYP3A4 > CYP2B6, but not at therapeutic dose.

1.4 Clinical Studies

A Phase I clinical study of TTYP01 Tablets has been completed in healthy subjects in Australia, including a single dose ascending study (60 mg, 120 mg, 180 mg, and 240 mg, Part A), a comparative study of bioavailability between TTYP01 Tablets and edaravone Injection (30 mg intravenous infusion within 30 minutes and 60 mg intravenous infusion within 60 minutes) (Part B), and a food impact study (Part C).

The Phase I clinical study of TTYP01 Tablets has been completed in Chinese healthy subjects,

including the bioavailability study of TTYP01 Tablets (60 mg), edaravone injection (30 mg) and TTYP01-I Tablets (original process, 60 mg) (Part I), study on safety, tolerability and pharmacokinetic characteristics (Part II, two dose groups of 60 mg BID and 120 mg QD), and food impact study (Part III: 120 mg).

1.4.1 Clinical Pharmacokinetics

1.4.1.1 TTYP01

1.4.1.1.1 Phase I clinical trial in Australia

The pharmacokinetic results of the single dose ascending study (60 mg, 120 mg, 180 mg, 240 mg, Part A) showed [6]: After a single oral dose of TTYP01, the median T_{max} of edaravone in the 60 mg-240 mg dose groups was 0.5-1.0 h, and the mean elimination half-life $t_{1/2}$ was 3.09-4.06 h. The mean C_{max} of edaravone in the 60 mg-240 mg dose groups was 712 to 3114 ng/mL, the mean AUC_{0-last} was approximately 634 to 5496 ng*h/mL, and the mean AUC_{0-inf} was approximately 645 to 5534 ng*h/mL. PK exposure (AUC and C_{max}) after single oral administration of TTYP01 from 60 mg to 240 mg increased with the increasing dose. After single oral administration of TTYP01, the mean CL/F and Vd/F of edaravone in the 60 mg to 240 mg dose groups were about 53 to 110 L/h and 273 to 480 L, respectively, which tended to decrease with increasing dose. The mean MRT was about 1.73 to 2.42 h. The increase in C_{max} of edaravone over the dose range of 60 mg to 240 mg was similar to that of the dose, and the increase in AUC was slightly more than dose proportion [6].

Results of the comparative bioavailability study (Part B) showed: Compared with intravenous infusion of 30 mg Radicut ®, the time to maximum concentration of edaravone was consistent after a single oral dose of 60 mg TTYP01, with slightly lower C_{max} and slightly higher AUC with a mean Fabs value of 59.84% after oral administration. Compared with intravenous infusion of 60 mg Radicut ®, after a single oral dose of 120 mg TTYP01, the time to peak was slightly earlier and the PK exposure levels (AUC and C_{max}) were higher for edaravone compared to that in the intravenous group, with a mean Fabs of 71.37%.

Results of the food impact study (Part C) in the Phase I clinical trial in Australian healthy population showed that the mean C_{max} was 1323.2793 ng/mL, the mean AUC_{0-t} was 1428.2053 h*ng/mL, the mean AUC_{0-inf} was 1449.5175 h*ng/mL, and the median T_{max} was 0.5 mg/mL following the oral administration of TTYP01 tablets in the fasting state; Oral administration of 120 mg of TTYP01 tablets after a high-fat meal resulted in a mean C_{max} of 282.1838 ng/mL, a mean AUC_{0-t} of 693.7419 h*ng/mL, a mean AUC_{0-inf} of 717.7111 h*ng/mL, and a median T_{max} of 1.5 h. The results showed that food significantly reduced the exposure level of TTYP01 (AUC and C_{max}) and delayed the time to maximum concentration T_{max}. Conclusion: There were statistically significant differences in AUC, C_{max}, and T_{max} under different fed conditions. The PK exposure

(AUC and C_{max}) of edaravone was significantly lower in the postprandial group compared to that in the fasting group, while T_{max} was slightly prolonged.

1.4.1.1.2 Phase I clinical trial in Chinese healthy population

The Phase I clinical study of TTYP01 Tablets has been completed in Chinese healthy subjects, including the bioavailability study of TTYP01 Tablets (60 mg), edaravone injection (30 mg) and TTYP01-I Tablets (original process, 60 mg) (Part I), study on safety, tolerability and pharmacokinetic characteristics (Part II, two dose groups of 60 mg BID and 120 mg QD), and food impact study (Part III: 120 mg). The test results are as follows:

Part I: Compared with intravenous infusion of edaravone injection at 30 mg, the mean Fabs of edaravone was 59.38% after a single oral dose of 60 mg TTYP01 Tablets; After a single oral administration of TTYP01-I Tablets at 60 mg, the mean Fabs for edaravone was 52.59%. The mean Frel for edaravone was 116.91% after a single oral dose of 60 mg TTYP01 Tablets compared to a single oral dose of 60 mg TTYP01-I Tablets.

Part II: Following multiple oral administration of TTYP01 tablets at 60 mg BID (N=9) and 120 mg QD (N=9) to healthy subjects, the mean (±SD) R_a (AUC) of edaravone was 1.00±0.00, 1.03±0.16 and the mean (±SD) R_a (C_{max}) was 1.20±0.42 and 1.06±0.49, respectively. The results showed no significant accumulation of edaravone following multiple consecutive oral doses of TTYP01 tablets at 60 mg BID or 120 mg QD.

Part III: Food significantly reduced edaravone exposure (C_{max} and AUC) and prolonged time to maximum concentration (T_{max}) following oral administration of 120 mg TTYP01 tablets under fasting or postprandial conditions.

1.4.1.2 Edaravone Injection

Healthy adult male subjects and healthy elderly subjects were given 0.5 mg/kg of edaravone injection, twice a day by intravenous drip over 30 minutes for 2 consecutive days. From the start of administration to 12 hours after administration, 0.7 to 0.9% of the parent drug and 71.0 to 79.9% of the metabolites were excreted in urine [9].

In a multi-center, double-blind, placebo-controlled clinical trial where European AIS patients aged 40-80 years with a National Institute of Health Stroke Scale (NIHSS) score of 3-15 and onset within 24 h were enrolled, subjects were divided into 2 groups: Group 1 received a loading dose of 0.08 mg/kg of edaravone injection followed by a continuous intravenous infusion of 0.2 mg/kg/h for 72 h, and Group 2 received a loading dose of 0.16 mg/kg followed by a continuous intravenous infusion of 0.4 mg/kg/h for 72 h. The geometric mean of plasma edaravone concentrations at the end of intravenous infusion was 391 ng/mL for patients in Group 1 and 1595 ng/mL for patients in Group 2 [10]. Edaravone does not accumulate in the body [9], and age, gender and race have no

influence on the pharmacokinetics of edaravone *in vivo* [11].

The human serum protein binding of edaravone is 92%, predominantly to albumin [11]. The metabolites of edaravone in both plasma and urine are predominantly sulfuric acid conjugates [9] The mean terminal elimination half-life of edaravone ranges from 4.5 to 6 hours and the half-life of the metabolites ranges from 2 to 2.8 hours [11].

1.4.2 Clinical Safety

1.4.2.1 TTYP01

1.4.2.1.1 Phase I clinical trial in Australia [12]

In the completed Phase 1 clinical study in healthy Australian subjects, safety results showed that single oral doses of TTYP01 tablets (60 mg, 120 mg, 180 mg, and 240 mg, Part A) were safe and well tolerated in healthy Australian subjects; During the comparative bioavailability study (Part B), oral administration of TTYP01 at 60 mg was comparable to the administration of edaravone injection at 30 mg in terms of safety and tolerability; Oral administration of TTYP01 tablets under both fasting and postprandial conditions was safe and well tolerated (Part C).

1.4.2.1.2 Phase I clinical trial in Chinese healthy population

The Phase I clinical study of TTYP01 tablets has been completed in Chinese healthy subjects. The safety results showed that a single oral dose of TTYP01 tablets at 60 mg was safe and well tolerated by Chinese healthy subjects, and was comparable to that of edaravone injection at 30 mg. Multiple oral doses of TTYP01 tablets at 60 mg BID or 120 mg QD were safe and well tolerated; Oral administration of TTYP01 tablets under both fasting and postprandial conditions was safe and well tolerated.

1.4.2.2 Edaravone Injection

In a Phase III clinical trial in Japanese patients with AIS, 252 subjects were enrolled and the results showed no significant difference in the frequency of adverse reactions in the edaravone group compared to that in the placebo group [13]. The clinical observation of more than 500,000 AIS patients in the four years after the approval of edaravone injection in Japan showed that the proportion of patients with adverse reactions was very low, and treatment of AIS using edaravone injection is safe.

1.4.3 Clinical Efficacy

1.4.3.1 TTYP01 Tablets

Currently, no clinical trial has been conducted for TTYP01 tablets in AIS patients, and no clinical efficacy information is available.

1.4.3.2 Edaravone Injection

In a multicenter, randomized, placebo-controlled, double-blind Phase III clinical trial, 252 patients

with AIS in Japan were enrolled where 125 were randomized to edaravone group and 127 were randomized to the placebo-control group. Patients within 72 hours of onset were treated with 30 mg twice daily for 14 consecutive days. Efficacy was assessed using the mRS scale in patients who were discharged within 3 months or in patients with 3 months of onset. A total of 250 patients (edaravone: control = 1:1) were included in the efficacy analysis set, and 2 patients in the placebo-control group were excluded due to subarachnoid hemorrhage and disseminated intravascular coagulation (DIC). The results showed significant efficacy in the edaravone group compared with that in the placebo-control group (p = 0.0382), as is shown in Table 2. The efficacy assessment was extended to 3, 6, and 12 months after onset, and the edaravone group still exhibited better efficacy, as is shown in Table3. In addition, the edaravone group had significant efficacy (p = 0.0001) when evaluated at 24 hours after treatment [13].

It has reported a real-world clinical study in Japan where 61048 AIS patients aged 18 years or older, with ≤14 days of hospitalization after onset were enrolled. In this study, patients were stratified according to ischemic stroke subtype, including large artery atherosclerosis, cardiogenic embolism, small vessel occlusion, and cryptogenic/undetermined. Stroke severity was assessed by the NIHSS score. The results showed an increase in ΔNIHSS (Discharge NIHSS-Admission NIHSS) scores in patients treated with edaravone compared to those with no use in all ischemic stroke subtypes. The mean ΔNIHSS was -0.46 with 95% confidence interval (CI) of -0.75 to -0.16 in the large artery atherosclerosis group, -0.64 with 95% CI of -1.09 to -0.2 in the cardiac embolization group, and -0.25 with 95% CI of -0.4 to -0.09 in the small vessel occlusion group. Edaravone improves the neurological symptoms [15].

Table 2 Assessment of patients discharged within 3 months or patients with 3 months of onset using the mRS score

Grading	0	1	2	3	4	5	Death	Statistics
Edaravone (n=125)	27	36	29	12	10	7	4	p=0.0382*
Placebo (n=125)	12	35	40	12	15	6	5	

Grade 0: No symptoms; Grade 1: No obvious symptoms of disability; Grade 2: Slight disability; Grade 3: Moderate disability; Grade 4: Serious disability; Grade 5: Severe disability.

Table3 Assessment of patients with 3, 6, and 12 months of onset using the mRS score

		0	1	2	3	4	5	Death	Statistics
3 months	Edaravone (n=115)	26	34	24	10	9	7	5	p=0.0481*
	Placebo (n=113)	10	39	26	11	14	7	6	
6 months	Edaravone (n=105)	27	35	15	11	3	8	6	p=0.0112*
	Placebo (n=103)	9	37	23	10	9	7	8	
12	Edaravone	27	31	14	8	4	6	10	p=0.0248*

months	(n=100)								
	Placebo (n=94)	8	35	19	7	11	5	9	

1.5 Risk/Benefit Assessment

1.5.1 Known Potential Risk

TTYP01 is an oral tablet with edaravone as the active pharmaceutical ingredient. TTYP01 is a Class 2 innovative chemical drug independently developed by Suzhou Auzone Biological Technology Co., Ltd. where the formulation of edaravone injection is modified.

The Phase I clinical study of TTYP01 tablets has been completed in Chinese healthy subjects. The safety results show that the main adverse reactions were neutropenia, elevated triglyceride, urinary occult blood, headache, elevated uric acid, premature beats, elevated total bilirubin, elevated direct bilirubin, and elevated indirect bilirubin.

The currently known risks for edaravone injection are the following: According to 569 clinical cases in Japan, 26 cases (4.57%) developed adverse reactions. The main manifestations were abnormal liver function in 16 cases (2.81%) and rash in 4 cases (0.70%). Of the 569 cases, 122 cases (21.4%) had abnormal changes in clinical test values, mainly abnormal liver function test values such as AST increased by 7.71% (43/558), ALT increased by 8.23% (46/559) and so on.

Serious adverse reactions include:

1. Acute renal failure (unspecified degree): During the course of medication, multiple renal function examination and close observation are required. In case of symptoms such as low renal function or oliguria, the invesigational drugs should be suspended and proper treatment should be provided.

2. Abnormal liver function and jaundice (unspecified degree): Accompanied by elevation of AST, ALT, ALP, γ-GTP, LDH and other abnormal liver function and jaundice. The liver function should be tested and closely observed during the course of medication. In case of any abnormality, the invesigational drugs should be suspended and proper treatment should be provided.

3. Thrombocytopenia (unspecified degree): manifestation of thrombocytopenia. During the course of medication, close observation is required. In case of any abnormality, the invesigational drugs should be suspended and proper treatment should be provided.

4. DIC (unspecified degree): DIC may appear, where regular examination is required during the course of administration. In case of laboratory manifestations and clinical symptoms suspected of DIC, the invesigational drugs should be suspended and proper treatment should be provided.

Other adverse reactions (incidence) and main manifestations include:

1. Anaphylaxis (0.1%–5%): Mainly manifested as rash, flushing, swelling, herpes, and itching;

2. Blood cell system (0.1%–5%): Mainly manifested as decreased red blood cell and increased

white blood cells, increased white blood cells, decreased white blood cells, decreased hematocrit value, decreased hemoglobin, increased platelets, and decreased platelets;

3. Injection site (0.1%–5%): Mainly manifested as rash, redness, and swelling at the injection site;

4. Liver (incidence > 5%): Mainly manifested as AST elevation, ALT elevation, LDH elevation, ALP elevation, and γ-GTP elevation; Liver (0.1%–5%): elevated total bilirubin, positive urobilinogen and bilirubinuria;

5. Kidney (0.1%–5%): Mainly manifested as elevated BUN, elevated serum uric acid, decreased serum uric acid, elevated proteinuria, hematuria, and creatinine (unspecified degree);

6. Digestive system (0.1%–5%): belching;

7. Others (0.1%–5%): fever, heat sensation, elevated blood pressure, elevated serum cholesterol, serum cholesterol decreased, elevated triglyceride, serum total protein decreased, elevated CK (CPK), CK (CPK) decreased, serum potassium decreased, and serum calcium decreased.

1.5.2 Known Potential Benefits

Direct potential benefit: All subjects enrolled in this trial can receive standard diagnosis and treatment and systematic examination of the disease by clinical experts, which will benefit the subsequent treatment of the disease. Subjects in the treatment group may experience improved conditions.

Long-term potential benefit: All subjects enrolled in this trial can receive standard diagnosis and treatment and systematic examination of the disease by clinical experts, which will benefit the subsequent treatment of the disease. On the success of this trial, more patients will be able to take the drug and obtain the corresponding treatment and benefits.

1.5.3 Evaluation of Potential Risks and Benefits

The study product is a modified new drug that has not been marketed either at home or abroad. According to Section 1.4 Clinical Studies, the efficacy of similar products was better than that of the placebo, with good safety and tolerability, and the quality of life was improved after long-term use. Compared with edaravone injection, TTYP01 tablets can be administered by patients themselves without relying on professional medical personnel, which improves compliance, reduces medical costs, and has significant clinical development potential. Therefore, the overall benefit to the patient is believed to outweigh the possible risks.

2. STUDY OBJECTIVES

To evaluate the efficacy and safety of TTYP01 tablets in the treatment of AIS through a multicenter, randomized, double-blind, parallel placebo-controlled design clinical trial.

3. STUDY DESIGN

3.1 Overall Design

A multicenter, randomized, double-blind, parallel, placebo-controlled trial design is used to evaluate the efficacy and safety of TTYP01 tablets in the treatment of patients with AIS.

It is estimated that 618 patients will be enrolled and randomly assigned to the treatment group and the placebo control group in a 1:1 ratio, with 309 patients in each group. Normally, the duration of this study is approximately 90 days. This trial will be divided into two periods, with a total of five visits: Treatment period: Day 1–Day 28 (the minimum length of in-hospital observation will be not less than 7 days), including three visits (V1–V3); Follow-up period: Day 29–Day 90, including two visits (V4: Day 60 ± 5 for telephone visit; V5: Day 90 ± 5, returning to hospital for End-of-Study visit).

3.2 Design Rationale

The rationale is as follows:

(1) *Technical Guidelines for Clinical Trials of Drugs for Acute Ischemic Stroke*, CFDA, February 09, 2018;

(2) Points *to Consider on Clinical Investigation of Medicinal Products for the Treatment of Acute Stroke*, EMEA, January 2001;

(3) *Guidelines for General Considerations in Clinical Trials of Drugs*, CFDA, January 18, 2017;

(4) *Standards and Procedures for Expedited Reporting of Safety Data during Drug Clinical Trials*, CFDA, April 27, 2018;

(5) *Guidelines for Prevention and Treatment of Stroke in China (2021)*;

(6) *The Fourth National Academic Conference on Cerebrovascular Diseases of the Chinese Medical Association · Diagnostic Points of Various Cerebrovascular Diseases (2021)*;

(7) *Guidelines for Diagnosis and Treatment of Acute Ischemic Stroke in China (2018)*;

(8) *2019 AHA/ASA Guidelines for Ischemic Stroke.*

According to the *Technical Guidelines for Clinical Trials of Drugs for the Treatment of Acute Ischemic Stroke* issued by the National Medical Products Administration (NMPA, formerly China Food and Drug Administration) in 2018, "At present, there is no sufficient clinical evidence to prove the efficacy of other drugs including neuroprotective agents, and placebo is recommended for clinical trials." "However, for other types of drugs such as neuroprotection, a superiority trial design (e.g., placebo-controlled) is recommended." Therefore, a superiority design is adopted for this trial with placebo as control.

In terms of subject selection, in accordance with China's *Good Clinical Practice* and *Ethical Review Methods for Biomedical Research Involving Human*, it is required that “for subjects with incapacity or limited capacity, project investigators shall obtain written informed consent from their guardian or legal acceptable representative”, and “children, pregnant women, mentally retarded

persons, mental patients, prisoners and other vulnerable groups shall be specially protected". The inclusion/exclusion criteria for this trial comply with regulations protecting vulnerable populations and the informed consent of the guardian will be obtained for patients who are unable to consent during the informed consent process.

3.3 Number of Subjects

A total of 618 subjects (309 in each treatment group) will be planned to be enrolled for the trial to ensure at least 274 subjects per each group (total of 548 subjects) complete the trial, assuming drop-out rate of ~20%.

3.4 Justification for Dose Selection

The dose selection for the Phase III clinical trial of TTYP01 tablets is primarily based on the results of both overseas and domestic Phase I clinical studies.

3.4.1 Phase I Clinical Trial in Australia

A phase I clinical study of TTYP01 tablets have been completed in healthy subjects in Australia, including a single-dose escalation study (60 mg, 120 mg, 180 mg, and 240 mg, Part A), a comparative bioavailability study of TTYP01 and edaravone injection (Part B), and a food effect study (120 mg, Part C). The safety results demonstrated that TTYP01 tablets exhibited good safety and tolerability.

3.4.2 Phase I Clinical Trial in China

A Phase I clinical study of TTYP01 tablets has been completed in healthy Chinese subjects. This study included a bioavailability assessment of TTYP01 tablets (60 mg), edaravone injection (30 mg), and TTYP01-I tablets (original process, 60 mg) in Part 1; a safety, tolerability, and pharmacokinetic profile study in Part 2 (which included two dose groups: 60 mg BID and 120 mg QD); and a food effect study in Part 3 (administering a dose of 120 mg). The test results are as follows:

3.4.2.1 Safety Results

A single oral dose of TTYP01 tablets (60 mg) was found to be safe and well tolerated in healthy Chinese subjects, comparable to edaravone injection (30 mg). Additionally, multiple oral doses of TTYP01 tablets administered at either 60 mg BID or 120 mg QD dosing regimens were also safe and well tolerated. Furthermore, the oral administration of TTYP01 tablets in both fasted and fed states demonstrated similar safety and tolerability.

3.4.2.2 Pharmacokinetic Results

Part I: Compared with intravenous infusion of edaravone injection at 30 mg, the mean Fabs of edaravone was 59.38% after a single oral dose of 60 mg TTYP01 tablets; After a single oral administration of TTYP01-I tablets at 60 mg, the mean Fabs for edaravone was 52.59%. Compared

to a single oral dose of 60 mg TTYP01-I tablets, the mean relative bioavailability (Frel) of edaravone was 116.91% after a single oral dose of 60 mg TTYP01 tablets.

Part II: After multiple consecutive oral doses of TTYP01 tablets at 60 mg BID (n = 9) and 120 mg QD (n = 9) in healthy subjects, the mean (± SD) edaravone area under the curve (AUC) was 1.00 ± 0.00 and 1.03 ± 0.16, respectively. The mean (± SD) values for the $R_{a(Cmax)}$ were 1.20 ± 0.42 and 1.06 ± 0.49, respectively. The results showed no significant accumulation of edaravone following multiple consecutive oral doses of TTYP01 tablets at 60 mg BID or 120 mg QD.

Part III: Food significantly reduced edaravone exposure (C_{max} and AUC) and prolonged time to maximum concentration (T_{max}) following oral administration of 120 mg TTYP01 tablets under fasting or postprandial conditions.

In summary, the recommended Phase III dosage of TTYP01 tablets is set at 60 mg BID, which corresponds to two TTYP01 tablets (60 mg) taken twice daily, administered 30–60 minutes before breakfast and dinner.

3.5 Definition of End-of-Study

A subject is considered to have completed the study if they have finished all phases, including the last visit or final study procedure as outlined in the Schedule of Study Activities. The end of the clinical trial is defined as the last visit of the last subject.

3.6 Randomization Method

This trial employed a block randomization method stratified by NIHSS score. SAS software (version 9.4 or above) will be utilized to generate the randomization numbers and assign the corresponding treatment groups. The randomization numbers will be then assigned through the central randomization system (IWRS) for clinical trials. The randomization list (blinded) will be sent to the project statistician by the randomization construction personnel (exported from the randomization system) at the end of the study and after database lock with the approval of the sponsor.

At screening, each subject will be assigned a screening number, formatted as S followed by five Arabic numerals (e.g., S01001, S02001). In this format, '01' in S01001 indicates the first site, while '001' denotes the first subject. Thus, S01001 refers to the first screened subject at site number 01, and so on. Randomization will occur on Day 1 of the trial, with subjects who pass the screening receiving randomization numbers in the order of their eligibility. The randomization number for the trial will be formatted as R followed by three Arabic numerals (e.g., R001).

Randomized subjects who withdraw or are withdrawn from the trial for any reason, whether or not they are taking study medication, will retain their randomization number and will not be allowed to re-enter the trial.

3.7 Blinding

This trial adopts a double-blind design. Investigational drugs will be manufactured, packaged, and supplied by the sponsor in accordance with the double-blind principle.

(1) Blinding: The on-site blinding of the drug will be conducted by personnel from the statistical unit and the sponsor, who are not involved in this trial. The drug packaging numbers and corresponding treatment group will be generated using SAS software (version 9.4 or above). The drug package number for the investigational drug or the control drug will be affixed to the label. Investigational drugs will be packaged as per subject, with a single package for each subject.

(2) Blinding record: The on-site drug blinding process will be documented in a blinding record, which is stored as part of the clinical trial documentation.

(3) Emergency letter: In this trial, electronic emergency letters will be used, with each random number corresponding to an electronic emergency letter that records the treatment group. The emergency letter is used for emergency unblinding and authorization to the investigator. The IWRS will maintain the protocol for emergency unblinding operations.

(4) Emergency unblinding: In case of emergency, if the investigator determines that knowing the drug used by the subject will be beneficial for treating an adverse event, emergency unblinding can be conducted through the IWRS.

(5) Unblinding: The trial unblinding will be conducted after the finalization of the statistical analysis plan and data review report, and database locked. The project statistician will request the unblinding, and upon approval by the sponsor, the treatment groups corresponding to the random numbers will be revealed for grouped statistical analysis of all data.

4. STUDY POPULATION

4.1 Inclusion Criteria

Subjects eligible for enrollment in this trial must meet all of the following inclusion criteria:

1. Patient whose age is ≥ 18 and ≤ 80 years;
2. Patients diagnosed with AIS according to the Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association;
3. Patients who refuse thrombolysis or thrombectomy, or do not meet the indications for thrombolysis or thrombectomy;
4. Patients who are within ≤ 24 hours after the symptom onset. For patients with uncertain onset time due to wake-up stroke, aphasia, consciousness disorders, etc., the last known asymptomatic time is considered as the onset time;

5. Patients with a first stroke, or a recurrent stroke who recovered well from a previous stroke (mRS score ≤ 1);
6. Patients whose NIHSS score is ≥ 6 and ≤ 20, and the sum of upper limb and lower limb scores in item 5 and 6 is ≥ 2 at the initial diagnosis;
7. Patients or their legal guardians are informed of and voluntarily participate in this study, and have signed a written informed consent form.

4.2 Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from the study:

1. Patients with any contraindications for CT or MRI examination such as metal implants, pacemakers, claustrophobia, etc.;
2. Patients with intracranial hemorrhage confirmed by CT or MRI including but not limited to cerebral hemorrhage, epidural hematoma, subdural hematoma, intracranial hematoma, ventricular hemorrhage, subarachnoid hemorrhage, traumatic cerebral hemorrhage, hemorrhagic transformation in this stroke, etc.;
3. Patients with massive cerebral infarction (i.e., the infarcted area > 1/3 of the cerebral hemisphere as indicated by CT or MRI);
4. Patients with consciousness disorders (Ia > 1 in NIHSS), posterior circulation infarction, or transient ischemic attack (TIA);
5. Patients who are unable or unwilling to cooperate with the study due to comorbidity such as severe mental disorders, depression, Alzheimer's disease, Parkinson's dementia, and Louis body dementia;
6. Patients who are scheduled for or have already received intravenous thrombolysis after the onset of this stroke, or those who plan to receive endovascular therapy urgently or within following 90 days;
7. Patients with a history of malignant tumors or parasitic diseases in the brain;
8. Patients with a history of severe traumatic brain injury or intracranial infection and with a mRS score > 1 before this stroke onset;
9. Patients with severe hypertension (systolic blood pressure ≥ 220 mmHg or diastolic blood pressure ≥ 120 mmHg) before the first administration, which cannot be controlled by treatment;
10. Patients with blood glucose < 2.7 mmol/L and being untreated, or blood glucose > 22.2 mmol/L at initial diagnosis;
11. Patients with dysphagia;
12. Patients with a history of rheumatic heart disease or atrial fibrillation, or with heart rate < 40 or > 120 beats/min, or with second- or third-degree atrioventricular block and being untreated,

or with other kinds of malignant arrhythmia, or with acute myocardial infarction or heart failure (grade III–IV according to the New York Heart Association [NYHA]) or cardiac intervention within the past 6 months;

13. Patients with other serious systemic or organ diseases which, in the opinion of the investigator, may hinder the evaluation of efficacy or make it unlikely for the patient to complete the expected course of treatment and follow-up (e.g., malignancy with the life expectancy < 90 days);
14. Patients with severe liver or kidney diseases, or significant abnormalities in renal or hepatic function tests such as alanine aminotransferase [ALT] or aspartate aminotransferase [AST] ≥ 3.0 × upper limit of normal [ULN], or serum creatinine [Cr] > 2.0 × ULN;
15. Patients with bleeding tendencies (e.g., the platelet count [PLT] < 75.0×10^9/L), or severe bleeding within 90 days before this stroke onset;
16. Patients with contraindications for antiplatelet or statin therapy;
17. Patients who are allergic to edaravone or excipients;
18. Patients with a suspected or proven history of alcohol or drug abuse;
19. Female patients who are pregnant, lactating, having a recent pregnancy plan, or unwilling to use contraception;
20. Patients who have participated in other clinical trials within 90 days before this stroke onset or are participating in other clinical trials;
21. Patients who, in the opinion of the investigator, are not suitable to participate in the clinical trial.

4.3 Withdrawal Criteria

4.3.1 Withdrawal/withdrawal as determined by the investigator

Investigator-initiated withdrawal refers to the fact that the investigator decides to withdraw the enrolled subject from the trial when the conditions that make the further study unsuitable occur in the subject during the study process.

1. Subjects who experience other comorbidities, complications, or special physiological changes during the study, such as recurrent stroke or symptomatic intracranial hemorrhage (sICH), classified as PH I or II according to ECASS criteria;
2. Subjects with poor compliance, such as failure to take investigational drugs after enrollment, lack of records for follow-up visits, unauthorized self-dressing changes, or the use of prohibited drugs specified in the protocol, which may adversely affect the assessment of the efficacy and safety;
3. Subjects who are taking other concurrent treatments that compromised the evaluation in this trial;
4. Serious adverse events (SAEs) or significant adverse events (AEs) may occur, making it

inappropriate to continue the trial;

5. The occurrence of intolerable toxicity related to the drug may include any of the following criteria: (1) serum ALT or AST levels > 8× ULN; (2) ALT or AST levels > 5 × ULN for two consecutive weeks; (3) ALT or AST levels > 3 × ULN with total bilirubin (TBIL) > 2 × ULN; (4) ALT or AST levels > 3 × ULN accompanied by progressively worsening fatigue and gastrointestinal symptoms, and/or eosinophilia (> 5%); (5) serum creatinine (Cr) levels reaching three times the baseline value or higher;
6. Subjects who do not meet the inclusion criteria or who meet any of the exclusion criteria are mistakenly included in this trial;
7. Subjects who participate in another intervention study during this trial;
8. Because of changes in the subject's medical condition unrelated to the investigational drug that occurred during treatment period, such as severe nosocomial infections, heart failure (graded III–IV according to NYHA), and epilepsy that was difficult to control with medication, the investigator determines that it is not in the subject's best interest to continue participation in this trial;
9. Other conditions that, in the opinion of the investigator, necessitate withdrawal from this trial.

4.3.2 Subjects' voluntary withdrawal from the trial

According to the informed consent form, the subject or his/her guardian has the right to withdraw from the trial during any phase, or the subject/guardian may be considered to have withdrawn if the subject no longer accepts investigational drugs or examinations and is lost to follow-up without revoking the consent (also referred to as withdrawal, or dropout).

1. Subjects who are unwilling or unlikely to continue in this trial may request withdrawal to the investigator;
2. Subjects may withdraw spontaneously for various reasons, including perceived poor efficacy, intolerance to certain adverse reactions, economic factors, or other unexplained reasons. The reason for withdrawal should be recognized as far as possible and documented;
3. Subjects who, although not explicitly withdrawing from the trial, no longer undergo visits and are lost to follow-up.

If a subject is unable to determine whether they should withdraw from the trial, the legal guardian has the obligation to make the decision. For subjects who withdraw from the trial for any reason, the investigator should record the reason for their withdrawal as much as possible, and complete and report all observation results in detail.

If a subject withdraws from the trial due to an adverse event (AE) or an abnormal laboratory test result, this important special event and test result should be recorded in the Case Report Form

(eCRF).

Except for cases where the subject withdraws consent or is lost to follow-up, attempts should be made to collect post-termination visit data up to the end of the study as specified in the protocol.

For subjects who drop out or are lost to follow-up, the investigator should take active measures to complete the last visit, ensuring their efficacy and safety data can be analyzed. The reasons for dropout should be recorded in detail, and efforts should be made to obtain safety and efficacy data of the subject. All data should be preserved complete and available for review.

Subjects who sign the informed consent form and have been randomized cannot be replaced.

All subjects who withdraw (drop out) from the trial are expected to complete the corresponding observations specified in the protocol. The reason for termination should be noted in the source document. If a subject withdraws due to an adverse event, the final examination and follow-up specified in the protocol should be conducted. Additionally, appropriate treatment should be provided, and the adverse event should be recorded.

4.4 Trial Suspension Criteria

Trial suspension refers to the termination of the study process before the end of the last visit as per the protocol. The main purpose of trial suspension is to protect the rights and interests of subjects, ensure the quality of the trial, and avoid unnecessary economic losses. All parties to the study should be notified of the suspension in time. Conditions under which a clinical trial may be suspended include, but are not limited to:

(1) When serious safety issues are identified during the study process, and the investigator considers that the safety of subjects may be compromised;

(2) If the investigational drug is determined to have no clinical value during the study process;

(3) If a major error is identified in the design of the trial protocol, making it difficult to evaluate the drug, or if there is a significant deviation in the implementation of the protocol that affects the final evaluation of the drug;

(4) If the sponsor requires the trial to be halted for reasons such as funding constraints or administrative issues;

(5) If drug regulatory authorities order the trial to be stopped for any reason;

(6) If other factors prevent the continuation of the trial.

Once the trial is suspended, the investigator should immediately inform subjects or the guardians, report to the Ethics Committee (EC) and the clinical trial office, explain the reason for the suspension of the trial, and inform the subjects in writing of any potential risks to their health.

5. STUDY INTERVENTION

5.1 Study Drug Information

Table 4 Study Drug Information Form

Item	Investigational drug	Control drug
Name	TTYP01 tablets	Placebo (Simulant TTYP01 Tablets)
Dosage form	Tablet	Tablet
Strength	30 mg	0 mg
Ingredients	Edaravone, polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus), sodium bisulfite, microcrystalline cellulose, polyvinylpyrrolidone, magnesium stearate	Microcrystalline cellulose, magnesium stearate
Storage conditions	Store at room temperature	Store at room temperature
Shelf life	18 months tentatively	18 months tentatively
Manufacturer	STA Pharmaceutical Co. Ltd.	STA Pharmaceutical Co. Ltd.
Supplier	Suzhou Auzone Biological Technology Co., Ltd.	Suzhou Auzone Biological Technology Co., Ltd.

5.2 Study Drug Dosing Regimen

Treatment group (TTYP01 tablets):

Subjects in the TTYP01 group are orally administrated with two TTYP-01 tablets (edaravone 30 mg per tablet) twice daily for consecutive 28 days (56 times in total). The first dose should be given within 1 hour after randomization and remaining doses should be administrated within 30–60 minutes before breakfast or dinner. In case of postprandial randomization, the second dose should not be earlier than 6 hours after the first dose.

Control group (placebo, simulant TTYP01 Tablets):

Subjects in the control group are orally administrated with two placebo tablets (edaravone 0 mg per tablet) twice daily for consecutive 28 days (56 times in total). The first dose should be given within 1 hour after randomization and remaining doses should be administrated within 30–60 minutes before breakfast or dinner. In case of postprandial randomization, the second dose should not be earlier than 6 hours after the first dose.

5.3 Packaging and Labeling of Investigational Drugs

The investigational drug should be provided by the sponsor with appropriate labeling and packaging. The sponsor shall label all investigational drugs in accordance with GCP requirements. The label shall be reviewed and approved according to the sponsor’s review and approval process, and the research organization may keep copies of the labels. Complete records of all investigational drugs including lot numbers and shelf life, as well as drug labels, will be maintained in the sponsor file.

5.3.1 Drug packaging

According to the protocol-specified dosage, the sponsor should provide sufficient investigational drugs.

5.3.2 Drug labeling

Each box of drugs is labeled on the outer package and includes drug name, protocol number, strength, drug packaging number, packaging specification, storage conditions, shelf life, batch number, dosage and administration, sponsor information, and other relevant information. The label also prominently displays the phrase 'For Clinical Trial Only' along with any necessary precautions. The size of the bag label is smaller, with content only showing key information such as the name, s protocol number, strength, drug packaging number, storage conditions, dosage and administration and sponsor of the investigational product.

- **Sample of the outer packaging label**

TTYP01 Tablets for Clinical Study
(For clinical trials only)
Drug name: TTYP01 Tablet/Placebo
Protocol No.: TTYP01-III-AIS **Drug package number:** [here to apply label]
Strength: 30 mg /tablet
Packaging specification: 10 tablets per bag, 3 or 9 bags in total.
Dosage and administration: Administered 30-60 minutes before breakfast and dinner, bid, 2 tablets each time
Batch No.: Omitted, subject to actual situation
Expiry date: Omitted, subject to the actual situation
Storage conditions: Store at room temperature.
Sponsor: Suzhou Auzone Biological Technology Co., Ltd.
Note: To avoid accidental administration, leftover drugs and empty packages need to be collected.

- **Sample of the inner packaging label**

TTYP01 Tablets for Clinical Study
(For clinical trials only)
Drug name: TTYP01 Tablet/Placebo
Protocol No.: TTYP01-III-AIS **Drug package number:** [here to apply label]
Strength: 30 mg /tablet
Dosage and administration: Administered 30-60 minutes before breakfast and dinner, bid, 2 tablets each time
Storage conditions: Store at room temperature.
Sponsor: Suzhou Auzone Biological Technology Co., Ltd.
Note: To avoid accidental administration, leftover drugs and empty packages need to be collected.

5.4 Management of Investigational drugs

The shipment of investigational drugs is the responsibility of the sponsor, who will ship the qualified investigational drugs to each site via the cold chain of medical devices. The drug administrator designated by the clinical trial institutions and participating departments of each site shall receive and count the drugs and relevant documents, and make relevant records. In case of damage, loss, and other abnormalities, it should be recorded exactly and reported to the sponsor and monitor in time. At the same time, the site keeps the drug handover form and/or waybill as well as the temperature records of the whole process.

The investigational drug can only be used in this trial and can only be administered by a designated person authorized by the investigator. The investigator/pharmacist in charge of drug management dispenses and retrieves the investigational drug and keep accurate record according to the study procedures. Records of investigational drug management should include date, quantity,

batch/serial numbers, shelf life, and allocation codes, signatures, etc. The investigator should keep records of the amount and dose of investigational drugs used in each subject. The amount of the used and the remaining investigational drug should match the amount provided by the sponsor.

After the end of the trial, the drug administrator is responsible for the recovery and accountability of the remaing unused drugs or the expired drug, which are returned to the sponsor, and destroyed by the sponsor in a concentrated way.

In order to strictly manage and use the investigational drug, each participating hospital should establish a strict management system, specifically a system for managing and dispensing investigational drugs by specific personnel in drug storage facility and trial department of the clinical trial institution. The investigational drug should be stored at room temperature.

5.5 Concomitant Medication

5.5.1 Permitted Concomitant Drugs During the Trial

5.5.1.1 Basic medication

Basic drugs are administered according to the *Guideline for the Prevention and Treatment of Stroke in China (Version 2021)*, including general treatment, antiplatelet therapy, etc. However, drugs prohibited in this trial, such as neuroprotective agents, should be excluded.

5.5.1.2 Other permitted drugs during the trial

In case of adverse reactions, the investigator should decide whether symptomatic treatment is needed, and should record the drug name, administration route, dosage, and starting and ending time of administration in the original records and eCRF.

All other treatments between the enrollment and the End-of-Study are considered concomitant therapies, and the drugs are recorded in the eCRF in the generic name. Concomitant drugs during the trial must be essential for the subject, and the investigator should ensure concomitant drugs do not interfere with the investigational drug. The dose should be kept at the minimum level.

5.5.1.3 Non-pharmacological treatment

It’s necessary to accurately and thoroughly document any permitted non-pharmacological treatments during the trial, such as rehabilitation therapy.

5.5.2 Prohibited Drugs During the Trial

The combination use of neuroprotective agents listed in the *Chinese Guideline for Diagnosis of Acute Ischemic Stroke 2018*, the *Guideline for the Prevention and Treatment of Stroke in China (Version 2021)*, or the *Chinese Guideline for the Secondary Prevention of Ischemic Stroke and Transient Ischemic Attack (Version 2010)* is prohibited during the study, such as commercial available edaravone, edaravone dexborneol, nimodipine, gangliosides, phosphatidylcholine, piracetam, olactam, butylphthalide, urinary kallindinogenase, cinepazide, nerve growth factor,

cerebrolysin, brain protein hydrolysate, deproteinised calf serum injection, and deproteinisd calf blood extract injection, as well as herbs with neuroprotective effects stated in instructions, etc. Other drugs in trial or unmarketed are also prohibited in this study.

5.5.3 Study Treatment Compliance

During the trial, investigational drugs should be administered according to the administration method specified in the protocol. If deviations from the administration method occur (such as deviations in administration time or dosage from the protocol), it is necessary for the investigator to record them in the source documents and eCRF. These deviations should serve as the basis for assessing the medication compliance. Before locking the database for statistical analysis, the principal investigator and statistical experts should decide on a case-by-case basis whether to include the data in the data analysis and to which data set they belong.

6. Study Procedure

6.1 Treatment Period (D1 to D28; V1 to V3)

V1 (D1):

- Signing the informed consent form;
- Assessment based on inclusion and exclusion criteria;
- Demographic data collection, including initials, sex, date of birth, ethnicity, height, weight, education level, etc.;
- Recording of the relevant medical history, including history of present illness, past history (including dementia/cognitive dysfunction, depression, etc.), medication history, allergy history, smoking history, history of alcohol consumption, etc.;
- Vital signs, including body temperature, blood pressure, pulse, and respiratory rate;
- Physical examination, including general condition, head, face, skin, lymph nodes, eyes, ears, nose, throat, mouth, respiratory system, cardiovascular system, abdomen, muscles, bones, and nervous system;
- 12-lead electrocardiogram (ECG);
- Laboratory tests: Blood routine, blood biochemistry, coagulation function, urine routine, etc.;
- Head CT or MRI scanning (T1, T2, FLAIR, and DWI + ADC);
- Blood or urine pregnancy test (for pre-menopausal women only);
- Randomization;
- Hospitalization;
- Distribution of investigational drugs: Distribution of investigational drugs of day 1 to day 7;
- mRS score;
- NIHSS score;

- BI score;
- Recording concomitant therapies and drugs;
- Collection and evaluation of adverse events.

V2 (D7):

- Vital sign examination;
- Physical examination;
- 12-lead ECG;
- Laboratory tests: Blood routine, blood biochemistry, coagulation function, urine routine, etc.;
- Distribution of investigational drugs: Distribution of investigational drug for the period until the next visit;
- Distribution of subject diary cards: Distribution of subject diary cards required at the next visit;
- mRS score;
- NIHSS score;
- BI score;
- MoCA score: Completing baseline assessment within 7 days (including day 7) after enrollment;
- HDRS score: Completing baseline assessment within 7 days (including day 7) after enrollment;
- Record concomitant therapies and concomitant drugs;
- Collection and evaluation of adverse events.

V3 (D28 + 3):

- Vital sign examination;
- Physical examination;
- 12-lead ECG;
- Laboratory tests: blood routine, blood biochemistry, coagulation function, urine routine, etc.;
- Head MRI scanning: selecting some eligible sites to perform post-stroke head MRI scanning (T1, T2, and FLAIR);
- Return of investigational drugs: subjects returning unused drugs and/or empty packages to investigators;
- Collection and assessment of subject diary cards: subjects submitting completed diary cards to investigators for assessment;
- Distribution of subject diary cards: Distribution of subject diary cards required for the next return visit;
- mRS score;
- NIHSS score;
- BI score;

- MoCA score;
- HDRS score;
- Recording concomitant therapies and drugs;
- Collection and evaluation of adverse events.

Note:

1. V1 should be conducted during this episode prior to the first administration of the treatment.
2. If the subject has completed some protocol-required examinations (e.g., physical examination, 12-lead ECG, laboratory tests, and blood or urine pregnancy tests) at the site within 24 hours of admission, and the investigator deems these examinations sufficient to support the screening of the subject, they may not be repeated. This consideration is based on the potential health risks associated with conducting these examinations in a short time, which could also significantly impact the subject's treatment schedule.
3. Selected sites with specific medical conditions will conduct head MRI scans on Day 2 and Day 5 (including T1, T2, FLAIR, and DWI + ADC) and on Day 28 + 3 (T1, T2, and FLAIR). It is expected to collect MRI data from approximately 75% of all subjects and perform centralized reading to compare changes in MRI variables (infarct volume and area). If a participant undergoes an MRI scan on Day 1, the MRI scan on Day 2 can be skipped and only the remaining MRI tests are required.
4. Dosing regimen during the treatment period: Subjects in the TTYP01 group or control group are orally administrated with two TTYP01 tablets (edaravone 30 mg per tablet) or two placebo tablets (edaravone 0 mg per tablet) twice daily for consecutive 28 days (56 times in total), respectively. The first dose should be given within 1 hour after randomization and remaining doses should be administrated within 30–60 minutes before breakfast or dinner. In case of postprandial randomization, the second dose should not be earlier than 6 hours after the first dose.

6.2 Follow-up Period (D29–D90; V4–V5)

V4 (D60 ± 5, telephone visit):

- mRS score;
- Recording concomitant therapies and concomitant drugs;
- Collection and evaluation of adverse events.

Note:

The investigator will record the telephone visiting for the subject or his/her guardian on Day 60 ± 5.

V5 (D90 ± 5, returning to hospital for End-of-Study visit):

- Vital sign examination;
- Physical examination;
- 12-lead ECG;
- Laboratory tests: Blood routine, blood biochemistry, coagulation function, urine routine, etc.;
- Blood or urine pregnancy tests (pre-menopausal women only);
- mRS score;
- NIHSS score;
- BI score;
- MoCA score;
- HDRS score;
- Collection and assessment of subject diary cards: Subjects submitting completed diary cards to the investigator for the assessment;
- Recording concomitant therapies and concomitant drugs;
- Collection and evaluation of adverse events.

Note:

Efficacy and safety data for early withdrawal subjects are collected as much as possible by performing the same processes and examination items as V5.

7. Study Evaluation

7.1 Efficacy Evaluation

7.1.1 Primary Outcome Measure

Proportion of subjects with mRS score ≤ 1 on Day 90 after stroke onset.

7.1.2 Secondary Outcome Measure

(1) mRS score on day 90 after stroke onset;

(2) Proportion of patients with a mRS score ≤ 2 on Day 90 after stroke onset;

(3) Changes from baseline in mRS score on Day 7, 28, 60, and 90 after stroke onset;

(4) Proportion of patients with an improvement of ≥ 4 in NIHSS score on Day 7, 28, and 90 after stroke onset;

(5) Proportion of patients with a NIHSS score of 0–1 on Day 7, 28, and 90 after stroke onset;

(6) Changes from baseline in NIHSS score on Day 7, 28, and 90 after stroke onset;

(7) Proportion of patients with Barthel Index (BI) score ≥ 95 on Day 7, 28, and 90 after stroke onset;

(8) Changes from baseline in BI score on Day 7, 28, and 90 after stroke onset;

(9) MoCA score on Day 7, 28, and 90 after stroke onset;

(10) HDRS score on Day 7, 28, and 90 after stroke onset;

(11) Changes from baseline in brain MRI variables (e.g., infarct volume and area) on Day 5 and 28 after stroke onset.

7.2 Safety Evaluation

Safety endpoints include the occurrence of adverse events (AEs) from the start of study to the end of follow-up period, including treatment-emergent adverse events (TEAEs), adverse drug reactions (ADRs), serious adverse events (SAEs), and TEAEs leading to subject termination from the trial.

(1) Proportion of all-cause deaths within 90 days after stroke onset;

(2) Proportion of symptomatic intracranial hemorrhages within 90 days after stroke onset;

(3) Proportion of recurrent ischemic strokes within 90 days after stroke onset;

(4) Proportion of AEs within 90 days after stroke onset;

(5) Proportion of SAEs within 90 days after stroke onset;

(6) Discontinuation due to any AE within 90 days after stroke onset;

(7) Discontinuation due to any other non-AE within 90 days after stroke onset.

8. Adverse Event

8.1 Definition

8.1.1 Adverse event

An AE refers to any adverse medical event that occurs after the subject in the trial receives the investigational drug. An AE does not necessarily have a causal relationship with the treatment. AEs include adverse, abnormal signs (including abnormal tests, examination findings), symptoms or illnesses that are temporally related to the use of the (experimental) medication, whether or not they are related to the (experimental) medication. An AE can therefore be a new occurrence, an exacerbation or an increase in frequency of the underlying diseases, or the exacerbation of an abnormal diagnostic test result including an abnormal laboratory value, etc.

Medical events that occur after signing informed consent should be collected and recorded. Medical events that occur after signing the informed consent form and before the first dose of investigational drugs are recorded in the eCRF as medical history/concomitant illnesses and are not recorded as AEs, unless they are relevant to the screening process.

For abnormal test results, the following criteria should be used to determine whether an abnormal laboratory test result should be reported as an AE:

(1) Symptoms accompanied with the test result, and/or;

(2) Test results requires further diagnostic testing or medical/surgical intervention, and/or;

(3) Test results lead to the dosage modification of the investigational drug (beyond the protocol-specified dosage) or;

(4) In the opinion of the investigator or sponsor, the test results constitute an AE.

The repetition of abnormal tests alone does not constitute an AE if it does not meet any of the above criteria. Test results reported in error do not need to be reported as AEs.

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as AEs. However, the disease for which the procedure be performed meets the definition of an AE, it should be reported as an AE. For example, acute appendicitis that occurs during the AE reporting period should be reported as an AE, and appendectomy performed to treat the condition should be recorded as the treatment for the AE.

8.1.2 Serious adverse event

Serious adverse event (SAE) refers to adverse medical events such as death, life-threatening (refer to those in which the subject is at immediate risk of death at the time of the event, rather than indicating a potential risk of death in more severe situations), permanent or severe disability or loss of function, the condition that causes the subject to be hospitalized or to prolong the hospitalization, and congenital abnormalities or birth defects after the subject receives the investigational drug.

Significant Adverse Event: Significant haematological and other laboratory abnormalities (except for SAE) and any event leading to the intervention, including cessation of trial/investigational product treatment, dose reduction, or important other concomitant treatment, except those reported as SAE.

Other important medical events: Medical and scientific judgment must be exercised in deciding whether expedited reporting in other situations, such as important medical events may not be immediately life-threatening, death, or hospitalization but may also be considered serious if medical treatments is needed to prevent one of the situations listed above. Examples include the intensive treatment in an emergency room, allergic bronchospasm or convulsions occurring at home, a development of drug dependency or addiction, etc.

(1) Unlisted (unexpected) adverse event/reference safety information

For investigational drugs, the expectation of an AE will be judged based on whether or not it is listed in the Investigator's Brochure.

The following conditions are not considered hospitalizations or SAEs: Hospital observation for less than 24 hours; hospitalization for routine examinations (hospitalization for less than 24 hours); hospitalization due to social reasons (such as lack of caregiver support); hospitalization for scheduled surgery agreed upon prior to the study; hospitalization for a pre-existing condition that is not associated with a new AE or exacerbation of a pre-existing adverse condition (e.g., examination

of persistent pre-treatment laboratory abnormalities); pre-planned treatment or surgery for the entire study protocol and/or for an individual subject should be recorded in the baseline documentation; hospitalization or prolongation of hospitalization as required by the protocol, such as hospitalization for dosing or completion of protocol-required examinations does not qualify as an AE.

(2) Serious adverse event start time

The onset date of the AE upgraded to SAE is taken as the start time of the SAE.

(3) Serious adverse event end time

The end time of the AE is considered to be that the AE is resolved, stabilized, and reasonably explained. The time shall be as exact as date, month and year, or as month and year if the information is not collected completely. If the AE is judged to have caused "death" directly or as the primary cause, the end time is the time of the subject's death.

8.1.3 Suspected unexpected serious adverse reaction

The suspected unexpected serious adverse reaction (SUSAR) refer to suspected and unexpected serious adverse reactions whose clinical manifestations in terms of nature and severity exceed the existing information such as the Investigator's Brochure of the investigational drug, the instructions of the marketed drug, or the summary of product characteristics.

8.2 Risk of drug use

According to the Investigator's Brochure and previous reports on edaravone injection, the following AEs are classified as identified or potential risks during the trial.

It mainly includes acute renal failure (unspecified degree), abnormal hepatic function, jaundice (unspecified degree), thrombocytopaenia (unspecified degree), disseminated intravascular coagulation (DIC) (unspecified degree).

8.2.1 Acute renal failure (unspecified degree)

During the study, if a subject's serum Cr, eGFR and other renal indicators increase to different degrees in a short period (refer to RIFLE or AKIN criteria), acute kidney injury or renal failure is very likely to occur, and the drug should be stopped immediately. According to the severity, the investigator should promptly give targeted standard medical treatment and symptomatic treatment as per the operation process of the hospital, and closely monitor the renal function and its outcome through serum creatinine and urine output.

8.2.2 Abnormal hepatic function, jaundice (all unspecified degree)

Drug induced liver injury (DILI) refers to the direct or indirect liver damage to different degrees caused by the drug itself or its metabolites during the use of drugs within the range of doses in normal therapy or clinical trials.

During the trial, if a subject is accompanied by abnormal liver function such as increase of AST,

ALT, ALP, γ-GTP and/or LDH, and jaundice, or even gastrointestinal symptoms such as asthenia, decreased appetite, oil anorexia, distending pain in the liver area and epigastric discomfort, it is very likely that acute liver injury occurs, and the investigational drug dose should be immediately stopped or reduced. The investigator should promptly give targeted standard medical treatment and symptomatic treatment according to the severity of liver injury as per the operation process of the hospital, and closely monitor the outcome of the liver injury.

8.2.3 Thrombocytopenia (unspecified degree)

Thrombocytopenia (unspecified degree) is associated with a decreased platelet count. Close observation should be made during the administration course of the investigational drug. It should to stop the adiministration of the investigational drug and treat it properly in case of abnormal conditions.

8.2.4 Disseminated intravascular coagulation (DIC) (unspecified degree)

There may be manifestations of disseminated intravascular coagulation. It should be detected regularly during the administration course of the investigational drug. If laboratory tests and clinical symptoms suspected of disseminated intravascular coagulation occur, the investigational drug should be stopped and proper treatment should be given.

8.3 Observation and recording of adverse events

All adverse events spontaneously reported by subjects or elicited in response to open-ended questions by the investigator,"Since your last visit/or since last question, have you experienced any health problems?", should be collected and recorded in the eCRF, as well as adverse events identified through observation. The details of the adverse events should be specifically documented, including the starting time , severity, ending time, intervention, and outcome.

When collecting adverse event data, it is preferable to record the diagnosis (if possible) rather than the series of signs and symptoms, i.e., the name of the adverse event should be the medical terminology and the medical diagnosis should be used in preference. However, if a diagnosis is known but the patient still has other signs or symptoms that do not match the diagnosis, each sign or symptom should be recorded respectively. If the laboratory test abnormality is part of the syndrome, the syndrome or medical diagnosis should be recorded (e.g., anemia with decreased hemoglobin should be recorded as “anemia” rather than “hemoglobin decreased” alone).

8.3.1 Follow-up of adverse events

When an AE occurs to a subject during the trial, the clinician shall take necessary treatment measures according to the specific situation, and notify the sponsor if necessary. The investigator will follow the AEs that have occurred, and follow up the AEs that have not resolved after the end of the trial until the event has resolved, stabilized, or until there is a reasonable explanation, or until

no further follow-up is required as judged by the investigator, or the subject is lost to follow-up, or the subject withdraws from the trial early. The treatment process, follow-up data, and outcome (including recovery, improvement, persistence, aggravation, and death) should be fully recorded in the study medical record and eCRF, and the relevant examination report should be attached.

The sponsor reserves the right to request further information, if necessary, from any subject regarding an ongoing AE/SAE after the end of the trial.

(1) Follow-up endpoints

A subject treated with investigational drug will need to be followed if:

(1) The subject has been followed up as per protocol until day 90 ± 5 after stroke onset;

(2) The subject is dead during the follow-up;

(3) Serious deviations of the inclusion/exclusion criteria are found after enrollment, and follow-up termination is deemed necessary by both the investigator and sponsor;

(4) The subject withdraws from the trial due to various reasons, and the follow-up endpoint is the last visit time;

(5) The subject needs to be withdrawn from the clinical trial at the discretion of the investigator, and the follow-up endpoint is the last visit time.

(2) Post-trial events

After the subject's final visit or the permanent discontinuation from the trial, the investigator is no longer obligated to actively inform the subject of any new AEs or SAEs that occur after the safety follow-up. However, if the investigator learns of any SAE (including death) in a subject after the subject has been permanently discontinued from the study, and there is a reasonable possibility that the event is related to the investigational drug, the investigator should notify the sponsor's pharmacovigilance team or representative.

(3) Variables

The following variables will be collected for each AE:

(1) AE term (verbatim);

(2) Starting and ending dates;

(3) Common terminology criteria for adverse events (CTCAE) classification;

(4) Whether it constitutes a SAE;

(5) Investigator's assessment of causal relationship between the AE and investigational drug;

(6) Action taken to the investigational drug;

(7) Treatment of the AE;

(8) Outcomes.

8.4 Adverse event assessment

8.4.1 Judgment for the severity of adverse events

Attention should be paid to distinguishing between SAE and the severity of AE. According to the definition of SAE, AEs that are severe in severity do not necessarily have to be SAEs. For example, vomiting lasting several hours may be considered severe vomiting but not a SAE. A stroke that results in only minor functional impairment may be considered a mild stroke but is a SAE.

The investigator should assess the severity of each AE and SAE reported during the trial. AEs and SAEs should be assessed and graded according to NCI-CTCAE v5.0.

For adverse events not included in the criteria, refer to the following criteria:

Grade	Expression
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated;
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting ageappropriate instrumental activities of daily living (ADL) (refer to preparing meals,shopping for groceries or clothes, using thetelephone, managing money, etc.).
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (refer to bathing, dressing andundressing, feeding self, using the toilet, taking medications, and not bedridden).
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

Note: The semicolon (;) used in the description of the grade refers to “or”.

8.4.2 Determination of the causal relationship of adverse events to the investigational drug

8.4.2.1 Judgment basis of adverse event causality:

(1) Whether there is a reasonable temporal relationship between the initiation of the drug and the occurrence of the suspected AEs;

(2) Whether the suspected adverse event is consistent with the type of known AEs of the investigational drug;

(3) Whether the suspected AEs can be explained by the effects of concomitant medication, the clinical condition of the patient or the influence of other therapies;

(4) Whether the suspected AE disappeared or abated after discontinuation or dose reduction;

(5) Whether the same reaction occurs again after exposure to suspected drugs.

8.4.2.2 Criteria for causality determination:

Make judgment according to the above 5 judgment indexes.

Judgment result	Judgment index				
	1	2	3	4	5
Definitely related	+	+	–	+	+
Probably related	+	+	–	+	?
Possibly related	+	+	±	±	?
Possibly unrelated	+	–	±	±	?
Not related	+	–	+	–	–

Description: + positive, – negative, ± hard to confirm positive or negative, and ? unknown situation. According to the above table, the relationship of the following Grade 5 adverse events to the drug is judged: Where “definitely related”, “probably related” and “possibly related” are summed as the numerator for

adverse reaction incidence calculation, and the denominator is the total number of subjects used for safety evaluation.

Note: The judgment of the relationship between adverse events and drug is the result of individual judgment of the investigator. When summarizing safety data in the clinical trial, the principal investigator should also make a general judgment according to the composition of the formulation, the results of non-clinical safety studies, the frequency, severity, trend of adverse events, and the occurrence of adverse events in the control group.

8.5 Subject risk control measures

Strengthen the informed consent, fully inform the subjects of the possible risks of using the drug, especially the SAEs that may exist when using the drug at a high dose, and ensure that the subject is informed fully and voluntarily signs the informed consent form.

For the important risk events of possible adverse reactions, the medical personnel shall be trained according to the corresponding treatment methods specified by the clinical study unit, and the facilities and equipment, including medical personnel and drugs required for emergency treatment of the above-mentioned serious adverse events are available in the hospital. The first-aid treatment and medical care conditions of the hospital should be evaluated typically in the selection of the research unit. In case of serious adverse events, the principal investigator should participate in the treatment and make medical treatment decisions, and contact experts in other medical departments for consultation and treatment if necessary.

Investigators shall closely observe and strictly record all adverse events during the study. For SAE meeting the requirements of laws and regulations, the sponsor shall be notified and reported in a timely manner, and then detailed and written follow-up reports shall be provided in a timely manner. The sponsor shall strengthen the collection and analysis of adverse events through investigators and monitors, identify suspected and unexpected serious adverse reactions (SUSARs) and submit them according to regulatory requirements; conduct summary analysis on the safety data of the investigational product on a regular basis, and submit a safety update report to the Center for Drug Evaluation (CDE) of the NMPA once a year according to regulatory requirements.

Strengthen the follow-up management of subjects, and strengthen the contact for subjects who withdraw or drop out due to AEs, and correctly complete the corresponding last follow-up content.

In view of the risks of the clinical trial, in order to protect the rights and interests of the subjects, and to provide timely treatment and handling during the trial due to AEs or SAEs, as well as reasonable compensation or indemnity, the sponsor will purchase clinical trial liability insurance covering each subject participating in the clinical trial.

8.6 Reporting procedure

8.6.1 Reporting procedures of serious adverse events

Except for the SAEs that do not need to be reported immediately as specified in the trial protocol or

other documents (such as the Investigator's Brochure), the investigator shall report all SAEs to the sponsor (or authorized representative) in writing within 24 hours after learning of the SAE, and then provide a detailed and written follow-up report in a timely manner. SAE reports and follow-up reports should indicate the identification code of the subject in the clinical trial, instead of the subject's real name, resident ID number, address and other identification information. The time limit of follow-up report is the same as that of the first report. AEs and abnormal laboratory values deemed important for safety evaluation, as specified in the trial protocol, should be reported to the sponsor according to the requirements and time limits outlined in the trial protocol.

For reports involving deaths, the investigator should provide the sponsor and EC with other required materials, such as autopsy reports and final medical reports.

For all SAEs occurring during the clinical trial, whether in initial or follow-up report, the investigator must complete the "Serious Adverse Event Report Form" provided by the sponsor, sign and date it. In the source documents, it should be documented when, how, and to whom the SAEs be reported.

When reporting an SAE, the following points should be noted:

(1) Potential diagnoses or syndromes should be reported as the primary SAE term rather than signs or symptoms (signs and symptoms may be described in the narrative).

(2) The event term of "death" should not be reported as an SAE but should be recorded as a result of the specific SAE term. The event term "death" may be used initially until the actual cause of death is known. If an autopsy is performed, an autopsy report should be provided.

8.6.2 Reporting procedure for SUSARs

Upon receipt of safety-related information from any source, the sponsor should promptly analyze and assess it, including severity, relevance to the investigational drug, and whether it is an expected event. The sponsor shall promptly report suspected and unexpected serious adverse reactions to all investigators participating in the clinical trial, clinical trial institutions and EC; the sponsor shall report suspected and unexpected serious adverse reactions to the drug regulatory authorities and the competent health authorities.

The sponsor shall report to CDE as soon as possible upon first learning about the SUSAR. For fatal or life-threatening unexpected serious adverse reactions, the report should be reported within 7 days, with follow-up information completion within the subsequent 8 days. For non-fatal or non-life-threatening unexpected serious adverse reactions, the report should be submitted within 15 days. The investigator should sign the SUSAR provided by the sponsor and report it to the EC.

The contact information for reporting SUSARs is as following table.

Suspected unexpected serious adverse reactions (SUSAR) reporting unit

Unit	Contact person	Contact number	Fax/email
Sponsor: Suzhou Auzone Biological Technology Co., Ltd.	Hou Xiaolong	15895583778	houxl@szauzone.com
CRO: Guoxin Pharmaceutical Technology (Beijing) Co., Ltd.	Pharmacovigilance Center	15201580570	pv@gxcro.com
National Health Commission		010-68792201	saefax@163.com
Center for Drug Evaluation (CDE) NMPA		010-68585566	010-68584181
Note: Center for Drug Evaluation (CDE) NMPA Address: No. 128, Jianguo Road, Chaoyang District, Beijing, Tel: 010-68585566			

8.6.3 Reporting procedures for development safety update reports (DSURs)

The sponsor shall submit the drug development safety update report to CDE on a regular basis (annually) according to relevant regulatory requirements. The drug development safety update report provided by the sponsor shall include the assessment of the risks and benefits of the clinical trial, and relevant information should be informed to all investigators participating in the clinical trial, clinical trial institutions, and EC.

Note: Provided that the GCP requirements are met, the reporting procedures for SAEs, SUSARs, and DSURs can be adjusted and implemented according to the safety event reporting requirements of different sites.

8.6.4 Reporting of pregnancy

If pregnancy occurs in a female subject or female partner of a male subject during the trial, the investigator should immediately discontinue the subject from the clinical trial and discontinue the administration of the investigational drug. The investigator should communicate scientifically and rigorously with the subject based on the medication information, inform the subject of the possible effects and risks of the investigational drug on the pregnant woman and fetus, and decide whether to terminate the pregnancy or continue the pregnancy at the subject's discretion.

Within 24 hours after the occurrence of the confirmed pregnancy, the investigator should fill out the *Pregnancy Report Form* upon discovery of the pregnancy, and report to the sponsor (or authorized representative), drug clinical trial institution and the EC of the hospital within 24 hours, and record it in the eCRF (if necessary). Pregnancy itself is not considered to be an AE or SAE, but any complicated situation that occurs during the course of a pregnancy or situations where pregnancy is terminated for medical reasons will be recorded, reported, and followed up as specified in the protocol as “adverse event” or “serious adverse event”. If the outcome of the pregnancy meets the criteria for an SAE, such as spontaneous abortion (including threatened abortion, inevitable abortion, incomplete abortion, complete abortion, missed abortion, etc.), stillbirth, neonatal death, or congenital anomaly, the investigator should report it according to the SAE reporting procedure.

If the subject decides to continue the pregnancy, follow-up is required until the end of the

pregnancy or for three months post-delivery. The investigator shall complete the pregnancy follow-up report within 24 hours of each follow-up pregnancy and report to the sponsor, drug clinical trial institution and the EC of the hospital.

All neonatal deaths that occur within one month of life, regardless of the cause of death, should be reported as SAE. In addition, any infant death after one month of life should also be reported if the investigator considers the death to be possibly related to the investigational product.

8.6.5 About lack of efficacy

Lack of efficacy per se cannot be reported as an adverse event and any symptoms/signs or sequelae resulting from lack of efficacy should only be reported if they meet the criteria for an adverse event/serious adverse event.

8.6.6 Independent Data Monitoring Committee

One interim analysis will be planned to be conducted at the timing of 50% subjects complete the trial. Independent Data Monitoring Committee (IDMC) will be established for the assurance of patient safety and trial integrity and monitoring the effectiveness data. The purpose of IDMC, IDMC members and their responsibilities, meeting frequency and etc. will be described in the IDMC Charter.

8.7 Risk control measures for protocol deviations

Strengthen the implementation of the protocol, report the protocol deviation to the EC in time and strengthen the training for investigators. In case of adverse events, priority should be given to the rights and safety of the subjects in the treatment. If necessary, deviation from the protocol is allowed for treatment. For critical procedures such as drug administration, dedicated Standard Operating Procedures (SOPs) should be developed, and relevant personnel should receive necessary training to ensure proper execution without deviations, thereby ensuring the scientific integrity of the protocol implementation.

Strengthen the training and authorization of researchers, especially drug management, to ensure that special personnel are responsible for each link and provide original records of any one link.

Any change involving the investigator during the study shall be timely trained and authorized before participating in the clinical trial.

In order to ensure the quality of drugs, a professional cold chain transportation company is responsible for the whole transportation of drugs and provides temperature records throughout the process. During the storage period at the study site, the drug should be stored according to the drug storage conditions, and if necessary, a drug refrigerator meeting the storage conditions should be provided. Drug management shall be managed by a specially-assigned person.

9. Data Management and Statistical Analyses

9.1 Data management

Electronic data management is used for this trial. The main data management processes are listed below, and other details are detailed in the Data Management Plan (DMP).

As a guidance document for data management, the DMP is written by the data manager (DM) and approved by the sponsor. The data management will be conducted according to the timing, content and methods defined in the DMP.

9.1.1 Data management of EDC

- Electronic Case Report Form (eCRF): The data manager designs and constructs the eCRF according to the trial protocol, sets up logical checks based on the Data Validation Plan (DVP), and releases it for use after testing and sponsor approval.
- Data entry: The eCRF data are derived from the source records. The data entry personnel inputs the subject visit data into EDC in a timely manner according to the eCRF completion instructions.
- Source data verification (SDV): The monitor performs the consistency verification between eCRF data and source data, and can raise questions if any issues are identified.
- Data query and solution: The queries come from the system query generated by EDC logic check, as well as manual queries from monitor, data manager, etc., and the investigator needs to answer the questions in time. Data managers and monitors review and respond to queries, and if necessary, additional queries may be issued until the data is free of discrepancies.
- Investigator's signature: After the data entry is completed and SDV is performed, the investigator will conduct an electronic signature review and confirmation. After signing, any data revisions require re-signing.
- Database lock: After the principal investigator, sponsor, statistical analysis personnel and data management personnel jointly sign the database lock application, the data manager will lock the database.
- Database submission: the data manager submits the database to the statistician.
- eCRF archiving: A PDF electronic file will be generated for each subject's eCRF.
- EDC closure: After the statistical analysis is completed, the data manager closes the database.

9.1.2 External data transfer

External data transfer agreements will be developed and external data management will be performed according to the DMP.

9.1.3 Medical coding

Adverse events will be coded using MedDRA (version 26.0 or higher) and concomitant medications will be coded using WHODD (V2022 SEP or above).

9.2 Statistical analyses

9.2.1 Statistical hypotheses

The following hypothesis will be tested for the primary efficacy endpoint:

- Null hypothesis (H_0): $P_{TTYP01} - P_{placebo} \leq 0$
- Alternative hypothesis (H_1): $P_{TTYP01} - P_{placebo} > 0$,

P: proportion of subjects who achieved mRS ≤1 on Day 90.

No formal hypothesis tests will be set up for the secondary efficacy endpoints; all the secondary efficacy endpoints will be just used to provide supportive evidence, and therefore, no adjustments for multiplicity will be made.

9.2.2 Sample size determination

A sample size of 274 subjects per each treatment group (total of 548 subjects) will provide at least 90% power to detect a difference of 15% (60% in the TTYP01 group versus 45% in the placebo group) for the primary endpoint the proportion of subjects who achieved mRS ≤1 on Day 90 with a one-sided α value of 0.025. Assuming drop-out rate of ~20%, 618 subjects (309 in each group) will be planned to be enrolled for the trial.

9.2.3 General considerations

All statistical analyses will be performed using SAS version 9.4 or above. Detailed statistical methods will be described in the SAP and finalized prior to database lock.

In general, all study data will be pooled and summarized/analyzed by treatment group: descriptive statistics (n, mean, median, first quartile (Q1), third quartile (Q3), standard deviation, minimum and maximum values) will be used for continuous variables; categorical variables will be described using frequency tables (frequencies and percentages).

All statistical hypothesis tests will use a 2-sided test at $\alpha = 0.05$ and two-sided 95% confidence intervals (CIs) will be calculated, unless otherwise specified.

9.2.4 Analysis sets

(1) Full analysis set (FAS): FAS will included all the randomized subjects who receive at least one dose of investigational drug and who have at least one post-dose primary efficacy assessment (i.e., mRS).

(2) Per-protocol set (PPS): As the subset of FAS, PPS will include all the subjects who have the primary efficacy endpoint available on D90, with treatment compliance of 80% to 120% and who have no major protocol deviations affecting the primary efficacy assessment (e.g. not meeting important inclusion criteria, use of prohibited medications impacting the primary efficacy outcomes during the trial).

(3) Safety set (SS): SS will include all the subjects who receive at least one dose of investigational

drug.

9.2.5 Subject disposition

A summary of the number and percentage of subjects who completed/discontinued study will be presented respectively by treatment group along with the primary reasons for study withdrawal. In addition, the number and percentage of subjects included in each analysis set will be presented.

9.2.6 Demographic and baseline characteristics

Demographic data (Age and Age Group (<65, ≥65), Race, Gender, Height, Weight, Body Mass Index (BMI)) will be analyzed with descriptive statistics as appropriate, respectively. In addition, baseline disease characteristics including AIS history, time from onset of AIS to treatment, baseline NIHSS, concomitant illness with AIS (hypertension, diabetes, hyperlipidemia and cardiac disease), alcohol history, smoking history and etc. will be also summarized.

9.2.7 Extent of exposure and treatment compliance

A descriptive summary of total exposure duration (days), total exposure amount (tablets), and treatment compliance (%) will be provided:

- Exposure duration (days) = date of last dose of study drug - date of first dose of study drug + 1;
- Exposure amount = actual total cumulative dose;
- Treatment compliance = actual total dose/study planned dose × 100%; planned dose will be calculated as 28 * 2 * 2 = 112 tablets.

The number of subjects with treatment compliance as <80%, 80% to 120%, >120% will be also tabulated.

9.2.8 Efficacy analyses

9.2.8.1 Primary efficacy endpoint

The number and percentage of subjects achieving mRS≤1 on Day 90 and the corresponding 95% CI will be summarized (Clopper-Pearson interval) by treatment group. The treatment group difference between TTYP01 and placebo group will be tested using Chi-square test, and the 95% CI of the difference will be estimated. In addition, odds ratio (OR) with the 95% CI as well as p-value will be also reported using logistic regression. Multiple imputation will be used to account for missing mRS; Death will be scored as 6 in mRS and thus there will be no missing mRS values due to death.

If the data are applicable, supplementary or sensitivity analyses will be performed to assess the robustness of the primary analysis results.

Further more, subgroup analyses will be conducted based on the demographic and baseline characteristics of subjects as applicable.

9.2.8.2 Secondary efficacy endpoints

The shift in Day 90 mRS as a 6-category ordinal scale will be analysed using ordinal logistic

regression, common OR, the associated 95% CI as well as the p-value will be reported. Similar statistical methods as the primary endpoint will be applied for binary secondary endpoints including mRS ≤2 on Day 90, NIHSS improvement ≥4 or NIHSS ≤1 on Day 7, 28 and 90 and etc. For the continuous secondary outcomes such as change in NIHSS from baseline, the least-square mean differences with 95% CI between treatment groups will be estimated by analysis of covariance (ANCOVA) or analysis of variance (ANOVA) as appropriate.

9.2.9 Safety analyses

AEs will be coded using Medical Dictionary for Regulatory Activities (MedDRA). AE analysis will be based on TEAE. Based on SS, the number and percentage of subjects in each treatment group with overall and various types of TEAEs including all TEAEs, all ADRs, CTCAE grade ≥3 TEAEs/ADRs, SAEs, serious ADRs, TEAEs/ADRs that leading to treatment discontinuation will be counted and also summarized by System Organ Class (SOC) and Preferred Term (PT), respectively.

The proportions of all AEs, symptomatic intracranial hemorrhage, recurrent symptomatic stroke (cerebral infarction), all-cause mortality, serious adverse events, discontinuation due to any adverse event and etc. and the corresponding 95% CI will be summarized by treatment group. The relative risk of the treatment group compared to the control group and the associated 95% CI will be also reported.

Cross-tabulation tables before and after treatment will be provided to analyze the post-treatment changes in the laboratory tests, 12-lead ECG, and other applicable safety parameters. Vital signs and the changes from baseline will be analyzed using descriptive statistics by treatment group.

9.2.10 Interim analysis

An interim analysis will be planned at the timing of 50% subjects complete D90 or early terminate, whichever occurs first, for the assurance of patient safety and trial integrity and monitoring the effectiveness data. To maintain an overall one-sided α of 0.025, the α-spending function to approximate O'Brien-Fleming will be implemented.

The interim analysis will be monitored by Independent Data Monitoring Committee (IDMC). The purpose of IDMC, IDMC members and their responsibilities, meeting frequency and etc. will be described in the IDMC Charter.

10. Quality control and quality assurance

10.1 Clinical monitoring

In accordance with relevant regulations, GCP, and sponsor procedures, the sponsor has commissioned a CRO to perform all monitoring activities for this clinical study. Monitors will work according to SOPs established by the sponsor or CRO, and CRO monitors will have the same rights

and responsibilities as sponsor monitors. The monitor will establish and maintain regular contact between the investigator and the sponsor.

At the time of contact, the monitor will:

- Check the progress of the trial;
- Review the collected data;
- Perform source document verification;
- Identify and resolve the issues.

This is to confirm that:

- The data are authentic, accurate, and complete;
- The safety and rights of the subjects are protected;
- The study is conducted in compliance with the currently approved protocol (and any supplements), GCP, and all relevant regulatory requirements;
- The investigator agrees to allow the monitor to check all relevant documents directly and arranges personal and staff time to discuss findings and all relevant issues with the monitor.

10.2 Audit

The audit shall be conducted by relevant personnel not directly involved in this trial. When the relevant personnel conduct the audit, the trial site must timely provide the materials to be inspected.

10.3 Quality assurance of the clinical trial process

To ensure the quality of the trial, a clinical study protocol will be jointly discussed and prepared by the sponsor and the investigators before the formal start of the trial. Before the start of the clinical trial, the investigator should carry out the training of the trial protocol to have a full understanding and awareness of the clinical trial protocol. GCP training is required for all relevant study personnel participating in the trial. During the trial, the investigator should carefully perform clinical operations and other work in accordance with the institutional SOPs and trial protocol requirements, and record it in a true, timely, complete and standardized manner. Quality control personnel shall check the basic conditions of the clinical trial to ensure that the conditions of clinical trial can meet the requirements of the protocol. Quality control personnel conduct quality checks on the trial process and corresponding source records. After the trial, the study unit sorts out the corresponding project documents, and archives them after checking by the quality control personnel. The quality assurance department of the clinical research unit performs quality control on the implementation of the test. When non-conformities are identified, the investigator and the head of the unit for correction ahould be notified promptly and the status of the correction should be tracked.

Each site must manage investigational drug according to SOPs, including receipt, storage, dispensing, retrieval, and destruction, as applicable. In accordance with GCP guidelines, necessary

steps will be taken during the design and implement of the study to ensure that the data collected are accurate, consistent, complete, and credible. All observed results and abnormal findings in the clinical trial should be verified and recorded in a timely manner to ensure the reliability of the data. The instruments, equipment, reagents, standards, etc. used for various inspection items in the clinical trial shall meet relevant requirements and ensure that they work under normal conditions.

The drug regulatory authorities, Institutional Review Board or Independent EC, monitor and/or auditor of the sponsor may systematically check the clinical trial related activities and documents to determine whether the trial is conducted in accordance with the trial protocol, SOPs and relevant regulations, and whether the trial data are accurate, true and complete.

10.4 Recording and retention of study data

In accordance with the principles of GCP, the investigator should keep all the detailed source documents of the subjects, including the contents of the trial process, medication administration, laboratory test data, safety data and efficacy evaluation, and the recorded data should be complete, timely and clear. Source documents, medical records and other relevant documents should be clear, detailed and easily identifiable by the personnel participating in the clinical trial.

Any modifications to the original data collection sheet and source data must not obliterate the source data. The correct modification method is to draw a single line on the original data, and then write the modified data next to the original data, and sign the date and the name or initials of the person who modified it.

The sponsor shall keep the clinical trial data related to the sponsor, and other data obtained by some relevant units participating in the clinical trial shall also be kept in the essential documents of the clinical trial as sponsor-specific data.

If the principal investigator moves or retires, or ceases to perform his or her study duties, the sponsor must be notified so that appropriate measures can be taken regarding the trial data.

10.5 Storage, management and archiving of test data

In order to ensure the evaluation and supervision of the clinical trial by the NMPA and the sponsor, the investigator shall agree to keep the original study data of all subjects participating in the trial, including the confirmation of the subjects (can effectively check different records, such as eCRF and original records of the hospital), the original signed informed consent form, and the detailed records of drug distribution, etc. The paper and electronic documents generated in the clinical study shall be properly kept to ensure timely traceability, and shall be kept for at least 5 years after the investigational drug is approved for marketing. All materials of this clinical trial are owned by Suzhou Auzone Biological Technology Co., Ltd., and the investigator shall not provide to any third party in any form without the written consent of the sponsor, except as required by the NMPA. If

otherwise specified in the contract, it shall be executed in accordance with the contract.

11. Ethical and Regulatory Requirements

11.1 GCP and ethical requirements

This trial is conducted in accordance with the Good Clinical Practice (2020), Declaration of Helsinki, relevant laws and regulations of China, and the opinions of the EC of this drug clinical trial institution.

Before the start of the trial, the investigator/trial institution should obtain the written approval opinion from EC of the investigator's members, investigator's brochure, trial protocol/protocol amendment, informed consent form, subject recruitment procedures, etc. During the clinical investigation, any modifications to the trial protocol and informed consent form must be approved or documented by the EC. After the end of the trial, the EC should be notified that the trial has ended.

11.2 Informed consent procedure

The principal investigator at each site will:

- Ensure that each subject or his/her guardian is provided with comprehensive information, orally or in writing, about the nature, purpose, possible risks and benefits of the study;
- Ensure that each subject or his/her guardian is informed that he/she may voluntarily discontinue participation in the study at any time;
- Ensure that each subject or his/her guardian is given the opportunity to ask questions and allow sufficient time to consider the information provided;
- Ensure that each subject or his/her guardian provides a signed and dated informed consent form (ICF) before any study-related procedures are performed;
- Ensure that the original signed ICF is maintained in the investigator study file;
- Ensure a copy of the signed ICF is provided to the subject;
- Ensure that any benefits to the subjects participating in the study as well as harm may occur to the subject are described in the ICF approved by the EC.

11.3 Protocol deviation

If a protocol deviation occurs, the investigator must inform the monitor and the significance of the deviation must be reviewed and discussed. Any protocol deviation must be documented either as a response to a query in the eCRF, or in the protocol violation report, or a combination of both. The protocol deviation report form will be kept by the sponsor. Protocol deviation reports with supporting documentation must be retained in the investigator site file.

11.4 Suspension or termination of study/ closure of site

The sponsor reserves the right to close the site or terminate the study at any time for any reason, if

appropriate. The party suspending or terminating the study shall provide written notification to the subjects, investigators, and the regulatory authorities of the clinical trial (NMPA, clinical trial institutions of the investigational site and the EC), and shall record the reasons for the suspension or termination of the study. If the investigator prematurely terminates or suspends the study, the principal investigator shall promptly inform the subjects, the EC, and the sponsor with the reason for the termination or suspension. If applicable, the investigator should contact the subject and notify the change in the visit schedule. Reasons for suspension or termination of the study/site closure include, but are not limited to, the following:

(1) Major errors in the clinical trial protocol are found in the trial, making it difficult to evaluate the drug;

(2) Determination of an unexpected, significant, or unacceptable risk to subjects;

(3) Inability to comply with the requirements of the protocol;

(4) Data are incomplete and/or insufficient for evaluation;

(5) Determination that the primary endpoint has been met;

(6) Due to reasons related to the sponsor itself or changes in national pharmaceutical development policies;

(7) The request of the drug regulatory authority or EC to terminate an approved study.

The suspension may be temporary or permanent. The study can only resume if issues related to safety, protocol compliance, and data quality are addressed and meet the requirements of the sponsor, EC, NMPA. Once the decision is made to terminate/close an investigational site, the sponsor shall properly handle the relevant matters after terminating/closing the investigational site, and promptly notify the investigational site, EC or NMPA. After the decision is made to terminate/close the investigational site, the archived study records specified in GCP shall be retained for future inspection, and other relevant study materials (including completed, partially completed and blank study materials and investigational medicinal products, etc.) shall be delivered to the sponsor according to the relevant requirements in GCP.

11.5 Confidentiality and privacy

The informed consent form may contain elements that comply with relevant data protection and privacy rights. In some cases, these will be documented in a separate companion document.

Participant privacy will be kept strictly confidential to the investigator, staff involved in the study, the sponsor and the respective treatments. The protocol, records, data, and all other information generated will be kept in strict confidence. Study information or data will not be divulged to any unauthorized third party without the written consent of the sponsor.

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

The study monitor, authorized representatives of the sponsor, representatives of EC, regulatory authorities, or the pharmaceutical company supplying investigational drug may inspect all documents and records required to be maintained by the investigator, including, but not limited to, medical records (clinic, outpatient, or hospital) and drug records of study subjects. The clinical site will allow the above-mentioned individuals to access to these records.

Study subject contact information will be kept strictly by each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for a period determined by the EC, site policy or sponsor requirements.

11.6 Revision of study protocol and informed consent form

Study procedures may be modified only after mutual consultation between the principal investigator and the sponsor.

If there are any significant changes to the protocol, these changes must be documented in a protocol amendment to the most recent version of the clinical study protocol, if required. The amendment should be approved by the appropriate EC and, if applicable, the national regulatory authority prior to implementation. The sponsor will distribute the most current version of the protocol and any amendments to each principal investigator.

If a protocol amendment requires changes to the central ICF, the sponsor and the central EC will approve the ICF amendment before the amendment can be used. Any administrative changes should be communicated to and approved by each EC.

12. Right to Publish Privacy

All data provided by Suzhou Auzone Biological Technology Co., Ltd. and all data generated during the course of the study (except the subject's medical records) should be kept confidential by the investigator and other study site personnel. Neither the investigator nor other staff at the study site may use the materials, data, or records for any purpose other than those related to the study. These restrictions do not apply to: (1) Data that has been publicly disclosed not due to errors by the investigator or research center staff; (2) Data that must be disclosed for the purpose of academic or EC review to assess this study; (3) Data that must be disclosed to provide appropriate healthcare to participants in the study.

The summary, submission and publication by the research institution must be reviewed by the sponsor and the principal investigator of the clinical research institution.

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14. Appendix

14.1 Appendix 1. Modified Rankin Scale (mRS)

The modified Rankin Scale is used to measure functional recovery after stroke. Formal definitions for each level are shown in bold. Further guidance is given in italics with a view to minimizing possible errors between different observers, but there are no requirements for the structure of the interview. Please note that only symptoms that have occurred since the stroke are considered. A person is considered to be able to walk independently if he or she can walk without outside help and with the help of some assistive device.

If both levels appear to be equally applicable to the patient, and further questioning is unlikely to make an absolutely correct choice, the more severe level should be selected.

Patient's condition	Scoring Criteria
No symptoms	0
No significant disability despite symptoms: able to carry out all usual duties and activities	1
Slight disability: unable to carry out all previous activities, but able to look after own affairs without assistance	2
Moderate disability: requiring some help, but able to walk without assistance	3
Moderately severe disability: unable to walk without assistance, unable to attend to needs without assistance	4
Severe disability: bed-ridden, incontinent, and requiring constant nursing care and attention	5

Note:

0 - No symptoms

Although there may be mild symptoms, the patient has not noticed any newly occurring functional limitations or symptoms since the stroke.

1 - No significant disability despite symptoms: able to carry out all usual duties and activities

The patient has certain symptoms caused by the stroke, either physical or cognitive (e.g., affecting speech, reading, or writing; or body movement; or sensation; or vision; or swallowing; or emotion), but can continue to engage in all the work, social, and leisure activities that he or she was engaged in before the stroke. The key question used to differentiate between Levels *1* and *2* (see below) could be, "Are there things you used to do regularly, but you can no longer do them until after the stroke?" Activities that are more frequent than once a month are considered "frequent".

2 - Slight disability: unable to carry out all previous activities, but able to look after own affairs without assistance

Certain activities that could be accomplished before the stroke (such as driving, dancing, reading, or working) can no longer be performed by the person after the stroke, but the person is still able to take care of himself/herself on a daily basis without assistance. The patient is able to dress, walk, eat, go to the bathroom, prepare simple food, shop, and travel locally without assistance. The patient lives without supervision. It is envisioned that patients at this level could be left alone in their

homes unattended for a week or more.

3 - Moderate disability: requiring some help, but able to walk without assistance

At this level, the patient can walk (with the aid of walking machinery), dress, go to the bathroom, eat, etc. independently, but more complex tasks need to be done with assistance. For example, other people are required to do shopping, cooking, or cleaning instead, and to visit the patient more than once a week to ensure completion of these activities. Assistance is needed not only to take care of the body, but also to give advice: for example, patients at this level will need supervision or encouragement to handle finances.

4 - Moderately severe disability: unable to walk without assistance, unable to attend to needs without assistance

The patient needs someone else to help with daily life, whether walking, dressing, going to the bathroom, or eating. The patient needs to be cared for at least once a day, usually two or more times a day, or must live in close proximity to the caregiver. To distinguish between levels *4* and *5* (see below), consider whether the patient is able to routinely live alone for an appropriate amount of time during the day.

5 - Severe disability: bed-ridden, incontinent, and requiring constant nursing care and attention

Although trained nurses are not required, someone will need to attend to them several times throughout the day and night.

14.2Appendix 2. National Institutes of Health Stroke Scale (NIHSS)

Note that for each NIHSS score, record the actual time of rating, score according to the table, and record the results. Do not change the score. The score reflects the patient's actual condition, not what the doctor thinks the patient should be. Quick check and record results. Do not train the patient (e.g., repeatedly ask the patient to make a certain effort) unless necessary instructions are given. If some items are not rated, they should be detailed on the form.

	Items	Scale Definition	Score
1a	Level of consciousness: The investigator must choose a response, even if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.	0 = Alert; keenly responsive 1 = Not alert, but arousable by minor stimulation to obey, answer, or respond 2 = Not alert, requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped) 3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, areflexic	___
1b	LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.	0 = Answers both questions correctly. 1 = Answers one question correctly. 2 = Answers neither question correctly.	___
1c	LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to them (pantomime) and score the result (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.	0 = Performs both tasks correctly 1 = Performs one task correctly 2 = Performs neither task correctly	___
2	Best gaze: Only horizontal eye movements were tested. Voluntary or reflexive (oculocephalic) eye movements will be scored but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an	0 = Normal 1 = Partial gaze palsy. This score is given when gaze is abnormal in one or both eyes, but where forced deviation or total gaze paresis are not present. 2 = Forced deviation, or total gaze	___

	Items	Scale Definition	Score
	isolated peripheral nerve paresis (CN III, IV or VI) score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness or other disorder of visual acuity or fields should be tested with reflexive movements and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.	paresis not overcome by the oculocephalic maneuver.	
3	Visual: Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat as appropriate. Patient must be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia is found. If patient is blind from any cause score 3. Double simultaneous stimulation is performed at this point. If there is extinction patient receives a 1 and the results are used to answer question 11.	0 = No visual loss 1 = Partial hemianopia 2 = Complete hemianopia 3 = Bilateral hemianopia (blind including cortical blindness)	___
4	Facial Palsy: Ask, or use pantomime to encourage the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or noncomprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barrier obscures the face, these should be removed to the extent possible.	0 = Normal symmetrical movement 1 = Minor paralysis (flattened nasolabial fold, asymmetry on smiling) 2 = Partial paralysis (total or near total paralysis of lower face) 3 = Complete paralysis of one or both sides (absence of facial movement in the upper and lower face)	___
5	Motor Arm: The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder, should it be recorded as untestable (NT), and the examiner must clearly write the explanation.	0 = No drift, limb holds 90 (or 45) degrees for full 10 seconds 1 = Drift, Limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support 2 = Some effort against gravity, limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity 3 = No effort against gravity, limb falls 4 = No movement UN = Amputation, joint fusion explains: _____	5a Left Arm ___ 5b Right Arm ___
6	Motor Leg: The limb is placed in the appropriate position: the leg 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and	0 = No drift, leg holds 30 degrees position for full 5 seconds 1 = Drift, leg falls by the end of the 5 second period but does not hit	6a Left leg ___ 6b Right

	Items	Scale Definition	Score
	pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, should it be recorded as untestable (NT), and the examiner must clearly write the explanation.	bed 2 = Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity 3 = No effort against gravity, leg falls to bed immediately 4 = No movement UN = Amputation, joint fusion explains: _____	leg ___
7	Limb Ataxia: This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, insure testing is done in intact visual field. The finger-nose-finger and heelshin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, should it be recorded as untestable (NT), and the examiner must clearly write the explanation. In case of blindness test by touching nose from extended arm position.	0 = Absent 1 = Present in one limb 2 = Present in two limbs UN = Amputation, joint fusion explains:	___
8	Sensory: Sensation or grimace to pin prick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas [arms (not hands), legs, trunk, face] as needed to accurately check for hemisensory loss. A score of 2, "severe or total," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will therefore probably score 1 or 0. The patient with brain stem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic score 2. Patients in coma (item 1a=3) are arbitrarily given a 2 on this item.	0 = Normal; no sensory loss 1 = Mild to moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick but patient is aware he/she is being touched 2 = Severe to total sensory loss; patient is not aware of being touched in the face, arm, and leg	___
9	Best Language: A great deal of information about comprehension will be obtained during the preceding sections of the examination. The patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet, and to read from the attached list of sentences. Comprehension is judged from responses here as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to repeat, and produce speech. The intubated patient should be asked to write. The patient in coma (question 1a=3) will arbitrarily score 3 on this item. The examiner must choose a score in the patient with stupor or limited cooperation but a score of 3 should	0 = No aphasia: normal 1 = Mild to moderate aphasia; some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided material difficult or impossible. For example, in conversation about provided materials examiner can identify picture or naming card from patient's response	___

	Items	Scale Definition	Score
	be used only if the patient is mute and follows no one step commands.	2 = Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response 3 = Mute, global aphasia; no usable speech or auditory comprehension	
10	Dysarthria: If patient is thought to be normal an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barrier to producing speech, it be recorded as untestable (UN), and the examiner must clearly write an explanation for not scoring. Do not tell the patient why he/she is being tested.	0 = Normal 1 = Mild to moderate; patient slurs at least some words and, at worst, can be understood with some difficulty. 2 = Severe; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric UN = Intubated or other physical barrier, explain: _____	___
11	Neglect: Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.	0 = No abnormality 1 = Visual, tactile, auditory, spatial, or personal inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities 2 = Profound hemi-inattention or hemi-inattention to more than one modality. Does not recognize own hand or orients to only one side of space	___
Total Scores			___

14.3 Appendix 3. Barthel Index (BI) of Activities of Daily Living

Scale Definition	Scoring and Rationale			
	Independent	**Minor help**	**Major help**	**Dependent**
Fedding	10 points Able to eat independently	5 points Minor help	0 point Requires major assistance or total dependence on others, or requires modified diet	
Bathing	5 points Once the bathwater is ready, they can independently complete the bathing process	0 point Need help during bathing		
Grooming	5 points Can independently complete	0 point Need help during grooming		
Dressing	10 points Able to dress independently	5 points Minor help	0 point Requires major assistance or total dependence on others	
Bowels	10 points Continent	5 points Occasional accident or need prompts from others	0 point Incontinent	
Bladder	10 points Continent	5 points Occasional accident or need prompts from others	0 point Incontinent, or indwelling catheter	
Toilet Use	10 points Able to dress independently	5 points Minor help	0 point Requires major assistance or total dependence on others	
Transfer (bed to chair and back)	15 points Able to dress independently	10 points Minor help	5 points Major help	0 point Completely dependent on others
Mobility (on level surfaces)	15 points Able to walk independently on flat ground for 45 m	10 points Minor help	5 points Major help	0 point Completely dependent on others
Stairs	10 points Able to independently go up and down 8 flights of stairs	5 points Minor help	0 point Requires major assistance or total dependence on others	

14.4 Appendix 4. Montreal Cognitive Assessment Scale (MoCA) (Beijing version)

The Montreal Cognitive Assessment (MoCA) was designed as a rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, abstraction, calculations, and orientation. Time to administer the MoCA is approximately 10 minutes. The total possible score is 30 points; a score of 26 or above of the original English version is considered normal.

1: Alternating Trail Making

Administration: " The examiner instructs the subject: "Please draw a line going from anumber to a letter in ascending order. Begin here [point to (1)] and draw a line from 1then to A then to 2 and so on. End here [point to (E)]."

Scoring: One point is allocated if the subject successfully draws the following pattern:1- A- 2- B- 3- C- 4- D- 5- E, without drawing any lines that cross. Any error that is not immediately self-corrected earns a score of 0.

2: Visuoconstructional Skills (Cube):

Administration: pointing to the cube: "Copy this drawing as accurately as you can, in the space below".

Scoring: One point is allocated for a correctly executed drawing.

- Drawing must be three-dimensional
- All lines are drawn
- No line is added
- Lines are relatively parallel and their length is similar (rectangular prisms are accepted)

A point is not assigned if any of the above-criteria are not met.

3: Visuoconstructional Skills (Clock):

Administration: "Draw a clock. Put in all the numbers and set the time to 10 past 11".

Scoring: One point is allocated for each of the following three criteria:

- Contour (1 pt.): the clock face must be a circle with only minor distortion acceptable (e.g., slight imperfection on closing the circle);
- Numbers (1 pt.): all clock numbers must be present with no additional numbers; numbers must be in the correct order and placed in the approximate quadrants on the clock face; Roman numerals are acceptable; numbers can be placed outside the circle contour;
- Hands (1 pt.): there must be two hands jointly indicating the correct time; the hour hand must be clearly shorter than the minute hand; hands must be centred within the clock face with their junction close to the clock centre. A point is not assigned for a given element if any of the above-criteria are not met.

4: Naming

Administration: Beginning on the left, point to each figure and say: “Tell me the name of this animal”.

Scoring: One point each is given for the following responses: (1) lion (2) rhinoceros or rhino (3) camel or dromedary.

5: Memory

Administration: The examiner reads a list of 5 words at a rate of one per second, giving the following instructions: “This is a memory test. I am going to read a list of words that you will have to remember now and later on. Listen carefully. When I am through, tell me as many words as you can remember. It doesn’t matter in what order you say them”. Mark a check in the allocated space for each word the patient produces on this first trial. When the patient indicates that (s)he has finished (has recalled all words), or can recall no more words, read the five words again with the following instructions: “I am going to read the same list for a second time. Try to remember and tell me as many words as you can, including words you said the first time.” Put a check in the allocated space for each word the patient recalls after the second trial.

At the end of the second trial, inform the patient that (s)he will be asked to recall these words again by saying, “I will ask you to recall those words again at the end of the test.”

Scoring: No points are given for Trials One and Two.

6: Attention

Forward Digit Span: Administration: Give the following instruction: “I am going to say some numbers and when I am through, repeat them to me exactly as I said them”. Read the five number sequence at a rate of one digit per second.

Backward Digit Span: Administration: Give the following instruction: “Now I am going to say some more numbers, but when I am through you must repeat them to me in the backwards order.” Read the five number sequence at a rate of one digit per second.

Scoring: Allocate one point for each sequence correctly repeated, (N.B.: the correct response for the backwards trial is 2-4-7).

Vigilance: Administration: The examiner reads the list of letters at a rate of one persecond, after giving the following instructions: “I am going to read a sequence of letters.Every time I say the letter A, tap your hand once. If I say a different letter, do not tapyour hand.”.

Scoring: One point is allocated if there is zero to one error (an error is a tap on a wrongletter or a failure to tap on letter A).

Serial 7s: Administration: The examiner gives the following instructions: “Now, I willask you to count by subtracting 7 from 60, and then, keep subtracting 7 from your answeruntil I tell you to

stop.” The subject must perform a mental calculation, therefore, (s) he may not use his/her fingers nor a pencil and paper to execute the task. The examiner maynot repeat the subject’s answers. If the subject asks what her/his last given answer was orwhat number (s) he must subtract from his/her answer, the examiner responds byrepeating the instructions if not already done so.

Scoring: This item is scored out of 3 points. Give no (0) points for no correctsubtractions, 1 point for one correct subtraction, 2 points for two or three correctsubtractions, and 3 points if the subject successfully makes four or five correctsubtractions. Each subtraction is evaluated independently; that is, if the subject respondswith an incorrect number but continues to correctly subtract 7 from it, each correctsubtraction is counted. For example, a subject may respond “52 - 45 - 38 - 31 – 24”where the “52” is incorrect, but all subsequent numbers are subtracted correctly. This isone error and the task would be given a score of 3.

7: Sentence repetition

Administration: The examiner gives the following instructions: “I am going to read you asentence. Repeat it after me, exactly as I say it [pause]: The child walked his dog in thepark after midnight.” Following the response, say: “Now I am going to read you anothersentence. Repeat it after me, exactly as I say it [pause]: The artist finished his painting atthe right moment for the exhibition.”Scoring: One point is allocated for each sentence correctly repeated. Repetitions must beexact. Be alert for omissions (e.g., omitting "right"), substitutions/additions (e.g.,substituting "after" for "at"), grammar errors/altering plurals (e.g. "his painting" for "hispaintings"), etc.

8: Verbal fluency

Administration: The examiner gives the following instructions: “Now, I want you to tellme as many words as you can think of that begin with the letter B. I will tell you to stopafter one minute. Proper nouns, numbers, and different forms of a verb are not permitted.Are you ready? [Pause] [Time for 60 sec.] Stop.” If the subject names two consecutive words that begin with another letter of the alphabet, the examiner repeats the target letterif the instructions have not yet been repeated.

Scoring: One point is allocated if the subject generates 11 words or more in 60 seconds.The examiner records the subject’s responses in the margins or on the back of the testsheet.

9: Abstraction

Administration: The examiner asks the subject to explain what each pair of words has incommon, starting with the example: “I will give you two words and I would like you totell me to what category they belong to [pause]: an orange and a banana.” If the subjectresponds correctly the examiner replies: ‘‘Yes, both items are part of the categoryFruits.’’ If the subject answers in a concrete manner, the examiner gives one additionalprompt: “Tell me another category to which these items belong to.” If the subject doesnot give the appropriate response (fruits), the examiner

says: “Yes, and they also bothbelong to the category Fruits.” No additional instructions or clarifications are given.After the practice trial, the examiner says: “Now, a hammer and a screwdriver.”Following the response, the examiner administers the second trial by saying: “Now,matches and a lamp.” A prompt (one for the entire abstraction section) may be given ifnone was used during the example.

Scoring: Only the last two pairs are scored. One point is given for each pair correctlyanswered. The following responses are acceptable:

- hammer- screwdriver = tools, carpentry, construction, work instruments,
- matches- lamp = light, lighting, illumination

The following responses are not acceptable:

- hammer- screwdriver = instruments, have handles, metallic objects,
- matches- lamp = fire, hot objects, produce heat

10: Delayed recall

Administration: The examiner gives the following instructions: “I read some words toyou earlier, which I asked you to remember. Tell me as many of those words as you canremember.” The examiner makes a check mark (√) for each of the words correctlyrecalled spontaneously without any cues, in the allocated space.

Scoring: One point is allocated for each word recalled freely without any cues.

Memory index score (MIS):
Administration: Following the delayed free recall trial, the examiner provides a category (semantic) cue for each word the subject was unable to recall. Example: ‘‘I will give you somehints to see if it helps you remember the words, the first word was a body part.’’ If the subject isunable to recall the word with the category cue, the examiner provides him/her with a multiplechoice cue. Example: “Which of the following words do you think it was, HAND, LEG, orFACE?” All non-recalled words are prompted in this manner. The examiner identifies the wordsthe subject was able to recall with the help of a cue (category or multiple-choice) by placing acheck mark (√) in the appropriate space. The cues for each word are presented below:
Category and/or multiple-choice hint
FACE: part of the body: nose, face, hand
VELVET: type of fabric: cotton, denim, velvet
CHURCH: type of building: church, school, hospital
DAISY: type of flower: rose, daisy, peony
RED: a colour: red, blue, green
Scoring: No points are allocated for words recalled with a cue. A cue is used for clinical information purposes only and can give the test interpreter additional information about the type of memory disorder. For memory deficits due to retrieval failures, performance can be improved with a cue. For memory deficits due to encoding failures, performance does not improve with a cue.

11: Orientation

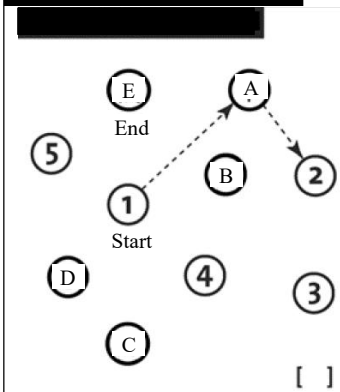
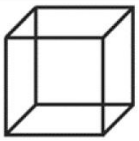
Administration: The examiner gives the following instructions: “Tell me today’s date.” Ifthe subject does not give a complete answer, the examiner prompts accordingly bysaying: “Tell me the [year, month, exact date, and day of the week].” Then the examinersays: “Now, tell me the name of this place, and which city it is in.”

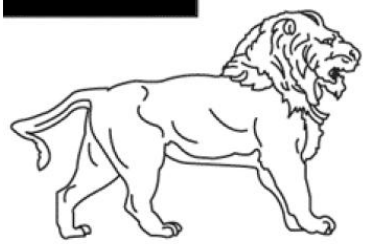
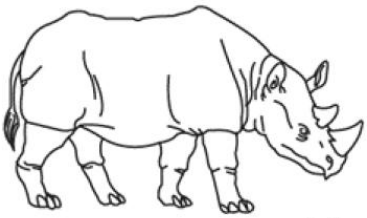
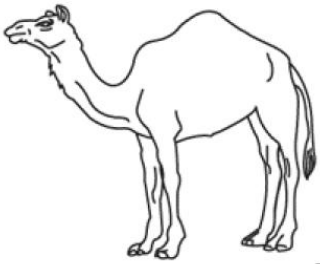
Scoring: One point is allocated for each item correctly answered. The date and place (name ofhospital, clinic, office) must be exact. No points are allocated if the subject makes an error ofone day for the day and date

TOTAL SCORE: Sum all subscores listed on the right-hand side. Add one point for subject whohas 12 years or fewer of formal education, for a possible maximum of 30 points. A final totalscore of 26 and above is considered normal. There is no Chinese and Beijing norm and reliability and validity analysis. Therefore, the original boundary value can be referred to temporarily.

Montreal Cognitive Assessment (MoCA) Beijing Version

Date of birth:
Education: Name:
Sex: Date:

VISUOSPATIAL / EXECUTIVE				POINTS
 [ ]	 Copy Cube [ ]	Draw CLOCK (Ten past eleven) (3 points) [ ] Contour [ ] Numbers [ ] Hands		_/5

NAMING				
 [ ]	 [ ]	 [ ]		_/3

MEMORY	Read list of words, patient must repeat them. Do 2 trials, even if 1st trial is successful Do a recall after 5 minutes		FACE	VELVET	CHURCH	DAISY	RED	NO POINTS
		1st TRIAL						
		2nd TRIAL						

ATTENTION	Read list of digits and ask the patient repeated (1 digit / sec.).	Patient has to repeat them in the forward order [ ]21854 Patient has to repeat them in the backward order [ ]742	_/2
	Read list of numbers. The patient must tap with his hand when number 1 appears. No points if ≥ 2 errors	[ ]52139411806215194511141905112	_/1
	Serial 7 subtraction starting at 100	[ ]93 [ ] 86 [ ] 79 [ ] 72 [ ]65 4 or 5 correct subtractions: 3 pts, 2 or 3 correct: 2 pts, 1 correct: 1 pt, 0 correct: 0 pt	_/3
LANGUAGE	Repeat: All I know is that Zhang Liang has come to help [ ] The cat always hid under the couch when dogs were in the room [ ]		_/2
	Language Fluency. Name maximum number of animals in one minute [ ] _____ (N≥11 names)		_/1
ABSTRACTION	Similarity between e.g. banana - orange = fruit [ ] train - bicycle [ ] watch - ruler		_/2

DELAYED RECALL	Has to recall words with no cue	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for uncued recall only	_/5
Options	Category hint							
	multiple-choice hint							

ORIENTATION	[ ] Date [ ] Month [ ] Year [ ] Day of Week [ ] Place [ ] City	-/6

Beijing version 26 August,2006 translated by Wei Wang & Hengge Xie
www.mocatest.org

Total Scores -/30

14.5 Appendix 5. Hamilton Depression Rating Scale (HDRS)

Hamilton Depression Rating Scale - 21-item scale

1. DEPRESSED MOOD (sadness, hopeless, helpless, worthless)	0 - Absent 1 - These feeling states indicated only on questioning 2 - These feeling states spontaneously reported verbally 3 - Communicates feeling states non-verbally, i.e. through facial expression, posture, voice and tendency to weep 4 - Patient reports virtually only these feeling states in his/her spontaneous verbal and non-verbal communication (facial expressions, gestures)
2. FEELINGS OF GUILT	0 - Absent 1 - Self reproach, feels he/she has let people down 2 - Ideas of guilt or rumination over past errors or sinful deeds 3 - Present illness is a punishment. Delusions of guilt 4 - Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations
3. SUICIDE	0 - Absent 1 - Feels life is not worth living 2 - Wishes he/she were dead or any thoughts of possible death to self 3 - Ideas or gestures of suicide 4 - Attempts at suicide
4. INSOMNIA (EARLY IN THE NIGHT)	0 - No difficulty falling asleep 1 - Complains of occasional difficulty falling asleep, i.e. more than 1⁄2 hour 2 - Complains of nightly difficulty falling asleep
5. INSOMNIA (MIDDLE OF THE NIGHT)	0 - No difficulty falling asleep 1 - Patient complains of being restless and disturbed during the night 2 - Waking during the night – any getting out of bed for any reason except for purposes of voiding
6. INSOMNIA (EARLY HOURS OF THE MORNING)	0 - No difficulty falling asleep 1 - Waking in early hours of the morning but goes back to sleep 2 - Unable to fall asleep again if he/she gets out of bed
7. WORK AND ACTIVITIES	0 - No difficulty falling asleep 1 - Thoughts and feelings of incapacity, fatigue or weakness related to activities, work or hobbies 2 - Loss of interest in activity, hobbies or work – either directly reported by the patient or indirect in listlessness, indecision and vacillation (feels he/she has to push self to work or activities) 3 - Decrease in actual time spent in activities or decrease in productivity. If the patient does not spend at least three hours a day in activities (job or hobbies) excluding routine chores 4 - Stopped working because of present illness. If patient engages in no activities except routine chores, or if patient fails to perform routine chores unassisted
8. RETARDATION (slowness of thought and speech, impaired ability to concentrate, decreased motor activity)	0 - Normal speech and thought 1 - Slight retardation during the interview 2 - Obvious retardation during the interview 3 - Interview difficult 4 - Complete stupor (unable to answer questions)
9. AGITATION	0 - Absent 1 - Fidgetiness 2 - Playing with hands, hair, etc. 3 - Moving about, can’t sit still 4 - Hand wringing, nail biting, hair-pulling, biting of lips
10. ANXIETY PSYCHIC	0 - Absent 1 - Subjective tension and irritability

	2 - Worrying about minor matters 3 - Apprehensive attitude apparent in face or speech 4 - Fears expressed without questioning
11. ANXIETY SOMATIC	(Physiological concomitants of anxiety, such as: gastro-intestinal – dry mouth, abdominal distension, indigestion, diarrhea, cramps, eructation; cardio-vascular – palpitations, headaches; respiratory – hyperventilation, sighing; pollakiuria; sweating) 0 - Absent 1 - Mild 2 - Moderate 3 - Severe 4 - Incapacitating (Significantly impacting daily life and activities)
12. SOMATIC SYMPTOMS GASTRO-INTESTINAL	0 - None 1 - Loss of appetite but eating without staff encouragement 2 - Difficulty eating without staff urging
13. GENERAL SOMATIC SYMPTOMS	0 - Absent 1 - Heaviness in limbs, back or neck. Backaches, headaches, muscle aches. Loss of energy and fatigability 2 - Any clear-cut symptom
14. GENITAL SYMPTOMS (symptoms such as loss of libido, menstrual disturbances)	0 - Absent 1 - Mild 2 - Severe
15. HYPOCHONDRIASIS	0 - Absent 1 - Self-absorption (bodily) 2 - Preoccupation with health 3 - Frequent complaints, requests for help, etc. 4 - Hypochondriacal delusions
16. INSIGHT	0 - Acknowledges being depressed and ill, or currently not experiencing depression 1 - Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc. 2 - Denies being ill at all
17. LOSS OF WEIGHT (based on medical history score)	0 - No weight loss 1 - Probable weight loss associated with present illness 2 - Definite (according to patient) weight loss
18. WEIGHT FLUCTUATION PATTERN (up during the day, down at night)	0 - Absent 1 - Mild 2 - Severe
19. DEPERSONALIZATION AND DEREALIZATION	0 - Absent 1 - nihilistic ideas 2 - feelings of unreality 3 - Feel unreal 4 - Feeling like one is not living as a person in the world
20. PARANOID SYMPTOMS	0 - Absent 1 - Suspicion or doubt 2 - Speculating that others intend to harm him/her 3 - Delusion that others intend to harm him/her and are actively trying to do so 4 - Fantasy that others are attempting to harm him/her
21. OBSESSIONAL SYMPTOMS	0 - Absent 1 - Admitting to having these symptoms 2 - Obsessive thoughts and compulsions against which the patient struggles
Total score:	Severe depression with a score of >30 Moderate depression with a score of >20 Mild depression with a score of >17

14.6 Appendix 7. Protocol Version Development and Amendment Schedule

Version No.	Version Date	Description of Change and Brief Rationale
V1.0	October 28, 2022	
V2.0	June 30, 2023	1. Dosing Regimen is improved: ***The first dose should be given within 1 hour after randomization and remaining doses should be administrated within 30–60 minutes before breakfast or dinner. In case of postprandial randomization, the second dose should not be earlier than 6 hours after the first dose***. 2. The inclusion criteria No. 3 is improved: "***Patients who refuse thrombolysis or thrombectomy, or do not meet the indications for thrombolysis or thrombectomy;***". 3. The inclusion criteria No. 4 is improved: "Patients who are within ≤ 24 hours after the symptom onset. ***For patients with uncertain onset time due to wake-up stroke, aphasia, consciousness disorders, etc., the last known asymptomatic time is considered as the onset time;***". 4. The exclusion criteria for dementia patients No. 5 is improved: "***Patients who are unable or unwilling to cooperate with the study due to comorbidity such as severe mental disorders, depression, Alzheimer's disease, Parkinson's dementia, and Louis body dementia;***". 5. The withdrawal criteria No.7 is improved: ***(7) Subjects who participate in another intervention study during this trial***. 6. IQCODE score is deleted, as this assessment will not be performed in the trial. 7. In study procedure, "***Continued administration of the investigational drug treatment***" is deleted from V1, V2 and V3. The description is modified due to ambiguity. 8. The blood chemistry text parameters-schedule of activities note 10 and 11 are improved:". 1) "***or blood urea nitrogen (BUN)***" is added; 2) "***Assessments of high-density lipoprotein (HDL), low-density lipoprotein (LDL) and D-dimer will be performed on D1, D7, D28 + 3 and D90 ± 5 or upon early withdrawal***" is added. 9. The relevant provision on urinalysis is supplemented-schedule of Activities note 9: ***If results of the urine routine test are not available due to emergency, investigatonal drugs can be administered first. If the subject has no urine temporarily, the test results of first urine sample collected upon admission will serve as baseline data.*** 10. According to the actual clinical execution: "V3 (D28 ± 3)" is revised as "V3 (D28 + 3)" throughout the text.