Suzhou Auzone Biological Technology Co., Ltd.

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

TTYP01-III-AIS

Statistical Analysis Plan

Version: V1.0

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Table of Contents

List of Abbreviations

Abbreviation	Definition
ADR	Adverse Drug Reaction
AE	Adverse Event
AIS	Acute Ischemic Stroke
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ATC	Anatomical Therapeutic Chemical Classification System
BI	Barthel Index of Activities of Daily Living
BMI	Body Mass Index
CI	Confidence Interval
CS	Clinically Significant
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CRF	Case Report Form
DWI	Diffusion-Weighted Imaging
FAS	Full Analysis Set
FCS	Fully Conditional Specification
FLAIR	Fluid-Attenuated Inversion Recovery
HDRS	21-Item Hamilton Depression Rating Scale
IDMC	Independent Data Monitoring Committee
ITT	Intention-to-Treat
LSM	Least-squares Mean
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
NCS	Not Clinically Significant
NIHSS	National Institutes of Health Stroke Scale
OR	Odds Ratio
PPS	Per Protocol Set
PT	Preferred Term
Q1	First Quartile
Q3	Third Quartile
RD	Rate Difference
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOC	System Organ Class
SS	Safety Set
TEAE	Treatment Emergent Adverse Event

1. Introduction

This Statistical Analysis Plan (SAP) provides detailed descriptions of statistical analysis methods and data handling principles of the study TTYP01-III-AIS. The SAP is developed based on study protocol TTYP01-III-AIS version 2.0 (Date: June 30, 2023) and Case Report Form (CRF) version 2.0 (Date: January 31, 2024).

2. Study Overview

2.1. Study Objective

To evaluate the efficacy and safety of TTYP01 Tablets in the treatment of acute ischemic stroke (AIS).

2.2. Study Drugs

Item	Investigational drug	Control drug
Name	TTYP01 tablets	Placebo (Simulation TTYP01 Tablets)
Dosage form	Tablet	Tablet
Strength	30 mg	30 mg
Ingredients	Avondale, polyvinyl prophylactic-polyvinyl acetate-polyethylene glycol graft co polymer (Voluptuous), sodium bisulfite, microcrystalline cellulose, polyvinylpyrrolidone, magnesium stearate	Microcrystalline cellulose, magnesium stearate
Storage conditions	Store at room temperature	Store at room temperature
Shelf life	May 29, 2024	May 29, 2024
Manufacturer	STA Pharmaceutical Co. Ltd.	STA Pharmaceutical Co. Ltd.
Supplier	Suzhou Auzone Biological Technology Co., Ltd.	Suzhou Auzone Biological Technology Co., Ltd.

2.3. Overall Study Design

This study adopts a multi-center, randomized, double-blind, parallel, placebo-controlled trial design to evaluate the efficacy and safety of TTYP01 Tablets in the treatment of patients with acute ischemic stroke.

It is expected that 618 subjects will be enrolled and randomized 1:1 to TTYP01 group and placebo control group, with 309 subjects in each group. The study duration is approximately 90 days. The study is divided into 2 periods, with a total of 5 visits: treatment observation period: D1-D28 (of which the minimum inpatient observation time is not less than 7 days), including 3 visits (V1-V3); follow-up period: D29-D90, including 2 visits (V4: telephone follow-up on D60±5; V5: return to hospital for end-of-study visit on D90±5). The detailed study design is provided in the protocol.

One interim analysis will be planned to be conducted by an independent data monitoring committee (IDMC) 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. Final analysis will be conducted after all the subjects complete the trial.

2.4. Randomization and Blinding

2.4.1. Randomization

Stratified block randomization is applied, all the eligible subjects will be randomized to TTYP01 group and placebo control group in a 1:1 ratio stratified by baseline National Institutes of Health Stroke Scale (NIHSS) score: 6-15 and 15-20.

2.4.2. Blinding

The study is double-blinded, all the investigators, subjects, and all other teams who are involved in

the study are not aware of the treatment assignment of the subjects prior to database lock.

2.5. 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.

3. Study Endpoints

3.1 Efficacy Endpoints

3.1.1 Primary Efficacy Endpoint

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

3.1.2 Secondary Efficacy Endpoint

1. mRS on day 90 after stroke onset;
2. Proportion of subjects with mRS≤2 on Day 90 after stroke onset;
3. Change from baseline in mRS on Day 7, 28, 60, and 90 after stroke onset;
4. Change from baseline in NIHSS on Day 7, 28, and 90 after stroke onset;
5. Proportion of subjects with NIHSS improvement≥4 on Day 7, 28, and 90 after stroke onset;
6. Proportion of subjects with NIHSS of 0-1 on Day 7, 28, and 90 after stroke onset;
7. Change from baseline in Barthel Index (BI) on Day 7, 28, and 90 after stroke onset;
8. Proportion of subjects with BI≥95 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. Change from baseline in brain MRI (e.g., infarct volume and area) on Day 5 and 28 after stroke onset.

3.2. Safety Endpoints

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.

4. 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.

5. Analysis Sets

(1) Intention-to-Treat set (ITT): ITT will include all the randomized subjects who receive at least one dose of investigational drug.

(2) 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).

(3) 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).

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

6. Statistical Methods

6.1. General Considerations

All statistical analyses will be performed using SAS version 9.4 or above.

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. Except for the primary efficacy endpoint, no formal hypothesis tests will be setup for the secondary endpoints, all the secondary efficacy endpoints will be just used to provide supportive evidence, and therefore, no adjustments for multiplicity will be made. Furthermore, statistical tests for secondary endpoints are just descriptive.

An interim analysis will be planned to be conducted at the timing of 50% subjects complete D90 or early terminate, whichever occurs first. To maintain an overall one-sided α of 0.025, the α-spending function to approximate O'Brien-Fleming will be implemented.

Baseline is defined as the last non-missing assessment/examination on or prior to the first dose of investigational drug. Only data from scheduled visits will be included in the statistical analyses by visit unless otherwise specified; But for summaries in respective of maximum change, minimum change, abnormalities, and clinically significant findings, data from both scheduled and unscheduled visits will be included.

6.2 Data Handling Conventions

6.2.1. Early Withdrawal and Missing Data

All the subjects who discontinued the study prematurely will be presented along with the reason for premature withdrawal in the final clinical study report (CSR). Where possible, all the available data from these subjects will be listed and included in the analyses as applicable.

The handling of missing efficacy data is described in section 6.5. Unless otherwise specified, all the missing safety data will be treated as missing and no data will be imputed or carried forward.

Missing/Partial AE Dates

The following imputation methods will be used for missing or partial AE dates. The imputation will be only for the classification of AEs and TEAEs; the raw dates will still be presented in all AE relevant data listings.

- AE start date
  - ✓ If the year and month are known and the year and month are earlier than the first administration of the investigational drug, the last day of the known month will be used for imputation.
  - ✓ If the year and month are known and the year and month are the same as the first administration of the investigational drug, the AE start date will be set to the date of the first administration of the investigational drug.
  - ✓ If the year and month are known and the year and month are later than the first administration of the investigational drug, the first day of the known month will be used for imputation.
  - ✓ If only the year is known and the year is earlier than the year of the first administration of the investigational drug, "December 31" will be used for imputation.
  - ✓ If only the year is known and the year is the same as the year of the first administration of the investigational drug, the AE start date will be set to the date of the first administration of the investigational drug.
  - ✓ If only the year is known and the year is later than the year of the first administration of the investigational drug, "January 01" will be used for imputation.
  - ✓ If the year, month, and day are all missing, the date of the first administration of the investigational drug will be used as the corresponding start date.
  - ✓ Other cases will be considered missing.
- AE end date
  - ✓ If the year and month are known, the last day of the known month will be used for imputation.
  - ✓ If only the year is known, "December 31" will be used for imputation.
  - ✓ If the imputed start date is after the end date, the end date will be taken as the corresponding start date.
  - ✓ Other cases will be considered missing.

6.2.2. Visit Window

All analyses will be performed based on scheduled visits, regardless of deviations from visit window.

For subjects who withdraw early from the study, if the "V5_Follow-up Period (D90) / Early Withdrawal" visit falls within the scheduled visit window as per the protocol, it will be remapped to the corresponding scheduled visit for inclusion in the analysis; if the remapped visit overlaps with an existing scheduled visit, the "V5_Follow-up Period (D90) / Early Withdrawal" will still be considered an early withdrawal visit and will not be included in the analysis unless specifically stated. The specific mapping windows are as follows:

V1_Treatment Observation Period (D1): assessment/examination date - first dosing date + 1 = 1

V2_Treatment Observation Period (D7): assessment/examination date - first dosing date + 1 = 7

V3_Treatment Observation Period (D28): 28 ≤ assessment/examination date - first dosing date +

1 ≤ 31 (25 ≤ assessment/examination date - first dosing date + 1 ≤ 31 instead for subjects who follow protocol v1.0)

V4_Follow-up Period (D60): 55 ≤ assessment/examination date - first dosing date+ 1 ≤ 65

V5_Follow-up Period (D90): 85 assessment/examination date - first dosing date + 1 ≤ 95

Early Withdrawal: Other time not within the scheduled window visit.

6.2.3. Other Data Handlings

With the exception of the calculation of certain study endpoints based on data collected in the CRF, no other data will be derived or transformed.

Extreme values are reported to data administrator as data queries and resolved or confirmed before database lock; no special data handling is performed.

6.3. Planned Analyses

An interim analysis will be performed by IDMC at the timing of 50% subjects complete D90 or early terminate, whichever occurs first.

The final analysis will be performed at the end of the study.

6.4. Study Subjects

6.4.1 Subject Disposition

The number of all screened subjects, the number and percentage of screening failures along with the reason will be reported, and will not otherwise be accounted for.

The number of subjects who are randomized, subjects who are randomized but not treated and subjects who are randomized and treated are summarized by treatment group, respectively. Furthermore, based on the subjects who are randomized and treated, a summary of the number and percentage of subjects who completed the study and who discontinued study in treatment period or follow-up period will be presented respectively by treatment group along with the primary reasons for study withdrawal.

The subject disposition is also summarized by investigational site.

A listing of the subject disposition for all screened subjects will be provided.

6.4.2. Protocol Deviations

Protocol deviations will be rated as either minor or major. The final list of major protocol deviations with the identification of subject exclusion from the analysis sets will be discussed in the blind data review meeting and finalized prior to database lock.

Based on the ITT, the protocol deviations will be summarized by the severity and the category of the deviations, and all protocol deviations will be listed.

6.4.3. Analysis Sets

Based on all randomized subjects, the number and percentage of subjects included in each analysis set will be presented along with the reasons for exclusion; the distribution of analysis sets and the reason(s) for exclusion from analysis sets will be listed.

6.4.4 Demographics and Baseline Characteristics

The demographics and baseline characteristics will be summarized based on ITT and FAS, respectively.

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.

The demographics and baseline characteristics of all randomized subjects will be listed.

6.4.5. Medical History, Prior and Concomitant Medications, and Non-drug Therapy

The medical history, prior and concomitant medications and non-drug treatments will be summarized based on the ITT analysis set and data of all randomized subjects will be listed, respectively:

- Medical history data will be coded using MedDRA version 26.0 or higher, and a summary by system organ class (SOC) and preferred term (PT) will be provided;
- Prior and concomitant medications will be coded using World Health Organization Drug Dictionary (WHO Drug Global Chinese) and will be summarized by Anatomical Therapeutic Chemical (ATC) classification system (Level 1) and preferred term (PT), respectively;
- Non-drug therapy will be coded using MedDRA version 26.0 or higher and will be summarized by SOC and PT.

6.4.6. Drug Exposure and Compliance

Based on the ITT, 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.

The drug exposure listing will be provided.

6.5. Efficacy Analyses

The primary analysis of the efficacy endpoints will be evaluated based on FAS, the evaluation with ITT and PPS will be provided as supportive as appropriate.

As very few subjects are included in the randomization stratum ‘Baseline NIHSS: 16-20’, stratified analyses by baseline NIHSS will be no more employed, unstratified analyses will be used instead. And furthermore, randomization stratification factor will not be included in any statistical models.

6.5.1 Primary Efficacy Endpoint

6.5.1.1 Main Estimand

Target population: All AIS patients who aged 18 to 80 years (inclusive), with onset within 24 hours of the first stroke or a recurrent stroke after a good recovery (mRS 0-1) from the previous stroke.

Study treatment: TTYP01 or placebo: 30 mg twice daily for consecutive 28 days.

Target variable: Proportion of subjects with mRS ≤1 on D90.

Intercurrent events and handling strategies:

Table 1 Intercurrent Events and Handling Strategies

Intercurrent Event	Management Strategy	Note

#1 Failure to complete protocol-defined treatment duration due to compliance issue (including treatment out of window, actual dose not compliant with the planned dose, poor compliance)	Treatment-policy strategy	Reflecting clinical practice
#2 Failure to complete protocol-defined treatment duration due to other non-compliance reasons (including poor efficacy, AE, subject's unwillingness or inability to continue, and withdrawal deemed necessary by the investigator, etc.)	Composite strategy, i.e. subject considered as 'non-responder'	Conservative estimation, treated as non-response
#3 Use of concomitant medications which have no disruptive effect on the investigational drug deemed by the investigator	Treatment-policy strategy	Reflecting clinical practice
#4 Use of protocol-prohibited drugs which have a significant impact on the primary efficacy endpoint (based on the final discussion and decision of the data review meeting)	Composite strategy, i.e. subject considered as 'non-responder'	Intercurrent events suggestive of poor efficacy, treated as non-response
#5 Use of protocol-prohibited drugs which have no significant impact on the primary efficacy endpoint (based on the final discussion and decision of the data review meeting)	Treatment-policy strategy	Reflecting clinical practice

Population-level summary:

Difference in the proportion of subjects with mRS≤1 on D90 between the two treatment groups and its 95% confidence interval.

6.5.1.2 Primary 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 with the 95% CI as well as p-value will be also reported using logistic regression including treatment in the model.

For subjects with intercurrent events, if the treatment-policy strategy is applied, the actual reported mRS on Day 90 will be used; if the composite strategy is applied, the reported mRS on D90 will be not used and the subject will be directly considered as 'non-responsder' for mRS ≤1on D90. Death will be scored as 6 in mRS and thus there will be no missing mRS values due to death. For the subjects who are still missing of mRS on D90, multiple imputation will be used to account for missing values for D90 mRS:

The multiple imputation model will include treatment group, baseline mRS, mRS at each post-baseline scheduled visit (i.e., D7, D28, D60, D90), baseline NIHSS, history of stroke, diabetes, hypertension, hyperlipidemia, heart disease (coronary artery disease), age, and sex. Multiple imputation will be performed using PROC MI in SAS and fully conditional specification (FCS) method will be used in this SAS procedure. A total of 20 imputations will be conducted with random seed 253543, each result in a complete dataset for all subjects.

The point and interval estimate of the difference in proportion will be obtained using PROC MIANALYZE; p-value will be also obtained using PROC MIANALYZE after Wilson-Hilferty transformation of Chi-square statistics for standardization. The odds ratio and its interval estimate as well as p-value from the Logistic models will be obtained using PROC MIANALYZE.

6.5.1.3 Sensitivity Analyses

The following sensitivity analyses will be performed accounting for different missing data imputation methods. 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 using exact method. And the odds ratio with the 95% CI as well as p-value will be also reported using logistic regression model:

1. No missing data will be imputed, the analysis will be only based on the observed values;

2. All the subjects with missing mRS on 90 will be considered as 'non-responder' of mRS ≤1 on D90;

3. All the subjects with missing mRS on 90 will be considered as 'responder' of mRS ≤1 on D90;

4. Last observation carried forward (LOCF) will be used to impute missing mRS on 90.

Furthermore, tipping point analysis (TPA) method will be also used to evaluate the robustness of the primary analysis results. The treatment group difference in proportion and the associated interval estimates as well as p-values will be reported to account for all possible combinations of missing data.

6.5.1.4 Supplementary Analyses

The following supplementary analyses will be performed:

1. Repeat primary analysis based on ITT or PPS;

2. To evaluate the impact of intercurrent events, different handling strategies might be employed:

- All the intercurrent events will be handled with treatment-policy strategy,
- Hypothetical strategy will be used for intercurrent event #2 and #4 (Table 1), only study data prior to the intercurrent events will be used for analysis,

repeat primary analysis and also apply multiple imputation for missing data.

6.5.1.5 Subgroup Analyses

Subgroup analyses will be performed to assess the similarities and differences of primary efficacy endpoint across different subjects subgroups. If data permit, the subgroups including sex, age (<65 years, ≥65 years), AIS history, baseline NIHSS (<median, ≥ median), baseline NIHSS (<10, ≥10), time from AIS onset to treatment (<median, ≥ median), concomitant illness with AIS (hypertension, diabetes, hyperlipidemia and cardiac disease). The subgroups might be adjusted based on the actual data, and more subgroups might be also explored.

Based on FAS, repeat the primary analysis, the treatment group difference between TTYP01 and placebo group and the associated 95% CI, odds ratio with the 95% CI will be reported for each subject subgroup.

6.5.2 Secondary Efficacy Endpoints

6.5.2.1 Estimands

Table 2 Intercurrent Events and Handling Strategies for Secondary Endpoints

Variables	Population	Treatment	Intercurrent Events and Handling Strategies	Population-Level Summary
mRS on day 90 after stroke onset	Same as primary estimand	Same as primary estimand	Same as primary estimand; For the events handled with composite strategy, assign to the worst value of	Common OR and 95% CI

			all the visits (including baseline and post-baseline) prior to the event	
Proportion of subjects with mRS≤ 2 on day 90 after stroke onset			Same as primary estimand	Treatment difference in the proportion and 95% CI
Change from baseline in NIHSS on day 7, 28, and 90 after stroke onset			● Death: composite strategy, i.e, assign to the worst value of all the visits (including baseline and post-baseline) prior to the event ● Other intercurrent events: same as primary estimand	Least-squares mean (LSMean) difference and 95% CI
Proportion of subjects with NIHSS improvement≥4 on day 7, 28, and 90 after stroke onset			● Death: composite strategy, i.e., subject directly treated as 'non-responsder' after death ● Other intercurrent events: same as primary estimand	Treatment difference in the proportion and 95% CI
Proportion of subjects with an NIHSS score of 0-1 on D90 after stroke onset			Same as estimand 'Proportion of subjects with a improvement of ≥4 in NIHSS on day 7, 28, and 90 after stroke onset'	
Change from baseline in BI on day 7, 28, and 90 after stroke onset			Same as estimand 'change from baseline in NIHSS on day 7, 28, and 90 after stroke onset'	
Proportion of subjects with BI ≥95 on day 7, 28, and 90 after stroke onset			Same as estimand 'Proportion of subjects with a improvement of ≥4 in NIHSS on day 7, 28, and 90 after stroke onset'	

For all the other secondary endpoints not listed in Table 2, treatment-policy strategy will be employed for all the intercurrent events.

6.5.2.2 mRS on Day 90

Based on FAS, the shift in Day 90 mRS as a 6-category ordinal scale will be analyzed using ordinal logistic regression; common OR, the associated 95% CI as well as p-value will be reported. For the subjects who are still missing of mRS on D90, the similar imputation methods as the primary efficacy endpoint will be used.

Repeat above analyses in ITT and PPS.

6.5.2.3 Proportion of Subjects with mRS≤2 on Day 90

Based on FAS, the proportion of subjects with mRS≤2 will be analyzed in the same manner as primary efficacy endpoint, the similar imputation methods as the primary efficacy endpoint will be used to impute missing data. 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 with the 95% CI as well as p-value will be also reported using logistic regression including treatment in the model.

Repeat above analyses in ITT and PPS.

6.5.2.4 Change from Baseline in mRS

Death will be scored as 6 in mRS.

Based on FAS, the absolute values and change from baseline will be summarized with descriptive statistics (number of subjects, mean, SD, median, Q1, Q3, minimum and maximum). Two-sample t-test or Wilcoxon rank-sum test will be performed for comparisons between treatment groups, paired t-test or signed rank test will be performed for change from baseline within each treatment group, respectively.

Furthermore, analysis of covariance (ANCOVA) will be used for treatment comparison to assess the treatment effects on change from baseline in mRS, the model will include baseline mRS as a covariate, with treatment group as factor; the differences in LSM and the associated 95% CIs will be reported.

Repeat above analyses in ITT and PPS.

6.5.2.5 Change from Baseline in NIHSS

Based on FAS, change from baseline in NIHSS on day 7, 28 and 90 will be summarized in the same manner as change from baseline in mRS.

Repeat above analyses in ITT and PPS.

6.5.2.6 Proportion of Subjects with NIHSS Improvement≥4 or NIHSS of 0-1

Based on FAS, the number and percentage of subjects with NIHSS improvement≥4 or NIHSS of 0-1 will be calculated and the corresponding 95% CI will be summarized (Clopper-Pearson interval) at each post-baseline scheduled visit, respectively. 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 with the 95% CI as well as p-value will be also reported using logistic regression including treatment in the model.

Repeat above analyses in ITT and PPS.

6.5.2.7 Change from Baseline in BI

Based on FAS, change from baseline in BI on day 7, 28 and 90 will be summarized in the same manner as change from baseline in mRS.

6.5.2.8 Proportion of Subjects with BI≥95

Based on FAS, the proportion of subjects with BI≥95 will be summarized similarly as proportion of subjects with NIHSS improvement ≥4 or NIHSS of 0-1.

6.5.2.9 MoCA or HDRS

Based on FAS, the absolute values of MoCA or HDRS will be summarized with descriptive statistics (number of subjects, mean, SD, median, Q1, Q3, minimum and maximum), respectively. Two-sample t-test or Wilcoxon rank-sum test will be performed for comparisons between treatment groups. Furthermore, the differences in LSM and the associated 95% CIs will be reported using analysis of variance (ANOVA) including treatment in the model.

6.5.2.10 Change from Baseline in MRI

Based on FAS, the infarct volume data by MRI Flair (Fluid-Attenuated Inversion Recovery) and DWI (Diffusion-Weighted Imaging) will be summarized for deep, lobar and total region, respectively:

The absolute values at D1, D5 and D28, D5/D28 change and percent change from baseline, D28 change and percent change from D5 will be summarized as data permit. In addition, D28 data by Flair compared to D1 or D5 DWI data and D5 data by Flair compared to D1 DWI data, including both change and percent change will be also summarized for the subjects with data available. Two-sample t-test or Wilcoxon rank-sum test will be performed for comparisons between treatment groups, paired t-test or signed rank test will be performed for change from baseline within each treatment group as

appropriate. Furthermore, ANCOVA will be performed for treatment comparison, the differences in LSM and the associated 95% CIs will be reported.

Repeat above analyses for the infarct area data.

6.6. Safety Analyses

All the safety analyses will be on basis of SS.

6.6.1 Adverse Events

AEs will be coded using MedDRA version 26.0 or higher; The severity of AEs will be graded by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v5.0.

Treatment-Emergent Adverse Event (TEAE) is defined as any AE that newly occurred or worsened throughout the period from the first dose of the investigational drug to the end of follow-up.

Adverse drug reaction (ADR) is defined as AE that is judged to be "definitely related", "probably related", and "possibly related" to the investigational drug.

The overview of AEs will be tabulated, the number of AE cases, the number and percentage of subjects with AEs for each of the following categories will be reported, respectively:

- All AEs
- All TEAEs
- All ADRs
- TEAEs with CTCAE Grade ≥3
- ADRs with CTCAE Grade ≥3
- TEAEs Leading to Drug Withdrawn
- ADRs Leading to Drug Withdrawn
- TEAEs Leading to Study Withdrawal
- ADRs Leading to Study Withdrawal
- Serious TEAEs
- Serious ADRs
- TEAEs Leading to Death
    - On-Treatment (defined as death occurred between the first and the last dose of investigational drug)
    - Follow-up (defined as death occurred after the last dose of investigational drug)
- ADRs Leading to Death.

The total number of AE cases, the number and percentage of subjects with above types of TEAEs will be summarized by SOC and PT. Subjects with multiple TEAEs in the same SOC or PT will be counted only once within that SOC or PT.

The number and percentage of subjects with TEAEs will be also summarized by SOC, PT and severity (CTCAE Grade 1~5) or causality, respectively. Subjects with multiple TEAEs in the same SOC or PT will be counted only once within that SOC or PT at the maximum severity or the closet relationship to the investigational drug (i.e., definitely related>probably related>possibly related>possibly unrelated>definitely unrelated).

Listings will be provided for all AEs, all ADRs, SAEs, AEs leading to drug withdrawn and AEs leading to study withdrawal and etc., respectively.

Furthermore, 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 TTYP01 compared to placebo control group and the associated 95% CI will be also reported.

6.6.2. Laboratory tests

Laboratory tests include hematology, blood chemistry, coagulation and urinalysis, etc..

The absolute values and change from baseline of laboratory tests at baseline and all scheduled post-baseline visits will be summarized with descriptive statistics (number of subjects, mean, SD, median, Q1, Q3, minimum and maximum).

A shift table will be prepared to compare baseline to the worst post-baseline visit (the order of clinical significance from worst to best: abnormal clinically significant (CS)>abnormal not clinically significant (NCS)>normal), using a categorization of normal, abnormal NCS, and abnormal CS as judged by the Investigator.

All the laboratory tests will be listed. In addition, the laboratory tests with at least one abnormal CS results will be listed separately, all the visits data of this laboratory test of this subject will be listed.

6.6.3. Vital signs

The vital signs and change from baseline will be summarized with descriptive statistics (number of subjects, mean, SD, median, Q1, Q3, minimum and maximum) by visits.

A shift table will be prepared to compare baseline to the worst post-baseline visit, using a categorization of normal, abnormal NCS, and abnormal CS as judged by the Investigator.

All the vital sign tests will be listed. In addition, the vital signs with at least one abnormal CS results will be listed separately, all the visits data of this vital sign of this subject will be listed.

6.6.4. Physical examination

A shift table will be prepared to compare baseline to the worst post-baseline visit, using a categorization of normal, abnormal NCS, and abnormal CS as judged by the Investigator.

All the physical examination data will be listed. In addition, the physical examinations with at least one abnormal CS results will be listed separately, all the visits data of this physical examination of this subject will be listed.

6.6.5. ECG

The ECG parameters and change from baseline will be summarized with descriptive statistics (number of subjects, mean, SD, median, Q1, Q3, minimum and maximum) by visits.

A shift table will be prepared to compare baseline to the worst post-baseline visit, using a categorization of normal, abnormal NCS, and abnormal CS as judged by the Investigator.

All the ECG data will be listed. In addition, the subjects with at least one abnormal CS results will be listed separately, all the visits data of this subject will be listed.

6.7. Major Changes in the Planned Analyses in Protocol

Add ITT set for statistical analyses as supportive; Except this, no major changes of planned analyses in the protocol are made in this statistical analysis plan.

7. Interim Analysis

One interim analysis will be planned to be conducted by IDMC 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.

Analysis timing and alpha allocation for interim analysis and final analysis are described as below:

	Analysis Timing	**Nominal Alpha (One-Sided)**
Interim analysis	50% subjects complete the trial	0.00153
Final analysis	100% subjects complete the trial	0.02450

The nominal alpha will be adjusted based on the actual timing of interim analysis.

8. Statistical Programming Deliverable

All statistical analyses, tables, listings and figures (TLFs) will be generated in SAS (version 9.4 or higher) with appropriate documentation and programming validation provided.

9. Statistical TLFs Template

TLFs templates will be provided as separate supporting documents to this SAP. The TLFs numberings will correspond to Section 14 and Appendix 16 of the ICH E3 Structure and Content of Clinical Study Reports.

10. Version History

Version No.	Date	Description
V1.0	July 01, 2024	