ARGATROBAN PLUS R-TPA FOR ACUTE ISCHAEMIC STROKE (ARAIS): A PROSPECTIVE, RANDOMISED, OPEN-LABEL, BLINDED-END POINT, MULTI-CENTRE, TRIAL

STATISTICAL ANALYSIS PLAN

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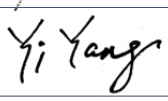
Prof Yi Yang (IDMC Chair)		

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ABBREVIATIONS

Abbreviation	Explanation
AE	Adverse Event
AIS	Acute ischaemic stroke
ARTSS	Argatroban With Recombinant Tissue Plasminogen Activator for Acute Stroke
CI	Confidence Interval
CRF	Clinical Research Form
GLM	Generalised Linear Model
GMR	Geometric Mean Ratio
HR	Hazard Ratio
IDMC	Independent Data Monitoring Committee
mITT	Modified Intent-to-treat
mRS	Modified Rankin Scale
NIHSS	National Institute of Health Stroke Scale
OR	Odds Ratio
PP	Per-protocol
RD	Risk Difference
RR	Risk Ratio
r-tPA	Recombinant tissue-type plasminogen activator
SAP	Statistical Analysis Plan

1. INTRODUCTION

1.1. Purpose of the statistical analysis plan

The purpose of this Statistical Analysis Plan (SAP) is to define the outcome variables, statistical methods, and analysis strategies to address the study's objectives in a multicentre, open-label, blinded-end point, randomised controlled trial to compare argatroban plus recombinant tissue-type plasminogen activator (r-tPA) with r-tPA alone treatment for acute ischaemic stroke (AIS): the ARIAS trial (Protocol version 1.0, 28/10/2018).

1.2. Background to the study

Vessel recanalisation is associated with lower mortality and improved functional outcome in patients with acute ischaemic stroke (AIS).[1] Intravenous thrombolysis with recombinant tissue plasminogen activator (r-tPA) has strongly been recommended in the early management of AIS.[2] However, only 20–30% of patients who received r-tPA achieved complete recanalisation.[3] Although the efficacy of endovascular therapy has been demonstrated in acute ischaemic stroke with large vessel occlusion,[4-9] the treatment largely depends on devices available at hospitals and experienced clinicians, limiting its use in clinical practice. Therefore, it is necessary to explore an effective and simple way to achieve vessel recanalisation in acute ischaemic stroke patients.

Argatroban, a selective thrombin inhibitor, directly inhibits free and clot-associated thrombin and thrombin-induced activities. Preclinical and clinical studies have shown that argatroban is safe and may offer benefits in patients with AIS.[10-14] In animal studies, argatroban plus r-tPA reportedly enhanced and sustained arterial recanalisation with thrombolysis using r-tPA[15], indicating the promise of adjunctive therapy in improving the prognosis after stroke. In patients with AIS, both ARTSS (Argatroban With Recombinant Tissue Plasminogen Activator for Acute Stroke)-1 and ARTSS-2 trials have suggested that the combination of argatroban with r-tPA is potentially safe and might have a favorable outcome[16-18].

However, ARTSS-1 and ARTSS-2 were exploratory in nature, and no definitive conclusions could be drawn because of the small sample size. Our trial aims to investigate the efficacy and safety of argatroban plus r-tPA in patients with AIS. We hypothesise that argatroban plus r-tPA might be superior to r-tPA alone in improving the functional outcomes without increasing the risk of intracerebral haemorrhage.

Based on the above discussion, this study intends to investigate the efficacy and safety of the combined application of alteplase and argatroban in the treatment of AIS.

2. STUDY OBJECTIVES AND OUTCOMES

2.1. Study Objectives

2.1.1. Primary Objective

To test the hypothesis that argatroban plus r-tPA will be superior to r-tPA alone in improving excellent functional outcomes in patients with AIS.

2.1.2. Secondary Objectives

1. To determine the proportion of favorable functional outcome at 90 days by treatment group.
2. To determine occurrence of early neurological improvement at 48 hours by treatment group.
3. To determine occurrence of early neurological deterioration at 48 hours by treatment group.
4. To determine change in neurological function at 14 days by treatment group.
5. To determine occurrence of stroke or other vascular events at 90 days by treatment group.

2.2. Outcomes

2.2.1. Primary outcome

The primary outcome is excellent functional outcome at 90 days(binary outcome), defined as a score of 0–1 on the mRS (modified Rankin Scale) for the evaluation of neurological disability assessed in person or, if an in-person visit was not possible, by personnel certified in the scoring of the mRS at 90 days after randomisation through telephone.

2.2.2. Secondary outcomes

1. Proportion of mRS (0-2) at 90 days (binary outcome)
2. Occurrence of early neurological improvement at 48 hours (binary outcome)
3. Occurrence of early neurological deterioration at 48 hours (binary outcome)
4. Change in NIHSS (National Institute of Health Stroke Scale) score compared with baseline at 14 days (continuous outcome)
5. The occurrence of stroke or other vascular events at 90 days (time-to-event outcome)

2.2.3. Safety outcomes

1. Proportion of symptomatic intracerebral haemorrhage;

2. Proportion of parenchymal hematoma type 2;

3. Proportion of major systemic bleeding.

2.2.4. Case ascertainment and case definitions

(1) Deaths

All deaths during the study period will be recorded. Cause of death will be clinically ascertained by the study physicians (participants will not receive post-mortems). Mortality by treatment group will be analysed with all-cause mortality within 90 days as the secondary outcome.

(2) Early neurological improvement

Early neurological improvement was defined as more than 2 NIHSS scores decrease [19], compared with baseline at 48 hours.

(3) Early neurological deterioration

Early neurological deterioration was defined as more than 4 NIHSS scores increase [20], but not result of cerebral haemorrhage, compared with baseline at 48 hours.

(4) Stroke

Stroke was defined as an acute focal central neurological deficit lasting >24 hours that resulted in irreversible brain damage or body impairment by a vascular cause [21].

(5) Other vascular events

Other vascular events include pulmonary embolism, peripheral vessel incident, and cardiovascular incident.

3. STUDY DESIGN

3.1. Design

This is a multicentre, open-label, blinded-end point, randomised controlled trial in patients with AIS.

3.2. Trial Sites

Trial recruitment will take place at about fourty hospitals nationwide. The trial sites build on prior successful collaborations, and have been selected due to their proven ability to successfully execute clinical trials of acute ischaemic stroke, and to reflect a spectrum of China health care settings.

3.3. Treatments

Trial arms:

The study regimens are:

argatroban plus r-tPA group: patients were administrated with intravenous r-tPA (0·9 mg/kg; maximum dose 90 mg, 10% administered as 1-minute bolus, the remaining infused over 1 hour; Boehringer Ingelheim Co., Ltd) and 100 μg/kg intravenous argatroban (Tianjin Institute of Pharmaceutical Research Co, Ltd) bolus over 3 to 5 minutes within 1 hour of the r-tPA bolus followed by argatroban infusion of 1·0 μg/kg per minute for 48 hours.

r-tPA alone group: patients were administrated with introvenous r-tPA (0·9 mg/kg; maximum dose 90 mg, 10% administered as 1-minute bolus, the remaining infused over 1 hour; Boehringer Ingelheim Co., Ltd).

3.4. Randomisation

A block randomisation, of which block size was 4, was performed on a 1:1 ratio using a computerized random sequence generation that was centrally administrated via a password-protected, web-based program at http://console.tt.zhinanmed.com (Beijing Zhinan Medical Technology Co., Ltd). The electronic data capture guarantees to make the selection in the natural order of the list filtering by study site only. Once a selection is made, the randomization record is tagged with the patient study allocated identifier, date and time of randomistion and other electronic data capture system audit values (username, machine name, etc). A tagged record cannot be selected more than once.

3.5. Sample Size

According to previous study [7], the proportion of expected excellent prognosis (mRS 0–1) at 90 days in control group is estimated to be about 21%. The proportion in the experimental group is estimated to be a 9% increase, compared with the proportion in the control group. Using power = 80% and two-sided alpha = 0.05 to carry out the unilateral test, the calculated sample size is 734 (367 per group). In consideration of 10% lost to follow-up, the total sample size is 808. Therefore, this study included 808 subjects, with 404 patients in each group.

4. ANALYSIS POPULATIONS

4.1. Study population data sets

The membership of each analysis set will be determined and documented and the reasons for exclusion will be given prior to database lock. A summary table will list the individual subjects sorted by treatment group and describe their protocol deviation/violation. Two study populations will be considered in the analysis to determine efficacy and safety, as follows:

Modified Intention-to-treat (mITT) population

All participants with valid informed consent will be included in the mITT population according to the treatment to which they are randomised, regardless of whether they prematurely discontinue treatment or are otherwise protocol violators/deviators. Participants lost to follow-up or withdrawn will not be included in the mITT population.

Per-protocol (PP) population

The PP population will be deemed as a sub-population of the mITT population and participants will be **excluded from the mITT population** if they:

- Did not adhere to study treatment (e.g. treated with uncompleted argatroban treatment, or unplaned discharge).

This population will be used for the supportive analyses.

Safety population

This population consists of all randomised subjects who receive at least one dose of study drug. Analysis for safety endpoints will utilize this analysis set.

4.2. Analysis Close Date

The analysis close date is the date on which the last participant completed 90-day follow-up.

Last contact date (also referred to as Trial reference end date): the date of the last trial related procedure. For survival subjects it is defined as the maximum of

- Date of last office visit (scheduled or unscheduled visit)
- Date of the last follow-up contact (including last date on subject survival status recorded)
- Date of the last known adverse event (AE) status or lab results reported on the AE or lab clinical research from (CRF) pages, respectively

4.3. Data cleaning

The data will then be checked to ensure that there are no erroneous entries and that all missing data is properly coded. Any changes will be made on the electronic data capture database.

4.4. Data download

For each time point, once all data have been inputted and checked, the database will be locked and a data download request made. The data will be downloaded into SAS, SPSS and STATA formats for statistical analyses.

5. STATISTICAL ANALYSES

The analyses will be carried out by the trial statistician and the primary analysis will be reviewed by a second statistician. The principle of mITT will be the main strategy of the analysis adopted for the primary outcome and all the secondary outcomes.

5.1. Primary Outcome Analysis

5.1.1. mITT analysis of the primary outcome - the primary analysis

The primary outcome is a binary outcome: excellent functional outcome defined as mRS (0-1) at 90 days. The primary analysis will be based on the mITT population as defined above.

The primary end point will be summarised by number (%) of participants that have excellent functional outcome by treatment group. A formal statistical analysis will be performed as a generalised linear model (GLM). In the GLM, the occurrence of excellent functional outcome at 90 days will be treated as the response variable and the treatment as the only predictor. Three GLMs will be used. They will have a binomial distribution, and logit, log, identity and logit link functions, which will generate odds ratio (OR), risk ratio (RR), risk difference (RD) of having a primary outcome between argatroban plus r-tPA and r-tPA alone together with two-sided 95% confidence intervals (CIs) and p-values.

5.1.2. PP analysis of the primary outcome

A supportive analysis of the primary outcome will also be performed on the PP population. Statistical methods will be the same as used in the Section 5.1.1.

5.1.3. Sensitivity analysis of the primary outcome

A sensitivity analysis of the primary outcome will be performed to assess whether the estimated treatment effect sensivity to the missing primatry outcome. For that, the missing values in the primary outcome will be imputed using the last observation carried forward method.

Sensitivity analysis will be performed on both mITT and PP populations.

5.1.4. Covariate adjusted analysis of the primary outcome

Adjusted analyses will also be carried out on the analysis of the primary endpoint to determine whether the treatment effect estimate is affected with the inclusion of covariables. The covariables that will be included in the adjusted GLMs are:

- Age (Year, continuous);
- Sex (Male/Female);
- NIHSS score at randomisation (continuous);
- Endovascular therapy (Yes/No);
- Large artery occlusion (Yes/No);
- Time from the onset of symptom to thrombolytic time (Minute, continuous);
- Premorbid function (mRS score, continuous);
- History of stroke or transient ischaemic attack (Yes/No).

The two covariates “Premorbid function” and “History of stroke or transient ischaemic attack” were not considered in the orginal protocol but added in the statistical analysis plan because it is believed they are related to the primary outcome.

From the above GLM models, the adjusted OR, RR and RD and 95% CI comparing the argatroban plus r-tPA to r-tPA alone will be derived. For the above adjusted GLM model analyses, a covariate will be excluded from the analysis if the proportion of subjects with missing value is more than 30% or the proportion of subjects with Yes or No is less than 5%.

Imputation for baseline missing covariates (see description below **7.4 missing data**) will be made for covariate adjusted analysis.

The above GLM may not converge when all covariates are introduced into the model simultaneously. To avoid non-convergence issue, we will first calculate a propensity score with treatment as the dependent variable (1 for argatroban plus r-tPA and 0 for r-tPA alone) and all covariates listed above as independent variables throught a logistic regression model, and then include the calculated propensity score (continuous variable) as a covariate in the GLM.

Covariate adjusted analysis will be performed on both mITT and PP populations.

5.1.5. Subgroup analysis of the primary outcome

Subgroup analysis of the primary endpoint will be performed using GLM model on the following subgroup variables:

- Age (< and ≥65 years)
- Sex (Male/Female)
- NIHSS score at randomisation (6-9 and >9)
- Endovascular therapy (Yes/No)
- Large artery occlusion (Yes/No)
- Time from the onset of symptom to thrombolytic time (< 3 and 3-4.5 hours)
- Premorbid function (mRS score, 0 and 1)
- History of stroke or TIA (Yes/No)

The unadjusted GLM model will be performed separately for each category of a subgroup covariate as done for the analysis of primary endpoint in Section 5.1.1 and OR with 95%CI will be presented. Subgroup analysis will be based on actual observed values of covariates and no imputation will be made for missing covariates.

Assessment of the homogeneity of treatment effect by a subgroup variable will be conducted by a GLM with the treatment, subgroup variable, and their interaction term as predictors, and the *P*-value presented for the interaction term.

Subgroup analysis will be performed on both mITT and PP populations.

5.2. Secondary Outcome Analysis

Secondary outcome analyses will be based on the mITT and PP populations.

5.2.1 Analysis of binary outcomes

Proportion of mRS (0-2) at 90 days, occurrence of early neurological improvement at 48 hours, and early neurological deterioration at 48 hours will be treated as a binary outcome and will be summarised by number (%) of participants with event by treatment group and analysed in a similar way as the primary end point by means of GLM. The OR, RR and RD with their two-sided 95% CIs between argatroban plus r-tPA and r-tPA alone will be estimated.

The analysis of ordinal outcome such as the mRS score at 90 days will also use GLM with treatment as the only predictor. The GLM model will have a multinomial distribution and cumulative logit link function, from which OR with their two-sided 95% CI comparing two treatment arms will be derived.

5.2.2 Analysis of time-to-event outcomes

The time-to-event outcomes (e.g. time from randomisation to the occurrence of stroke and other vascular events at the end of 90 days) will be summarised by number (%) of participants with event and incidence rate by treatment arm.

Survival curves will be plotted using Kaplan-Meier method and compared using the log-rank test. Cox regression model will be used to derive hazard ratio (HR) and its 2-sided 95%CI for comparing two treatment groups.

5.2.3 Analysis of secondary outcomes with repeated measurements

The NIHSS score is measured at admission and 14 days later.

These data will be managed according to the following procedures and rules before being analysed:

We will calculate the change in log (NIHSS score) for each patient between randomisation and 14 days, and used a GLM with log (NIHSS score) as response variable, treatment as predictor, baseline measurement of log (NIHSS score) as covariate to calculate geometric mean ratio (GMR) of NIHSS score between the two groups.

5.3. Exploratory Analysis

Other statistical methods may be used if deemed necessary but was considered as exploratrory.

6. SAFETY ANALYSES

6.1. Safety Variables

Safety analyses will summarise the number of safety events, including symptomatic intracerebral haemorrhage, parenchymal hematoma type 2, and major systemic bleeding occurring after randomisation.

In addition, summaries of the suspected relationship with trial treatment, suspected trial treatment or other cause, duration of recovered safety events, seriousness criteria, event outcome, DAIDS grade and safety events, will be presented by treatment received and overall.

Line listings of all reported safety events for each participant will also be presented by treatment received. They will include (where appropriate):

- Randomised treatment
- DAIDS grade
- Event description
- Seriousness criteria
- Suspected relationship to the trial medications
- Suspected products
- Other causality
- Expectedness
- Date of randomisation
- Date of onset
- Date event became serious (serious events only)
- Date of recovery
- Outcome
- Details of the treatment received

7. GENERAL CONSIDERATIONS FOR DATA ANALYSES

SPSS® (version 23) will be used to perform all data analyses. R may also be used for some data analyses and generate the majority of data displays.

7.1. Covariates Analyses

Covariate analyses will be performed on the primary outcome and secondary outcomes on the mITT and PP populations. Other covariate analyses will be performed if deemed necessary.

7.2. Subgroup Analysis

Subgroup analyses will be performed for the primary outcome on the mITT and PP populations. Assessment of the homogeneity of treatment effect by a subgroup variable will be conducted by a binary logistic regression with the treatment, subgroup variable, and their interaction term as predictors, and the *P*-value presented for the interaction term.

7.3. Multiplicity

Analyses of secondary outcomes and additional analyses for the primary outcome are regarded as exploratory in nature, therefore, multiplicity adjustment will not apply to the primary and secondary outcome analyses.

7.4. Missing data

7.4.1 Baseline covariates

Missing values in covariate adjusted analysis will be imputed according to the proportion of missingness.

If the missing values for a particular covariate be less than 5%, simple imputation methods will be used to impute the missing value for the covariate. For continuous variable, missing values will be imputed from random values assuming a normal distribution with mean and standard deviation calculated from the available sample. For categorical variables, missing values will be imputed from random values from a uniform distribution with probabilities $p_1, p_2, \dots, p_k$, again from the available sample. For a count variable, missing values will be imputed from random values from a Poisson distribution with mean λ estimated from the available sample.

If the proportion of missing observations for a covariate is larger than 5% but less than 30%, multiple imputation method will be used. Multiple imputations will be based on the statistical distributions of the variables pre-specified in the covariate adjusted analysis measured at the baseline assessment.

The seed for the imputations will be 123456.

7.4.2 Efficacy outcomes

Missing efficacy data will be treated as missing at random and no imputation will be made in the primary analysis. For the sensitivity analysis, primary and secondary outcomes will be imputed using the last observation carried forward method.

7.5. Further Exploratory Analyses

Further exploratory analyses may be carried out should they be deemed necessary; this will be at the discretion of the trial management group. These will be added to the analysis plan as an amendment along with justification, where appropriate.

7.6. Data Summaries

Continuous variables will be summarised according to number of subjects with non-missing data (n), mean, standard deviation, median, minimum, and maximum. The confidence interval will be added on summaries of continuous effectiveness variables.

Categorical variables will be summarised according to the absolute frequency and percentage of subjects (%) in each category level. The denominator for the percentages is the number of subjects in the treatment arm with data available, unless noted otherwise. Event rates per 100 person years will also be reported for time-to-event clinical outcomes and adverse events of special interest.

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