Supplement 2

Part	Item	Version	Date
1	Statistical Analysis Plan	1.0	August 03, 2018
2	Statistical Analysis Plan	2.0	May 06, 2020
3	Detail of Statistical Analysis Plan Change	-	-

ANTIPLATELET VS R-TPA FOR ACUTE MILD ISCHEMIC STROKE (ARAMIS): A PROSPECTIVE, RANDOMIZED, OPEN-LABEL, BLINDED-ENDPOINT, MULTI-CENTRE TRIAL

STATISTICAL ANALYSIS PLAN

ClinicalTrials.gov registration number: NCT03661411

Protocol version and date: Version 1.0 of August 03, 2018

Chief Investigators: Prof Hui-Sheng Chen ***Trial statisticians:*** Dr Yu Cui, Prof Duolao Wang ***SAP authors:*** Dr Yu Cui, Prof Duolao Wang, Prof Hui-Sheng Chen

SAP version history		
Version Date	SAP Version #	Details of Changes
August 03, 2018	*1.0*	*first version*

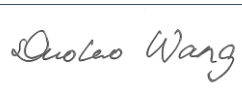
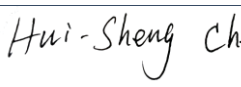
Signatures		
	Signature	Date
Dr Yu Cui (Trial Statistician)		August 03, 2018
Prof Duolao Wang (Trial Statistician)		
Prof Hui-Sheng Chen (Chief Investigator)		
Prof Yi-Long Wang (IDMC Chair)		

TABLE OF CONTENTS

1. ABBREVIATIONS

Abbreviation	Explanation
AE	Adverse Event
AIS	Acute ischemic stroke
CI	Confidence Interval
CRF	Case Report Form
GLM	Generalized Linear Model
HR	Hazard Ratio
IDMC	Independent Data Monitoring Committee
mRS	Modified Rankin Scale
NIHSS	National Institute of Health Stroke Scale
RD	Risk Difference
RR	Risk Ratio
OR	Odds Ratio
PP	Per-protocol
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TIA	Transient Ischemic Attack
TMG	Trial Management Group

2. INTRODUCTION

2.1. 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 prospective, randomized, open-label, blinded-endpoint, multi-centre trial to compare dual antiplatelet treatment with intravenous alteplase for nondisabling acute minor ischaemic stroke: the ARAMIS trial (Protocol version 1.0 of August 03, 2018).

2.2. Background to the study

Acute ischemic stroke (AIS) is one of common diseases with significant morbidity, mortality, and disability. A wide array of studies confirms that intravenous thrombolytic therapy with alteplase can effectively improve the functional prognosis in AIS [1], thus all guidelines recommended the intravenous thrombolytic therapy with alteplase for AIS within 4.5 h from stroke onset [2].

Minor stroke is usually defined as NIHSS score ≤ 3 or 5, that accounts for 1/2-2/3 of AIS [3-4], but the evidence of intravenous thrombolysis of those without clearly disabling deficits is still insufficient [5-6]. A study from Canada shows that 28.5% of patients with minor stroke and without receiving alteplase therapy were unable to walk independently when discharged [7]. The PRISMS study is designed to further compare the efficacy and safety of intravenous alteplase vs. aspirin alone in patients with minor stroke (NIHSS ≤ 5) and without clearly disabling deficits [8]. Unfortunately, the study has been very early terminated due to the sponsorship reason in 2018, with only 313 cases enrolled. The preliminary results show that there is no significant difference of the 90-day neurological function between the two groups, while the treatment group with alteplase has a higher rate of symptomatic intracranial hemorrhage than the control group with aspirin alone. Furthermore, the guidelines recommend that once the patients received thrombolysis, antithrombotic therapy cannot be given within 24 hours after thrombolysis. The recommendation makes clinical doctors puzzled to treat the early neurological deterioration, especially in minor stroke patients.

The CHANCE trial in 2013 shows that the efficacy of the combination of aspirin with clopidogrel is superior to aspirin alone with minor stroke (NIHSS ≤ 3) or TIA (ABCD2 ≤ 4) [9]. The post hoc

analysis of the CHANCE trial in 2017 indicates that bleeding risk outweighs benefit after the 10th day [10]. The POINT study in 2018 further confirmed the efficacy and safety of intensive antiplatelet therapy in minor stroke within 12 hours of onset [11].

This study intends to demonstrate that dual antiplatelet have similar effect with alteplase on 90-day functional outcome in nondisabling mild stroke population, and have more less symptomatic intracranial hemorrhage.

3. STUDY OBJECTIVES AND OUTCOMES

3.1. Study objectives

3.1.1. Primary objective

To test the hypothesis that dual antiplatelet have similar effect with alteplase on 90-day excellent functional outcome in nondisabling mild stroke population.

3.1.2. Secondary objectives

a) To determine the proportion of favorable functional outcome at 90 days by treatment group.
b) To determine change in neurological function at 24 hours by treatment group.
c) To determine occurrence of early neurological improvement at 24 hours by treatment group.
d) To determine occurrence of early neurological deterioration at 24 hours by treatment group.
e) To determine occurrence of stroke or other vascular events at 90 days by treatment group.
f) To determine all-cause mortality at 90 days by treatment group.

3.2. Outcomes

3.2.1. Primary outcome

The primary outcome is the occurrence of mRS (0-1) at 90 days (binary outcome), defined as a score of 0–1 on the mRS 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.

3.2.2. Secondary outcomes

a) Occurrence of mRS (0-2) at 90 days (binary outcome);
b) Change in National Institute of Health Stroke Scale (NIHSS) score compared with baseline at 24 hours;
c) Occurrence of early neurological improvement (binary outcome);
d) Occurrence of early neurological deterioration (binary outcome);
e) Occurrence of stroke or other vascular events at 90 ± 7 days (time-to-event outcome);
f) Occurrence of all-cause mortality at 90 ± 7 days (binary outcome);

3.2.3. 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 deterioration was defined as more than or equal to 2 NIHSS scores decrease, compared with baseline at 24 hours.

(3) Early neurological deterioration

Early neurological deterioration was defined as more than or equal to 2 NIHSS scores increase, but not result of cerebral hemorrhage, compared with baseline at 24 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 [12].

(5) Other vascular events

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

(6) Additional Safety Variables

Adverse events (AE) is any adverse medical event that occurs in the course of the study. All information about AEs should be recorded on the AEs page of the case report, and whether the unexpected AE is associated with the early antiplatelet treatment will be further adjudicated by principal investigator.

4. STUDY DESIGN

4.1. Design

This is a multi-centre, randomized, open-label, blinded-endpoint, noninferiority trial in patients with nondisabling acute minor ischaemic stroke.

4.2. Trial sites

Trial recruitment will take place at about 20 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.

4.3. Treatments

Trial arms:

The study regimens are:

Exprement group: orally 300-mg clopidogrel on the first day followed by 75 mg daily for 10–14 days and 100-mg aspirin on the first day immediately followed by 100 mg daily for 10–14 days and then given standard guideline-based antithrombotic treatment from 14 days to 90 days.

Control group: intravenous alteplase with standard dose of 0.9 mg/kg, up to a maximum of 90 mg, followed by guideline-based antithrombotic treatment 24 hours after thrombolysis until to 90 days.

4.4. Randomisation

A randomisation method with minimization algorithm 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://aramis.medsci.cn (Shanghai Meisi Medical Technology Co., Ltd). The EDC 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 randomization and other EDC system audit values (username, machine name, etc). A tagged record cannot be selected more than once.

4.5. Sample Size

This trial mainly detects a 10% absolute difference in the proportion of the dual antiplatelet group with favorable outcome with 80% power and a one-sided type I error rate of 0.025 to test the non-inferiority, and the proportion of 65% of the dual antiplatelet group will experience a favorable outcome referring to previous report [13]. Thus, the sample size of 716 subjects resulted from these assumptions. According to the ITT principle, the maximum sample size is 752 subjects with 5% lost. Finally, a total of 760 subjects are expected to include in this study, with 380 patients in each group.

5. ANALYSIS POPULATIONS

5.1. 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:

Full analysis set population

All participants with valid informed consent will be included in the full analysis set 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 full analysis set population.

Per-protocol (PP) population

Per protocol population will be deemed as a sub-population of the full analysis set population and participants will be **excluded from the full analysis set population** if they:

a) Did not adhere to study treatment (e.g. unplaned discharge).
b) Switched treatment (e.g. a participant is randomised to experment group but received intravenous alteplase treatment)

The PP population will be used for the supportive analyses.

Safety population

A patient should be included if, and only if, they actually received a study treatment. This set of patients are grouped for analysis according to the treatment they actually received, as opposed to the treatment they were allocated to receive at randomisation. The Safety Population is used for the analysis of safety, including adverse events, toxicity and laboratory evaluations.

5.2. 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

a) Date of last office visit (scheduled or unscheduled visit);
b) Date of the last follow-up contact (including last date on subject survival status recorded);
c) Date of the last known adverse event (AE) status or lab results reported on the AE or lab case report from (CRF) pages, respectively.

5.3. 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 EDC database.

5.4. 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.

6. 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 full analysis set will be the main strategy of the analysis adopted for the primary outcome and all the secondary outcomes.

6.1. Primary outcome analysis

6.1.1. Full analysis set 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 full analysis set population as defined above.

The primary endpoint will be summarised by number (%) of participants that have excellent functional outcome by treatment group. A formal statistical analysis will be performed as a generalized linear model (GLM) with binomial distribution and identity link function (binomial-identity regression model). 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. From this model, risk difference (RD) in the proportion of the primary outcome between dual antiplatelet and intravenous alteplase together with the two-sided 95% confidence interval (CI) (equivalent to the one-sided 97.5% CI). In addition, p-value for one-sided noninferiority test will be calculated. Furthermore, risk ratio with their two-sided 95%CI will be calculated using GLM with binomial distribution and log link fuction (binomial-log regression model).

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 6.1.1

6.1.2. Sensitivity analysis of the primary outcome

To assess the influence of the missing primary endpoints on the treatment effect estimate, sensitivity analyses will be performed using the same statistical methods as described in Section 6.1.1, considering several situations:

(1) The last observation carried forward method.

Missing mRS score at 90 days will be imputed using the value of NIHSS measured at 24 hours-12 days using the following relationship. NIHSS 0-3 at 24 hours, or NIHSS 0-5 at 7-12 days will correspond to mRS 0-1 at 90 days, while others correspond to mRS 2-6 at 90 days.

(2) Worst-case scenario:

All patients with a missing primary endpoint will be considered as a failure (mRS 2-6) in both treatment groups.

(3) Best-case scenario:

All patients with a missing primary endpoint will be considered as a success (mRS 0-1) in both treatment groups.

6.1.3. Covariate adjusted analysis of the primary outcome

Adjusted binomial-identity model 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 analyses are:

a) Age (Continuous)
b) History of diabetes mellitus (Yes/No)
c) Time from the onset of symptom to administration time (Minute, Continuous)
d) Stroke etiology
e) Degree of vascular stenosis (≤50% vs. >50%)
f) Location of responsible vessel (anterior circulation/posterior circulation)

From the above model, the adjusted RD and 95% CI comparing the dual antiplatelet treatment to the intravenous alteplase will be derived. In addition, binomial-log regression model will be used to calculate RR and 95% CI.

The above binomial-identity regression model 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 dual antiplatelet treatment and 0 for intravenous alteplase) 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 binomial-identity regression model.

Imputation for baseline missing covariates (see description below **8.4 missing data**) will be made for covariate adjusted analysis. If a covariate has over 50% value missing, the variable will be dropped from covariate adjusted analysis.

6.1.4. Subgroup analysis of the primary outcome

These followed covariates by age (<65 years or ≥65 years), diabetes (present or not present), time from onset to treatment (<2 hours or ≥2 hours), stroke etiology (arteriosclerosis vs small vessel lesion), degree of vascular stenosis (≤ 50% vs. > 50%), location of index vessel (anterior circulation vs. posterior circulation) will be included in subgroup analyses by performing the above unadjusted binomial-log regression model analysis separately for each category of a subgroup covariate. RD and RR and their 95% CIs will be presented for subgroup analysis.

Assessment of the homogeneity of treatment effect measured with RR by a subgroup variable will be conducted by a binomial-log regression model with the treatment, subgroup variable, and their interaction term as predictors, and the p-value presented for the interaction term.

6.2. Secondary outcome analysis

Secondary outcome analyses will be based on the full analysis set and PP populations.

6.2.1 Analysis of binary outcomes

Proportion of mRS (0-2) at 90 days, occurrence of early neurological improvement at 24 hours, and occurrence of early neurological deterioration at 24 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 endpoint by means of GLMs. The RD and RR and their two-sided 95% CIs between early antiplatelet and Control will be estimated.

The analysis of other binary outcomes will also use binomial-identity regression models with treatment as the only predictor. RD and RR with their two-sided 95% CIs comparing two treatment arms will be derived from the GLM models.

6.2.2 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 two-sided 95% CI for comparing two treatment groups.

6.2.3 Analysis of continuous outcome

The NIHSS score is measured at admission and 24 hours later.

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

We will calculate the change of NIHSS score for each patient between randomisation and 24 hours, and used a GLM with normal distribution and identity link function to compare the means in the change from baseline between the 2 groups. Log transformation may be performed if normality or variance homogeneity assumptions for residuals are violated after visual inspection of their histogram and scatterplots.

6.3. Exploratory analysis

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

7. SAFETY ANALYSES

7.1. Safety variables

All safety analyses will be performed on the safety population.

Adverse events (AEs) will be restricted to those occurring during the 90 days after randomisation.

AEs will be summarised using the number of AEs, the number (%) of participants with AEs by treatment arms. The number of patients with any AE or SAEs will be analysed using logistic regression model from which odds ratios (OR) and its two-sided 95% CI will be calculated.

Safety analyses will summarise the number of any adverse medical events, serious adverse events (SAEs), and deaths occurring after randomisation.

Summaries of the total number of reported AEs/SAEs and number of participants reporting at least one AEs/SAE will be presented by treatment received and overall. In addition, summaries of the suspected relationship with trial treatment, suspected trial treatment or other cause, duration of recovered SAEs, seriousness criteria, event outcome, DAIDS grade and SAE, will be presented by treatment received and overall.

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

a) Randomised treatment
b) DAIDS grade
c) Event description
d) Seriousness criteria
e) Suspected relationship to the trial medications
f) Suspected products
g) Other causality
h) Expectedness
i) Date of randomisation
j) Date of onset
k) Date event became serious (serious events only)
l) Date of recovery
m) Outcome
n) Details of the treatment received

8. GENERAL CONSIDERATIONS FOR DATA ANALYSES

SPSS® (version 20) will be used to perform all data analyses.

8.1. Covariates analyses

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

8.2. Subgroup analysis

Subgroup analyses will be performed for the primary outcome on the full analysis set 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.

8.3. 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.

8.4. Missing data

All efforts will be made to minimize the amount of missing data, particularly the 90-day outcome assessment.

8.4.1. Baseline covariates

Missing baseline covariates will be imputed using simple imputation methods in the covariate adjusted analysis based on the covariate distributions, should the missing values for a particular covariate be less than 5%. For a continuous variable, missing values will be imputed from random values from a normal distribution with mean and SD calculated from the available sample. For a categorical variable, missing values will be imputed from random values from a uniform distribution with probabilities P_1, P_2, …, and P_k from the sample.

If the missing values for a covariate are ≥5% but less than 50%, then they will be imputed using multiple imputation (M=10). If a covariate has over 50% value missing, the variable will be dropped from covariate adjusted analysis. The seed for the imputations will be 030818.

8.4.2. Efficacy outcomes

Sensitivity analyses based on different hypotheses about the missingness pattern of the primary outcome will be performed to test for the robustness of the primary analysis results. Please refer to Section 6.1.2.

8.5. Further exploratory analyses

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

8.6. Data summaries

Continuous variables will be summarised according to number of subjects with non-missing data (n), mean, standard deviation (SD), 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 number of subjects in the treatment arm with data available, unless noted otherwise. Event rates per 100 person years will be reported for time-to-event clinical outcomes and adverse events of special interest.

8.7. Interim analysis

An independent Data Monitoring Committee (DMC) was set up to ensure ongoing review of safety data, especially bleeding events. An interim analysis will be performed after 50% of subjects have completed follow-up.

9. REFERENCES

1. Wardlaw JM, Zoppo G and Yamaguchi T. Thrombolysis for acute ischaemic stroke. Cochrane Database Syst Rev 2003; 3: CD000213.
2. Hacke W, Kaste M, Bluhmki E, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 2008; 359: 1317–1329.
3. Reeves M, Khoury J, Alwell K, et al. Distribution of national institutes of health stroke scale

in the Cincinnati/Northern Kentucky stroke study. Stroke 2013; 44: 3211–3213.

4. Dhamoon MS, Moon YP, Paik MC, et al. Long-term functional recovery after first ischemic stroke. Stroke 2009; 40: 2805–2811.
5. Demaerschalk BM, Kleindorfer DO, Adeoye OM, et al. Scientific rationale for the inclusion and exclusion criteria for intravenous alteplase in acute ischemic stroke. Stroke 2016; 47: 581–641.
6. Jauch EC, Saver JL, Adams HP, et al. Guidelines for the early management of patients with acute ischemic stroke. Stroke 2013; 44: 870–947.
7. Barber PA, Zhang J, Demchuk AM, Hill MD and Buchan AM. Why are stroke patients excluded from tPA therapy? An analysis of patient eligibility. Neurology 2001; 56: 1015–1020.
8. Pooja KH, Dawn OK, Thomas D, et al. Effect of alteplase vs aspirin on functional outcome for patients with acute ischemic stroke and minor nondisabling neurologic deficits: the PRISMS randomized clinical trial. JAMA 2018; 320: 156–166.
9. Wang Y, Wang Y, Zhao X, et al. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. N Engl J Med 2013; 369: 11–19.
10. Pan Y, Jing J, Chen WQ, et al. Risks and benefits of clopidogrel-aspirin in minor stroke or TIA: time course analysis of CHANCE. Neurology 2017; 88: 1906–1911.
11. Johnston SC, Easton JD, Farrant M, et al. Clopidogrel and aspirin in acute ischemic stroke and high-risk TIA. N Engl J Med 2018; 379: 215–225.
12. Sacco RL, Kasner SE, Broderick JP, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2013;44: 2064-2089.
13. Yeatts SD, Broderick JP, Chatterjee A, et al. Alteplase for the treatment of acute ischemic stroke in patients with low National Institutes of Health Stroke Scale and not clearly disabling deficits (Potential of rtPA for Ischemic Strokes with Mild Symptoms PRISMS): Rationale and design. Int J Stroke 2018; 13: 654-661.

ANTIPLATELET VS R-TPA FOR ACUTE MILD ISCHEMIC STROKE (ARAMIS): A PROSPECTIVE, RANDOMIZED, OPEN-LABEL, BLINDED-ENDPOINT, MULTI-CENTRE TRIAL

STATISTICAL ANALYSIS PLAN

ClinicalTrials.gov registration number: NCT03661411

Protocol version and date: Version 2.0 of May 6, 2020

Chief Investigators: Prof Hui-Sheng Chen ***Trial statisticians:*** Dr Yu Cui, Prof Duolao Wang ***SAP authors:*** Dr Yu Cui, Prof Duolao Wang, Prof Hui-Sheng Chen

SAP version history		
Version Date	SAP Version #	Details of Changes
May 6, 2020	*2.0*	*first version*

Signatures		
	Signature	Date
Dr Yu Cui (Trial Statistician)	Yu Cui	May 06, 2020
Prof Duolao Wang (Trial Statistician)	Duolao Wang	
Prof Hui-Sheng Chen (Chief Investigator)	Hui-Sheng Chen	
Prof Yi-Long Wang (IDMC Chair)		

TABLE OF CONTENTS

1. ABBREVIATIONS

Abbreviation	Explanation
AE	Adverse Event
AIS	Acute ischemic stroke
CI	Confidence Interval
CRF	Case Report Form
GLM	Generalized Linear Model
HR	Hazard Ratio
IDMC	Independent Data Monitoring Committee
IPW	Inverse Probability Weighting
mRS	Modified Rankin Scale
NIHSS	National Institute of Health Stroke Scale
RD	Risk Difference
RR	Risk Ratio
OR	Odds Ratio
PP	Per-protocol
AT	As Treated
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TIA	Transient Ischemic Attack
TMG	Trial Management Group

2. INTRODUCTION

2.1. 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 prospective, randomized, open-label, blinded-endpoint, multi-centre trial to compare dual antiplatelet treatment with intravenous alteplase for nondisabling acute minor ischaemic stroke: the ARAMIS trial (Protocol version 2.0 of May 6, 2020).

2.2. Background to the study

Acute ischemic stroke (AIS) is one of common diseases with significant morbidity, mortality, and disability. A wide array of studies confirms that intravenous thrombolytic therapy with alteplase can effectively improve the functional prognosis in AIS [1], thus all guidelines recommended the intravenous thrombolytic therapy with alteplase for AIS within 4.5 h from stroke onset [2].

Minor stroke is usually defined as NIHSS score ≤ 3 or 5, that accounts for 1/2-2/3 of AIS [3-4], but the evidence of intravenous thrombolysis of those without clearly disabling deficits is still insufficient [5-6]. A study from Canada shows that 28.5% of patients with minor stroke and without receiving alteplase therapy were unable to walk independently when discharged [7]. The PRISMS study is designed to further compare the efficacy and safety of intravenous alteplase vs. aspirin alone in patients with minor stroke (NIHSS ≤ 5) and without clearly disabling deficits [8]. Unfortunately, the study has been very early terminated due to the sponsorship reason in 2018, with only 313 cases enrolled. The preliminary results show that there is no significant difference of the 90-day neurological function between the two groups, while the treatment group with alteplase has a higher rate of symptomatic intracranial hemorrhage than the control group with aspirin alone. Furthermore, the guidelines recommend that once the patients received thrombolysis, antithrombotic therapy cannot be given within 24 hours after thrombolysis. The recommendation makes clinical doctors puzzled to treat the early neurological deterioration, especially in minor stroke patients.

The CHANCE trial in 2013 shows that the efficacy of the combination of aspirin with clopidogrel is superior to aspirin alone with minor stroke (NIHSS ≤ 3) or TIA (ABCD2 ≤ 4) [9]. The post hoc

analysis of the CHANCE trial in 2017 indicates that bleeding risk outweighs benefit after the 10th day [10]. The POINT study in 2018 further confirmed the efficacy and safety of intensive antiplatelet therapy in minor stroke within 12 hours of onset [11].

This study intends to demonstrate that dual antiplatelet have similar effect with alteplase on 90-day functional outcome in nondisabling mild stroke population, and have more less symptomatic intracranial hemorrhage.

3. STUDY OBJECTIVES AND OUTCOMES

3.1. Study objectives

3.1.1. Primary objective

To test the hypothesis that dual antiplatelet have similar effect with alteplase on 90-day excellent functional outcome in nondisabling mild stroke population.

3.1.2. Secondary objectives

a) To determine the proportion of favorable functional outcome at 90 days by treatment group.
b) To determine an ordinal shift of the full range of mRS scores at 90 days.
c) To determine change in neurological function at 24 hours by treatment group.
d) To determine occurrence of early neurological improvement at 24 hours by treatment group.
e) To determine occurrence of early neurological deterioration at 24 hours by treatment group.
f) To determine occurrence of stroke or other vascular events at 90 days by treatment group.
g) To determine all-cause mortality at 90 days by treatment group.

3.2. Outcomes

3.2.1. Primary outcome

The primary outcome is the occurrence of mRS (0-1) at 90 days (binary outcome), defined as a score of 0–1 on the mRS 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.

3.2.2. Secondary outcomes

a) Occurrence of mRS (0-2) at 90 days (binary outcome);
b) An ordinal shift of the full range of mRS scores at 90 days (ordinal outcome);
c) Change in National Institute of Health Stroke Scale (NIHSS) score compared with baseline at 24 hours;
d) Occurrence of early neurological improvement (binary outcome);
e) Occurrence of early neurological deterioration (binary outcome);
f) Occurrence of stroke or other vascular events at 90 ± 7 days (time-to-event outcome);
g) Occurrence of all-cause mortality at 90 ± 7 days (binary outcome);

3.2.3. 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 deterioration was defined as more than or equal to 2 NIHSS scores decrease, compared with baseline at 24 hours.

(3) Early neurological deterioration

Early neurological deterioration was defined as more than or equal to 2 NIHSS scores increase [12], but not result of cerebral hemorrhage, compared with baseline at 24 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 [12].

(5) Other vascular events

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

(6) Additional Safety Variables

Adverse events (AE) is any adverse medical event that occurs in the course of the study. All information about AEs should be recorded on the AEs page of the case report, and whether the unexpected AE is associated with the early antiplatelet treatment will be further adjudicated by principal investigator.

4. STUDY DESIGN

4.1. Design

This is a multi-centre, randomized, open-label, blinded-endpoint, noninferiority trial in patients with nondisabling acute minor ischaemic stroke.

4.2. Trial sites

Trial recruitment will take place at about 20 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.

4.3. Treatments

Trial arms:

The study regimens are:

Exprement group: orally 300-mg clopidogrel on the first day followed by 75 mg daily for 10–14 days and 100-mg aspirin on the first day immediately followed by 100 mg daily for 10–14 days and then given standard guideline-based antithrombotic treatment from 14 days to 90 days.

Control group: intravenous alteplase with standard dose of 0.9 mg/kg, up to a maximum of 90 mg, followed by guideline-based antithrombotic treatment 24 hours after thrombolysis until to 90 days.

4.4. Randomisation

A randomisation method with minimization algorithm 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://aramis.medsci.cn (Shanghai Meisi Medical Technology Co., Ltd). The EDC 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 randomization and other EDC system audit values (username, machine name, etc). A tagged record cannot be selected more than once.

4.5. Sample size

According to our recent unpublished data, the proportion of expected excellent functional outcome (mRS 0-1) at 90 days in control group is estimated to be about 87%, and the proportion in the experimental group is estimated to be about 89.5% based on PRISMS [8]. The margin of non-inferiority was defined as 4.5% in our trial, which was based on the subset analysis of the third international stroke trial (IST-3) showing a 9% absolute difference in the proportion of favorable outcome in minor nondisabling patients treated versus untreated with intravenous alteplase [13]. Using power = 80% and $\alpha = 0.05$ to carry out the two-side test, the required sample size to test the non-inferiority hypothesis is 666. In consideration of 12% lost to follow-up, the total sample size is 757. Therefore, this study still included 760 patients, with 380 patients in each group.

5. ANALYSIS POPULATIONS

5.1. 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:

Full analysis set population

All participants with valid informed consent will be included in the full analysis set 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 full analysis set population.

Per-protocol (PP) population

Per protocol population will be deemed as a sub-population of the full analysis set population and participants will be **excluded from the full analysis set population** if they:

a) Did not adhere to study treatment (e.g. unplaned discharge).
b) Switched treatment (e.g. a participant is randomised to experment group but received intravenous alteplase treatment)

As-treated (AT) population

As-treated (AT) population is based on the treatment actually received. There is no single CRF question that determines the As-treated arm, and the arm will be determined by the trial statistician.

The PP and AT populations will be used for the supportive analyses.

Safety population

A patient should be included if, and only if, they actually received a study treatment. This set of patients are grouped for analysis according to the treatment they actually received, as opposed to the treatment they were allocated to receive at randomisation. The Safety Population is used for the analysis of safety, including adverse events, toxicity and laboratory evaluations.

5.2. 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

a) Date of last office visit (scheduled or unscheduled visit);

b) Date of the last follow-up contact (including last date on subject survival status recorded);
c) Date of the last known adverse event (AE) status or lab results reported on the AE or lab case report from (CRF) pages, respectively.

5.3. 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 EDC database.

5.4. 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.

6. 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 full analysis set will be the main strategy of the analysis adopted for the primary outcome and all the secondary outcomes.

6.1. Primary outcome analysis

6.1.1. Full analysis set 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 full analysis set population as defined above.

The primary endpoint will be summarised by number (%) of participants that have excellent functional outcome by treatment group. A formal statistical analysis will be performed as a generalized linear model (GLM) with binomial distribution and identity link function (binomial-identity regression model). 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. From this model, risk difference (RD) in the proportion of the primary outcome between dual antiplatelet and intravenous alteplase together with the two-sided 95% confidence interval (CI) (equivalent to the

one-sided 97.5% CI). In addition, p-value for one-sided noninferiority test will be calculated. Furthermore, risk ratio with their two-sided 95%CI will be calculated using GLM with binomial distribution and log link fuction (binomial-log regression model).

A supportive analysis of the primary outcome will also be performed on the PP and AT populations. Statistical methods will be the same as used in the Section 6.1.1

6.1.2. Sensitivity analysis of the primary outcome

To assess the influence of the missing primary endpoints on the treatment effect estimate, sensitivity analyses will be performed using the same statistical methods as described in Section 6.1.1, considering several situations:

(1) The last observation carried forward method.

Missing mRS score at 90 days will be imputed using the value of NIHSS measured at 24 hours-12 days using the following relationship. NIHSS 0-3 at 24 hours, or NIHSS 0-5 at 7-12 days will correspond to mRS 0-1 at 90 days, while others correspond to mRS 2-6 at 90 days.

(2) Worst-case scenario:

All patients with a missing primary endpoint will be considered as a failure (mRS 2-6) in both treatment groups.

(3) Best-case scenario:

All patients with a missing primary endpoint will be considered as a success (mRS 0-1) in both treatment groups.

6.1.3. Covariate adjusted analysis of the primary outcome

Adjusted binomial-identity model 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 analyses are:

a) Age (Continuous)
b) Sex (Male/Female)

c) History of diabetes mellitus (Yes/No)
d) NIHSS score at randomisation (Continuous)
e) Time from the onset of symptom to administration time (Minute, Continuous)
f) Stroke etiology
g) Degree of vascular stenosis (≤50% vs. >50%)
h) Location of responsible vessel (anterior circulation/posterior circulation)

From the above model, the adjusted RD and 95% CI comparing the dual antiplatelet treatment to the intravenous alteplase will be derived. In addition, binomial-log regression model will be used to calculate RR and 95% CI.

The above binomial-identity regression model 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 dual antiplatelet treatment and 0 for intravenous alteplase) 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 binomial-identity regression model.

Imputation for baseline missing covariates (see description below **8.4 missing data**) will be made for covariate adjusted analysis. If a covariate has over 50% value missing, the variable will be dropped from covariate adjusted analysis.

In addition, as a sensitivity analysis, inverse probability weighting (IPW) using propensity score will be used to calculate the adjusted RD and RR.

6.1.4. Subgroup analysis of the primary outcome

These followed covariates by age (<65 years vs. ≥65 years), sex (male vs. female), diabetes (present vs. not present), NIHSS score at randomisation (0-3 vs. 4-5), time from onset to treatment (<2 hours vs. ≥2 hours), stroke etiology (arteriosclerosis vs. small vessel lesion), degree of vascular stenosis (≤ 50% vs. > 50%), location of index vessel (anterior circulation vs. posterior circulation) will be included in subgroup analyses by performing the above unadjusted binomial-log regression model analysis separately for each category of a subgroup covariate. RD and RR and their 95% CIs will be presented for subgroup analysis.

Assessment of the homogeneity of treatment effect as measured with RR by a subgroup variable will be conducted by a binomial-log regression model with the treatment, subgroup variable, and their interaction term as predictors, and the p-value presented for the interaction term.

6.2. Secondary outcome analysis

Secondary outcome analyses will be based on the full analysis set, PP and AT populations.

6.2.1 Analysis of binary outcomes

Proportion of mRS (0-2) at 90 days, occurrence of early neurological improvement at 24 hours, and occurrence of early neurological deterioration at 24 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 endpoint by means of GLMs. The RD and RR and their two-sided 95% CIs between early antiplatelet and Control will be estimated.

The analysis of other binary outcomes will also use binomial-identity regression models with treatment as the only predictor. RD and RR with their two-sided 95% CIs comparing two treatment arms will be derived from the GLM models.

6.2.2 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 two-sided 95% CI for comparing two treatment groups.

6.2.3 Analysis of continuous outcome

The NIHSS score is measured at admission and 24 hours later.

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

We will calculate the change of NIHSS score for each patient between randomisation and 24 hours, and used a GLM with normal distribution and identity link function to compare the means in the change from baseline between the 2 groups. Log transformation may be performed if normality or variance homogeneity assumptions for residuals are violated after visual inspection of their histogram and scatterplots.

6.2.4 Analysis of ordinal outcome

The mRS score is measured at 90 days and will be summarised by number (%) of participants with event by treatment group. The ordinal logistic analysis via GLM will be used to derive odds ratio (OR) and its two-sided 95% CI for comparing two treatment groups.

6.3. Exploratory analysis

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

7. SAFETY ANALYSES

7.1. Safety variables

All safety analyses will be performed on the safety population.

Adverse events (AEs) will be restricted to those occurring during the 90 days after randomisation.

AEs will be summarised using the number of AEs, the number (%) of participants with AEs by treatment arms. The number of patients with any AE or SAEs will be analysed using logistic regression model from which odds ratios and its two-sided 95% CI will be calculated.

Safety analyses will summarise the number of any adverse medical events, serious adverse events (SAEs), and deaths occurring after randomisation.

Summaries of the total number of reported AEs/SAEs and number of participants reporting at least one AEs/SAE will be presented by treatment received and overall. In addition, summaries of the suspected relationship with trial treatment, suspected trial treatment or other cause, duration of recovered SAEs, seriousness criteria, event outcome, DAIDS grade and SAE, will be presented by treatment received and overall.

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

a) Randomised treatment
b) DAIDS grade
c) Event description
d) Seriousness criteria
e) Suspected relationship to the trial medications
f) Suspected products
g) Other causality
h) Expectedness
i) Date of randomisation
j) Date of onset
k) Date event became serious (serious events only)
l) Date of recovery
m) Outcome
n) Details of the treatment received

8. GENERAL CONSIDERATIONS FOR DATA ANALYSES

SPSS® (version 23) and SAS 9.4 will be used to perform all data analyses. R may also be used for some data analyses and generate the majority of data displays.

8.1. Covariates analyses

Covariate analyses will be performed on the primary outcome and secondary outcomes on the full analysis set, PP and AT populations. Other covariate analyses will be performed if deemed necessary.

8.2. Subgroup analysis

Subgroup analyses will be performed for the primary outcome on the full analysis set, PP, and AT 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.

8.3. 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.

8.4. Missing data

All efforts will be made to minimize the amount of missing data, particularly the 90-day outcome assessment.

8.4.1. Baseline covariates

Missing baseline covariates will be imputed using simple imputation methods in the covariate adjusted analysis based on the covariate distributions, should the missing values for a particular covariate be less than 5%. For a continuous variable, missing values will be imputed from random values from a normal distribution with mean and SD calculated from the available sample. For a categorical variable, missing values will be imputed from random values from a uniform distribution with probabilities P_1, P_2, …, and P_k from the sample.

If the missing values for a covariate are ≥5% but less than 50%, then they will be imputed using multiple imputation (M=10). If a covariate has over 50% value missing, the variable will be dropped from covariate adjusted analysis. The seed for the imputations will be 030818.

8.4.2. Efficacy outcomes

Sensitivity analyses based on different hypotheses about the missingness pattern of the primary outcome will be performed to test for the robustness of the primary analysis results. Please refer to Section 6.1.2.

8.5. Further exploratory analyses

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

8.6. Data summaries

Continuous variables will be summarised according to number of subjects with non-missing data (n), mean, standard deviation (SD), 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 number of subjects in the treatment arm with data available, unless noted otherwise. Event rates per 100 person years will be reported for time-to-event clinical outcomes and adverse events of special interest.

8.7. Interim analysis

An independent Data Monitoring Committee (DMC) was set up to ensure ongoing review of safety data, especially bleeding events. It was planned in the original protocol that an interim analysis will be performed after 50% of subjects have completed follow-up. However, the planned interim analysis is removed from the updated protocol (2.0 version) after the discussion of the Steering Committee with DMC for the following reasons: (1) there is a good safety profile in this trial to date, while adjustment for the ahlpa error spent on the interim analysis would lead to an increased sample size; (2) stopping rules for the interim analysis was not specified in the original protocol.

9. REFERENCES

1. Wardlaw JM, Zoppo G and Yamaguchi T. Thrombolysis for acute ischaemic stroke. Cochrane Database Syst Rev 2003; 3: CD000213.
2. Hacke W, Kaste M, Bluhmki E, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 2008; 359: 1317–1329.
3. Reeves M, Khoury J, Alwell K, et al. Distribution of national institutes of health stroke scale in the Cincinnati/Northern Kentucky stroke study. Stroke 2013; 44: 3211–3213.
4. Dhamoon MS, Moon YP, Paik MC, et al. Long-term functional recovery after first ischemic stroke. Stroke 2009; 40: 2805–2811.

5. Demaerschalk BM, Kleindorfer DO, Adeoye OM, et al. Scientific rationale for the inclusion and exclusion criteria for intravenous alteplase in acute ischemic stroke. Stroke 2016; 47: 581–641.

6. Jauch EC, Saver JL, Adams HP, et al. Guidelines for the early management of patients with acute ischemic stroke. Stroke 2013; 44: 870–947.

7. Barber PA, Zhang J, Demchuk AM, Hill MD and Buchan AM. Why are stroke patients excluded from tPA therapy? An analysis of patient eligibility. Neurology 2001; 56: 1015–1020.

8. Pooja KH, Dawn OK, Thomas D, et al. Effect of alteplase vs aspirin on functional outcome for patients with acute ischemic stroke and minor nondisabling neurologic deficits: the PRISMS randomized clinical trial. JAMA 2018; 320: 156–166.

9. Wang Y, Wang Y, Zhao X, et al. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. N Engl J Med 2013; 369: 11–19.

10. Pan Y, Jing J, Chen WQ, et al. Risks and benefits of clopidogrel-aspirin in minor stroke or TIA: time course analysis of CHANCE. Neurology 2017; 88: 1906–1911.

11. Johnston SC, Easton JD, Farrant M, et al. Clopidogrel and aspirin in acute ischemic stroke and high-risk TIA. N Engl J Med 2018; 379: 215–225.

12. Sacco RL, Kasner SE, Broderick JP, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2013;44: 2064-2089.

13. Khatri P, Tayama D, Cohen G, et al. Effect of Intravenous Recombinant Tissue-Type Plasminogen Activator in Patients With Mild Stroke in the Third International Stroke Trial-3: Post Hoc Analysis. Stroke 2015; 46: 2325-2327.

Detail of Statistical Analysis Plan Change

Page, Section	**Version 1.0**	**Version 2.0**
P1, Protocol version and date	Version 1.0 of August 03, 2018	**Change:** Version 2.0 of May 6, 2020
P2, Version Date SAP Version	 August 03, 2018 1.0	**Change:** May 6, 2020 2.0
P5, ABBREVIATIONS	-	**Add:** AT As Treated IPW Inverse Probability Weighting
P7, 3.1.2 Secondary objectives	-	**Add:** b) To determine an ordinal shift of the full range of mRS scores at 90 days.
P8, 3.2.2 Secondary outcomes	-	**Add:**

		b) An ordinal shift of the full range of mRS scores at 90 days (ordinal outcome);
P10, 4.5 Sample size	This trial mainly detects a 10% absolute difference in the proportion of the dual antiplatelet group with favorable outcome with 80% power and a one-sided type I error rate of 0.025 to test the non-inferiority, and the proportion of 65% of the dual antiplatelet group will experience a favorable outcome referring to previous report [13]. Thus, the sample size of 716 subjects resulted from these assumptions. According to the ITT principle, the maximum sample size is 752 subjects with 5% lost. Finally, a total of 760 subjects are expected to include in this study, with 380 patients in each group.	**Change:** According to our recent unpublished data, the proportion of expected excellent functional outcome (mRS 0-1) at 90 days in control group is estimated to be about 87%, and the proportion in the experimental group is estimated to be about 89.5% based on PRISMS [8]. The margin of non-inferiority was defined as 4.5% in our trial, which was based on the subset analysis of the third international stroke trial (IST-3) showing a 9% absolute difference in the proportion of favorable outcome in minor nondisabling patients treated versus untreated with intravenous alteplase [13]. Using power = 80% and $\alpha = 0.05$ to carry out the two-side test, the required sample size to test the non-inferiority hypothesis is 666. In consideration of 12% lost to follow-up, the total sample size is 757. Therefore, this study still included 760 patients, with 380 patients in each group. **Rationale:** (1) The trial was originally planned as a noninferiority study but the sample size calculation was erroneously made for a superiority trial. This error was found by the DMC statistician (Dr. Yi-Long Wang) on April 2020 and was corrected in this version.

		(2) We found that 65% of the estimated primary outcome in the original protocol was not correct regarding excellent functional outcome in the alteplase arm. Our cohort study found that among patients with minor stroke, 85% achieved excellent outcome at 90 days [1]. Based on this result and the nature of nondisabling minor stroke in the current trial, the proportion of excellent outcome was estimated as 87% in the alteplase arm in final protocol. Referring to the results of PRISMS trial showing numerically higher proportion of excellent outcome in the aspirin arm compared to the alteplase arm [2], the proportion of excellent outcome in DAPT arm was changed from 65% to 89.5% in the final protocol. (3) The assumption of a 10% absolute difference in the original protocol was not appropriate. Given the noninferiority design, the noninferiority margin of -4.5 percentage points was chosen in the final protocol, which was based on the Third International Stroke Trial, where subgroup analysis showed a 9% absolute difference in the proportion of favorable outcome as measured by the Oxfordfordshire Handicap Score (OHS 0 to 1) in patients with minor stroke who were treated with intravenous alteplase compared to standard medical treatment [3]. (4) The changes were not informed by review of the study data.

P11, 5.1 Study population data sets	-	**Add:** As-treated (AT) population As-treated (AT) population is based on the treatment actually received. There is no single CRF question that determines the As-treated arm, and the arm will be determined by the trial statistician.
P11, 5.1 Study population data sets	The PP population will be used for the supportive analyses.	**Change:** The PP and AT populations will be used for the supportive analyses.
P13, 6.1.3. Covariate adjusted analysis of the primary outcome	a) Age (Continuous) b) History of diabetes mellitus (Yes/No) c) Time from the onset of symptom to administration time (Hour, Continuous) d) Stroke etiology e) Degree of vascular stenosis (≤50% vs. >50%) f) Location of responsible vessel (anterior circulation/posterior circulation)	**Change:** a) Age (Continuous) b) Sex (Male/Female) c) History of diabetes mellitus (Yes/No) d) NIHSS score at randomization (Continuous) e) Time from the onset of symptom to administration time (Hour, Continuous) f) Stroke etiology g) Degree of vascular stenosis (≤50% vs. >50%) h) Location of responsible vessel (anterior circulation/posterior circulation)

P14, 6.1.3. Covariate adjusted analysis of the primary outcome		**Add:** If a covariate has over 50% value missing, the variable will be dropped from covariate adjusted analysis. In addition, as a sensitivity analysis, inverse probability weighting (IPW) using propensity score will be used to calculate the adjusted RD and RR.
P14, 6.1.4. Subgroup analysis of the primary outcome	-	**Add:** sex (male or female), NIHSS score at randomization (0-3 or 4-5),
P14, 6.2. Secondary outcome analysis	Secondary outcome analyses will be based on the full analysis set and PP populations.	**Change:** Secondary outcome analyses will be based on the full analysis set, PP and AT populations.
P15, 6.2. Secondary Outcome Analysis	-	**Add:** 6.2.4 Analysis of ordinal outcome The mRS score is measured at 90 days and will be summarized by number (%) of participants with event by treatment group. The ordinal logistic analysis via GLM will be used to derive odds ratio (OR) and its 2-sided 95% CI for comparing two treatment groups.

P16, 8. GENERAL CONSIDERATIONS FOR DATA ANALYSES	SPSS® (version 20) will be used to perform all data analyses.	**Change:** SPSS® (version 23) and SAS 9.4 will be used to perform all data analyses. R may also be used for some data analyses and generate the majority of data displays.
P17, 8.1. Covariates Analyses	Covariate analyses will be performed on the primary outcome and secondary outcomes on the full analysis set and PP populations.	**Change:** Covariate analyses will be performed on the primary outcome and secondary outcomes on the full analysis set, PP and AT populations.
P17, 8.2. Subgroup Analysis	Subgroup analyses will be performed for the primary outcome on the full analysis set and PP populations.	**Change:** Subgroup analyses will be performed for the primary outcome on the full analysis set, PP, and AT populations.
P 18, 8.7. Interim analysis	An independent Data Monitoring Committee (DMC) was set up to ensure ongoing review of safety data, especially bleeding events. It was planned in the original protocol that an interim analysis will be performed after 50% of subjects have completed follow-up.	**Change:** An independent Data Monitoring Committee (DMC) was set up to ensure ongoing review of safety data, especially bleeding events. It was planned in the original protocol that an interim analysis will be performed after 50% of subjects have completed follow-up. However, the planned interim analysis is removed from the updated protocol (2.0 version) after the discussion of the steering committee with DMC for the

		following reasons: (1) there is a good safety profile in this trial to date, while adjustment for the ahlpa error spent on the interim analysis would lead to an increased sample size; (2) stopping rules for the interim analysis was not specified in the original protocol.
P19, Reference	13. Yeatts SD, Broderick JP, Chatterjee A, et al. Alteplase for the treatment of acute ischemic stroke in patients with low National Institutes of Health Stroke Scale and not clearly disabling deficits (Potential of rtPA for Ischemic Strokes with Mild Symptoms PRISMS): Rationale and design. Int J Stroke 2018; 13: 654-661.	13. Khatri P, Tayama D, Cohen G, et al. Effect of Intravenous Recombinant Tissue-Type Plasminogen Activator in Patients With Mild Stroke in the Third International Stroke Trial-3: Post Hoc Analysis. Stroke 2015; 46: 2325-2327.

References:

1. Wang X, Li X, Xu Y, et al. Effectiveness of intravenous r-tPA versus UK for acute ischaemic stroke: a nationwide prospective Chinese registry study. Stroke Vasc Neurol 2021; 6: 603-609.
2. Khatri P, Kleindorfer DO, Devlin T, et al. Effect of Alteplase vs Aspirin on Functional Outcome for Patients With Acute Ischemic Stroke and Minor Nondisabling Neurologic Deficits: The PRISMS Randomized Clinical Trial. JAMA 2018; 320: 156-166.
3. Khatri P, Tayama D, Cohen G, et al. Effect of Intravenous Recombinant Tissue-Type Plasminogen Activator in Patients With Mild Stroke in the Third International Stroke Trial-3: Post Hoc Analysis. Stroke 2015; 46: 2325-2327.