STATISTICAL AND EPIDEMIOLOGICAL ANALYSIS PLAN (SEAP) FOR NON-INTERVENTIONAL STUDIES (NIS)

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NIS Lead [SEAP reviewer]	[redacted]
NIS Data Manager [SEAP reviewer]	[redacted]

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1. TABLE OF CONTENTS

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2. LIST OF ABBREVIATIONS

AIS	Acute Ischaemic Stroke
ASD	Absolute Standardised Difference
BI	Boehringer Ingelheim
CI	Confidence Interval
DMRP	Data Management and Review Plan
GPP	Good Pharmacoepidemiology Practice
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	Intravenous
IVT	Intravenous Thrombolysis
mRS	Modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
NIS	Non-Interventional Study(ies)
PS	Propensity Score
PSM	Propensity Score Matching
rt-PA	Recombinant Tissue Plasminogen Activator
SOP	Standard Operation Procedure
tPA	Tissue-type Plasminogen Activator
ZSQCC	Zhejiang Stroke Quality Control Centre

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3. RESPONSIBLE PARTIES

NIS Statistician [SEAP author]

[redacted]

SEAP reviewers are:

- BI NIS Lead [SEAP reviewer] (in all cases)
- NIS Data Manager [SEAP reviewer] (in all cases)
- RWE CoE [SEAP reviewer] (for all globally initiated studies and for local studies) involving BI products and Global NIS not involving BI products,
- TSTAT (for NISnd only)
- TM Epi [SEAP reviewer] (When BI NIS lead is not TM Epi; in all cases)

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4. PURPOSE AND SCOPE

This is a complete statistical and epidemiological analysis plan for the research about what are the 1-year clinical outcomes among AIS patients who were treated with IV rt-PA within 4.5 hours of symptom onset compared with those who arrived or were admitted to the hospital within 4.5 hours of symptom onset and did not receive any reperfusion treatment in a real-world clinical setting, including the 1-year all-cause mortality and neurological functional outcomes. More detailed and explicit statistical analysis methods and procedures are presented here, especially the Propensity Score Matching method.

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5. AMENDMENTS AND UPDATES

None

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6. RESEARCH QUESTION AND OBJECTIVE

Research question: In a real-world clinical setting, what are the 1-year clinical outcomes among AIS patients who were treated with IV rt-PA within 4.5 hours of symptom onset compared with those who arrived or were admitted to the hospital within 4.5 hours of symptom onset and did not receive any reperfusion treatment?

Primary objective:

- To compare the 1-year mortality of AIS patients treated with IV rt-PA within 4.5 hours of symptom onset versus those who arrived or were admitted to the hospital within 4.5 hours of symptom onset and did not receive reperfusion treatment.

Secondary objective:

- To compare the 1-year neurological functional outcome (as measured by Modified Rankin Scale [mRS]) of AIS patients treated with IV rt-PA within 4.5 hours of symptom onset versus those who arrived or were admitted to the hospital within 4.5 hours of symptom onset and did not receive reperfusion.

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7. RESEARCH METHODS

7.1 STUDY DESIGN

This study is a non-interventional study (NIS) based on existing data. We will analyse data of AIS patients aged ≥ 18 years in China collected from the ZSQCC platform.[P21-04780], [P21-04783], [P21-04784], [P21-04779], [P21-04782]

Approximately 11700 AIS patients in total are recorded in the ZSQCC platform and will be analysed in this study. There were approximately 8500 AIS patients (≥ 18 years of age) who were treated with IV rt PA and 3200 patients who were admitted to the hospital within 4.5 hours of symptom onset and did not receive reperfusion treatment from Jan 2017 to Mar 2020. In the China National Stroke Registry I (2007-2008), the 12-month mortality rate for post-stroke patients was approximately 14%.[R21-1852] For this study, it is assumed that 1-year mortality is 12% and 15% for the rt-PA treatment cohort and the cohort who did not receive reperfusion, respectively. In total, 6069 patients with a treatment ratio 2:1 are needed to observe 855 events and to detect the difference (hazard ratio=0.79) with a 2-sided alpha of 0.05 and 90% power. In addition, considering a treatment ratio 1:1 with the same assumption described above with power of 90% at a 2-sided, 5% significant level, roughly 5400 patients are needed to observe 756 events to detect the difference.

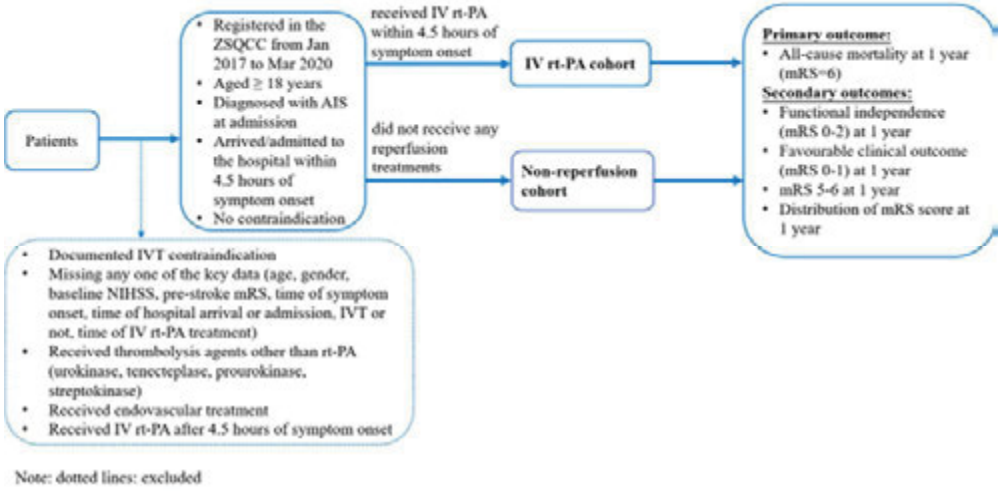
Patients meeting the inclusion and exclusion criteria will be divided into 2 cohorts:

- IV rt-PA cohort: Patients who received IV rt-PA within 4.5 hours of symptom onset
- Non-reperfusion cohort: Patients who arrived or were admitted to the hospital within 4.5 hours of symptom onset and did not receive any reperfusion treatment

Propensity score matching (PSM) will be used to match the baseline characteristics between the above 2 cohorts. The primary, secondary outcomes, as well as baseline characteristics will be compared between the matched cohorts.

The flow of data selection for each of the 2 reporting patient cohorts is depicted in Figure 1 below.

Figure 1 Patient cohorts selected for the non-interventional study



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7.2 SETTING

In this study, AIS patient data from 80 stroke centres in the ZSQCC platform from January 2017 to March 2020 will be used.

7.3 STUDY POPULATION

No sampling will be undertaken and all patients who meet all the inclusion criteria and none of the exclusion criteria will be included.

The inclusion and exclusion criteria are listed below:

Inclusion criteria:

- Patients registered in the ZSQCC platform from Jan 2017 to Mar 2020
- ≥ 18 years of age
- Diagnosed with AIS at admission
- Arrived or admitted to the hospital within 4.5 hours of symptom onset
- If treated with IV rt-PA: received IV rt-PA within 4.5 hours of symptom onset

Exclusion criteria:

- Documented IVT contraindication except age to IV rt-PA treatment according to the SmPC
- Missing any one of the key data (age, gender, baseline National Institutes of Health Stroke Scale [NIHSS], time of symptom onset, time of hospital arrival or admission, IVT or not, time of IV rt-PA treatment)
- Received thrombolysis agents other than rt-PA (urokinase, tenecteplase, recombinant plasminogen activator, prourokinase, streptokinase)
- Received endovascular treatment
- Received IV rt-PA after 4.5 hours of symptom onset

7.4 STUDY VISITS

Not applicable.

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8. VARIABLES

8.1 EXPOSURES

The exposure of this study is IV rt PA treatment within 4.5 hours of symptom onset.

8.2 OUTCOMES

8.2.1 Primary outcomes

- All-cause mortality at 1 year (mRS=6)

8.2.2 Secondary outcomes

- Functional independence (mRS 0-2) at 1 year
- Favourable clinical outcome (mRS 0-1) at 1 year
- mRS 5-6 at 1 year
- Distribution of mRS score at 1 year

8.3 COVARIATES

The covariates to be collected at baseline are as follows:

- Demographic and sociological characteristics
  - Age
  - Gender (male, female)
  - Medical insurance status (urban employee basic medical insurance, urban resident basic medical insurance, new rural cooperative medical insurance, other insurance, no insurance)
- Lifestyle related characteristics
  - Smoking status (current smoker, former smoker, never smoker)
- Stroke severity (baseline NIHSS)
- Pre-stroke mRS
- Time from symptom onset to hospital admission
- For patients in the IV rt PA cohort:
  - Time from symptom onset to treatment
  - Time from hospital admission to treatment
  - rt PA dosage (dichotomised as standard dosage and low dosage)
- Comorbidities at baseline
  - Diabetes
  - Coronary artery disease
  - Atrial fibrillation
  - Prior stroke/transient ischaemic attack
  - Hypertension
- Co-medication at baseline
  - Anti-platelet
  - Oral anticoagulation
  - Lipid lowering
- Hospital level
  - Grade 2
  - Grade 3

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Other covariates including:

- Duration of hospitalization
- Reasons for not being treated with rt-PA for rt-PA non treatment group

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9. DATA SOURCES

The current study will be conducted based on data from ZSQCC platform, which is a continuous comprehensive reporting system that collects approximately 100000 patient-level data from 80 stroke centres from January 2017 to March 2020.

All medical documents for consecutive acute stroke patients admitted in the stroke centre will be provided by investigators from ZSQCC. Only the de-identified scanned documents will be preserved as images in a safe information database. The local infrastructure and characteristics of each recruited centre will also be recorded.

The database includes patient demographic information, baseline clinical characteristics, indicators related to diagnosis and treatment during hospitalisation and follow-up after discharge. The platform staffs tried to follow up all discharged patients by a phone call at 1-year discharge. Telephone follow-up of the platform was conducted by platform staffs. The follow-up standards were unified, and telephone recordings were all kept. Neurologists conducted trainings at regular intervals for platform staffs and randomly checked the telephone recordings. Several studies based on this dataset were approved by the Second Affiliated Hospital of Zhejiang University Institutional Review Board (IRB) and were published in peer-reviewed journals.[P21-04780], [P21-04783], [P21-04784], [P21-04779], [P21-04782]

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10. DATA MANAGEMENT AND SOFTWARE/TOOLS

10.1 SOFTWARE/TOOLS

Propensity score matching (PSM) will be performed using R 4.0.5 (package: MatchIt) and other analyses will be conducted with SAS 9.4.

10.2 HANDLING OF MISSING VALUES

For comorbidities at baseline (including diabetes, coronary artery disease, atrial fibrillation, prior stroke/transient ischaemic attack, and hypertension) and co-medication at baseline (including anti-platelet, oral anticoagulation, and lipid lowering), missing values will be regarded as absence of those comorbidity and co-medication. For medical insurance status, smoking status, and hospital level, missing values will be assigned to a separate group. Medians will be imputed for missing values of Pre-stroke mRS.

10.3 HANDLING OF INCONSISTENCIES IN DATA AND OUTLIERS

Inconsistencies in Data and Outliers will be re-checked. If they are still abnormal (for example: age > 120; gender is neither male nor female), they will be removed from the analysis.

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11. DATA ANALYSIS

11.1 MAIN ANALYSIS

Descriptive data regarding patient demographic, lifestyle-related, and clinical characteristics will be summarized. For categorical measures, data will include the frequency (number of patients with specific cases [n]) and percentage (%) of total study patients observed in each category (N). All variables will be summarized descriptively by tabular displays of mean, standard deviations, median, inter-quartile ranges, and ranges of continuous variables, and frequency distributions of categorical variables. When necessary, continuous variables also will be categorized into intervals, with the distribution of patients (n, N, %) for each interval provided. We will compare the baseline characteristics of the 2 treatment cohorts (patients who received IV rt-PA and patients who did not receive reperfusion treatment) through the absolute standardized difference (ASD) method, where at least 0.1 ASD will be considered a significant difference.

To account for potential confounding, the 2 cohorts will be 1:1 or 2:1 matched by baseline characteristics using the Propensity Score Matching method. The feasibility of PSM will be evaluated based on available sample size and descriptive results. If patient characteristics between the 2 cohorts are significantly different, i.e., less than 50% of patients in the IV rt PA cohort can be matched to the non-reperfusion cohort based on PSM, the study design will be re-evaluated before proceeding to analysis. The PSM aims to balance the 2 cohorts on baseline covariates. The propensity score model will include age, gender, baseline NIHSS/ NIHSS categories (0-4, 5-10, 11-15, 16-21, and $\geqslant$ 22), pre-stroke mRS, medical insurance status, smoking status, hospital level, diabetes, coronary artery disease, atrial fibrillation, prior stroke/transient ischaemic attack, hypertension, anti-platelet, lipid lowering, oral anticoagulation, and time from symptom onset to hospital admission. The method of Nearest Neighbour matching will be used (a maximum caliper width equal to 0.2 of the standard deviation of the logit of the propensity score) to select the matched samples. Then we will compare the baseline characteristics of the 2 propensity-score-matched cohorts. The absolute standardized difference (ASD) between the 2 PS-matched cohorts will be calculated, where at least 0.1 ASD will be considered a significant difference. For baseline covariates that are not sufficiently balanced after PSM, they will be included in an appropriate multivariate model to adjust for those differences.

For the primary outcome, a comparison of the percentage of 1-year* all-cause mortality (mRS=6) between the 2 matched cohorts will be conducted using a Chi-square test. A Kaplan-Meier curve with log-rank test will be used to analyse the data. Cox regression models will be used to estimate hazard ratios (95% confidence interval) between the 2 matched cohorts.

For the secondary outcomes, descriptive summaries will be conducted in the PS-matched cohorts separately. For the categorical variables including functional independence (mRS 0-2), favourable clinical outcome (mRS 0-1), and mRS 5-6 at 1 year, the Chi-square test and conditional logistic regression model will be conducted. For the distribution of mRS score at 1 year, the Wilcoxon rank sum test and ordinal logistic regression model will be performed to calculate the odds ratio and 95% CI.

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The primary and secondary outcomes will be analysed using the same method described above in the following subgroups:

- Baseline NIHSS score (0-4, 5-10, 11-15, 16-21 and ⩾ 22)
- Onset to treatment time (⩽ 90 mins, 91-180 mins, and 181-270 mins)
- Age (18-80 years, > 80 years)
- rt PA dose (low dose of ~0.6 mg/kg, standard dose 0.9 mg/kg)

[redacted]

*Note: 1 year in this analysis is defined as 12±1 months.

11.2 SAFETY ANALYSIS

This study is a NIS based on existing data. From safety information collecting and reporting perspective, this study will not involve individual medical record review, thus no adverse event/adverse drug reaction information is required to collect. The analysis refers to Section 10.1.

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12. QUALITY CONTROL

The statistical analytic approach and programming code will be reviewed/repeated by a second analyst.

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13. REFERENCES

13.1 PUBLISHED REFERENCES

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13.2 UNPUBLISHED REFERENCES

None

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ANNEX 1. ADDITIONAL INFORMATION

None

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ANNEX 2. REVIEWERS AND APPROVAL SIGNATURES

The NIS SEAP must be sent for review to the following individuals **prior to approval**.

Reviewer	NIS involving BI product(s)	NIS not involving BI product(s)	
		Global NIS	Local NIS
NIS Lead	X	X	X
Global TM Epi*	X	X	X
NIS Data Manager	X	X	X
TSTAT (for NISnd only)	X	X	X
RWE CoE	X	X	

** When BI NIS lead is not TM Epi*

Study Title: 1-year clinical outcomes after intravenous rt-PA for Chinese AIS patients

Study Number: 0135-0350

Protocol Version: 1.0

I herewith certify that I agree to the content of the study SEAP and to all documents referenced in the study SEAP.

Position: _____ Name/Date: _____ Signature: _____

Position: _____ Name/Date: _____ Signature: _____