PROTOCOL

Version 1.0 of October 18, 2018

Argatroban plus r-tPA for Acute Ischaemic Stroke (ARAIS): a prospective, randomised, open-label, blinded-end point, multi-centre trial

Chief Investigators

Prof Hui-Sheng Chen

Department of Neurology

General Hospital of Shenyang Military Region

Shenyang, 110016, China

Tel: 86-13352452086

E-mail: chszh@aliyun.com

Prof Yi-Long Wang

Department of Neurology

Beijing Tiantan Hospital,

Beijing, 100070, China

Tel: 86-13911666571

E-mail: yilong528@aliyun.com

The protocol was drafted before the trial began, and was never amended after Version 1.0.

Table of Contents

Abstract

Title	Argatroban plus r-tPA for Acute Ischemic Stroke (ARAIS): a prospective, randomised, open-label, blinded-end point, multi-centre trial
Principle Centre	General Hospital of Shenyang Military Region
Sponsor	Cerebrovascular Disease Collaboration Innovation Alliance (CDCIA) – Liaoning
Objective	To explore the efficacy and safety of Argatroban plus r-tPA for ischaemic stroke
Efficacy Outcome	**Primary outcome：** 1. Proportion of modified Rankin Score (mRS, 0–1) at 90±7 days after randomisation. **Secondary outcome：** 1. Proportion of mRS (0–2) at 90±7 days after randomisation; 2. Incidence of early neurological improvement, defined as more than or equal to 2 NIHSS scores decrease, compared with baseline at 48 hours; 3. Incidence of early neurological deterioration, defined as more than or equal to 4 NIHSS scores increase, but not result of cerebral haemorrhage, compared with baseline at 48 hours; 4. Change in National Institute of Health Stroke Scale (NIHSS) score compared with baseline at 14 days; 5. Occurrence of stroke or other vascular events (transient ischaemic 6. attack, myocardial infarction, and vascular death) at 90±7 days;
Safety Outcome	1. Proportion of symptomatic intracerebral haemorrhage; 2. Proportion of parenchymal hematoma type 2; 3. Major systemic bleeding.
Trial Design	This is a prospective, randomised, open-label, blinded-endpoint, multicentre trial. Subjects included are randomly assigned into two groups: the experiment group and the control group. Follow-up was to be performed at baseline, 24 hours, 7±1 days, 14±2 days, and 90±7 days after randomisation.

	The primary outcome assessors were masked to the allocation assignment.
Trial Population	Patients with acute ischaemic stroke
Sample Size	808
Inclusion criteria	1. Male or female participants (18 ≤ age ≤ 80 years old); 2. Acute ischaemic stroke confirmed by head CT or MRI; 3. Time from symptom onset to the administration of r-tPA ≤ 4·5 h; 4. NIHSS ≥6 at the time of randomisation; 5. Signed informed consent.
Exclusion criteria	1. Serve neurological deficit before stroke onset (premorbid mRS ≥ 2) 2. History of stroke within 3 months; 3. History of intracranial haemorrhage; 4. Suspected subarachnoid haemorrhage; 5. Intracranial tumour, vascular malformation or arterial aneurysm; 6. Major surgery within 1 month; 7. Systolic pressure ≥180 mmHg or diastolic pressure ≥110 mmHg; 8. Platelet count < 10^5/mm^3; 9. Heparin therapy or oral anticoagulation therapy within 48 hours; 10. Abnormal APTT; 11. Thrombin or Xa factor inhibitor; 12. Severe disease with a life expectancy of less than 3 months; 13. Blood glucose < 50 mg/dL (2·7mmol/L); 14. Patients who have received any other investigational drug or device within 3 months; 15. Pregnancy; 16. Researchers consider patients inappropriate to participate in the registry.
Trial Cycle	All included patients were followed up at baseline, 24 hours, 48 hours, 7 ± 1 days, 14 ± 2 days, and 90 ± 7 days after randomisation, respectively.
Treatment Regimens	Eligible patients were randomly (a ratio of 1:1) assigned into the

	experimental group: argatroban (100 μg/kg intravenous argatroban bolus over 3 to 5 minute within 1 hour of the r-tPA bolus, followed by argatroban infusion of 1·0 μg/kg per minute for 48 h) plus r-tPA (0·9 mg/kg; maximum dose 90 mg, 10% administered as 1-minute bolus, the remaining infused over 1 hour) are administered, or the control group: r-tPA (0·9 mg/kg; maximum dose 90 mg, 10% administered as 1-minute bolus, the remaining infused over 1 hour) is administered.
Procedure	**Screening period:** On day 0 (baseline period), it is necessary to complete enrolment screening, and collect demographic characteristics, medical history (including history of hypertension, diabetes and drug treatment history, etc), brain imaging (computer tomography, computed tomography angiography or magnetic resonance imaging), neurological measurements (NIHSS score, mRS score), haematological examination (blood routine, blood glucose, hepatic and renal function, coagulation routine, urine routine, electrocardiogram, etc) and other information. **Treatment period:** The patients received argatroban plus r-tPA or r-tPA. **Follow-up period:** NIHSS score was assessed at 24 hours, 48 hours, 7 ± 1 days, and 14 ± 2 days after randomisation. The mRS score was assessed at 90 ± 7 days after randomisation. All concomitant medications, adverse events, stroke recurrence and other vascular events of each visit were recorded since the last visit. All the adverse events of included subjects should be recorded and tracked until properly resolved. All the serious adverse events of included subjects should be recorded and tracked, even if the subjects have finished the trial, until the events were resolved, or stabilization judged by the investigator.
Concomitant Treatment	Guideline-based treatment
Statistical Analysis	Intention-to-treat analysis will be used to compare the treatment effect between two groups and all the data will be analysed with Software Statistical Product and Service Solutions (version 23). The mean standard deviation will be used if the continuous data were normally distributed, and the median and interquartile range will be used if the continuous data were non-normally distributed. Categorical data will be expressed as number

	(percentage). When comparing the data of two groups, t test or Mann–Whitney test was used for continuous data, and chi-square test was used for categorical data. Logistic regression analysis will be performed for the proportion of an excellent outcome at 90 days, a favourable outcome at 90 days, early neurological improvement at 48 hours, early neurological deterioration at 48 hours and safety endpoints. An ordinal regression analysis will be conducted to investigate a treatment effect across the mRS scale. Generalised linear model was used to compare the decrease in NIHSS score from baseline to 14 days. The Cox regression analysis will be used for the risk of stroke recurrence or other vascular events at 90 days between two treatment groups. Statistical tests were considered significant when the two-sided *P* value was less than 0·05.
Sites Number	About 40
Duration	24 months

Abbreviation

Abbreviation	Full title
AE	Adverse Event
AIS	Acute Ischaemic Stroke
ALT	Alanine Aminotransferase
AST	Aspartate Transaminase
APTT	Activated Partial Thromboplastin Time
ARTSS	Argatroban With Recombinant Tissue Plasminogen Activator for Acute Stroke
BUN	Blood Urea Nitrogen
CDCIA	Cerebrovascular Disease Collaboration Innovation Alliance
Cr	Creatinine
CRF	Clinical Research Form
CT	Computed Tomography
DBIL	Direct Bilirubin
DMC	Data Monitoring Committee
ECG	Electrocardiogram
FIB	Human Fibrinogen
GCP	Good Clinical Practice
GLU	Glucose
HDL	High Density Lipoprotein
HGB	Haemoglobin
ID	Identification
IEC	Independent Ethics Committee
IQR	Inter-Quartile Range
IRB	Institutional Review Board
ITT	Intention to Treat
LDL	Low Density Lipoprotein
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale

NIHSS	National Institute of Health Stroke Scale
NRBC	Urine Red Blood Cell
NWBC	Urine White Blood Cell
RBC	Red Blood Cell
PLT	Platelets
PPS	Per Protocol Set
PRO	Urine Protein
PT	Prothrombin Time
r-tPA	recombinant tissue-type plasminogen activator
SAE	Serious Adverse Event
SD	Standard Deviation
SS	Safety Set
TBIL	Total Bilirubin
TC	Total Cholesterol
TG	Triglyceride
TOAST	Trial of Org 10 172 in acute stroke treatment
TT	Thrombin Time
WBC	White Blood Cell

1.Background and current state of knowledge

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.

2.Objectives of the trial

2.1 Hypothesis tested

This study intends to demonstrate that argatroban plus r-tPA will be superior to

r-tPA alone in improving functional outcomes in patients with AIS.

2.2 Primary objective

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

2.3 Secondary objectives

1. To determine the proportion of favourable 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.

3. Design and selection of patients

3.1 Trail plan

This is a prospective, randomised, open-label, blinded-endpoint, multicentre trial.

The patients were randomly assigned into the two groups:

Experimental group: intravenous argatroban (100 μg/kg bolus over 3 to 5 minutes) was administrated within 1 hour of the r-tPA bolus, followed by argatroban infusion of 1·0 μg/kg per minute for 48 hours after randomisation;

Control group: intravenous r-tPA standard dose of 0·9 mg/kg, up to a maximum of 90 mg;

Both groups of patients were given standard guideline-based treatment until to 90 days.

Follow-up was performed at baseline, 24 hours, 48 hours, 7 ± 1 days, 14 ± 2 days, and 90 ± 7 days after randomisation, respectively.

The primary outcome will be measured without knowledge of the allocation assignment by trained investigators in each trail site.

3.**2 Selection criteria:**

Inclusion criteria: in order to be eligible, the patients must meet all the following criteria:

1) Male or female participants (18 ≤ age ≤ 80 years old);
2) Acute ischaemic stroke confirmed by head CT or MRI;
3) Time from symptom onset to the administration of r-tPA ≤ 4·5 h;
4) NIHSS ≥6 at the time of randomisation;
5) Signed informed consent.

Exclusion criteria: in order to be included the patients must not have any of the following criteria:

1) Serve neurological deficit before stroke onset (premorbid mRS ≥ 2)
2) History of stroke within 3 months;
3) History of intracranial haemorrhage;
4) Suspected subarachnoid haemorrhage;
5) Intracranial tumour, vascular malformation, or arterial aneurysm;
6) Major surgery within 1 month;
7) Systolic pressure ≥180 mmHg or diastolic pressure ≥110 mmHg;
8) Platelet count < $10^5/mm^3$;
9) Heparin therapy or oral anticoagulation therapy within 48 hours;
10) Abnormal APTT;
11) Thrombin or Xa factor inhibitor;
12) Severe disease with a life expectancy of less than 3 months;
13) Blood glucose < 50 mg/dL (2.7mmol/L);

14) Patients who have received any other investigational drug or device within 3 months;

15) Pregnancy;

16) Researchers consider patients inappropriate to participate in the registry.

Suspension Criteria:

Trial suspension means that the clinical trial has not finished as planned, and all trials are stopped in the middle period. The purpose of trial suspension is to protect the rights and interests of subjects, ensure the quality of the trial, and avoid unnecessary economic losses:

1. The proportion of serious adverse events during the trial is higher than 10%, and an independent Data Monitoring Committee (DMC) has the right to terminate the study unconditionally.

2. Trial should be discontinued when one treatment is found to be significantly better than the other.

3.3 Duration of participation for each subject:

Each subject will be followed up in the trial for 3 months.

3.4 Randomization and measures to reduce bias:

In the trial, included patients were randomly assigned into the experiment group or control group using a randomisation (1:1) method with using block randomisation via a password-protected, web-based program at http://console.tt.zhinanmed.com (Beijing Zhinan Medical Technology Co., Ltd). The block size is four at a time. The number of randomised patients planned for this study is approximately 808.

NIHSS were performed by the same neurologists who were not blinded to treatment allocation in order to avoid potential bias as much as possible. To prevent the potential bias on the main results, 90-day clinical assessments including the primary endpoint (mRS) were evaluated in person by one qualified personnel who was blinded to treatment allocation according to a standardized procedure manual in each study centre. If an in-person visit was not possible, 90-day mRS was evaluated

through a structured interview for telephone assessment. To ensure validity and reproducibility of the evaluation, we held a training course for all investigators at each centre. Concomitant medications and adverse events within 90 days after randomisation will be recorded in detail by investigators. Central adjudication of clinical and safety outcomes was also done by assessors who were unaware of the randomised allocation assignment or clinical details.

4 Plan and conduct of the trial

4.1 Clinical trial flow chart

Item \ Period	Screening	Treatment	Follow-up			
Visit	1	2	3	4	5	6
Time	0 day[1]	24 hours after randomisation	48 hours after randomisation	7 ± 1 days after randomisation	14 ± 2 days after randomisation	90 ± 7 days after randomisation
Inclusion/Exclusion Criteria	x					
Sign informed consent	x					
Randomisation	x					
Demographic characteristics[2]	x					
Medical history	x					
Physical examination	x	x	x	x	x	x
Brain CT/MRI	x	x				
TOAST classification					x	
ECG（12 lead）	x				x	
24h ambulatory ECG[3]					x[3]	
Blood routine[4]	x				x	
Urine routine[5]	x					
Blood biochemistry[6]	x				x	
Coagulation routine[7]	x	x	x	x	x	
NIHSS score	x	x	x	x	x	
mRS score	x					x
Concomitant medication		x	x	x	x	x
Adverse events		x	x	x	x	x
Stroke recurrence and other vascular events[8]		x	x	x	x	x

1. Day 0: limited to the period from the onset of stroke to the time before randomisation;

2. Demographic characteristics: The age of the subjects is calculated based on the identification (ID) card information;
3. 24h ambulatory electrocardiogram: required to be completed within 7 days of admission;
4. Blood routine: including total number of red blood cells (RBC), total number of white blood cells (WBC), platelet count (PLT), haemoglobin (HGB). The results are measured within 24 hours before screening;
5. Urine routine: including urine red blood cells (NRBC), urine white blood cells (NWBC), urine protein (PRO), and urine sugar (GLU). The results are measured within 24 hours before screening;
6. Blood biochemistry: Hepatic function tests: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL); Renal function tests: serum urea nitrogen (BUN), Creatinine (Cr); Blood Glucose (GLU); Lipid, including total cholesterol (TC), triglycerides (TG), high density cholesterol (HDL), low density cholesterol (LDL). The results are measured within 24 hours before screening;
7. Coagulation examination: prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FIB), thrombin time (TT). The results are measured within 24 hours before screening;
8. Stroke recurrence and other vascular events: occurrence of stroke recurrence and other vascular events within 90 days from randomisation.

4.2 Study completion

The study is considered to be finished when the last visit of the last subject in the trial is completed.

4.3 Study termination

The sponsor reserves the right to close the study centre or suspend the study at any time. The study centre should be closed after the study is completed. The centre is considered closed after all required documentation and study supplies have been collected and a centre closure visit has been conducted.

Investigators can also request to suspend the study, but they must give reasonable reasons in advance.

Reasons for the sponsor or investigator to close the centre in advance may include, but are not limited to:

1. Investigator fails to comply with the requirements of the study protocol, IEC/IRB or local regulatory authorities, sponsor's operating procedures or GCP guidelines;
2. Security considerations;
3. Investigators are not recruiting enough subjects.

5. Study medications

5.1 Identification of study drugs

r-tPA (specification: 20 mg/ampoule or 50 mg/ ampoule, batch size: subject to the actual batch number of the drug issued, storage: sealed preservation, manufacturer: Boehringer Ingelheim Co., Ltd)

Argatroban is manufactured by Tianjin Institute of Pharmaceutical Research Co., Ltd.

5.2 Responsibilities of study drugs administration

The investigator of the clinical trial is responsible for ensuring that the study drugs received by the study centre are counted and recorded during the whole process of the study, and the distribution and recovery of the drug must be recorded in the Drug Dispensing and Recycling Record Form. The original packaging of the study drug must be returned, whether there is any remaining study drug in it. Returned study drugs should be kept at the site designated by the study centre and separate from unused study drugs. Do not destroy or mix medicines in different packages until inspectors have counted them. The use of the study drug must strictly follow the instructions in the protocol and the package label. The sponsor monitor must be allowed to count and reconcile unused and returned study drugs at each monitoring. Unused or returned study drugs to be destroyed must be recorded on the Drug Recycling Record Form and returned to the medication destruction centre.

6. Treatment administered to included subjects

6.1 Description of the treatment required to conduct the study

The experimental group received intravenous argatroban bolus (100 μg/kg) over 3 to 5 minute within 1 hour of the r-tPA bolus (0·9 mg/kg, up to a maximum of 90 mg), followed by argatroban infusion of 1.0 μg/kg/minute for 48 hours. This infusion rates of argatroban are adjusted to a target activated partial thromboplastin time (APTT) of 1·75 × baseline (±10%). A dosing algorithm was developed so that standardized increments or decrements of argatroban infusion rate took place in response to the APTT. Argatroban infusion was terminated immediately if major systemic bleeding or symptomatic intracerebral hemorrhage was suspected.

The control group received intravenous r-tPA (0·9 mg/kg, up to a maximum of 90 mg) followed by guideline-based treatment 24 hours after thrombolysis.

All patients were then given standard guideline-based therapy from 24 hours after intravenous thrombolysis to 90 days.

6.2 Permitted and prohibited medical drugs and treatments in the study

There are no specific medical treatments for this study, except that concomitant antithrombotic were not permitted during infusion of argatroban.

7. Outcome measurements

7.1 Primary efficacy outcome

Proportion of mRS (0−1) at 90±7 days after randomisation.

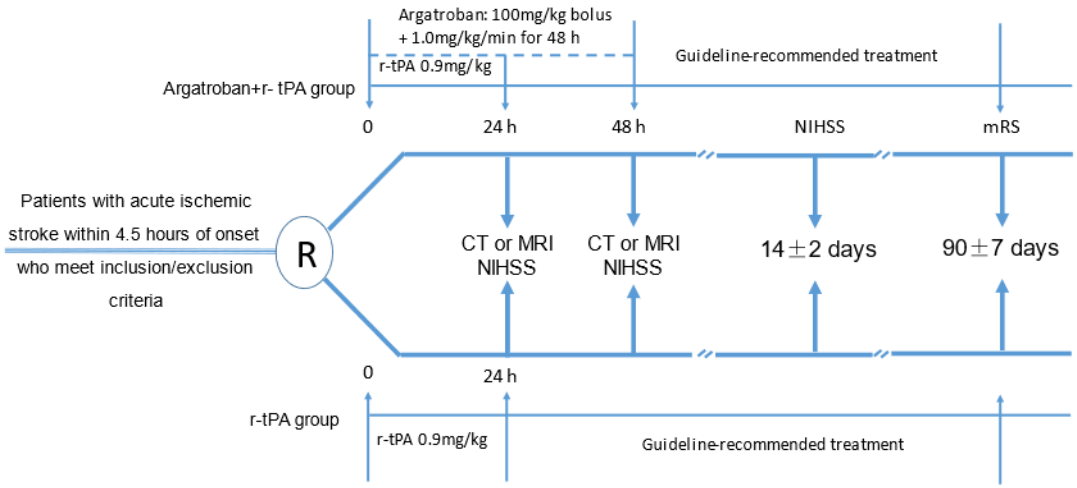
7.2 Secondary efficacy outcomes

1. Proportion of mRS (0−2) at 90±7 days after randomisation;
2. Incidence of early neurological improvement, defined as more than or equal to 2 NIHSS scores decrease, compared with baseline at 48 hours;
3. Incidence of early neurological deterioration, defined as more than or equal to 4 NIHSS scores increase, but not result of cerebral hemorrhage, compared with baseline at 48 hours;
4. Change in NIHSS score compared with baseline at 14±2 days;
5. Occurrence of stroke or other vascular events at 90±7 days;

7.3 Safety outcomes

1. Proportion of symptomatic intracerebral haemorrhage;
2. Proportion of parenchymal hematoma type 2;
3. Proportion of major systemic bleeding.

7.4 Study procedure



7.5 Study periods

Screening period: On day 0 (baseline period), it is necessary to complete enrolment screening, and collect demographic characteristics, medical history (including history of hypertension, diabetes and drug treatment history, etc), brain imaging (CT or MRI), neurological measurements (NIHSS score, mRS score), haematological examination (blood routine, blood glucose, hepatic and renal function, coagulation routine, urine routine, electrocardiogram, etc) and other information.

Treatment period: The two groups were given intravenous argatroban plus r-tPA with standard dose of 0·9 mg/kg (experimental group) or intravenous r-tPA with standard dose (control group). At the same time, patients were given standard guideline-based treatment. Brain CT or MRI were performed at 24 and 48 hours after randomisation.

Follow-up period: NIHSS score was assessed at 24 hours, 48 hours, 7 ± 1 days, and 14 ± 2 days after randomisation. The mRS score was assessed at 90 ± 7 days after randomisation. All concomitant medications, adverse events, stroke recurrence and other vascular events of each visit were recorded since the last visit.

All the adverse events of included subjects should be recorded and tracked until properly resolved.

All the serious adverse events of included subjects should be recorded and tracked, even if the subjects have finished the trial, until the events were resolved, or stabilization judged by the investigator.

8. Description of safety assessment parameters

8.1 Evaluate clinical safety:

Physical examinations, including neurological assessments, imaging studies, were performed at baseline, 24 hours, 48 hours, 7 ± 1 days, 14 ± 2 days, and 90 ± 7 days after randomisation; adverse event of each visit was collected after baseline visit.

Adverse events monitoring

All information on adverse events, whether mentioned by subjects, discovered by investigators, or discovered through physical examination, laboratory tests, and other methods, should be recorded on the adverse events page of the case report form and handled in accordance with appropriate regulations and report.

Adverse Event (AE)

The adverse event is any adverse medical event that occurs in a study subject during a study that is not necessarily related to this treatment. Thus, the adverse event can be any unfavourable and unexpected sign (including abnormal laboratory findings), symptom or disease temporally related to the use of study drugs, regardless of whether it is related to the study drugs or not.

It is included that any events that are new or that have worsened in severity and frequency from baseline, including abnormal results from diagnostic methods such as laboratory tests.

Note: Adverse event collection begins with signed informed consent.

Serious Adverse Event (SAE)

Serious adverse event is any adverse medical event at any dose that meets one or more of the following criteria:

- cause death (note: death is a consequence, not an event);
- Life-threatening (Note: "Life-threatening" means that the subject is in immediate danger of death at the time of the event, not the assumption that death would have occurred if the event is more severe);
- cause significant or permanent disability or impairment of organ function;
- Teratogenic and birth defects;
- Causing or prolonging hospitalisation;
- Significant medical event or need for intervention (occur without treatment)'

Note: Any event requiring hospitalisation (or prolonged hospitalisation) that occurs during the subject's participation in the study must be reported as a serious adverse event. The following circumstances leading to hospitalization are not considered serious adverse events:

- Hospitalization for social reasons other than adverse events
- Hospitalization for a scheduled surgery or other treatment or examination prior to study entry (must be recorded on the case report form)
- Hospitalization for elective surgery or treatment or examination due to anticipated disease progression

8.2 Safety assessment

For all adverse events in clinical studies, the following factors must be evaluated for safety:

- Severity criteria for SAE
- The causal relationship between the event and the investigational drug
- Severity of incident
- Anticipation of events

Causal relationship between events and the study drugs

Regardless of serious adverse events or non-serious adverse events, the investigator must evaluate the relevance of the event to the use of the study drugs according to the following criteria:

Evaluating association between adverse events and study treatments Criteria

5-level classification	Judgment criteria
Definitely related	The time of initiation of use is reasonably related to the time of occurrence of the adverse event; the adverse event corresponds to the known adverse reaction type of the study treatment; the occurrence of the adverse event cannot be explained by factors other than the study treatment (such as concomitant medication); the adverse events reduce or disappear after study treatment dose reduction or discontinuation; similar adverse events (non-essential) can occur with re-use of study treatment.
Probably related	The time of initiation of use is reasonably related to the time of occurrence of the adverse event; the adverse event corresponds to the known adverse reaction type of the study treatment; the occurrence of the adverse event can or cannot be explained by factors other than the study treatment (such as concomitant medication); the adverse event can or cannot reduce or disappear after study treatment dose reduction or discontinuation; it is not sure whether similar adverse events (non-essential) can occur with re-use of study treatment.
Probably unrelated	The time of initiation of use is not reasonably related to the time of occurrence of the adverse event; the adverse

5-level classification	Judgment criteria
	event corresponds to the known adverse reaction type of the study treatment; the occurrence of the adverse event can or cannot be explained by factors other than the study treatment (such as concomitant medication); the adverse event can or cannot reduce or disappear after study treatment dose reduction or discontinuation; it is not sure whether similar adverse events (non-essential) can occur with re-use of study treatment.
Definitely unrelated	The time of initiation of use is not reasonably related to the time of occurrence of the adverse event; the adverse event corresponds to the known adverse reaction type of the study treatment; the occurrence of the adverse event can or cannot be explained by factors other than the study treatment (such as concomitant medication); the adverse event can or cannot reduce or disappear after study treatment dose reduction or discontinuation; similar adverse events (non-essential) cannot occur with re-use of study treatment.
Unjudged	The judgment cannot be made due to incomplete, contradictory information or the fact that the information cannot be supplemented and verified.

Table of Correlation Evaluation Criteria for Adverse Events and Treatment Use

	Definitely related	Probably related	Probably unrelated	Definitely unrelated	Unjudged
Have a reasonable chronological order with the study drugs	+	+	+	−	?
For the known reaction type of the study drugs	+	+	−	−	?
Explanation for reasons other than available research equipment	−	±	±	+	?
Responses lessen after decompression of study drugs	+	±	±	−	?
The reaction reappears after the study drugs is reused	+ or ?	?	?	−	?

Note: "+" in the table is affirmative; "-" is negative; "±" is difficult to affirm or deny; "?" indicates that the situation is unknown.

Severity Criteria

Severity should be assessed according to the following graded descriptions:

- Mild: No symptoms or discomfort; does not interfere with daily activities and function; usually does not require medication to relieve symptoms.
- Moderate: Symptoms cause significant discomfort; daily activities and function are affected; study participation can be continued; intervention required to relieve symptoms.
- Severe: Severe causes severe discomfort; symptoms result in loss of function and significantly interfere with daily activities; in severe cases,

study treatment can be discontinued; symptomatic treatment and/or hospitalization are required.

The investigator should use clinical judgment to assess the severity of the event (e.g., abnormal laboratory results) from the subject's direct experience.

8.3 Safety report

All adverse events

All AEs, regardless of their severity, nature of severity, or their causal relationship to study treatment, from the time of signed informed consent until the last follow-up visit (90 days after first use of the drugs), are to be recorded in the original and recorded in medical terms on the clinical research form (CRF). When symptoms and signs are caused by common causes, a diagnosis of the disease should be given where possible (e.g., cough, runny nose, sneezing, sore throat, and headache should be reported as "upper respiratory tract infection"). The investigator must make a judgment on the causal relationship between the AE and the study treatment and record it on the clinical research form. All treatments for AEs should be documented in the original medical record and reported as requested by the sponsor.

All SAEs in clinical research must be reported to the ethics committee of the centre, the principal investigator of the centre, the sponsor, the contract research organization, the research team leader unit, and the adverse reaction monitoring centre within 24 hours; Report to the ethics committee and data and safety monitoring committee of the team leader unit within 7 natural days after being informed. Investigators must complete a SAE form to record the time, severity, duration, actions taken and outcomes of SAEs. Any follow-up information for SAEs should also be reported in writing within 24 hours according to the above process.

All SAE that do not resolve at study termination or subject early withdrawal must be followed up to any of the following:

1. Incident mitigation
2. Events are stable

3. If the baseline value is known, the event returns to the baseline value
4. Events can be attributed to other drugs or factors not related to study
5. It is unlikely that any further information will be available (subject or physician refuses to provide further information and remains lost to follow-up after various follow-up attempts)

Death

Death is the result of an event. The death of a subject in a clinical trial, regardless of whether the event was expected or drug-related, would be considered a SAE. Events leading to death should be recorded in medical terms and reported on the eCRF. All causes of death (death diagnoses) must be reported as SAEs. Investigators should make every effort to obtain and send death certificates and autopsy reports to designated personnel.

Pregnancy

If the researcher finds that the subject is pregnant during the research, the researcher should fill out the "Pregnancy Incident Report Form" within 24 hours after learning and report it to the clinical research supervisor, and notify the project leader by phone

Abnormal pregnancy results, considered SAEs, should be reported in accordance with the SAE reporting procedure.

Abnormal laboratory test results

During the study, when the results of laboratory tests meet the following conditions, they must be regarded as AEs and recorded in medical terms on the AEs of the CRF:

1. Accompanying clinical symptoms
2. Causing a change in the trial protocol (e.g., treatment interruption or discontinuation)
3. lead to a change in medical intervention or concomitant treatment
4. Clinically significant as judged by the investigator (medical and scientific methods should be used to judge whether an isolated laboratory abnormality is an AE)

When the AE meets the serious criteria, it should be reported in accordance with the SAE reporting procedure.

9. Statistics

9.1 Sample size

According to previous study,[7] the proportion of expected excellent functional outcome (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 proportion in the control group. Using power = 80% and $\alpha = 0.05$ to carry out the two-side test, the calculated sample size to test the superiority hypothesis is 734. In consideration of 10% lost to follow-up, the total sample size is 808. Therefore, this study included 808 patients, with 404 patients in each group.

9.2 Statistical analysis plan

Intention-to-treat (ITT) analysis will be used to analyse the therapeutic effects of the two groups and all the data will be analysed with SPSS 23 Software. The mean ± standard deviation (SD) will be used if the continuous data are normally distributed, and the median and quartile spacing (IQR) will be used if the continuous data are non-normally distributed. Categorical data are expressed as number (percentage). Difference of the primary endpoint and secondary endpoints such as mRS (0-2) at 90 days, incidence of early neurological improvement, incidence of early neurological deterioration, proportion of symptomatic intracerebral haemorrhage in 90 days, incidence of major bleeding events in 90 days will be compared using binary logistic regression. Change in NIHSS score between two groups will be compared using generalised linear model. Time-to-events of stroke recurrence and other vascular events will be compared using Cox regression. Statistical tests were considered significant when the two-sided *P* value was less than 0.05.

Stratification: The primary end point in the ARAIS study will further be stratified by age (<65 vs. ≥65), sex (male vs. female), NIHSS, endovascular therapy (yes vs no), and time from onset to treatment (0–3 h vs. >3 h). Differences of primary

end point in above specific stratifications will be assessed by testing for interaction of the pre-set baseline variable with primary end point.

9.3 Analysis population

Intention-To-Treat (ITT)

The ITT population includes all subjects who were randomly assigned to a treatment group on an intention-to-treat basis, including subjects who planned to receive treatment but did not actually receive it.

Per Protocol Set (PPS)

The protocol-compliant set is a subset of the FAS, which will include completion of all treatments or at least the determination of the primary end point as required by the research protocol, and good compliance (the ratio of the actual dose to the applied dose is between 80% and 120%). Subjects with no serious protocol violation (the definition of serious protocol violation will be specified in the Statistical Analysis Plan) constitute the PPS analysis set for this study.

Safety Set (SS)

The SS analysis population included all subjects who received at least one study protocol treatment and had at least one safety evaluation.

10. Data Management and Monitoring

10.1 Training of study site personnel

Before the first patient is entered into the study in each site, the sponsor (Cerebrovascular Disease Collaboration Innovation Alliance (CDCIA) – Liaoning) will organize and train all study site personnel (medical, nursing, and other staff), including the trial protocol, investigator's brochure, the evaluation of related scales, the use of electronic data capture system, drug management, SAE report procedure, etc. The Principal Investigator will ensure that appropriate training relevant to the study cover all these staff.

10.2 Monitoring of the study

During the study, a staff from CDCIA will have regular contacts with the study site, including visits to:

- Provide the related information and support to the Investigator(s)
- Confirm that the investigational team is adhering to the protocol and data are being accurately and timely recorded in the CRFs
- Confirm that the responsibility of study drug management is being implemented and the drugs are available
- Perform source data verification including deferred informed consent, laboratory results, neuroimaging data, clinical data, and neurological function evaluation (NIHSS, mRS) at baseline and follow-up

CDCIA staff will be available whenever the investigator or other personnel at the center needs information and advice about the study.

Appendix

Appendix 1：National Institutes of Health Stroke Scale (NIHSS)

	Check	Score	Point
1a	Level of Consciousness: The investigator must choose a response if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.	0 = Alert; keenly responsive. 1 = Not alert; but arousable by minor stimulation to obey, answer, or respond. 2 = Not alert; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped). 3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, and areflexic.	—
1b	LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier, or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.	0 = Answers both questions correctly. 1 = Answers one question correctly. 2 = Answers neither question correctly.	—
1c	LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute	0 = Performs both tasks correctly. 1 = Performs one task correctly. 2 = Performs neither task correctly.	—

	another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to him or her (pantomime), and the result scored (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.		
2	Best Gaze: Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored, but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI), score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness, or other disorder of visual acuity or fields should be tested with reflexive movements, and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence	0 = Normal. 1 = Partial gaze palsy; gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present. 2 = Forced deviation, or total gaze paresis not overcome by the oculocephalic maneuver.	—

	of a partial gaze palsy.		
3	Visual: Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat, as appropriate. Patients may be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia, is found. If patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction, patient receives a 1, and the results are used to respond to item 11.	0 = No visual loss. 1 = Partial hemianopia. 2 = Complete hemianopia. 3 = Bilateral hemianopia (blind including cortical blindness).	—
4	Facial Palsy: Ask – or use pantomime to encourage – the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barriers obscure the face, these should be removed to the extent possible.	0 = Normal symmetrical movements. 1 = Minor paralysis (flattened nasolabial fold, asymmetry on smiling). 2 = Partial paralysis (total or near-total paralysis of lower face). 3 = Complete paralysis of one or both sides (absence of facial movement in the upper and lower face).	—
5	Motor Arm: The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45	0 = No drift; limb holds 90 (or 45) degrees for full 10 seconds. 1 = Drift; limb holds 90 (or 45) degrees,	____ ____

	degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	but drifts down before full 10 seconds; does not hit bed or other support. 2 = Some effort against gravity; limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity. 3 = No effort against gravity; limb falls. 4 = No movement. UN = Amputation or joint fusion, explain: _____ 5a. Left Arm 5b. Right Arm	
6	Motor Leg: The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = No drift; leg holds 30-degree position for full 5 seconds. 1 = Drift; leg falls by the end of the 5-second period but does not hit bed. 2 = Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity. 3 = No effort against gravity; leg falls to bed immediately. 4 = No movement. UN = Amputation or joint fusion, explain: _____ 6a. Left Leg 6b. Right Leg	_____ _____
7	Limb Ataxia: This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual	0 = Absent. 1 = Present in one limb. 2 = Present in two limbs.	—

	defect, ensure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position.	UN = Amputation or joint fusion, explain: _____	
8	Sensory: Sensation or grimace to pinprick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas (arms [not hands], legs, trunk, face) as needed to accurately check for hemisensory loss. A score of 2, "severe or total sensory loss," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will, therefore, probably score 1 or 0. The patient with brainstem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic, score 2. Patients in a coma	0 = Normal; no sensory loss. 1 = Mild-to-moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched. 2 = Severe to total sensory loss; patient is not aware of being touched in the face, arm, and leg.	—

	(item 1a=3) are automatically given a 2 on this item.		
9	Best Language: A great deal of information about comprehension will be obtained during the preceding sections of the examination. For this scale item, the patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet and to read from the attached list of sentences. Comprehension is judged from responses here, as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in a coma (item 1a=3) will automatically score 3 on this item. The examiner must choose a score for the patient with stupor or limited cooperation, but a score of 3 should be used only if the patient is mute and follows no one-step commands.	0 = No aphasia; normal. 1 = Mild-to-moderate aphasia; some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response. 2 = Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response. 3 = Mute, global aphasia; no usable speech or auditory comprehension.	—
10	Dysarthria: If patient is thought to be normal, an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the	0 = Normal. 1 = Mild-to-moderate dysarthria; patient slurs at least some words and, at worst, can be understood with some difficulty.	—

	patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barriers to producing speech, the examiner should record the score as untestable (UN), and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.	2 = Severe dysarthria; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric. UN = Intubated or other physical barrier, explain:_____	
11	Extinction and Inattention (formerly Neglect): Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.	0 = No abnormality. 1 = Visual, tactile, auditory, spatial, or personal inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities. 2 = Profound hemi-inattention or extinction to more than one modality; does not recognize own hand or orients to only one side of space.	—

Appendix 2：Modified Rankin Score (mRS)

Grade	Description
0	No symptoms
1	Symptoms without any incapacity (able to perform all usual activities)
2	Mild incapacity (unable to perform all usual activities but able to look after his/her affairs alone)
3	Moderate incapacity (requires assistance but walks alone)
4	Severe incapacity (requires assistance for walking and physical body needs)
5	Severe incapacity (bedbound, incontinent, permanent surveillance required)
6	Death

Appendix 3：TOAST classification

1 Large artery atherosclerotic type: requires vascular imaging examination to confirm that the intracranial or extracranial large artery stenosis corresponding to the neurological deficit of cerebral infarction is more than 50% or occluded, and the vascular lesions are consistent with atherosclerotic changes; or there is intracranial or Indirect evidence of stenosis or occlusion of extracranial large arteries, such as imaging (CT or MRI) showing cerebral cortex, brainstem, cerebellum or subcortical infarction with a diameter of >1.5cm, and clinical manifestations are mainly signs of cortical damage, such as aphasia , changes in consciousness, body image disturbance, etc., or signs of brainstem and cerebellum damage. Evidence of at least one atherosclerotic stroke risk factor (such as advanced age, hypertension, hyperlipidaemia, etc.) or systemic atherosclerosis (such as plaque, coronary heart disease, etc.) is required. At the same time, cerebral infarction caused by cardio embolism should also be excluded, such as no acute infarction outside the stenosis > 50% or occlusion of intracranial or extracranial large arteries, and no high or moderate risk factors for cardioembolic stroke (see Brain Embolization chapter).

2 Cardiogenic embolism type: the clinical manifestations and imaging studies are the same as those of large atherosclerotic type. This classification is supported if there is more than one vessel innervation or multisystem embolism. The presence of at least one high or moderate risk factor for cardioembolic stroke was required.

3 Small artery occlusion type: There may be no obvious clinical manifestations or various lacunar syndromes, but no cerebral cortex involvement. The head CT or MRI is required to be normal, or the infarct diameter is less than 1.5cm.

4 Other etiological types: refer to other rare aetiologies other than the above three types of clear ethology. Such as blood coagulation disorders, changes in blood components, vasculitis due to various reasons, vascular malformations, connective tissue diseases, dissecting aneurysms, fibrous dystrophy and cerebral infarction.

5 Unexplained type: including two or more aetiologies, negative auxiliary examinations, no cause found, and insufficient auxiliary examinations.

Reference

1. Rha JH, Saver JL. The impact of recanalization on ischemic stroke outcome: a meta-analysis. Stroke 2007; 38:967-73.

2. Powers WJ, Rabinstein AA, Ackerson T, et al. 2018 guidelines for the early management of patients with acute ischemic stroke: A guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2018; 49:e46-e110.

3. Alexandrov AV, Grotta JC. Arterial reocclusion in stroke patients treated with intravenous tissue plasminogen activator. Neurology 2002; 59:862-7.

4. Jovin TG, Chamorro A, Cobo E, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. N Engl J Med 2015; 372:2296-306.

5. Dávalos A, Cobo E, Molina CA, et al. Safety and efficacy of thrombectomy in acute ischaemic stroke (REVASCAT): 1-year follow-up of a randomised open-label trial. The Lancet Neurology 2017; 16:369-76.

6. Berkhemer OA, Fransen PS, Beumer D, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. N Engl J Med 2015; 372:11-20.

7. van den Berg LA, Dijkgraaf MG, Berkhemer OA, et al. Two-year outcome after endovascular treatment for acute ischemic stroke. N Engl J Med 2017; 376:1341-9.

8. Goyal M, Demchuk AM, Menon BK, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. N Engl J Med 2015; 372:1019-30.

9. Campbell BCV, Mitchell PJ, Kleinig TJ, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. N Engl J Med 2015; 372:1009-18.

10. Lyden P, Pereira B, Chen B, et al. Direct thrombin inhibitor argatroban reduces stroke damage in 2 different models. Stroke 2014; 45:896-9.

11. Tamao Y, Kikumoto R. Effect of argatroban, a selective thrombin inhibitor, on animal models of cerebral thrombosis. Semin Thromb Hemost 1997; 23:523-30.

12. LaMonte MP, Nash ML, Wang DZ, et al. Argatroban anticoagulation in patients with acute ischemic stroke (ARGIS-1): a randomized, placebo-controlled safety study. Stroke 2004; 35:1677-82.

13. Wada T, Yasunaga H, Horiguchi H, et al. Outcomes of argatroban treatment in patients with atherothrombotic stroke: observational nationwide study in Japan. Stroke 2016; 47:471-6.

14. Ishibashi H, Koide M, Obara S, et al. High-dose argatroban therapy for stroke: novel treatment for delayed treatment and the recanalization mechanism. J Stroke Cerebrovasc Dis 2013; 22:656-60.

15. Jang IK, Gold HK, Leinbach RC, et al. In vivo thrombin inhibition enhances and sustains arterial recanalization with recombinant tissue-type plasminogen activator. Circ Res 1990; 67:1552-61.

16. Barreto AD, Alexandrov AV, Lyden P, et al. The argatroban and tissue-type plasminogen activator stroke study: final results of a pilot safety study. Stroke 2012; 43:770-5.

17. Sugg RM, Pary JK, Uchino K, et al. Argatroban tPA stroke study: study design and results in the first treated cohort. Arch Neurol 2006; 63:1057-62.

18. Barreto AD, Ford GA, Shen L, et al. Randomized, multicenter trial of ARTSS-2 (Argatroban With Recombinant Tissue Plasminogen Activator for Acute Stroke). Stroke 2017; 48:1608-16.