This supplement contains the following items:

1. Original protocol in English (page 2 to 100), final protocol in English (page 101 to 199), summary of changes in English (page 200 to 217).

2. Original statistical analysis plan in English (page 218 to 232), final statistical analysis plan in English (page 233 to 247), summary of changes in English (page 248 to 249).

Xiong Y, *et al. Stroke Vasc Neurol* 2025; 10:e003048. doi: 10.1136/svn-2023-003048

Original protocol

（V1.0，2020.10.13）

Xiong Y, *et al. Stroke Vasc Neurol* 2025; 10:e003048. doi: 10.1136/svn-2023-003048

Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II —A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

(Protocol No. : MK02-2020-01)

Clinical Trial Approval No. :2017L02308

Clinical Group Leader: Beijing Tiantan Hospital, Capital Medical University

Principal Investigator: Yongjun Wang

Sponsor: Guangzhou Recomgen Biotech Co., Ltd.

Confidentiality statement

All information contained in this protocol is the property of the Sponsor and, therefore, is provided only for the review of the investigator, co-investigator, ethics committee, clinical research associate, regulatory authorities and medical institutions. It is strictly prohibited to disclose any information to third parties unrelated to the study without the written approval of the Sponsor, except for the necessary explanation given to the subject who may participate in the study during the informed consent obtaining process with the subject.

Protocol Revision History

Protocol version number	Release date
V1.0	**2020.10.13**

Sponsor Signature Page

Sponsor's Statement

Title: Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

We will initiate, apply for, organize, fund and audit this clinical trial in accordance with the *Good Clinical Practice*, conscientiously perform my duties as a Sponsor in accordance with the *Measures for the Administration* of *Drug Registration* and other relevant provisions, and provide relevant materials, test drugs, funds, etc. in accordance with the clinical trial agreement.

Sponsor: Guangzhou Recomgen Biotech Co., Ltd.

Sponsor’s Address: No. 1, Jinfengyuan Road, Science City, Guangzhou Economic and Technological Development Zone

Project Leader (Name):	Medical Expert:
Position:	Position:
Tel:	Tel:
Signature: _____	Signature: _____
Date: _____	Date: _____

Investigator Signature Page

Investigator's Statement

Title: Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

I have read the protocol. I consent to conduct the clinical trial in accordance with the protocol design and regulations. I will conscientiously perform my duties as an investigator in accordance with the *Good Clinical Practice*. I will guarantee the accuracy, truthfulness, completeness and reliability of the test data, and accept the audit and inspection by the clinical research associates and auditors designated by the contract research organization/Sponsor and the regulatory regulatories.

Research Facility: Beijing Tiantan Hospital, Capital Medical University

Address: No. 119, South Fourth Ring West Road, Fengtai District, Beijing

Investigator (Name) : Yongjun Wang Position: Dean

Job title: Chief physician

Signature: _____

Date: _____

Signature Page of Data Management Facility

Data Management Facility's Statement

Title: Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

I have read the protocol. I will conscientiously fulfill my responsibilities as a data manager in accordance with the *Good Clinical Practice*. I agree to carry out data management according to the protocol design, ensure that the information and data our facility manages over with respect to this trial are all true, accurate, complete and correct, and will not be used for other clinical trials, nor be disclosed to any other individual or group without the written permission of the Sponsor.

Data Management Facility: Giant Med-Pharma Services, Inc.

Address: No. 5 Jianguomen North Street, Dongcheng District, Beijing

Data management director (name): Xuan Gong

Signature: _____

Date: _____

Signature Page of Statistical Analysis Facility

Statistical Analysis Facility's Statement

Title: Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

I have read the protocol. I will conscientiously perform my duties as a statistician in accordance with the *Good Clinical Practice*. I agree to carry out statistics according to the protocol design, ensure that the statistical information and data with respect to this trial are all true, accurate, complete and correct, and will not be used for other clinical trials, nor be disclosed to any other individual or group without the written permission of the Sponsor.

Statistical Facility: Clinical Research Institute of Peking University

Address: No. 38 Xueyuan Road, Haidian District, Beijing

Statistics (name) : Chen Yao

Signature: _____

Date: _____

Signature Page of Contract Research Organization

Contract Research Organization's Statement

Title: Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

I have read the protocol. I will conscientiously perform my duties as a clinical research associate in accordance with the *Good Clinical Practice*. I agree to carry out study monitoring according to the protocol design, ensure that all behaviors comply with the protocol and the recorded information and data with respect to this trial are all true, accurate, complete and correct, and will not be used for other clinical trials, nor be disclosed to any other individual or group without the written permission of the Sponsor.

Contract Research Organization: Giant Med-Pharma Services, Inc.

Address: No. 5, Jianguomen North Street, Dongcheng District, Beijing

Director of Contract Research Organization: Haibo Wu

Signature: _____

Date: _____

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Protocol summary

Protocol title	Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours
Version number /Release date	V1.0 / Qct. 13th, 2020
Sponsor	Guangzhou Recomgen Biotech Co., Ltd.
Indications	Acute ischemic stroke within 4.5h of onset
Study objectives	To evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) compared with standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset). **Primary study objectives:** To evaluate the difference in the proportion of subjects with 90-day MRS score of 0-1 in subjects with hyperacute ischemic stroke (within 4.5 h of symptom onset) treated with rhTNK-tPA (0.25 mg/kg) versus standard rT-PA (0.9mg/kg). **Secondary study objectives:** 1. To compare the difference in efficacy between rhTNK-tPA (0.25 mg/kg) and standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset) subjects: ① The proportion of subjects with an mRS score of 0-2 at 90±7 days ② MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution) ③ The proportion of subjects whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first) ④ Health-related quality of life (EQ-5d) at 90±7 days ⑤ The proportion of subjects with a Bathel score ≥ 95 points at 90±7 days 2. To compare the difference in safety between rHTNK-tPA (0.25 mg/kg) and standard rT-PA (0.9mg/kg) in the treatment of subjects with hyperacute ischemic stroke (within 4.5 h of symptom

	onset) : ① The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASSIII) ② The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASSIII) ③ The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS standards) ④ The incidence of any intracranial hemorrhage within 90 days ⑤ The incidence of significant hemorrhage events at other sites within 90 days (as defined by GUSTO) ⑥ 90-day overall mortality ⑦ Incidence rates of adverse events/serious adverse events within 90 days
Study design	Multicenter, prospective, centrally randomized, open label, blinded-endpoint, active controlled, non-inferiority trial.
Inclusion/exclusion criteria	**Inclusion criteria** (1) Age ≥18 years, male or female; (2) Time from onset to treatment < 4.5h; the time of symptom onset is defined as "the last time point at which the patient appears normal"; (3) Clinical diagnosis as ischemic stroke (the diagnosis following the *Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018*); (4) MRS score of 0-1 before onset; (5) 4 < NIHSS < 26 at baseline; (6) Subjects or their guardians voluntarily sign the informed consent. **Exclusion criteria** (1) Known to be allergic to rhTNK-tPA and/or rT-PA; (2) A history of intracranial hemorrhage; (3) A history of severe head trauma or stroke within 3 months; (4) A history of intracranial or spinal surgery within 3 months;

<table>
<tr><td></td><td>(5) A history of gastrointestinal or urinary tract hemorrhage within 3 weeks;
(6) A history of major surgery within 2 weeks;
(7) A history of arterial puncture at sites difficult for compression hemostasis within 1 week.
(8) Intracranial tumors (except neuroectodermal tumors, such as meningiomas), large intracranial aneurysms;
(9) Intracranial hemorrhage (including parenchymal hemorrhage, intraventricular hemorrhage, subarachnoid hemorrhage, subdural/extradural hematoma, etc.);
(10) Active visceral bleeding;
(11) Aortic arch dissection found.
(12) Uncontrollable hypertension upon active antihypertensive treatment: systolic blood pressure ≥180 mm Hg, or diastolic blood pressure ≥100 mm Hg;
(13) Propensity for acute bleeding, including platelet counts of less than 100×109/ L or otherwise;
(14) Blood glucose <2.8 mmol/L or >22.22 mmol/L;
(15) On oral warfarin anticoagulant treatment with INR>1.7 or PT >15s;
(16) Having received heparin treatment within 24 h;
(17) Having received thrombin inhibitors or factor Xa inhibitors within 48 h;
(18) Head CT or MRI shows a large infarction (infarcted area > 1/3 of the middle cerebral artery).
(19) Subjects who are unable or unwilling to cooperate due to hemiplegia (Todd's palsy) after epileptic seizure or other neurological/psychiatric disorders;
(20) Pregnant women, lactating women, or subjects who do not agree to use effective contraception during the trial;
(21) Participation in other clinical trials within 3 months prior to screening;
(22) Unsuitability for participation or participation in this study as judged by the investigator may result in exposure to greater risk.</td></tr>
<tr><td>Study drugs</td><td>Test drug:
<table><tr><td>Drug name</td><td>Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-TPA)</td></tr><tr><td>Trade name</td><td>MingFuLe</td></tr></table></td></tr>
</table>

	Strengths: 1.0 x 10^7 IU/16 mg/vial Storage conditions: Store below 25℃ away from light. This product should be used immediately after dissolution. If not used immediately, it should be stored in cold storage at 2 - 8℃ away from light and used within 24 hours. Shelf life: 24 months Manufacturer: Guangzhou Recomgen Biotech Co., Ltd. Supplier: Guangzhou Recomgen Biotech Co., Ltd. **Control drug:** Drug name: Alteplase for Injection (rt-PA) Trade name: Actilyse® Strengths: 50mg/vial, 20mg/vial Storage conditions: Keep in the original packaging. Store below 25℃ away from light. It is recommended to use the prepared solution immediately after preparation. It has been proved that the prepared solution can remain stable at 2-8℃ for 24h. Frezzing prohibited. Shelf life: 36 months Manufacturer: Boehringer Ingelheim Shanghai Pharmaceuticals Co. Ltd. Supplier: Guangzhou Recomgen Biotech Co., Ltd.
Methods of grouping and administration	Subjects will be randomly assigned to the following groups in a 1:1 ratio: **Treatment group (rhTNK-TPA 0.25mg/kg) :** RhTNK-TPA 0.25mg/kg: Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the subject, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds. **Control group (rt-PA 0.9mg/kg) :** RT-PA 0.9mg/kg: The product is dissolved in sterile water for injection to obtain a solution with a concentration of 1mg/ ml. The amount required for medication is calculated and measured according to the actual body weight of the subject. 10% of the solution is injected by intravenous bolus, and the remaining 90% is injected by intravenous drip within 1h.

Efficacy endpoints	**Primary efficacy endpoint:** The proportion of subjects with 90-day MRS score of 0-1 **Secondary efficacy endpoints:** ① The proportion of subjects with an mRS score of 0-2 at 90±7 days ② MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution) ③ The proportion of subjects whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first) ④ Health-related quality of life (EQ-5d) at 90±7 day ⑤ The proportion of subjects with a Bathel score ≥ 95 points at 90±7 days
Safety endpoints	① The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASSIII) ② The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASSIII) ③ The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS standards) ④ The incidence of any intracranial hemorrhage within 90 days ⑤ The incidence of significant hemorrhage events at other sites within 90 days (as defined by GUSTO) ⑥ 90-day overall mortality ⑦ Incidence rates of adverse events/serious adverse events within 90 days
Sample size	According to a paper published in the Lancet[7] in 2014 about meta-analysis of the incidence of 90-day MRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the data under age of 80 was collected with the incidence of rt-PA and Placebo in clinical trials initiated by European researchers, of which the meta-analysis result of RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the Guidelines for the Design of Non-inferiority Drug Clinical Trials [32], the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high priority index. Therefore, based on the lower CI limit of 1.14, together with a efficacy of 25% of the active control, the non-inferior threshold was determined as 0.906. Then assuming α=0.025 using one-sided test, power 1-β=0.8, treatment group (TNK) incidence of 64%, control group (rt-PA) incidence of 63%, real RR= 1.02, the

	sample size was calculated by PASS software to be 643 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each group, a total of 1430 cases.
Statistical analysis	Efficacy endpoints: Chi-square test or Fisher's exact probability method will be used for qualitative classification, and 95% confidence intervals for incidence differences between groups will be calculated. Qualitative grade data are analyzed by Wilcoxon rank sum test, and quantitative indicators are analyzed by two-sample t-test or rank sum test as appropriate, and 95% confidence intervals for score differences between groups are calculated. Statistical comparison between the primary efficacy indicators analysis groups with CMH chi-square considering the center effect, computational efficient RR OR RD 95% confidence interval, and the center effect, mRS baseline score of logistic regression model analysis, report comparing rhTNK - tPA and rt - PA OR 95% confidence interval, and the primary efficacy endpoint results will also according to whether bridge group subgroup analysis was carried out. Safety endpoints: Chi-square test or Fisher's exact probability method are used for qualitative classification, and 95% confidence intervals for incidence differences between groups are calculated. Qualitative grade data are analyzed by Wilcoxon rank sum test, and quantitative indicators are analyzed by two-sample t-test or rank sum test as appropriate, and 95% confidence intervals for score differences between groups are calculated.

Research Flow Chart

Measures		Screening/Baseline	Treatment period	follow-up			
		4.5 h. (Before thrombolysis)	0h (Thrombolytic Therapy)	24 h + 2 h	24h-36h	7±1 day or before discharge (whichever occurs first)	90±7 days/early end of visit
Signing Informed Consent		X					
Demographic characteristics		X					
Symptoms of this onset		X					
Medical history		X					
Prior medication[1]		X					
Concomitant medication/treatment[9]		X	X	X	X	X	X
Vital signs[2]		X	X	X		X	
mRS		X[3]					X
NIHSS		X		X		X	
BI scale						X	X
EQ - 5 D scale						X	X
Blood/urine pregnancy test[4]		X					
Laboratory examination[5]	Routine blood test	X		X		X	
	Blood biochemistry	X		X		X	
	Coagulation function	X		X		X	
	Routine urinalysis			X		X	
Electrocardiogram (ECG)[6]		X				X	
CT or MRI[7]		X			X		
randomization		X					
Thrombolysis information[8]			X				
Adverse Events/Serious Adverse Events			X	X	X	X	X

1. Prior medication is only recorded for drugs that fall into the restrictions in the inclusion/exclusion criteria.
2. The "blood pressure" of screening and enrollment is collected when vital signs are collected."0h" is regarded as the monitoring results of vital signs within 5min before thrombolysis. Vital signs include blood pressure, pulse, body temperature and respiratory

rate;

3. The mRS score at screening include pre-onset score and post-onset pre-thrombolysis score.
4. Blood/urine pregnancy test is applicable to fertile women only;
5. Laboratory examination:
    1) Baseline laboratory tests include routine blood test, blood biochemistry, and coagulation function; Subjects will not need to repeat the test if they have already performed some laboratory tests between the present onset and prior to thrombolysis. For baseline blood lipid tests (total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), triglyceride (TG)), the most recent fasting blood lipid is collected after the onset of the disease. Blood glucose test is performed at baseline. Peripheral blood glucose is measured as one of the inclusion and exclusion criteria, and venous blood is collected for blood glucose test. Blood biochemical results are allowed to be obtained after the study drug has been administered. If abnormal blood biochemical results are obtained, they will be handled by the study physician in accordance with the discipline guidelines and the clinical management pathway of the hospital.
    2) 24h laboratory examination after thrombolysis can accept the test results within 72h after onset (after administration), including routine blood test, blood biochemistry, coagulation function, routine urinalysis;
    3) Routine blood test, blood biochemistry, coagulation function and routine urinalysis 7±1 day or before discharge (according to the first occurrence);
6. If the subject has examined the ECG before thrombolysis after the present onset, no repeat examination is required;
7. Screening/baseline imaging examination is “CT or MRI”, which is used to exclude hemorrhagic stroke; the CT or MRI results generated by other hospitals can also be used as a screening basis, if the study physician deems them acceptable; if a subject has undergone a CT or MRI check post symptom onset before onset of thrombolysis, such examination may be omitted. The imaging examination during the follow-up period is "CT or MRI", which is used to detect hemorrhagic events and should be completed within 24 - 36h.
8. Thrombolysis information includes thrombolysis time of the treatment group and the control group (including intravenous infusion time of the treatment group, intravenous infusion time of the control group, and the start and end time of intravenous infusion), intravenous dose, intravenous infusion dose of the control group, and the occurrence of adverse events.
9. For concomitant medication/treatment, pay attention to collecting information about bridging therapy.

Abbreviations table

ACA	Anterior cerebral artery	HR	Heart rate
ADC	Apparent diffusion coefficient	ICH	Intracranial bleeding
AE	Adverse events	ICU	intensive care unit (ICU)
ASA	aspirin	INR	International standard ratio
BP	Blood pressure	IRB	Ethics committee
CK	Creatine kinase	IVH	Intraventricular hemorrhage
CK-MB	Creatine kinase -MB	IS	Ischemic stroke
COX	Ring oxidase	LDL	Low density lipoprotein
CRP	C-reactive protein	MCA	Middle cerebral artery
CRF	Case report form	MI	Myocardial infarction,
CRO	Contract Research Organization	MoCA	Montreal Cognitive Assessment
CT	Computerized tomography	MRA	Magnetic resonance angiography
CTA	CT angiography	MRI	Magnetic resonance imaging
DRF	Deviation resolution table	mRS	Improved Rankin score
DMC	Data Monitoring Committee	NIHSS	National Institutes of Health Stroke Scale
DWI	Diffusion-weighted imaging	PCA	Posterior cerebral artery
ECG	Electrocardiogram (ECG)	PWI	Perfusion Weighted Imaging
ED-5Q	Quality of Life Scale	RR	Relative risk
EMS	Emergency medical service	RRR	Relative risk reduction rate
ER	The emergency room	SAE	Serious Adverse Events
FLAIR	Liquid attenuation inversion recovery sequence	SAH	Subarachnoid hemorrhage
GCS	Glasgow Coma Score	SFDA	State Food and Drug Administration
GCP	Standardized management of drug clinical trials	sICH	Symptomatic intracranial hemorrhage
GPIIb/IIIa	Glycoprotein IIb/IIIa	TIA	Transient ischemic attack
GUSTO	Global Infarction-related Artery Opening Strategy	TCD	Transcranial Doppler ultrasound
		tPA	Tissue plasminogen activator

1. Research background

1.1 Introduction

The most effective drug treatment for Acute Ischemic Stroke (AIS) is intravenous thrombolysis of recombinant tissue plasminogen activator (rt-PA) for injection at an extremely early stage (< 4.5 h), which has been consistently recommended by domestic and foreign guidelines for cerebrovascular diseases[1-3]. Under the guidance of imaging, the time window of intravenous thrombolysis for partial anterior circulation cerebral infarction or post-awakening ischemic stroke can be extended to less than 9 hours, and rt-PA has good efficacy and safety[4-6]. Therefore, more patients with AIS can benefit from intravenous rt-PA thrombolysis.

However, the proportion of good prognosis of intravenous thrombolysis with rt-PA is relatively low[7], partly because of the second generation of intravenous thrombolysis drug rt - PA vessels take rate for medium and large vascular occlusion take rate lower (MCA telecentric end clogging of intravenous thrombolysis vessels take rate is 38% (95% CI 22-54%), near heart MCA occlusion of blood vessels take rate is 21% (95% CI 15-29%) of the internal carotid artery occlusion intravenous thrombolysis take rate is 4% (95% CI 1-8%), basilar artery occlusion intravenous thrombolysis take rate of 4% (95% CI 0-22%), and significantly related to vascular take rate and prognosis[8]. Five international RCT studies have confirmed that intravenous thrombolytic therapy combined with endovascular therapy is more beneficial than intravenous thrombolytic therapy for patients with great vessel occlusion[9-14]. However, the distribution of medical resources is not balanced, and endovascular surgery cannot meet the needs of AIS emergency treatment in many areas[15]. The time delay caused by referral may reduce the benefit of thrombectomy [16]. Some patients may not be able to tolerate anesthesia or surgery due to comorbidities or may not be able to undergo surgery due to medical costs. Therefore, there is an urgent need for effective treatment options other than endovascular treatment.

Compared with rt-PA, the half-life of the third generation intravenous thrombolytic drug TNK-tPA is 4 times that of rt-PA, the tissue specificity of fibrin is 15 times that of rt-PA, and it is less affected by plasminogen activator inhibitor PAI-1 (PAI-1)[17]. Different studies have shown that TNK-tPA treatment of AIS is superior to

rt-PA in patients with AIS complicated with great vessel occlusion, resulting in higher vascular opening rate or reperfusion and better prognosis[18-22].

In view of the current European and American guidelines and domestic guidelines, intravenous thrombolysis should be given to AIS after general CT examination within 4.5 hours of onset, and imaging examinations such as CTA/CTP/MRI are not recommended to guide intravenous thrombolysis to reduce time delay[1,2]. In the completed AIS study (sample size: 1100 cases) within 4.5 hours of onset, intravenous TNK-tPA or rt-PA thrombolysis nor-test is randomly administered based on general CT examination, and the superior efficacy of TNK-tPA has not been confirmed [23].Meta-analysis showed that the efficacy of TNK-tPA in the treatment of AIS is at least not inferior to rt-PA[24,25]However, there is heterogeneity in the study and there is a lack of direct evidence and Chinese data. Therefore, the hypothesis of this study is that the efficacy of TNK-tPA is at least not inferior to rt-PA in Chinese patients with AIS within 4.5 hours of onset. On the basis of the phase II clinical trial, this study used a national multicenter, prospective, centrally randomized, open label, blind endpoint evaluation, active controlled phase III clinical trial to verify the above hypothesis. On the basis of meeting the non-inferiority test, the superior efficiency will be further tested.

1.2 Background of test drug

Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK - tPA, trade name "MingFuLe"), developed by Guangzhou Recomgen Biotech Co., Ltd. (hereinafter referred to as " Recomgen Biotech"), is a recombinant protein produced using mammalian cells by genetic engineering technology, and on January 14, 2015, got approved by NMPA for marketing as a clinical thrombolytic therapy for acute myocardial infarction. RhTNK-tPA is the third generation of recombinant t-PA product, which is a mutant of alteplase: ① The mutation of T103 site to N103 makes it change from non-glycosylated site to glycosylated site, and prolongs its half-life;(2) The mutation of N117 to Q117 removes the glycosylation function of N103, repairs the adverse effect of N103 mutation on fibrin specificity, and also reduces the clearance rate in plasma. ③ K296, H297, R298 and R299 are mutated into A296, A297, A298 and A299, which enhanced the binding ability of fibrin and reduced the reaction of PAI-1, its inhibitor[26]. RhTNK - tPA, therefore,

compared to the rt - PA, has a longer half-life by four times, improved antagonism ability against the inhibitor PAI – 1 by 80 times, and higher specificity by 14 times, making it the most safe and effective recombinant t - PA thrombolytic drugs to date. Besides, rhTNK - tPA can use a single intravenous bolus injection, easing the trouble of intravenous drip or repeated intravenous push existing in other fibrinolytic drugs, easy to use, more suitable for first aid. RhTNK - tPA has the same pharmacological effects with alteplase, in theory can also be applied to the treatment of acute ischemic stroke, which has been preliminarily confirmed in preclinical animal experiments and the clinical trials before phase 3 for treatment of acute ischemic stroke with good efficacy.

The Phase II clinical study of rhTNK-tPA for acute ischemic stroke is titled "A phase 2, dose-finding, multicenter, prospective, randomized, open label, parallel, active controlled trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) for acute ischemic stroke within 3 hours. "This study was conducted in 22 research centers, and a total of 4 groups were set up, which were the treatment groups, rhTNK-tPA 0.10 mg/kg group, rhTNK-tPA 0.25 mg/kg group, rhTNK-tPA 0.32 mg/kg group and the control group rT-PA group. 60 subjects were included in each group, and 240 subjects were included in total. Drug administration method of rhTNK-tPA: Each vial was dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication was calculated and measured according to the actual body weight of the subject, 25mg at most. The solution was administered via single intravenous bolus injection in 5-10 seconds.. RT-PA administration method: 10% of the total dose (0.9mg/kg) was injected by intravenous bolus within 1 minute, and the remaining 90% was injected by intravenous drip within 1h after mixing well. The results showed that the primary efficacy endpoints, i.e. the proportion of subjects whose NIHSS score was ≤ 1 or was improved compared to the baseline over 4 at 14 days, were 63.33%(38/60), 77.19%(44/57), 66.67%(40/60), and 62.71%(37/59) in the rhTNK-tPA 0.10mg/kg group, the rhTNK-tPA 0.25mg/kg group, the rhTNK-tPA 0.32mg/kg group, and the rT-PA group, respectively, without significant statistical difference ($P > 0.05$). For secondary efficacy endpoints: i.e. the proportion of subjects capable of independent self-care at 90 days (good prognosis at 90 days defined as mRS 0-1), the change of mRS (continuity) at 90 days and the change rate from

baseline, the proportion of subjects with mRS score 0-2, NIHSS (change of NIHSS score at 90-day follow-up), the proportion of patients with a BI score ⩾ 95 at 90 days, the quality of life (EQ - 5 D scale), there was no significant statistical difference between the groups (P > 0.05). For events in the safety endpoints, the proportion of patients with symptomatic intracranial hemorrhage (sICH) at 36h was 5.00%(3/60 cases) in the rhTNK-tPA 0.10mg/kg group, 0.00%(0/57 cases) in the rhTNK-tPA 0.32mg/kg group, and 1.69%(1/59 cases) in the rT-PA group, respectively, without significant statistical difference (P > 0.05). There were no statistically significant differences in the overall mortality at 90 days, the proportion of patients with asymptomatic intracranial hemorrhage at 90 days, the proportion of patients with bleeding events at other sites at 90 days, and the proportion of patients with adverse events/serious adverse events within 90 days (P > 0.05). According to the system organ classification, adverse events with an incidence of ≥5% in each group are shown in the table below. The SAEs that occurred during the study that may be associated with the study drugs are shown in the following table.

AEs with incidences ≥ 5% in phase 2 clinical study

SOC	PT	RhTNK - tPA 0.10 mg/kg Number of cases (incidence)	RhTNK - tPA 0.25 mg/kg Number of cases (incidence)	RhTNK - tPA 0.32 mg/kg Number of cases (incidence)	rt-PA 0.9 mg/kg Number of cases (incidence)
Metabolism and Nutrition Disorders	Hypoproteinemia	6 (10.00%)	1 (1.75%).	5 (8.33%)	3 (5.08%)
	Hypokalemia	6 (10.00%)	2 (3.51%).	2 (3.33%).	3 (5.08%)
	Hyponatremia	2 (3.33%).	1 (1.75%).	3 (5.00%)	0 (0.00%)
	Hyperuricemia	2 (3.33%).	2 (3.51%).	5 (8.33%)	8 (13.56%)
	Hyperhomocysteinemia	12 (20.00%).	9 (15.79%)	4 (6.67%)	6 (10.17%)
	Hyperlipidemia	9 (15.00%)	6 (10.53%)	9 (15.00%)	11 (18.64%).
	Dyslipidemia	2 (3.33%).	3 (5.26%)	3 (5.00%)	0 (0.00%)
Hepatobiliary Disorders	Abnormal liver function	10 (16.67%)	3 (5.26%)	4 (6.67%)	6 (10.17%)
	Liver cyst	1 (1.67%).	1 (1.75%).	0 (0.00%)	4 (6.78%)
	Hepatic steatosis	2 (3.33%).	0 (0.00%)	2 (3.33%).	5 (8.47%)
Infections and Infestations	Lung infection	5 (8.33%)	2 (3.51%).	9 (15.00%)	5 (8.47%)
	Urinary tract infection	1 (1.67%).	2 (3.51%).	4 (6.67%)	3 (5.08%)
Investigations	With blood in his urine	0 (0.00%)	5 (8.77%)	5 (8.33%)	1 (1.69%).
	Potassium lower	1 (1.67%).	3 (5.26%)	2 (3.33%).	2 (3.39%).
	Elevated blood glucose	1 (1.67%).	1 (1.75%).	3 (5.00%)	3 (5.08%)
	Decreased	0 (0.00%)	0 (0.00%)	0 (0.00%)	3 (5.08%)

	fibrinogen				
	High blood pressure	2 (3.33%).	1 (1.75%).	0 (0.00%)	3 (5.08%)
	Abnormal lipoprotein	3 (5.00%)	2 (3.51%).	0 (0.00%)	1 (1.69%).
	Elevated lipid	0 (0.00%)	0 (0.00%)	3 (5.00%)	2 (3.39%).
Nervous System Disorders	Hemorrhagic cerebral infarction	1 (1.67%).	3 (5.26%)	1 (1.67%).	1 (1.69%).
	Cerebral artery stenosis	1 (1.67%).	5 (8.77%)	1 (1.67%).	3 (5.08%)
	Arteriosclerosis cerebri	2 (3.33%).	3 (5.26%)	0 (0.00%)	1 (1.69%).
	Brain infarction	6 (10.00%)	6 (10.53%)	2 (3.33%).	3 (5.08%)
	Carotid stenosis	1 (1.67%).	1 (1.75%).	2 (3.33%).	4 (6.78%)
	Carotid atherosclerosis	2 (3.33%).	0 (0.00%)	3 (5.00%)	4 (6.78%)
	Have a headache	2 (3.33%).	3 (5.26%)	4 (6.67%)	3 (5.08%)
	Dizzy	2 (3.33%).	1 (1.75%).	3 (5.00%)	1 (1.69%).
Injury, Poisoning and Procedural Complications	Cerebral hernia	4 (6.67%)	0 (0.00%)	0 (0.00%)	1 (1.69%).
Respiratory system, chest and mediastinal diseases	Epistaxis	1 (1.67%).	1 (1.75%).	3 (5.00%)	0 (0.00%)
	Lung inflammation	3 (5.00%)	0 (0.00%)	0 (0.00%)	2 (3.39%).
	Pulmonary lumps	0 (0.00%)	1 (1.75%).	4 (6.67%)	2 (3.39%).
	Pneumonia	4 (6.67%)	2 (3.51%).	3 (5.00%)	1 (1.69%).
	Respiratory failure	1 (1.67%).	1 (1.75%).	3 (5.00%)	1 (1.69%).
	Hemoptysis	0 (0.00%)	0 (0.00%)	0 (0.00%)	4 (6.78%)
	Pleural effusion	2 (3.33%).	0 (0.00%)	2 (3.33%).	3 (5.08%)
Psychiatric Disorders	Restlessness	1 (1.67%).	4 (7.02%)	1 (1.67%).	0 (0.00%)
	Insomnia	0 (0.00%)	1 (1.75%).	3 (5.00%)	5 (8.47%)
Skin and Subcutaneous Tissue Disorders	Subcutaneous bleeding	0 (0.00%)	2 (3.51%).	1 (1.67%).	3 (5.08%)
Renal and Urinary Disorders	Blood in the urine	3 (5.00%)	3 (5.26%)	2 (3.33%).	0 (0.00%)
Gastrointestinal Disorders	Constipation	13 (21.67%).	11 (19.30%).	11 (18.33%).	11 (18.64%).
	Abdominal pain	0 (0.00%)	1 (1.75%).	0 (0.00%)	3 (5.08%)
	Diarrhea	1 (1.67%).	1 (1.75%).	6 (10.00%)	2 (3.39%).
	Vomiting	1 (1.67%).	0 (0.00%)	3 (5.00%)	6 (10.17%)
	Gastrointestinal bleeding	0 (0.00%)	0 (0.00%)	3 (5.00%)	0 (0.00%)
	Bleeding gums	6 (10.00%)	12 (21.05%).	9 (15.00%)	9 (15.25%)
	Stress ulcer	4 (6.67%)	1 (1.75%).	3 (5.00%)	1 (1.69%).
Cardiac Disorders	Atrial fibrillation	0 (0.00%)	2 (3.51%).	5 (8.33%)	2 (3.39%).
	Heart failure	2 (3.33%).	1 (1.75%).	4 (6.67%)	5 (8.47%)
Vascular Disorders	Atherosclerosis	3 (5.00%)	5 (8.77%)	9 (15.00%)	4 (6.78%)
	Hypertension	1 (1.67%).	3 (5.26%)	4 (6.67%)	1 (1.69%).
Blood and Lymphatic Disorders	Clotting disorders	3 (5.00%)	0 (0.00%)	1 (1.67%).	2 (3.39%).

	anemia	5 (8.33%)	3 (5.26%)	3 (5.00%)	3 (5.08%)

SAE determined to be possibly related to the study drugs in Phase 2 clinical study

Grouping	Medical terms for SAE (diagnostics)	Severity of SAE	Measures taken on study drugs	SAE outcome	Sequelae	Relationship between SAE and study drugs
RhTNK - tPA 0.10 mg/kg	Right basal ganglia bleeding	Prolonged hospital stay	Not applicable	Symptoms persist		Definitely related
RhTNK - tPA 0.25 mg/kg	Multiple subcutaneous hematomas	Endangering life or death	Not applicable	Resolve	No	Definitely related
RhTNK - tPA 0.32 mg/kg	Cerebral hemorrhage	Endangering life or death	Not applicable	Resolve	Yes	Possibly related
	Gastrointestinal hemorrhage	Endangering life or death	Drug withdrawal	Symptoms persist		Possibly related
	Ischemic optic neuropathy of the left eye	Dysfunction	Drug withdrawal	Symptoms persist		Possibly related
	Hemorrhagic transformation after infarction	Endangering life or death	Not applicable	Death		Definitely related
Rt - PA 0.9 mg/kg	Large cerebral infarction with hemorrhage	Endangering life or death	Not applicable	Death		Possibly related

1.3 Overview of clinical studies at home and abroad

(1) NOR - TEST trial [23]

The purpose of the NOR-test was to compare the safety and efficacy of intravenous thrombolysis with tenecteplase and alteplase. NOR - TEST Trial for phase III, multicenter (13) research center, prospective, randomized, open label, end blinded clinical trial, the target population in super acute phase of ischemic stroke (< 4.5 h) patients or symptoms occur within 4.5 h after waking, suspected of acute ischemic stroke and suitable for thrombolysis or thrombectomy bridge before the treatment, according to the proportion of 1:1 randomly given intravenous injection of tenecteplase at 0.4 mg/kg (maximum 40 mg) or alteplase at 0.9 mg/kg (maximum 90 mg).The primary endpoint was the ratio of good prognosis at 90 days (mRS 0 to 1).A total of 1107 patients were enrolled in the study, 7 patients dropped out of the trial, and 1100 patients were randomly assigned to the tenecteplase group (n=549) or alteplase group (n=551). The median age was 77 years (64-79), and the median NIHSS score was 4 (2-8).A final diagnosis of non-ischemic stroke or non-TIA was

found in 99 (18%) patients in the tenecteplase group and 91 (17%) patients in the alteplase group. A total of 354 patients (64%) in the tenecteplase group and 345 patients (63%) in the alteplase group were included in the final outcome analysis.

The results showed as follows: ① The incidence of primary endpoints in the two groups was 64% and 63%(OR 1.08, 95%CI 0.84 to 1.38;P = 0.52);② The 90-day mortality rates were 5% and 5%(P=0.68), respectively.(3) The incidence of serious adverse events was similar in both groups (26%).

Conclusion: Tenecteplase is not superior to alteplase, and the safety of both is similar. However, in general, the majority of patients in this study were patients with mild stroke, and about 75% of the patients had NIHSS≤7 points before treatment, and the proportion of patients with great artery occlusion was small. The research on patients with severe stroke still needs to be continued.

(2) WAKE UP study[6]

G. Thomalla et al. from the Eibendorf Medical Center, University of Hamburg, Germany, conducted a WAK-UP trial to investigate the efficacy and safety of intravenous thrombolysis therapy with alteplase in patients with acute ischemic stroke with unknown onset time screened based on MRI images.

The WAKE-UP study is a multicenter, randomized, double-blind, placebo-controlled clinical trial. The vast majority of patients with post-awakening stroke have onset a few hours before awakening, and these patients may meet the time window for intravenous thrombolysis. For stroke patients with known onset time, DWI-FLAIR - mismatch on MRI often indicates that the onset of stroke occurs within 4.5 hours before imaging examination. The study was planned to include 800 patients, but 503 patients were included and the trial ended early due to lack of funding. Of these patients, 254 were randomized to alteplase and 249 were randomized to placebo. Patients scheduled for mechanical thrombectomy were excluded from the study. The primary endpoint was good prognosis at 90 days (MRS score of 0-1), and the secondary outcome was MRS score shift analysis.

Results: The ratio of good outcomes at 90 days in the alteplase group and placebo group was 131/246 (53.3%) and 102/244 (41.8%), respectively (adjusted OR, 1.61;95% CI, 1.09 - 2.36;P = 0.02). The median 90-day MRS score was 1 and 2,

respectively (adjusted OR, 1.62;95% CI, 1.17 - 2.23;P = 0.003).The mortality rates were 4.1% and 1.2%, respectively (OR, 3.38;95% CI, 0.92 - 12.52;P = 0.07).The rates of symptomatic intracranial hemorrhage were 2.0% and 0.4% (OR, 4.95;95% CI, 0.57 - 42.87;P = 0.15).

CONCLUSIONS: For stroke patients with unknown onset time and DWI-FLAIR mismatch on MRI, intravenous thrombolytic therapy with alteplase can improve functional outcomes at 90 days, but the incidence of intracranial hemorrhage is higher.

(3) ECASS - III study [27]

Intravenous thrombolysis with alteplase has only been approved for the treatment of acute ischemic stroke patients, but the efficacy and safety of this drug beyond the 3h time window remains unclear. The purpose of this study was to investigate the efficacy and safety of alteplase in patients with 3-4.5h acute ischemic stroke.

Results: A total of 821 patients were enrolled in this study, 418 in the alteplase treatment group and 403 in the placebo control group. The median duration of alteplase administration was 3 hours and 59 minutes. More patients in the treatment group had a good prognosis (52.4% vs. 45.2%, OR, 1.34, 95%CL, 1.02-1.76;P = 0.04).Overall, alteplase improved the prognosis of patients (OR, 1.28, 95%CI, 1.00-1.65, P < 0.05).The rate of intracranial hemorrhage in the alteplase treatment group was higher than that in the control group (all intracranial bleeding, 27.0% vs. 17.6%;P = 0.001;Symptomatic intracranial hemorrhage, 2.4% vs. 0.2%;There was no significant difference in mortality between the two groups (7.7% vs. 8.4%, P=0.68).There was no significant difference in serious adverse reactions between the two groups.

CONCLUSIONS: Intravenous alteplase is effective in patients with acute ischemic stroke 3-4.5h after onset, compared with controls, and significantly improves clinical outcomes, but symptomatic intracranial hemorrhage is common.

(4) TAAIS study[18]

The Australian TNK trial was designed to compare the clinical efficacy and safety of alteplase and tenecteplase. Patients with acute cerebral infarction within 6 hours of onset were enrolled in this Phase IIb randomized trial. Patients were randomly divided into alteplase group (0.9 mg/kg), low-dose tenecteplase group (0.1

mg/kg) and high-dose tenecteplase group (0.25 mg/kg) according to 1:1:1.In order to improve the benefit of thrombolysis, the CTP of all enrolled patients before treatment showed perfusion defect area at least 20% larger than the infarction core, with a volume of at least 20ml, and CTA showed corresponding major artery occlusion (ACA, MCA, and PCA), except ICA and VA occlusion patients. The primary endpoint of the study was the proportion of reperfusion and the rate of clinical symptom improvement (evaluated by NIHSS score) indicated by PVI-DWI after 24h.A total of 75 patients with acute cerebral infarction (ACI) were enrolled, with 25 patients in each group. The baseline mean NIHSS score was 14.4±2.6 and the median time from onset to treatment was 2.9±0.8h.

The results showed as follows: (1) Compared with the alteplase group, the two groups of patients who received tenecteplase after 24h had a higher recirculation rate (P<0.001), and there was no significant difference in the incidence of intracranial hemorrhage and other adverse events between the two groups; ② In all efficacy outcomes, higher doses of tenecteplase and lower doses of tenecteplase and alteplase had more benefits, including 90d non-severe disability rate (72% of tenecteplase vs 40% of alteplase, P=0.02).

Conclusions: Tenecteplase may lead to better vascular recanalization rates and clinical outcomes in patients selected by baseline CTP.

(5) Extend-Ia TNK trial [21-22]

Part 1:

The Extend-Ia TNK trial was also aimed at determining whether tenecteplase had a better reperfusion effect than alteplase prior to thrombectomy. The study was a phase II, multicenter, prospective, randomized, open label, blind endpoint, non-inferior-quality study. This study enrolled patients who received thrombolytic therapy for acute cerebral infarction within 4.5h. CTA indicated great artery occlusion (ICA, BA, MCA-M1, and MCA-M2), and thrombectomy could be performed within 6h of onset. They were randomly divided into intravenous tenecteplase 0.25 mg/kg group or alteplase 0.9 mg/kg group according to 1:1.Multimodal CT was performed before thrombolysis, and perfusion was evaluated by angiography (MTICI grade).The primary endpoint was mTICI 22/3 or the proportion of desirable thrombus

disappearance, the secondary endpoint was a 90-day MRS score, 72hNIHSS score decrease of ≥8 points or a ratio of 0-1, and the safety endpoint was SICH and death.

Results: A total of 202 stroke patients were enrolled in this study, 101 subjects received tenecteplase and 101 subjects received alteplase. Primary endpoint Good perfusion rate (Tenecteplase 22% vs. Alteplase 10%) (Ratio difference 12%, 95%CI,2-21, Ratio 2.2, 95%CI, 1.1-4.4; Non-inferiority P = 0.002;P = 0.03).90-day functional outcome of tenecteplase was better than that of alteplase (median mRS 2 vs 3, OR 1.7, 95%CI, 1.0-2.8; P = 0.04). Symptomatic cerebral hemorrhage occurred in 1% of patients in each group.

Conclusion: Tenecteplase has a better rate of vascular recanalization and reperfusion in patients with aortic occlusion.

Part 2:

The EXTEND - IA a successor Part 2 test is a launched by the investigators, multicenter, prospective, randomized, open label, blind end study, were incorporated into the internal carotid artery, basilar artery or 4.5 hours of stroke onset of middle cerebral artery occlusion for mechanical bolt of ischemic cerebral apoplexy patients, cerebral apoplexy before mRS 3 or less (no age limit) and intravenous (IV) thrombolysis no contraindications. Patients were randomly assigned to IV TNK0.4mg/kg (Max. 40mg) or 0.25mg/kg (Max.25mg) before thrombectomy. The primary clinical outcome was reperfusion of more than 50% of the affected ischemic area before thrombectomy, or an unrecoverable intracranial thrombus. Secondary clinical outcomes included MRS score at 90 days and early neurological improvement (NIHSS score at 3 days: NIHSS score improved by at least 8 points or 0 to 1); The primary safety outcomes were symptomatic intracerebral hemorrhage (SICH) and all-cause death.

Of the 300 patients, 150 were randomized to 0.4mg/kg TNK and 150 to 0.25mg/kg TNK. In the 0.4mg/kg group (29 /150) and the 0.25mg/kg group (29 /150) (19.3%) (unadjusted risk difference, 0.0% [95% CI, −8.9% to −8.9%];Adjusted risk ratio, 1.03[95%CI, 0.66-1.61];P = 0.89).There was no significant difference in any of the four functional outcomes between the 0.4mg/kg group and the 0.25mg/kg group among the six secondary clinical outcomes, with all-cause death (26 [17%] vs 22 [15%];Unadjusted Risk Difference, 2.7% [95%CI, −5.6% to 11.0%]) vs. SICH

(7[4.7%] vs. 2 [1.3%];Unadjusted risk difference, 3.3% [95%CI, −0.5% to 7.2%]) was also not significantly different.

The results of this study suggest that 0.40mg/kg tenecteplase compared with 0.25mg/kg did not significantly improve cerebral reperfusion prior to endovascular thrombectomy in patients with ischemic stroke with intracranial large vessel occlusion. Results showed that 0.40-mg/kg dose of TNK did not have an advantage over 0.25-mg/kg dose in patients with large vessel occlusive ischemic stroke who were scheduled to undergo endovascular thrombectomy.

(6) ATTEST trial [19]

The ATTEST trial was designed to compare the efficacy and safety of tenecteplase and alteplase in patients without baseline imaging selection. This phase II, prospective, randomized, open label, blind outcome trial included patients with acute cerebral infarction within 4.5h after onset, and were randomly assigned to alteplase group (0.9 mg/kg) or tenecteplase group (0.25 mg/kg).CTP and CTA were used to evaluate infarction and perfusion before and after treatment, and the primary endpoint was the ratio of penumbra rescue within 24 to 48 hours.

The results showed as follows: (1) A total of 104 patients were enrolled in the study, and 71 patients reached the primary endpoint. The proportion of penumbra saved in the two groups was basically the same (68% in the tenecteplase group vs. 68% in the alteplase group).② There was no significant difference between the two groups in symptomatic intracranial hemorrhage (1/52 vs 2/51, P=0.55) and total intracranial hemorrhage (3/52 vs 4/51, P=0.59).③ There was no difference in the severity of adverse events between the two groups.

Conclusion: There is no difference between tenecteplase and alteplase in nerve function evaluation and imaging evaluation.

1.4 Experimental design

In conclusion, this study will evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) compared with standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset). This is a multicenter, prospective, centrally randomized, open label, blinded-endpoint, active controlled, non-inferior phase III clinical trial.

The drug administration method of the test drug (rhTNK-TPA) is as follows: according to the body weight of the subjects, the final dose is determined at 0.25mg/kg; Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the subject, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds..

The administration method of active control drug (rt-PA) is as follows: The dose required for medication is calculated and measured according to the actual body weight of the subject based on a standard of 0.9 mg rt-PA/kg. 10% of the dose is injected by intraveno

us bolus, and the remaining 90% is injected by intravenous drip within 1h.

2. Study objectives

To evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) compared with standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset).

Primary study objectives:

To evaluate the difference in the ratio of 90-day MRS score of 0-1 between rhTNK-tPA (0.25 mg/kg) and standard rT-PA (0.9mg/kg) in the treatment of subjects with hyperacute ischemic stroke (within 4.5 h of symptom onset).

Secondary study objectives:

1. To compare the difference in efficacy between rhTNK-tPA (0.25 mg/kg) and standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset) subjects:

(1) The proportion of subjects with an mRS score of 0-2 at 90±7 days

(2) MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution)

(3) The proportion of subjects whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first)

(4) Health-related quality of life (EQ-5d) at 90±7 day

(5) The proportion of subjects with a Bathel score ⩾ 95 points at 90±7 days

2. To compare the difference in safety between rhTNK-tPA (0.25 mg/kg) and standard rT-PA (0.9mg/kg) in the treatment of subjects with hyperacute ischemic stroke (within 4.5 h of symptom onset):

① The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASSIII)

② The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASSIII)

③ The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS standards)

④ The incidence of any intracranial hemorrhage within 90 days

⑤ The incidence of significant hemorrhage events at other sites within 90 days (as defined by GUSTO)

⑥ 90-day overall mortality

⑦ Incidence rates of adverse events/serious adverse events within 90 days

3. Study design

3.1 Overall design

This study is a multicenter, prospective, centrally randomized, open label, blinded-endpoint, parallel active control, and non-inferiority design.

3.2 Grouping

The subjects are randomly divided 1:1 into two groups, the treatment group (rhTNK-tPA 0.25mg/kg) and the control group (rT-PA 0.9mg/kg), and are given treatment within 4.5h after symptom onset.

3.3 Visits

Study visit points: -4.5h-0h (before thrombolysis), 0h (thrombolytic therapy), 24h±2h, 24h-36h, 7±1 day, 90±7 days (end visit).

3.4 Measures to control bias

3.4.1 Randomization

The central randomization method is adopted in this study, and the central randomization system is used to set dynamic block randomization. The proportion of subjects in the treatment group and the control group is 1:1 in each center, and each center assigned random number to each enrolled subject in the way of competitive enrollment.

After the subjects complete all screening tests and the investigators determine that they meet the inclusion criteria or do not meet the exclusion criteria, the staff of the research center is responsible for entering the basic information of the patients (initials, age, sex, identification code, etc.) into the central randomization system based on the network. The system will automatically generate a random number and subject ID according to the above principles and feed back to the central investigator via the network whether the patient is assigned to the trial group (rhTNK-tPA 0.25mg/kg group) or the control group (rT-PA 0.9mg/kg group).Investigators at the research center received the results of randomization and then treated the patients according to the appropriate group.

3.4.2 Blinding

All endpoints in this study will be evaluated in a blind manner by a qualified study physician who is not aware of the treatment grouping. The study physician responsible for blind state evaluation will be authorized by the PI of each center after receiving unified training and passing the examination.

3.5 Academic committee

It consists of the Sponsor, medical experts designated by the Sponsor and key investigators, and will hold meetings (teleconferences or on-site meetings) as needed to review the progress of the study, and make appropriate guidance and major decisions on the study.

3.6 Data Monitoring Committee

It consists of independent statisticians and neurologists who are not involved in the study execution. The membership of the DMC will be determined by the DMC and the Steering Committee prior to the commencement of the trial, including membership, roles, and responsibilities. The DMC will monitor the progress of this

study on a regular basis to ensure that the study meets the highest standards of ethics and subject safety. The DMC will make recommendations on the safety data of the trial: terminate or continue the trial or modify the protocol to continue.

3.7 Clinical event committee

It consists of neurologists, cardiologists, etc., who are not involved in the execution of the study. Membership of the Clinical Event Committee (including qualifications, roles and responsibilities) shall be confirmed by the Academic Committee prior to the commencement of the trial.

Clinical endpoint events include: new vascular events/hemorrhagic stroke (ischemic stroke/mi/cardio-cerebral revascularization (including: carotid intima stripped, intracranial artery interventional therapy, intracranial artery bypass surgery, coronary intervention or bypass surgery)), symptomatic intracranial hemorrhage (ECASSIII definition), PH2 intracranial hemorrhage (SITS), other parts of the significant bleeding (GUSTO hemorrhage definition, severe and moderate bleeding) and death.

All clinical endpoints will be first determined by the investigator and then verified and identified by the Clinical Event Committee based on clinical, laboratory, original images, or documentation. It will be discussed over by the independent Clinical Event Committee.

4. Study population

4.1 Inclusion criteria

(1) Age ≥18 years, male or female;
(2) Time from onset to treatment < 4.5h; the time of symptom onset is defined as "the last time point at which the patient appears normal";
(3) Clinical diagnosis as ischemic stroke (the diagnosis following the *Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018*);
(4) MRS score of 0-1 before onset;
(5) 4 < NIHSS < 26 at baseline;
(6) Subjects or their guardians voluntarily sign the informed consent.

4.2 Exclusion criteria

(1) Known to be allergic to rhTNK-tPA and/or rT-PA;
(2) A history of intracranial hemorrhage;
(3) A history of severe head trauma or stroke within 3 months;
(4) A history of intracranial or spinal surgery within 3 months;
(5) A history of gastrointestinal or urinary tract hemorrhage within 3 weeks;
(6) A history of major surgery within 2 weeks;
(7) A history of arterial puncture at sites difficult for compression hemostasis within 1 week.
(8) Intracranial tumors (except neuroectodermal tumors, such as meningiomas), large intracranial aneurysms;
(9) Intracranial hemorrhage (including parenchymal hemorrhage, intraventricular hemorrhage, subarachnoid hemorrhage, subdural/extradural hematoma, etc.);
(10) Active visceral bleeding;
(11) Aortic arch dissection found.
(12) Uncontrollable hypertension upon active antihypertensive treatment: systolic blood pressure ≥180 mm Hg, or diastolic blood pressure ≥100 mm Hg;
(13) Propensity for acute bleeding, including platelet counts of less than 100×10^9/ L or otherwise;
(14) Blood glucose <2.8 mmol/L or >22.22 mmol/L;
(15) On oral warfarin anticoagulant treatment with INR>1.7 or PT > 15s;
(16) Having received heparin treatment within 24 h;
(17) Having received thrombin inhibitors or factor Xa inhibitors within 48 h;
(18) Head CT or MRI shows a large infarction (infarcted area > 1/3 of the middle cerebral artery).
(19) Subjects who are unable or unwilling to cooperate due to hemiplegia (Todd's palsy) after epileptic seizure or other neurological/psychiatric disorders;
(20) Pregnant women, lactating women, or subjects who do not agree to use effective contraception during the trial;
(21) Participation in other clinical trials within 3 months prior to screening;
(22) Unsuitability for participation or participation in this study as judged by the investigator may result in exposure to greater risk.

4.3 Withdrawal criteria

4.3.1 Withdrawal decided by the investigator

1) Subjects with poor compliance, failure to take medication as required or participate in study visits, which affects the judgment of efficacy or safety;
2) In the judgment of the Principal Investigator, the subject should withdraw from the study in any case for the benefit/risk of the subject.

4.3.2 Subjects' voluntary withdrawal

According to the provisions of the informed consent, the subject has the right to withdraw from the study midway; In case that the subject has not withdrawn the informed consent, but no longer accepts the medication and testing, it is also considered as withdrawal, also called drop-out. As far as possible, the reasons for their withdrawal should be known and recorded.

If a subject withdraws from the study due to AE or abnormal laboratory test results, this important special event and test results should be recorded in an electronic case report form (eCRF).

For cases that drop out of the trial or are lost to follow-up, investigators should actively take measures to complete the last examination as far as possible in order to analyze the efficacy and safety. Any dropout subject should be documented in detail, safety and efficacy data for the subject should be obtained as far as possible, and an evaluation at the end of all studies should be conducted with subject consent and compliance. All case data should be kept complete for future reference.

Subjects who have signed the informed consent and been randomly grouped may not be replaced.

4.4 Removal criteria

1) Cases who are found not meeting the inclusion criteria or meeting the exclusion criteria after enrollment;
2) Cases to be removed due to violation of the provisions of the clinical trial protocol for concomitant medication and/or treatment, as determined by the Academic Committee;
3) Cases without medication after enrollment;
4) Cases without evaluable records after administration;
5) Cases who fail to take stipulated medication with poor compliance, resulting in impossibility to judge efficacy or incomplete data adversely affecting the judgment of efficacy and safety.

Cases eliminated should be explained, and their data should be kept complete for future reference. If patients have received at least one study drug treatment, ITT analysis could be performed according to the data records. Patients who have received at least one treatment and have a safety record may also be included in the safety analysis as appropriate.

4.5 Discontinuation criteria

Trial discontinuation refers to the discontinuation of all tests in the course of a clinical trial that has not been completed according to the protocol. The purpose of discontinuing the trial is mainly to protect the rights and interests of subjects, ensure the quality of the trial and avoid unnecessary economic losses. If one of the following situations occurs during the trial, the trial should be discontinued.

1) Serious safety problems occur in the trial process;

2) In the course of the trial, the drug efficacy is too poor, being ineffective in 2/3 of the cases;

3) Significant errors in the clinical trial protocol are found, making it difficult to evaluate drug response;

4) Significant deviations occur in the implementation of the trial protocol, making it difficult to evaluate the drug response if it continues;

5) The Sponsor requests the discontinuation or the drug regulatory authority orders the discontinuation of the experimental person for some reason.

The discontinuation of testing can be temporary or permanent. When the trial is discontinued, all trial records should be kept for future reference.

4.6 Termination criteria

1) The Sponsor and/or the investigator deem it inappropriate to proceed with the trial on medical or ethical grounds.

2) The Sponsor and/or the investigator deem it inappropriate to proceed with the study on the basis of scientific reasons or on the basis of factual, accurate or normative considerations for the conduct of the study. For example, major errors in the study protocol (including evaluation criteria, operation, analysis methods, etc.) are found in the trial, so it is difficult to evaluate the drug response; Or a well-designed program, with significant deviations during implementation (including incomplete or unassessable important data), continues, making it difficult to evaluate drug response.

3) Due to various reasons (such as funding reasons, management reasons or changes in medical development policies, etc.), the Sponsor decides to terminate the study under the premise of fully protecting the safety and rights of volunteers;

4) In accordance with the GCP guidelines and local laws and regulations, the National Medical Products Administration, the Ethics Committee, the investigator or the Sponsor, for any reason, should discontinue the study or discontinue the study in which the volunteers participated, taking into account their rights, the safety and health of the volunteers.

5. Study drugs

5.1 Name of study drugs

Test drug:

Drug name	Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-TPA)
Trade name	MingFuLe
Strengths	1.0 x 10^7 IU/16 mg/vial
Storage conditions	Store below 25℃ away from light. This product should be used immediately after dissolution. If not used immediately, it should be stored in cold storage at 2 - 8℃away from light and used within 24 hours.
Shelf life	24 months
Manufacturer	Guangzhou Recomgen Biotech Co., Ltd.
Supplier	Guangzhou Recomgen Biotech Co., Ltd.

Control drug:

Drug name	Alteplase for Injection (rt-PA)
Trade name	Actilyse®
Strengths	50mg/vial, 20mg/vial
Storage conditions	Keep in the original packaging. Store below 25℃ away from light. It is recommended to use the prepared solution immediately after preparation. It has been proved that the prepared solution can remain stable at 2-8℃ for 24h. Frezzing prohibited.
Shelf life	36 months
Manufacturer	Boehringer Ingelheim Pharma GmbH&Co.KG
Supplier	Guangzhou Recomgen Biotech Co., Ltd.

5.2 Labeling of study drugs

The test drug "Recombinant Human TNK Tissue-type Plasminogen Activator for Injection" will be produced and packaged by Guangzhou Recomgen Biotech Co., Ltd. (Sponsor), and the control drug "Alteplase for Injection" will be purchased and provided by Sponsor, and distributed to each clinical research center by the Sponsor.

Both the outer and inner packing box of the test drug shall be labeled as follows:

Code:

Product name: Recombinant Human TNK Tissue-type Plasminogen Activator for Injection

Strengths: 1.0×10^7 IU/16mg/vial

Batch number:

Production date:

Expiration date:

Storage condition: Store below 25℃ away from light

Note: For use in rhTNK-tPA III clinical trial only.

Guangzhou Recomgen Biotech Co., Ltd.

Note: Please fill in the subject random number at "code", specify the strength (number of vials or dose of single vial) according to different packages, and complete the batch number, production date and expiration date of the test drug according to the actual situation.

The packing box of the control drug should be labeled as follows:

Code:

Product name: Alteplase for injection

Strengths: □20mg/vial / □50mg/vial

Batch number:

Production date:

Expiration date:

Storage condition: Store below 25℃ away from light

Note: For use in rhTNK-tPA III clinical trial only.

Guangzhou Recomgen Biotech Co., Ltd.

Note: Please fill in the subject random number at "code", select the strength according to the actual dose (20mg/vial or 50mg/vial), and complete the batch number, production date and expiration date of the control drug according to the actual situation.

5.3 Method of administration

Treatment group (rhTNK-TPA 0.25mg/kg, 25mg at most):

RhTNK-TPA 0.25mg/kg: Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the subject, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds.

Control group (rt-PA 0.9mg/kg) :

RT-PA 0.9mg/kg: The product is dissolved in sterile water for injection to obtain a solution with a concentration of 1mg/ ml. The amount required for medication is calculated and measured according to the actual body weight of the subject. 10% of the solution is injected by intravenous bolus, and the remaining 90% is injected by intravenous drip within 1h.

5.4 Drug storage conditions

Study drugs will arrive at the research centers prior to the launch of the center, and then sufficient study drugs will be supplied to the Center on a regular basis based on the actual number of enrolled subjects and the amount of drugs to be used. The study drug will be stored in a secure location under the direct responsibility of the investigator or other authorized person and will be stored in accordance with the conditions described on the label.

5.5 Drug distribution and inventory

The investigator must keep accurate records of the number of boxes/bags of the drug being studied. In this study, the drug is administered intravenously, and the study drug had to be administered by the investigator.

5.6 Drug recovery and destruction

Investigators are required to record information on all study drug dispensations, including date, quantity, drug lot number, and subject number. Used and unused drugs must be recovered and destroyed in accordance with relevant regulations and procedures.

6. Treatment

6.1 Treatment administered

Study drugs should be administered as soon as possible after randomization (less than 4.5h from onset of disease to onset of thrombolysis).

Thrombolysis time (intravenous infusion time in the treatment group, intravenous infusion time in the control group, and the start and end time of intravenous infusion), administration dose (intravenous infusion dose in the treatment group, intravenous infusion dose in the control group, and intravenous infusion dose), vital signs and the occurrence of adverse events during administration should be recorded.

6.2 Concomitant treatment

All enrolled subjects should be treated in the Acute Stroke Unit or, if needed, in the Intensive Care Unit (ICU). All subjects will be enrolled according to the *Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018*.[28] Subsequent standardized early treatment management and secondary prophylactic medication are performed. Subjects are treated with thrombolysis according to relevant guidelines (*Chinese Guidelines for Endovascular Treatment of Acute Ischemic Stroke 2018*).[29], *Chinese Guidelines for Early Intravascular Interventional Diagnosis and Treatment of Acute Ischemic Stroke 2018*[30]), and it is up to the investigator to make the final decision on whether or not to perform endovascular treatment, and to record the treatment method and drug combination.

Concomitant drugs (including anticoagulants, defibrillators, antiplatelet agents, lipid-lowering agents, antihypertensive agents, hypoglycemic agents, etc.) before and after randomization and during hospitalization and follow-up should be recorded. Non-therapeutic drugs such as contrast agents, pre-flushing tube heparin, etc., are not recorded as concomitant drugs.

(1) If the subject does not receive mechanical thrombotomy, no antiplatelet drugs, anticoagulants, defibrillators, thrombolytic drugs or drugs that may affect platelet and clotting function are allowed to be used within 24 hours after the study drugs is used.

(2) If the subjects have received mechanical thrombotomy but do not receive emergency stent implantation, the investigators will determine whether the concomitant use of antiplatelet and anticoagulant therapy is necessary according to

relevant guidelines within 24h after the use of the study drugs, and the concomitant use of drugs will be recorded.

(3) If the subjects have received emergency stent implantation, antiplatelet agents and anticoagulants can be used within 24 hours after the study drugs is used.

(4) Any medication other than those listed above (including blood pressure lowering, blood glucose lowering, lipid-lowering drugs, etc.) is allowed. If necessary, the investigator should carefully consider administering a relatively stable and safe dose to the subject.

During the study period, any treatment and/or prescriptions issued prior to randomization or any medication changes during the study period should be recorded on an electronic case report form.

6.3 Medication compliance

In the recruitment and screening stage, the purpose of the trial, the basic information of the study drugs, the protocol, the trial process, the administration regimens, clinical observation, the potential risk if participating in the study, compensation, etc., will be introduced in detail, so that subjects could be fully informed and voluntarily participate in the study to improve the compliance of the study. This study uses single dose, to be administered by the study nurse. Before administration, subject number, administration table and dosage should be carefully checked; after administration, the remaining quantity of study drugs, empty packages and administration equipment should be carefully checked.

7. Study process

7.1 Screening/baseline assessment

1) Demographic information: sex, age, ethnicity.

2) The time of present onset as well as the symptoms (the present onset symptoms are mainly reflected in NIHSS score).

3) Risk factors (past medical history and history of present illness) : high blood pressure, diabetes, cerebral infarction, TIA, coronary heart disease, myocardial infarction, arrhythmia, always oral medication (aspirin, clopidogrel, and warfarin, antihypertensive drugs, lipid-lowering drugs, oral glucose-lowering drugs, drug adjust heart rate), dementia, peripheral artery

disease, lipid metabolic disorders, liver and kidney function is not complete, and neck intracranial vascular stenosis or deformity, smoking, drinking alcohol.

4) Prior and concomitant medication.

5) Vital signs (blood pressure, pulse, respiratory rate and body temperature).

6) MRS score before onset, MRS score post onset before onset of thrombolysis, NIHSS score post onset before onset of thrombolysis.

7) Electrocardiogram (If the subject has had an electrocardiogram before thrombolysis after the onset of the disease, no repeat examination is required).

8) Imaging (CT or MRI).

Screening/baseline imaging examination is “CT or MRI”, which is used to exclude hemorrhagic stroke; the CT or MRI results generated by other hospitals can also be used as a screening basis, if the study physician deems them acceptable; if a subject has undergone a CT or MRI check post symptom onset before onset of thrombolysis, such examination may be omitted. CT or MRI evaluation should include: cerebral hemorrhage, acute severe symptomatic ischemia in the area of blood supply, or other pathological changes.

9) Laboratory examination:

Routine blood test:

Red blood cell count (RBC), white blood cell count (WBC), neutrophil count (NEU), lymphocyte count (LY), hematocrit (HCT), hemoglobin (Hb), platelet count (PLT).

Blood biochemistry:

Creatinine (GREA), UREA (or blood urea nitrogen BUN), glucose (GLU), uric acid (UA), lactate dehydrogenase (LDH), creatine phosphokinase isoenzyme (CK-MB), alkaline phosphatase (ALP), glutamyltranspeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), triglyceride (TG);

For baseline blood lipid tests (total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), triglyceride (TG)), the most recent fasting blood lipid after the onset of the disease should be measured. Blood glucose test is performed at baseline using peripheral blood glucose measured in venous blood as one of the inclusion and exclusion criteria.

Coagulation function test:

Prothrombin time (PT), activated partial prothrombin time (APTT), thrombin time (TT), fibrinogen (FIB), international standardized ratio (INR).

Blood/urine pregnancy test:

Only for fertile female subjects.

Note: The study population is acute ischemic stroke subjects. For the reason of time, subjects, after signing written informed consent by themselves or their guardians, can be randomly grouped to receive treatment first; but for safety reasons, investigators must learn routine blood test, blood glucose levels (peripheral blood glucose results) and urine pregnancy test (if applicable) results to determine the suitability for participation into the study, if warfarin has been used, including coagulation function results. Blood biochemical and coagulation tests should be completed before administration, and the results can be provided after enrolment. Blood biochemical results are allowed to be obtained after the study drug has been administered. If abnormal blood biochemical results are obtained, they will be handled by the study physician in accordance with the discipline guidelines and the clinical management pathway of the hospital. Subject will not need to repeat the test if they have already performed some laboratory tests between the present onset and prior to thrombolysis.

7.2 Randomization

If the patients agree to participate in the trial, after the patients are screened and agree to be enrolled, the investigator will use the central randomization system to generate randomization numbers and group information for the patients according to the order of their enrollment, and the randomization number will be recorded on the enrolment screening table and the CRF table.

7.3 Treatment

Study drugs should be administered as soon as possible after randomization (less than 4.5h from onset of disease to onset of thrombolysis).

Thrombolysis time (intravenous infusion time in the treatment group, intravenous infusion time in the control group, and the start and end time of intravenous infusion), administration dose (intravenous infusion dose in the treatment group, intravenous infusion dose in the control group, and intravenous infusion dose), vital signs and the occurrence of adverse events during administration should be recorded.

Vital signs (blood pressure, pulse, body temperature, respiratory rate) should be measured within 5min before thrombolysis.

Concomitant medication and adverse events should be recorded.

7.4 Follow-up

7.4.1 24 + 2 h

During the follow-up:

NIHSS score.

Vital signs (blood pressure, pulse, body temperature, respiratory rate).

Laboratory examination (test results obtained within 72 hours after onset (after thrombolysis) are acceptable):

Routine blood test, blood biochemistry and coagulation tests are to be performed, including the same items as during screening period.

Routine urinalysis: pH value, protein, occult blood, glucose, bilirubin, nitrite, ketone body.

Concomitant medication and adverse events are recorded.

7.4.2 24 - 36 h

During the follow-up:

Imaging: CT or MRI, used to detect hemorrhagic events.

Concomitant medication and adverse events are recorded.

7.4.3 7±1 day or before discharge (whichever occurs first)

During the follow-up:

NIHSS score, BI scale and EQ-5D scale.

Vital signs (blood pressure, heart rate, body temperature, respiratory rate).

Laboratory examination:

Routine blood test, blood biochemistry and coagulation tests are to be performed, including the same items as during screening period.

Routine urinalysis: pH value, protein, occult blood, glucose, bilirubin, nitrite, ketone body.

Electrocardiogram.

Concomitant medication and adverse events are recorded.

7.4.4 90 + 7 days

During the follow-up:

MRS score, BI scale and EQ-5D scale are evaluated.

Concomitant medication and adverse events are recorded.

8. Efficacy endpoints

8.1 Primary efficacy endpoints

The proportion of subjects with 90-day MRS score of 0-1: Proportion of subjects with 90-day MRS score of 0-1 to all enrolled subjects. Calculation formula:

The proportion of subjects with 90-day MRS score of 0-1 = the number of subjects with 90-day MRS score of 0-1 / the number of subjects enrolled in this group ×100%

8.2 Secondary efficacy endpoints

1) The proportion of subjects with an mRS score of 0-2 at 90±7 days
2) MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution)
3) The proportion of subjects whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first)
4) Health-related quality of life (EQ-5d) at 90±7 day

5) The proportion of subjects with a Bathel score ⩾ 95 points at 90±7 days

8.3 Safety endpoints

1) The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASSIII)
2) The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASSIII)
3) The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS standards)
4) The incidence of any intracranial hemorrhage within 90 days
5) The incidence of significant hemorrhage events at other sites within 90 days (as defined by GUSTO)
6) 90-day overall mortality
7) Incidence rates of adverse events/serious adverse events within 90 days.

9. Observation and treatment of adverse events

9.1 Adverse events

9.1.1 Definition of adverse events and adverse reactions

Adverse Event (AE) refers to all adverse medical events that occur after the subject receives the study drugs, which may be manifested as symptoms, signs, diseases or abnormal laboratory tests, but does not necessarily have a causal relationship with the study drugs.

Adverse drug reaction (ADR) refers to any harmful or undesirable reaction that occurs during a clinical trial and may be related to the study drugs with at least a reasonable possibility of a causal relationship between the study drugs and the adverse event, i.e., an association cannot be ruled out.

9.1.2 Recording of adverse events

Adverse event reporting period: adverse event recording starts immediately after the subjects receive the study drug, until the last follow-up after completion of the study. All AE findings must be recorded by the investigator (or designee) in the AE section of the eCRF. The record includes a description of the AE, the time of occurrence and resolution, severity, relationship with the study drugs, measures taken, and outcomes.

9.1.3 Severity assessment

The severity of adverse events should be assessed according to CTCAE version 5.0. In case of adverse events not listed in the table, the following criteria can be used:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate: minimal, local or noninvasive intervention indicated; limiting age- appropriate instrumental Activities of Daily Living.

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

9.1.4 Assessment of association between adverse events and study drugs

All adverse events in the clinical study, including any abnormal symptoms, signs, laboratory tests, or other special tests, should be documented in detail and evaluated for association. The investigator must follow up and obtain sufficient information to determine whether the cause of an adverse event is related to the study drugs. According to the criterion of causality between drugs and adverse events, the correlation between adverse events and the application of the test drug is divided into five grades: definitely related, probably related, possibly related, unlikely related, and definitely not related. Any adverse reactions assessed to be definitely related, probably related, possibly related and unlikely related are listed as adverse drug reactions. The total number of ADR cases is taken as the numerator, and all selected cases for ADR assessment are taken as the denominator to calculate the incidence of ADR.

The judgment criteria are as follows:

Definitely related: consistent with the known type of reaction of the suspected drug, forming a reasonable time sequence between onset and drug administration, reduction or disappearance of the adverse event after dose reduction or withdrawal, and reoccurrence of the adverse event after administration.

Probably related: consistent with the known type of reaction of the suspected drug, forming a reasonable time sequence between onset and drug administration, reduction or disappearance of the adverse event after dose reduction or withdrawal,

but may be resulted from the subject's clinical status or other reasons.

Possibly related: consistent with the known type of reaction to the suspected drug, forming a reasonable time sequence between onset and drug administration, reduction or insignificant improvement of the adverse event after dose reduction or withdrawal, but may be explained by the subject's clinical status or other reasons.

Unlikely related: not consistent with the known type of reaction to the suspected drug, not forming a reasonable time sequence between onset and drug administration and may be resulted from the subject's clinical status or other reasons.

Definitely not related: not consistent with the known type of reaction to the suspected drug, not forming a reasonable time sequence between onset and drug administration, may be resulted from the subject's clinical status or other reasons, reduction or disappearance of the adverse event after the exclusion of clinical symptoms or other causes.

9.2 Serious adverse events

9.2.1 Definition of serious adverse events

A serious adverse event (SAE) is a medical event that occurs after a subject receives the study drugs, such as death, life-threatening, permanent or severe disability or loss of function, requiring hospitalization or prolonged hospitalization, or leading to a congenital abnormality or birth defect, excluding the following:

Non-therapeutic hospitalization, such as: rehabilitation hospitalization, nursing facility hospitalization, transactional hospitalization, hospitalization for examination only, planned elective hospitalization, etc.

9.2.2 Recording and reporting of serious adverse events

When an SAE occurs during the clinical trial, the investigator should report it in writing to the Sponsor as soon as he becomes aware of it, followed by a detailed, written follow-up report in a timely manner. If a death is involved, the investigator shall immediately submit a written report to the Ethics Committee of the center, as well as provide other necessary information, such as the autopsy report and the final medical report.

Upon receipt of the SAE report, the Sponsor or designee shall immediately analyze, evaluate and promptly report suspected unexpected serious adverse events

(SUSAR) to all investigators participating in the clinical trial, the clinical trial facility, the Ethics Committee, the drug regulatory authority and the health authority.

Reporting unit	Contact	Phone	Email
Ethics Committee of Beijing Tiantan Hospital	'	010-59978555	ttyyirb@163.com
Guangzhou Recomgen Biotech Co., Ltd.	School of medicine	18926139511 020-82209991	yangqiao@recomgenbio.com
PV contact	Zhao Qianna	010-51281119	PV@giantcro.com

9.3 Reporting of suspected unexpected serious adverse reaction (SUSAR) [31]

Suspected unexpected serious adverse reactions refer to suspected unexpected serious adverse reactions whose nature and severity of clinical manifestations exceed the available data such as the investigator's brochure of the study drugs, the package insert of a marketed drug, or the summary of product characteristics.

All unexpected serious adverse reactions that are definitely related to or suspected to the study drugs occurring during clinical trials (both within and outside China) should be promptly reported to the national drug evaluation body within the specified time limit. The contents of individual safety reports of unexpected severe adverse events should be reported in accordance with the relevant requirements of ICH E2B (R3): *Clinical Safety Data Management: Data Elements for Transmission of Individual Case Safety Reports*. Relevant terms shall be coded in the ICH M1: *Medical Dictionary for Regulatory Activities* (MEDDRA).

a) Start and end times for expedited reporting of SUSAR

The start time of expedited reporting is the clinical trial approval date/the start date of the implied approval of the national drug evaluation body, and the end time is the end date of the last subject follow-up in China. (Unexpected serious adverse reactions occurring from the end of the clinical trial follow-up to the conclusion of the review and approval should also be subject to expedited reporting.)

b) Time limit for expedited reporting of SUSAR

Fatal or life-threatening: first report within 7 days, followed by follow-up within 8 days.

Non-fatal or life-threatening: first report within 15 days.

c) The electronic transmission mode of SUSAR

Submit through GATEWAY

Submit as an XML file

9.4 Pregnancy events

If a subject becomes pregnant during the study period, the investigator should report to the relevant authorities (Sponsor, hospital Ethics Committee) within 24h after being informed. Subjects should stop medication immediately after pregnancy is found and continue safety follow-up until the end of pregnancy or termination of pregnancy. Pregnancy events will be reported in accordance with SAE reporting requirements and timelines.

9.5 Possible adverse drug reactions

Adverse events in clinical trials of this product are the same as other thrombolytic drugs. The most common adverse reactions in clinical studies of this product are bleeding, including intracranial hemorrhage and other minor bleeding adverse events. The specific data are shown in the table below.

Hemorrhagic adverse events in clinical trials of this product

	rhTNK-tPA 16mg (n=124)	**rt-PA 50mg (n=127)**
Intracranial bleeding	**1 (0.81%).**	**1 (0.79%).**
Adverse events of minor bleeding:		
Urinary tract hemorrhage	**8 (6.45%)**	**6 (4.72%)**
Bleeding gums	**6 (4.84%)**	**4 (3.15%)**
Subcutaneous ecchymosis	**4 (3.22%)**	**4 (3.15%)**
Gastrointestinal hemorrhage	**3 (2.42%)**	**5 (3.94%)**
RBC indexes such as hemoglobin decreased	**2 (1.61%).**	**3 (2.36%)**
Hematoma at puncture site	**1 (0.81%).**	**4 (3.15%)**
A bleeding point on tongue tip	**0**	**1 (0.79%).**

Thrombolytic therapy should be discontinued when a potential tendency to hemorrhage, especially intracranial hemorrhage, is found.

Allergic reaction: No patients have been found to have allergic reaction after using this product in the clinical trial. Once an allergic reaction occurs, antiallergic

treatment is required.

Other adverse events: The following adverse events have been reported in patients who received this product in clinical trials. The effect of this product on the incidence of these adverse events is unclear. These adverse events include cardiogenic shock, heart failure, cardiac rupture and electromechanical separation, ventricular fibrillation, and cardiac rupture (3.23%); increase in aminotransferase, myocardial enzyme, blood lipid and blood glucose, as well as nausea, vomiting, fever and cough, all mild. The majority of these adverse events are determined not to be related to the test drug, but to underlying and/or comorbidities, as well as concomitant drugs.

Clinical adverse reactions of similar products authorized in the United States and the European Union:

In an international multicenter, double-blind clinical trial (ASSENT-2), TNK-tPA (generic name: Tenecteplase, trade name in the United States: TNKase®, trade name in the European Union: Metalyse®) and rt-PA was compared. The incidence rates of intracranial hemorrhage in patients treated with TNKase® and rt-PA were both 0.9%, and the incidence rates of stroke were 1.8% and 1.7%, respectively. The incidence of stroke in both groups, including intracranial hemorrhage, increased with age. The data of non-intracranial hemorrhagic adverse events in the ASSENT-2 study are shown in the table below.

Non-intracranial hemorrhagic events in the ASSENT-2 study

	TNK-tPA (n=8461)	**rt-PA (n=8488)**	**The relative risk (95% CI)**
Bleeding events			
Severe bleeding event[a]	**4.7%**	**5.9%**	**0.78 (0.69, 0.89)**
Minor bleeding event	**21.8%**	**23.0%**	**0.94 (0.89, 1.00)**
Blood transfusion unit			
all	**4.3%**	**5.5%**	**0.77 (0.67, 0.89)**
1 to 2	**2.6%**	**2.6%**	**-**
> 2	**1.7%**	**2.2%**	**-**

Note A: Severe bleeding events are defined as those that require blood transfusion, or those that cause hemodynamic harm.

Patients treated with TNKase had a lower incidence of non-intracranial bleeding and requiring blood transfusion. The main types of bleeding in 1% or more of patients

are hematoma (1.7%) and gastrointestinal bleeding (1%). The main types of bleeding in 1% or less of patients are urinary tract bleeding, bleeding at the site of puncture (including cardiac catheter bleeding), retroperitoneal bleeding, respiratory tract bleeding, and bleeding at unspecified sites. The types of minor bleeding in 1% or more patients are hematoma (12.3%), urinary tract bleeding (3.7%), bleeding at the site of puncture (including cardiac catheter bleeding) (3.6%), pharyngeal bleeding (3.1%), gastrointestinal bleeding (1.9%), nasal bleeding (1.5%), and unspecified site (1.3%).

Anaphylaxis: Few (< 1%) patients treated with TNKase have reported anaphylaxis (e.g., hypersensitivity, angioedema, laryngeal edema, rash, urticaria). < 0.1% of patients treated with TNKase experienced anaphylaxis, but causality is uncertain. When an allergic reaction occurs, conventional treatment is usually used.

Other adverse events: The following adverse events are observed in patients treated with TNKase in clinical trials. These reactions are often sequelae of the underlying disease, and the effect of TNKase on their incidence is unclear. These adverse events include cardiogenic shock, arrhythmias, atrioventricular block, pulmonary edema, heart failure, cardiac arrest, recurrent myocardial ischemia, recurrent myocardial infarction, cardiac rupture, tamponade, pericarditis, pericardial effusion, mitral insufficiency, thrombosis, embolism, and electromechanical separation of the heart. These events can be life-threatening and may result in death. Nausea and/or vomiting, low blood pressure and fever have also been reported.

9.6 Management and follow-up of adverse events

All adverse events, including laboratory abnormalities, must be followed up to normalization or remission, or return to baseline, or when follow-up is determined by the investigator to be unnecessary, or subjects are lost to follow-up, so as to ensure the safety of the subjects.

If a subject develops any serious adverse event during the clinical trial, the investigator shall immediately take appropriate treatment for the subject, regardless of whether the event is related to the clinical trial or the study drugs. The investigator should immediately make a comprehensive evaluation of the incident and complete the serious adverse event report form and report it to the relevant authorities. Severe adverse events are followed up to normalization or remission, or return to baseline, or when follow-up is determined by the investigator to be unnecessary or subjects are

lost to follow-up. A summary and/or follow-up serious adverse event report form is required.

9.7 Emergency response plan for adverse events

1) Bleeding event management plan

In order to prevent the occurrence of bleeding events, the inclusion and exclusion criteria of this study protocol should be strictly followed to exclude the subjects with potential high blood risk. During thrombolytic therapy, close attention should be paid to the changes of symptoms and signs of subjects so as to detect bleeding events in time. The treatment of bleeding events should follow the clinical routine of the hospital and be based on the clinical judgment of doctors. The following measures can be referred to:

1. If the patient has neurological symptoms and/or abnormal signs within 24 hours after the start of treatment, intracranial hemorrhage should be suspected, and the thrombolytic, antiplatelet and anticoagulant drugs being used should be discontinued, and imaging examination should be performed immediately to confirm the site of bleeding;

2. If intracranial hemorrhage or other clinically visible bleeding (such as hematemesis, hematochezia, hemoptysis, etc.) is confirmed, the following measures are recommended:

(1) Completely discontinue thrombolytic, antiplatelet and anticoagulant drugs;

(2) Invite relevant professional physicians for consultation to jointly determine the symptomatic treatment plan;

(3) Closely monitor the vital signs and timely treat complications;

(4) Fresh frozen plasma infusion;

(5) Use antifibrinolytic drugs such as tranexamic acid within 24 hours after thrombolytic therapy.

(6) If normal heparin therapy is used at the same time, protamine may be used within 4 hours to exert an antagonistic effect of heparin (1mg of protamine to antagonize 100U of normal heparin);

(7) Blood transfusion: Blood transfusion may be required to correct hemodynamic abnormalities (hypotension caused by bleeding) or anemia. The indications for blood transfusion should be mastered according to the clinical judgment of the

doctor. While strictly grasping the indications for blood transfusion, attention should be paid to the adverse effects of severe anemia on acute myocardial infarction.

(8) Evaluate the feasibility of surgical intervention when necessary;

3. Minor bleeding, such as skin and mucosal bleeding, nosebleed, gingival bleeding, microscopic hematuria, positive stool occult blood, etc., can be symptomatic in advance, and thrombolysis, antiplatelet and anticoagulant therapy should be continued according to the bleeding degree.

In particular, symptomatic intracranial hemorrhage (2%-7%).The vast majority of symptomatic intracranial hemorrhage occur within 24 hours of thrombolytic therapy. The symptoms include sudden changes in the state of consciousness, single or multiple location signs of the nervous system, coma, headache, nausea, vomiting and seizures, hypertensive emergency, and rapid death in some cases. When this happens, active measures should be taken:

(1) Stop thrombolysis, antiplatelet and anticoagulant therapy immediately.

(2) Perform imaging examination (emergency CT or MRI) to determine the location, amount and classification of intracranial bleeding.

(3) Measure erythrocyte volume, hemoglobin, prothrombin, activated partial thromboplastin time, platelet count, fibrinogen, D-dimer, blood group and perform a cross-match test.

(4) Reduce intracranial pressure, including proper control of blood pressure, raising the head of the bed by 30 degrees, intravenous mannitol, endotracheal intubation and auxiliary ventilation, surgical hematoma removal, decompression of bone flap and ventricle puncture and drainage when necessary.

(5) If necessary, use reverse thrombolysis, antiplatelet and anticoagulant drugs: 2U of fresh frozen plasma should be given every 6 hours within 24h; it is recommended to neutralize with protamine (1mg protamine to neutralize 100U of normal heparin) for patients who have used normal heparin therapy within 4h. If the bleeding time is abnormal, 6-8U of platelets can be transfused.

(6) Properly control the blood pressure.

2) Allergic reactions

In the clinical trials of this product, no patients have been found to have allergic reaction after using this product. In the event of anaphylaxis, antiallergic treatment is required.

3) Other expected adverse events

The following adverse events have been reported in patients who received this product in clinical trials. The effect of this product on the incidence of these adverse events is unclear. These adverse events include cardiogenic shock, heart failure, cardiac rupture and electromechanical separation, ventricular fibrillation, and cardiac rupture (3.23%); increase in aminotransferase, myocardial enzyme, blood lipid and blood glucose, as well as nausea, vomiting, fever and cough, all mild. The majority of these adverse events are determined not to be related to the test drug, but to underlying and/or comorbidities, as well as concomitant drugs.

The relationship between the above events and TNK-TPA is not clear, and most of them are potential combined diseases. If the above phenomena occur, appropriate treatment should be taken according to the situation.

4) Other adverse reactions

Other adverse reactions should be analyzed by the investigator according to the specific situation, and given symptomatic treatment according to the corresponding treatment guidelines or clinical routine procedures.

10. Data management

10.1 Source data/source files

Source data refers to all information recorded in the original records or copies (certified copies) of the clinical trial, including clinical findings, observations, and other relevant activity records necessary for reconstruction and evaluation of the clinical trial.

Source documents refer to the original medical records, medical documents and data generated during the clinical trial. Source file contains the source data, such as the hospital medical records, medical images, laboratory records, clinical trial related memorandum, clinical trial subjects the diary or assessment form, hair medicine,

instruments and automatic recording of data and save the prescription pharmacy, laboratory and the department of medical and related documents and records, including duplicated or copied certified copy thereof. Source files can be paper and/or electronic.

The investigator and the medical institution to which he/she works permit the Sponsor to supervise or audit the clinical trial; The health administration and drug supervisory and regulatory authorities have the right to inspect clinical trials and have direct access to source data/source documents.

10.2 Database establishment and data entry

(1) Selection of EDC system: In this clinical study, EDC system is used to collect eCRF data.

(2) Database establishment: build eCRF interface according to protocol and CRF table, and set logical review limit conditions; Input the test data to carry on the trial operation of the database, and then establish the special database system for the trial.

(3) Data entry: each user will get an independent user name and password. The designated personnel of the research center will input the original data into the eCRF through the secure network. The system will automatically check the data deviation in the eCRF, generate the corresponding prompt message, and allow the personnel of the research center to modify the input data.

The clinical research associate should confirm that all data in the eCRF is consistent with the original record. Any errors or omissions found will be notified to the staff of the Research Centre in the form of a query sheet requesting modifications to the relevant data in the eCRF. Before the database is locked, the investigators will confirm and electronically sign off to ensure the accuracy of the data recorded.

10.3 Data monitoring

Monitoring of eCRF data and process files:

(1) During the study, the clinical research associate shall come to the research center to check the informed consent and screening and enrollment of the subjects.

(2) Confirm that all eCRFs are filled correctly and consistent with the original data.

(3) Adverse events of each subject shall be confirmed and recorded.

(4) The withdrawal of the subject shall be explained in the eCRF.

(5) Confirm that all AE's have been documented and that SAE has made a report for the record.

(6) Verify whether the study drugs are supplied, stored, distributed and recovered in accordance with relevant regulations, and make corresponding records of the storage conditions, time and records of the remaining study drugs in accordance with relevant requirements.

ECRF data, analytical data and process documents will be stored on paper or electronically. After the database is locked, the locked data will be transferred to the Sponsor.

10.4 Quality assurance of research data

To ensure the accuracy, integrity and reliability of the data, the investigator needs to record all trial data and procedures, including informed consent signing, screening, medication and blood sample collection, and laboratory analysis data. If necessary, the investigator should provide the original data to the Sponsor, the relevant regulatory authorities, and the Ethics Committee.

The Sponsor will designate relevant personnel to complete the following work:

(1) Provide appropriate instructional research documents and materials to the research center.

(2) The Sponsor will organize a kick-off meeting to train the investigators and study coordinators before the study begins. This meeting will explain the study protocol, eCRF filling and study procedure.

(3) Regular follow-up research center for related quality control.

(4) Keep in touch with the Research Centre at all times by telephone, mail and/or fax.

(5) Check and evaluate eCRF data in a timely manner, analyze quality, or use standardized computer editing to detect errors in data collection.

In addition, the Sponsor or designated representative will visit the study center periodically to check that the subject data is consistent with the original records and may audit the study at any time.

10.5 Data review and locking

The data management facility reviews the data recorded in the eCRF in accordance with the standard operating procedures. For any questions in the data, the

data management personnel will question the research center personnel in the form of an electronic query sheet, requesting the personnel designated by the research center to answer the questions and make some necessary modifications to the data. All data changes and related operations need to be documented. The data administrator shall write the data audit report according to the final version study protocol, data audit standard and database. The project manager will organize the Sponsor, major investigators, statisticians and data administrators to hold a data review meeting to review the data, and the representatives of all parties will sign the review resolution. After the parties approve the database locking, the data administrator organizes the database locking work and submits the locked database to the statistician for statistical analysis.

11. Sample size

In this study, rt-PA active control will be used based on a statistical hypothesis test of non-inferiority, and the proportion of subjects with 90-day MRS score of 0-1 will be used as the primary efficacy endpoint.

According to a paper published in the Lancet[7] in 2014 about meta-analysis of the incidence of 90-day MRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the Guidelines for the Design of Non-inferiority Drug Clinical Trials [32], the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high priority index. Therefore, based on the lower CI limit of 1.14, together with an efficacy of 25% of the active control, the non-inferior threshold was determined as 0.906. Then assuming α=0.025 using one-sided test, power 1-β=0.8, treatment group (TNK) incidence of 64%, control group (rt-PA) incidence of 63%, real RR= 1.02, the sample size was calculated by PASS software to be 643 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each group, a total of 1430 cases.

12. Statistical plan

12.1 Statistical analysis

Full analysis set (FAS), per protocol set (PPS) and safety analysis set (SAS) will

be established according to the NMPA regulations for data statistical analysis. Strict quality control will be carried out in the whole process of data collection to ensure the authenticity of the evaluation data.

Full analysis set (FAS): a data set consisting of all subjects randomized to receive at least one study drug according to the basic principle of Intention To Treat.

Per protocol set (PPS): PPS includes all subjects who complete the stipulated treatment without missing primary efficacy endpoints or serious violations of the protocol. Serious protocol violation will be finally defined during data review and may generally include (but not limited to) the following situations: subjects violating inclusion criteria or exclusion criteria, poor compliance in stipulated medication, concomitant medication that affects outcome indicators, and out of visit window.

Safety analysis set (SS): a set of all subjects participating in the trial, receiving at least one study drug, and having at least one safety evaluation.

FAS and PPS are established as sensitive analysis groups; Efficacy analysis is performed on the basis of FAS and PPS. In efficacy evaluation of FAS, missing data of efficacy endpoints will be carried forward using the last observed data after administration (LOCF). For PPS, missing data of efficacy will not be processed. Safety endpoints will be performed on the safety set and safety missing data will not be filled. All baseline demographic data analyses will be performed on the basis of the FAS; illogic data needs to be investigated by the data management facility, investigators or Sponsor together to identify possible reasons, and restrained by the parties in a data review meeting after full discussion: illogic data, if sufficient reasons have been found to indicate its incorrectness, should be removed, or otherwise, should be remained and subjected to analysis and the results obtained without such removed data should be treated as sensitivity analysis; If there is data collected in the study that is not in accordance with the program requirements such as planned external visits, it will be presented in a list, and descriptive or comparative analysis will be decided at the data review meeting depending on the specific situation of the data.

12.2 General principles

All statistical analysis will be conducted by SAS9.4 or higher version statistical software. The content of statistical analysis in the protocol is the general principle of statistical analysis method, and the specific statistical analysis process will be carried out according to the pre-formulated statistical analysis protocol.

Statistical description: quantitative indicators describe mean, standard deviation, minimum, maximum, median, quartile, etc. Qualitative indicators describe the number and percentage of cases in each category or grade.

Statistical inference: the two-sample t-test or non-parametric test is used for the comparison of quantitative indicators between groups depending on the data situation, the non-parametric test is used for the comparison of grade indicators, and the Chi-square or Fisher exact probability method is used for qualitative indicators. Evaluation of efficacy endpoints will be considered for inclusion in baseline or center effect analyses depending on the specific situation.

Statistical hypothesis: Comparison of the primary efficacy endpoint between groups is conducted by a one-sided test, with a significance level of one-sided α=0.025, null hypothesis H0: RR $\leq \Delta$ and alternative hypothesis H1: RR > - Δ; when P < 0.025 in statistical tests, or lower limit of 95% CI of RR > - Δ, it indicates that the test drug is not inferior to control drug; RR is the ratio of incidence of the treatment group and the control group, Δ is the non-inferior threshold, 0.906 in this trial. All other statistical analyses are conducted by two-sided tests, with a significance level of two-sided α=0.05. When P≤0.05, it indicates a statistically significant difference. The confidence interval is 95% on both sides. Specific non-inferiority hypothesis tests are as follows:

$$H_0: RR_{试验组/对照组} \leq \Delta \quad H_0: RR_{试验组/对照组} \leq \Delta$$
$$H_1: RR_{试验组/对照组} > \Delta \quad H_1: RR_{试验组/对照组} > \Delta$$

12.3 Analysis content

The actual number of subjects enrolled in each group, completed and dropped out from the study, removed, deviated from or violated the protocol, demographic and other baseline characteristics, compliance, efficacy analysis, and safety analysis. Statistical analysis includes (1) the case number of completed and that of dropped out in each research center, etc.; (2) The demographic and baseline characteristics of each group at the time of enrollment, necessary for comparability analysis between the treatment group and the control group; 3) Efficacy evaluation and safety evaluation, as described below.

12.4 Efficacy evaluation

Primary efficacy endpoints

Description method: Patients with 90-day MRS score of 0-1 among all enrolled patients in the corresponding data set is statistically described in the form of number and percentage respectively.

Analysis methods: in this protocol, the primary endpoint is the proportion of subjects with 90-day mRS score of 0-1; for such endpoint, CMH test with controlled center effect will be applied to statistically compare the treatment group and control group, and the 95% confidence interval of efficacy, and corresponding RR or RD and corresponding 95% confidence interval will be calculated; Logistic regression model analysis will also be considered to report the 95% confidence intervals of OR for TNK-TPA versus rt-PA. Adjustment for covariates with inter-group differences or center-group interactions will be made as appropriate.

Analysis of secondary efficacy endpoints

Include:

1) The proportion of subjects with an mRS score of 0-2 at 90±7 days
2) MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution)
3) The proportion of subjects whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS $\geqslant$ 4 at 24±2h, 7±1d or before discharge (whichever comes first)
4) Health-related quality of life (EQ-5d) at 90±7 day
5) The proportion of subjects with a Bathel score $\geqslant$ 95 points at 90$\pm$7 days

The analysis of secondary endpoints refers to the general principles of statistical analysis. For secondary efficacy outcomes, common odds ratio with its 95% confidence interval was calculated using ordinal logistic regression for the outcome of ordinal 90-day mRS score, and odds ratios with their 95% confidence intervals were calculated using binary logistic regression for other secondary efficacy outcomes.

Subgroup analysis: In this study, bridging therapy is performed within 24h of thrombolysis. Because bridging therapy may be a potential outcome influencing factor, bridging therapy is predesigned as a covariate in the subgroup analysis, and the RR values and 95% confidence intervals of the incidence of the trial and control groups in

each subgroup are calculated respectively.

12.5 Safety evaluation

The number and incidence of safety endpoints in each group are statistically described. The number of cases and events, and incidence of adverse events and adverse reactions in each group are statistically described, and the specific list of adverse events is described. Chi-square or Fisher tests are used to compare whether there are differences between groups in safety endpoints. Laboratory data and other safety data will be descriptive statistics, and the data before and after treatment will be compared by paired t test, mainly to analyze and list the specific situation of normal cases before treatment but abnormal cases after treatment or abnormal cases before treatment but aggravated cases after treatment.

Primary safety evaluation endpoints:

Include:

1) The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASSIII)
2) The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASSIII)
3) The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS standards)
4) The incidence of any intracranial hemorrhage within 90 days
5) The incidence of significant hemorrhage events at other sites within 90 days (as defined by GUSTO)
6) 90-day overall mortality
7) Incidence rates of adverse events/serious adverse events within 90 days;

The analysis of the primary safety endpoints refers to the general statistical analysis principles. The number and incidence of various bleeding events and adverse events in the primary safety endpoints are described. The incidence of events between the two groups is compared using the chi-square test or Fisher's exact probability method, and a 95% confidence interval for the difference in incidence between the groups is calculated.

Other safety endpoints:

① Adverse events

The number of cases and events of AE, adverse reactions, AE causing drop-out and SAE are calculated respectively, and a detailed list is provided. The incidence is calculated using the number of people in each safety dataset as the denominator, and the Chi-square test or Fisher's exact probability method is used for inter-group comparison when necessary.

The number of cases and events of the events mentioned in the above endpoints are counted according to the systematic organ classification and preferred terms encoded by MEDDRA, as detailed in the Statistical Analysis protocol (SAP).

② Evaluation of laboratory test indicators

Include routine blood test, routine urinalysis, blood biochemistry and lipid indexes.

The number of cases is described for each kind of clinical judgment result (normal, abnormal without clinical significance, abnormal with clinical significance and not tested) of laboratory examination results before and after treatment in the form of a cross table.

The patients who are judged to be abnormal after the treatment are described in detail by nature (with clinical significance, without clinical significance, or not tested) in the form of lists.

③ Evaluation of vital signs

The mean, standard deviation, median, minimum and maximum are used to describe the changes of vital signs from each visit to the baseline.

④ Evaluation of physical examination

The physical examination results of each visit are described and the number and percentage of normal and abnormal cases are calculated.

⑤ ECG evaluation

Refer to the evaluation section of laboratory examination indicators.

13. Ethics and regulatory procedures

13.1 Ethical principles

This study protocol has been rigorously reviewed. It contains information that is

consistent with current knowledge of the risks and benefits of test drugs and is consistent with the ethical, ethical and scientific principles of clinical trials set out in the *Declaration of Helsinki* 2013, as well as the guidelines of Chinese *Good Clinical Practice*.

13.2 Laws and regulations

The management of this clinical trial will be in accordance with international laws and regulations, and the laws and regulations of China where the trial is conducted, as well as any guidelines on its application.

13.3 Informed consent

Informed consent obtaining is the process by which subjects confirm their consent to voluntarily participate in the clinical trial after being informed of all aspects that may influence their decision to participate in the trial. This process shall be documented by a written, signed and dated informed consent.

To implement informed consent, investigators should abide by the ethical principles of the *Declaration of Helsinki* and meet the following requirements:

(1) The investigator shall use the latest version of the informed consent form approved by the IRB and other information provided to the subject. If necessary, subjects during the clinical trial should re-sign the informed consent.

(II) When the investigator obtains new information that may affect the continued participation of subjects in the study, he/she shall timely inform the subjects or their guardians and make corresponding records.

(3) The investigator shall not influence the subjects to participate in or continue the clinical trial by coercion, inducement for profit or other improper means.

(4) The Investigator or the designated study-related personnel shall fully inform subjects of all matters related to the clinical trial, including written information and the consent of the IRB.

(5) Informed consent form and other oral and written information provided to the subjects shall be easy to understand, so that the subjects or their guardians and witnesses can easily understand.

(6) Before signing the informed consent, the investigator or the designated study-related personnel shall give sufficient time and opportunity for subjects or their guardians to understand the detailed information of the clinical trial and answer

questions raised by subjects or their guardians in detail related to the clinical trial.

(7) The subject or his/her guardian, as well as the investigator performing the informed consent shall sign and indicate the date on the informed consent. If not signed by the subject, the relationship shall be indicated.

(8) If the subject or his/her guardian lacks reading ability, an impartial witness should be present to witness the whole informed consent process. The investigator shall explain in detail the contents of the informed consent and other written materials to the subjects or their guardians and witnesses. Such as participants or their guardians oral agreed to participate in the trial, in the case of ability should sign the informed consent, as witnesses shall also sign the informed consent form and indicate the date, to prove that the participants or their guardians of informed consent and other written materials investigators accurately explain, and understand the relevant content, agreed to participate in clinical trials.

(9) The subject or his/her guardian shall receive the signed and dated original or copy of the informed consent and other written information provided to the subject, including the original or copy of the updated informed consent and the revised version of other written information provided to the subject.

(10) If the subject has no capacity for civil conduct, it shall obtain the written informed consent of its guardian; If the subject is a person with limited capacity for civil conduct, the subject shall obtain the written informed consent of the subject and his/her guardian. When the guardian gives informed consent on behalf of the subjects, the guardian shall inform the subjects of the relevant information of the clinical trial to the extent understandable to the subjects, and try to have the subjects personally sign the informed consent and indicate the date.

(11) Under emergency circumstances, if informed consent of subjects cannot be obtained before participating in the clinical trial, their guardians can give informed consent on behalf of the subjects. If their guardians are not present, the method of enrollment of subjects shall be clearly stated in the trial protocol and other documents, and written consent of the ethics committee shall be obtained; At the same time, informed consent should be obtained as soon as possible for subjects or their guardians to continue to participate in the clinical trial.

(XII) The specific time and personnel of informed consent of the subject shall be recorded in the medical history.

(13) Informed consent should be given in the research center to avoid

infringement/disclosure of the subject's privacy.

13.4 Review board/independent ethics committee (IRB/IEC)

Before the trial begins, the investigator or Sponsor must submit the clinical trial protocol to the IRB, and the investigator must submit a copy of the IRB's signed written consent to the protocol to the Sponsor.

Clinical trials (study number, study protocol name, version number), reviewed documents (including clinical study protocol, informed consent, investigator operation manual, investigator resume, etc.), voting member qualification and review date should be clearly recorded in the IRB consent form.

The study drugs shall not be released to the study center before the trial has begun, and the clinical study shall not begin until a copy of the written consent received and dated by the Sponsor is obtained.

During the course of a clinical trial, any modification of all protocol should be reported to the IRB and approved before implementation. Any event occurring during the study that may affect the safety of subjects or the continuation of the clinical trial, in particular changes in safety, shall be reported to the ERB. Updates to the Investigator Practice Manual should be submitted to the IRB.

A progress report of the clinical trial and a summary of the clinical results at the end of the clinical trial should be submitted to the IRB annually, if required.

14. Quality control and assurance

The investigator is committed to following the clinical trial protocol and the ICH guidelines for the management of clinical trials of medicinal products, as well as the applicable laws and regulations, when conducting clinical trials.

The investigator must properly handle all data obtained during the course of the clinical study to ensure the rights and privacy of participants in the clinical study. The investigator must consent to access and review of the clinical study data by the clinical research associate/auditor/inspector in order to verify the accuracy of the source data and to understand the progress of the study. If it is not possible to verify the original records, the Investigator agrees to assist the Inspector/Inspector/Inspector in further verifying the quality of the data.

The study hospital site visits will be conducted regularly by the clinical research associate during the clinical trial to ensure that all aspects of the study protocol are

strictly followed, and the source data will be checked to ensure that they are consistent with the eCRF. Specific quality control measures shall be included in the design protocol.

Data recorded directly on the EDC (i.e., data without prior written or electronic records) and the identification of data considered as raw data will be specified and specified in the monitoring protocol in advance according to the protocol. Otherwise, it is deemed to lack raw data.

15. Protocol deviation and violation

All requirements specified in the study protocol must be strictly implemented. Any intentional or unintentional deviation from or violation of the study protocol and the principles of the GCP can be classified as a deviation from or violation of the protocol. In the process of supervision, if the clinical research associate finds any deviation from the program, the investigator or the clinical research associate shall fill in the violation program record, and record in detail the time of discovery, the time and process of occurrence of the event, the reason and the corresponding treatment measures. The investigator shall sign the record and notify the ethics committee. In the data statistics and summary report, investigators analyze and report the impact of the deviation or violation of the protocol on the final data and conclusions.

An assessment should be made when a serious protocol violation occurs. If necessary, the Sponsor may terminate the study in advance.

16. Data and data preservation

The investigator should maintain all study data, including confirmation of all subjects (enabling effective verification of different records, such as study medical records and original hospital records, all original signed informed consents, detailed records of drug distribution, and electronic records of all test indicators (documents requiring traceability). Retain for at least 5 years after the test drug is approved for marketing.

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Appendix 1: Procedures for rhTNK-TPA intravenous thrombolytic therapy

(For reference only)

I. RhTNK -tPA intravenous thrombolytic therapy procedure

1. RhTNK-TPA injection: Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the subject, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds.

2. Monitoring vital signs and changes in neurological function.

- Blood pressure measurement q15min×2h, q60min×22h thereafter (or q30min×6h, q60min×16h thereafter)
- Pulse and respiratory rate are measured q1h×12h, followed by q2h×12h
- The neurological function score is Q1h ×6h and Q3h ×18h
- Neurological examination is performed daily after 24h
- Blood pressure is controlled below 185/110mmHg before thrombolysis and maintained below 185/100 mmHg for at least the first 24 h after rhTNK-tPA administration.

3. Central venipuncture and arterial puncture should be avoided in the first 24 hours after thrombolysis; Try to avoid indwelling catheters during or after thrombolysis for at least 30 minutes. The nasogastric feeding tube should be avoided in the first 24 hours. In patients with thrombolysis, two venous channels should be opened as far as possible.

4. Cranial images are reexamined 24h after rhTNK-TPA injection to guide the use of antiplatelet or anticoagulant agents.

5. Check the tongue and lips 45 minutes after medication to determine if there is vasogenic edema. If vasogenic edema is found, antihistamines and

glucocorticoids should be given.

6. Insulin can be given when blood glucose exceeds 10 mmol/L. Blood glucose monitoring should be strengthened, and hyperglycemia patients can be controlled within 7.8 - 10 mmol/L. When blood glucose is below 3.3mmol /L, 10% - 20% glucose can be given orally or by injection. The goal is to get normal blood glucose.

II. Treatment of exacerbation after thrombolysis

If, within 24 h after thrombolysis, the symptom is aggravating, imaging shall be conducted first to determine the presence of sICH. In case the imaging shows asymptomatic or hemorrhagic infarction, no special intervention is required but continue with the antiplatelet therapy as stipulated in the *Guidelines*; in case the imaging shows sICH or parenchymal hematoma, suspend or stop the antiplatelet therapy, control the blood pressure, and remove the hematoma through surgery when necessary. For symptoms worsened by non-bleeding causes after thrombolysis, or worsened after improvement, clinical, laboratory and neuroimaging examinations should be conducted to clarify the causes as far as possible, and targeted interventions should be taken. For patients with major artery occlusion or failed intravenous thrombolysis, remedial intra arterial thrombolysis or intravascular therapy can be considered.

Xiong Y, *et al. Stroke Vasc Neurol* 2025; 10:e003048. doi: 10.1136/svn-2023-003048

Appendix 2: Clinical Event Definition

<table>
<tr><td>Symptomatic intracranial hemorrhage</td><td>ECASS-III Criterion: sICH is defined as any apparently extravascular blood in the brain or within the cranium associated with clinical deterioration, defined by an increase of 4 points or more on the NIHSS from the baseline or within 7 days; or that led to death and that was identified as the predominant cause of the neurologic deterioration.</td></tr>
<tr><td>PH2 type intracranial hemorrhage</td><td>Sits Criterion: Within 36 hours after thrombolysis, the NIHSS increases by ⩾4 from the baseline or the lowest NIHSS, and the imaging shows a hematoma occupying 30% or more of the infarcted tissue, with obvious mass effect.

Type — Description
HI-1 — Scattered small petechiae
HI-2 — Confluent petechiae
PH-1 — Hematoma within infarcted tissue, occupying < 30%, no substantive mass effect
PH-2 — Hematoma occupying 30% or more of infarcted tissue, with obvious mass effect</td></tr>
<tr><td>Any intracranial hemorrhage</td><td>All types of intracranial hemorrhage.</td></tr>
<tr><td>Bleeding in other places</td><td>GUSTO bleeding: 1. Severe or life-threatening bleeding: Intracranial bleeding or bleeding that causes substantial hemodynamic compromise requiring treatment
2. Moderate bleeding: Bleeding which needs blood transfusion
3. Minor bleeding: Other bleeding, neither requiring transfusion nor causing hemodynamic compromise</td></tr>
<tr><td>death</td><td>Vasogenic and non-vasogenic deaths.</td></tr>
<tr><td>New vascular events</td><td>Ischemic stroke/hemorrhagic stroke/myocardial infarction/cardio-cerebral revascularization (including: carotid endarterectomy, intracranial and extracranial artery interventional therapy, intracranial and extracranial artery bypass grafting, coronary interventional therapy or bypass grafting)</td></tr>
</table>

Annex 3: Commonly Used Scales

1. Modified Rankin Scale (MRS)

The modified Rankin Scale is used to measure the neurological recovery of patients after stroke. The bold text shows the formal definition of each level, and the italics give further guidance to reduce possible variations between different observers, but do not describe the structure of the interview. Please note that only symptoms that have occurred since stroke are considered. If the patient is able to walk without help from humans or aid tools, he or she is considered able to walk independently.

If both grades seem to be equally applicable to the patient, and further questioning is unlikely to make an absolutely correct choice, the more severe grade should be chosen.

0: No symptoms at all

Although mild symptoms may be present, the patient has no new functional limitations or symptoms since the stroke.

1: No significant disability despite symptoms; able to carry out all usual duties and activities

The patient had some of the symptoms of stroke, both physical and cognitive (e.g., affecting speech, reading, writing; Or physical movement; Or feeling; Or visual; Or swallowing; Or emotional), but may continue to engage in all pre-stroke work, social and leisure activities. The key question to distinguish between levels 1 and 2 (see below) could be, "Are there things you used to do that you couldn't do until after the stroke?". Activities that occur more than once a month are considered regular.

2: Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance

Certain activities that were previously possible after stroke (such as driving, dancing, reading, or working), patients are no longer able to do after stroke, but can

still take care of themselves daily without assistance. Patients are able to dress, walk, eat, go to the bathroom, prepare simple food, shop, and travel locally without assistance. Patients live without supervision. Imagine patients at this level being able to stay at home unattended for a week or more.

3: Moderate disability; requiring some help, but able to walk without assistance

At this grade, patients can walk independently (with walking AIDS), dress themselves, go to the bathroom, eat, etc., but more complex tasks require assistance. For example, the need for someone else to do the shopping, cooking, or cleaning, and visiting the patient more than once a week to ensure that these activities are completed. Help is needed not only to take care of the body, but also to give advice, such as: patients at this level will need supervision or encouragement to manage their finances.

4: Moderately severe disability; unable to walk and attend to bodily needs without assistance

Sufferers need other people to help with daily life, whether it's walking, dressing, going to the bathroom or eating. Patients need to be cared for at least once a day, often twice or more, or must live close to their carers. To distinguish between levels 4 and 5 (see below), consider whether the patient is routinely able to live alone at appropriate times of the day.

5. Severe disability; bedridden, incontinent, and requiring constant nursing care and attention

There is no need for trained nurses, but someone will be required to watch them several times throughout the day and night.

6. Dead

2. National Institutes of Health Stroke Scale (NIHSS)

project	Scoring criteria	score
1a. Level of consciousness (LOC): 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: (It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.) 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.	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 of a partial gaze palsy.	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	
3. Visual fields	0 = no visual loss	

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	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 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using	0 = no drift; limb holds 90 (or 45) degrees for full 10 seconds 1 = drift; limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support 2 = some effort against gravity; limb	

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.	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 9 = 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. Onlyin the case of amputation or joint fusion at the hip, the examiner should record the score as 9, 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 9 = 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 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 9, and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position.	0 = absent 1 = present in one limb 2 = present in two limbs Ataxia present: Left arm 1 = Yes 2 = No 9 = amputation or joint fusion, explain: _____ Right arm 1 = Yes 2 = No 9 = amputation or joint fusion, explain: _____ Left leg 1 = Yes 2 = No 9 = amputation or joint fusion, explain: _____ Right leg 1 = Yes 2 = No 9 = 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	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,	

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 (item 1a=3) are automatically given a 2 on this item.	and leg	
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	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; 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	

should be used only if the patient is mute and follows no one-step commands.		
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 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 9, and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.	0 = normal 1 = mild-to-moderate dysarthria; patient slurs at least some words and, at worst, can be understood with some difficulty 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. 9 = 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 inattentionor 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	
Total		

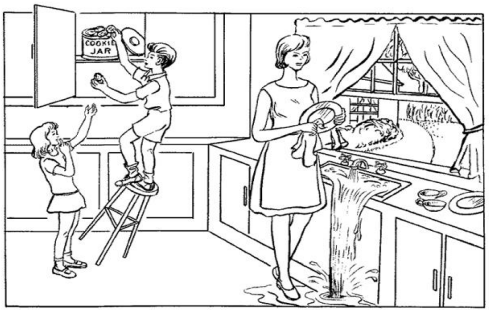
Note:

Grade patients in strict accordance with the above table and record the score. No modification is allowed. The score shall reflect the actual situation of patients, not what the doctor thinks the patient should be. Check patients in the shortest time and record the results. Do not train the patient (e.g., repeatedly asking the patient to perform a certain effort) except give some necessary instructions.

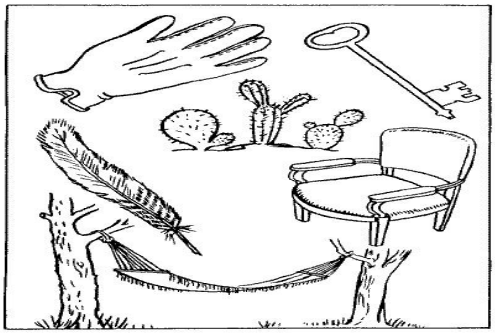
If some scale items are not used, a detailed explanation should be made in the form.

Attached: Pictures for the test of Best language (item 9) and Dysarthria (item 10)

To describe what's happening in Figure 1



To name the items in Figure 2



To read the following sentences in Figure 3

Please read the following sentences:

知道(Zhi Dao; You know how)

下楼梯(Xia Lou Ti; Down the stairs)

回家做饭(Hui Jia Zuo Fan; go home to cook dinner)

在学校复习(Zai Xiao Fu Xi; Review at school)

发表精彩演讲(Fa Biao Jing Cai Yan Jiang; Give a great speech)

To read the following words in Figure 4

Please read out the following words:

妈妈(MAMA)

大地(Da Di; The Earth)

飞机飞机(Fei Ji Fei Ji; The aircraft)

丝绸(Si Chou; silk)

按时开工(An Shi Kai Gong; Start on time)

吃葡萄不吐葡萄皮(Chi Pu Tao Bu Tu Pu Tao Pi; Eat grapes without spitting grape skin)

Basic principles and precautions of NIHSS scoring

Basic principles:

Record the first response of the patient, even though the later ones are better;

Record only what the patient has done, not what you think he can do;

Record while checking, avoid inducing patients as far as possible;

For the untestable items, please record the score as "9", and the computer statistics will automatically process it as a default value;

Principle of "sameness": The same evaluation standard should be applied for multiple follow-up visits for the same patients.

How to score for patients in comma at NIHSS scoring

When the score on item 1a is less than 3, the patient shall go through all the items of NIHSS one by one.

A 3 is scored on item 1a only if the patient makes no movement (other than reflexive posturing) in response to such noxious stimulation as sternum rub and orbital pressure.

If the score = 3 on item 1a, then the score on other items shall be as follows:

On item 1b: 2

On item 1c: 2

On item 2: 1, if the patient can overcome the reflex of the head and eyes; otherwise, 2.

On item 3: depending on the results with visual threat

On item 4: 3

On items 5 and 6: 4 for each

On item 7: Ataxia is scored only if present. The score shall be 0 if the patient fails to finish the finger-nose-finger and heel-shin tests.

On item 8: 2

On item 9: 3

On item 10: 2

On item 11: 2 since a patient in coma has no cognitive competence.

How to calculate the total score of NIHSS?

In calculating the total score, the following items should not be included in the total score:

The score on items 5 and 6 when it is 9 in case of amputation or joint fusion

The score on item 7 in presence of ataxia when "Left arm; 1 = Yes, 2 = No; 9 = amputation or joint fusion, explain: _____" (not required at the time of registration).

3.Barthel Index (BI)

Explanation	project	Scoring criteria	score
Occasional: once a week	Bowels	0 = incontinent 5 = occasional accident 10 = continent	
Occasional: < once per day. A 10 can be scored for a catheterized patient if he/she is continent with the catheter	Bladder	0 = incontinent 5 = occasional accident 10 = continent	
A 5 can be scored even the implements are provided by the carer, for example, prepasted toothburush, water, etc.	Grooming	0 = needs to help with personal care 5 = independent face/hair/teeth/shaving	
Toilet use means the patient can walk to a toilet and go back all by himself/herself. A 5 is scored when the patient still need some help	Toilet use	0 = dependent 5 = needs some help, but can do something alone 10 = independent	
Feeding relates to anormal diet (not just soft diet), though the food can be cooked or served by others. A 5 is scored when the patient feed by himself while the dishes and rice are taken by others.	Feeding	0 = unable 5 = needs help taking dishes and rice 10 = independent	
Transfer means transfer from bed to chair and back. A 0 is scored when the patient can sit up only with physical support of two people; a 5 is scored when the patient can stand up with support of one strong/trained people or two untrained people.	Transfers	0 = unable, no sitting balance 5 = major help (two people, physical), can sit 10 = minor help (one people, verbal or physical) 15 = independent	

Mobility means the (on level surfaces on level surfaces indoor. Aid tools are allowed. If a wheelchair is used, the patients shall be able to corner and go through doors all by himself/herself. A 10 is scored when the patient can walk with the help of the untrained carer or supervisor	Mobility	0 = immobile 5 = independent in wheelchair 10 = walks with help of one person (verbal or physical) 15 = independent (but may use any aid; for example, stick)	
Dressing relates to all kinds of clothes. A 5 is scored when the patients can put on the clothes but needs help with the buttons or zips.	Dressing	0 = dependent 5 = needs help but can do about half unaided 10 = independent (including buttons, zips, laces, etc.)	
A 10 is scored when the patient is able to go upstairs with aid tools	Stairs	0 = unable 5 = needs help (verbal, physical, carrying aid) 10 = independent	
A 5 is scored when the patients can enter and exit the bathroom and scrub his/her body all alone, when the patient can take showers all alone without assistance or supervision	Bathing	0 = dependent 5 = independent (or in shower)	

4. EQ - 5D-3L scale

Under each heading, please tick the ONE box that best describes your health TODAY.

Mobility

☐ I have no problems in walking about

☐ I have some problems in walking about

☐ I'm confined to bed

Self-care

☐ I have no problems with self-care

☐ I have some problems washing or dressing myself

☐ I am unable to wash or dress myself

Usual Activities (e.g., work, study, housework, family or leisure activities)

☐ I have no problems with performing my usual activities

☐ I have some problems with performing my usual activities

☐ I am unable to perform my usual activities

Pain/discomfort

☐ I have no pain or discomfort

☐ I have moderate pain or discomfort

☐ I have extreme pain or discomfort

Anxiety/Depression

☐ I am not anxious or depressed

☐ I am moderately anxious or depressed

☐ I am extremely anxious or depressed

We would like to know how good or bad your health is TODAY. This scale is numbered from 0 to 100. 100 means the best health you can imagine. 0 means the worst health you can imagine. Please mark an X on the scale to indicate how your health is TODAY. Now, write the number you

marked on the scale in the box below

The best health you can imagine

The worst health you can imagine

Your health TODAY = ☐

5. sICH definition

ECASS-III Criterion: sICH is defined as any apparently extravascular blood in the brain or within the cranium associated with clinical deterioration, defined by an increase of 4 points or more on the NIHSS from the baseline or within 7 days; or that led to death and that was identified as the predominant cause of the neurologic deterioration.

6. Definition of PH2 intracranial hemorrhage

Sits Criterion: Within 36 hours after thrombolysis, the NIHSS increases by ≥4 from the baseline or the lowest NIHSS, and the imaging shows a hematoma occupying 30% or more of the infarcted tissue, with obvious mass effect.

Type	Description
HI-1	Scattered small petechiae
HI-2	Confluent petechiae
PH-1	Hematoma within infarcted tissue, occupying < 30%, no substantive mass effect
PH-2	Hematoma occupying 30% or more of infarcted tissue, with obvious mass effect

7. Gusto bleeding definition

① Severe or life-threatening bleeding: Intracranial bleeding or bleeding that causes substantial hemodynamic compromise requiring treatment

②Moderate bleeding: Bleeding which needs blood transfusion

③ Minor bleeding: Other bleeding, neither requiring transfusion nor causing hemodynamic compromise

Xiong Y, *et al. Stroke Vasc Neurol* 2025; 10:e003048. doi: 10.1136/svn-2023-003048

Final protocol approved by the Ethics committee

Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II (TRACE II)

—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

(Protocol No. : MK02-2020-01)

Clinical Trial Approval No. :2017L02308

Clinical Group Leader: Beijing Tiantan Hospital, Capital Medical University

Principal Investigator: Yongjun Wang

Sponsor: Guangzhou Recomgen Biotech Co., Ltd.

Confidentiality statement

All information contained in this protocol is the property of the Sponsor and, therefore, is provided only for the review of the investigator, co-investigator, ethics committee, clinical research associate, regulatory authorities and medical institutions. It is strictly prohibited to disclose any information to third parties unrelated to the study without the written approval of the Sponsor, except for the necessary explanation given to the subject who may participate in the study during the informed consent obtaining process with the subject.

Protocol Revision History

Protocol version number	Release date
V1.0	**2020.10.13**
V2.0	**2020.12.31**
V2.1	**2021.01.22**
V2.2	**2021.04.20**

Sponsor Signature Page

Sponsor's Statement

Title: Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

We will initiate, apply for, organize, fund and audit this clinical trial in accordance with the *Good Clinical Practice*, conscientiously perform my duties as a Sponsor in accordance with the *Measures for the Administration* of *Drug Registration* and other relevant provisions, and provide relevant materials, test drugs, funds, etc. in accordance with the clinical trial agreement.

Sponsor: Guangzhou Recomgen Biotech Co., Ltd.

Sponsor’s Address: No. 1, Jinfengyuan Road, Science City, Guangzhou Economic and Technological Development Zone

Project Leader (Name):	Medical Expert:
Position:	Position:
Tel:	Tel:
Signature: _____	Signature: _____
Date: _____	Date: _____

Investigator Signature Page

Investigator's Statement

Title: Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

I have read the protocol. I consent to conduct the clinical trial in accordance with the protocol design and regulations. I will conscientiously perform my duties as an investigator in accordance with the *Good Clinical Practice*. I will guarantee the accuracy, truthfulness, completeness and reliability of the test data, and accept the audit and inspection by the clinical research associates and auditors designated by the contract research organization/Sponsor and the regulatory regulatories.

Research Facility: Beijing Tiantan Hospital, Capital Medical University

Address: No. 119, South Fourth Ring West Road, Fengtai District, Beijing

Investigator (Name) : Yongjun Wang Position: Dean

Job title: Chief physician

Signature: _____

Date: _____

Signature Page of Data Management Facility

Data Management Facility's Statement

Title: Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

I have read the protocol. I will conscientiously fulfill my responsibilities as a data manager in accordance with the *Good Clinical Practice*. I agree to carry out data management according to the protocol design, ensure that the information and data our facility manages over with respect to this trial are all true, accurate, complete and correct, and will not be used for other clinical trials, nor be disclosed to any other individual or group without the written permission of the Sponsor.

Data Management Facility: Giant Med-Pharma Services, Inc.

Address: No. 5 Jianguomen North Street, Dongcheng District, Beijing

Data management director (name): Xuan Gong

Signature: _____

Date: _____

Signature Page of Statistical Analysis Facility

Statistical Analysis Facility's Statement

Title: Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

I have read the protocol. I will conscientiously perform my duties as a statistician in accordance with the *Good Clinical Practice*. I agree to carry out statistics according to the protocol design, ensure that the statistical information and data with respect to this trial are all true, accurate, complete and correct, and will not be used for other clinical trials, nor be disclosed to any other individual or group without the written permission of the Sponsor.

Statistical Facility: Clinical Research Institute of Peking University

Address: No. 38 Xueyuan Road, Haidian District, Beijing

Statistics Director (name) : Chen Yao

Signature: _____

Date: _____

Signature Page of Contract Research Organization

Contract Research Organization's Statement

Title: Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

I have read the protocol. I will conscientiously perform my duties as a clinical research associate in accordance with the *Good Clinical Practice*. I agree to carry out study monitoring according to the protocol design, ensure that all behaviors comply with the protocol and the recorded information and data with respect to this trial are all true, accurate, complete and correct, and will not be used for other clinical trials, nor be disclosed to any other individual or group without the written permission of the Sponsor.

Contract Research Organization: Giant Med-Pharma Services, Inc.

Address: No. 5, Jianguomen North Street, Dongcheng District, Beijing

Director of Contract Research Organization: Haibo Wu

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Date: _____

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Protocol summary

Protocol title	Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours
Project code	TRACE II
Version number /Release date	V2.2 / April 20th, 2021
Sponsor	Guangzhou Recomgen Biotech Co., Ltd.
Indications	Acute ischemic stroke within 4.5h of onset
Study objectives	To evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) compared with standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset). **Primary study objectives:** To evaluate the difference in the proportion of subjects with 90-day MRS score of 0-1 in subjects with hyperacute ischemic stroke (within 4.5 h of symptom onset) treated with rhTNK-tPA (0.25 mg/kg) versus standard rT-PA (0.9mg/kg). **Secondary study objectives:** 2. To compare the difference in efficacy between rhTNK-tPA (0.25 mg/kg) and standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset) subjects: ① The proportion of subjects with an mRS score of 0-2 at 90±7 days ② MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution) ③ The proportion of subjects whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first) ④ Health-related quality of life (EQ-5d) at 90±7 days ⑤ The proportion of subjects with a Bathel score ≥ 95 points at 90±7 days

	2. To compare the difference in safety between rHTNK-tPA (0.25 mg/kg) and standard rT-PA (0.9mg/kg) in the treatment of subjects with hyperacute ischemic stroke (within 4.5 h of symptom onset) : ① The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASSIII) ② The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASSIII) ③ The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS standards) ④ The incidence of any intracranial hemorrhage within 90 days ⑤ The incidence of significant hemorrhage events at other sites within 90 days (as defined by GUSTO) ⑥ 90-day overall mortality ⑦ Incidence rates of adverse events/serious adverse events within 90 days
Study design	Multicenter, prospective, centrally randomized, open label, blinded-endpoint, active controlled, non-inferiority trial.
Inclusion/exclusion criteria	**Inclusion criteria** (1) Age ≥18 years, male or female; (2) Time from onset to treatment < 4.5h; the time of symptom onset is defined as "the last time point at which the patient appears normal"; (3) Clinical diagnosis as ischemic stroke (the diagnosis following the *Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018*); (4) MRS score of 0-1 before onset; (5) 4 < NIHSS < 26 at baseline; (6) Subjects or their guardians voluntarily sign the informed consent. **Exclusion criteria** (1) Patients scheduled to receive endovascular therapy; (2) NIHSS score 1A > 2; (3) Known to be allergic to rhTNK-tPA and/or rT-PA;

	(4) A history of intracranial hemorrhage; (5) A history of severe head trauma or stroke within 3 months; (6) A history of intracranial or spinal surgery within 3 months; (7) A history of gastrointestinal or urinary tract hemorrhage within 3 weeks; (8) A history of major surgery within 2 weeks; (9) A history of arterial puncture at sites difficult for compression hemostasis within 1 week. (10) Intracranial tumors (except neuroectodermal tumors, such as meningiomas), large intracranial aneurysms; (11) Intracranial hemorrhage (including parenchymal hemorrhage, intraventricular hemorrhage, subarachnoid hemorrhage, subdural/extradural hematoma, etc.); (12) Active visceral bleeding; (13) Aortic arch dissection found. (14) Uncontrollable hypertension upon active antihypertensive treatment: systolic blood pressure ≥180 mm Hg, or diastolic blood pressure ≥100 mm Hg; (15) Propensity for acute bleeding, including platelet counts of less than 100×109/ L or otherwise; (16) Blood glucose <2.8 mmol/L or >22.22 mmol/L; (17) On oral warfarin anticoagulant treatment with INR>1.7 or PT >15s; (18) Having received heparin treatment within 24 h; (19) Having received thrombin inhibitors or factor Xa inhibitors within 48 h; (20) Head CT or MRI shows a large infarction (infarcted area > 1/3 of the middle cerebral artery). (21) Subjects who are unable or unwilling to cooperate due to hemiplegia (Todd's palsy) after epileptic seizure or other neurological/psychiatric disorders; (22) Pregnant women, lactating women, or subjects who do not agree to use effective contraception during the trial; (23) Participation in other clinical trials within 3 months prior to screening; (24) Unsuitability for participation or participation in this study as judged by the investigator may result in exposure to greater risk.
Study drugs	**Test drug:**

	<table><tr><td>Drug name</td><td>Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-TPA)</td></tr><tr><td>Trade name</td><td>MingFuLe</td></tr><tr><td>Strengths</td><td>1.0 x 10^7 IU/16 mg/vial</td></tr><tr><td>Storage conditions</td><td>Store below 25℃ away from light. This product should be used immediately after dissolution. If not used immediately, it should be stored in cold storage at 2 - 8℃ away from light and used within 24 hours.</td></tr><tr><td>Shelf life</td><td>24 months</td></tr><tr><td>Manufacturer</td><td>Guangzhou Recomgen Biotech Co., Ltd.</td></tr><tr><td>Supplier</td><td>Guangzhou Recomgen Biotech Co., Ltd.</td></tr></table> **Control drug:** <table><tr><td>Drug name</td><td>Alteplase for Injection (rt-PA)</td></tr><tr><td>Trade name</td><td>Actilyse®</td></tr><tr><td>Strengths</td><td>50mg/vial, 20mg/vial</td></tr><tr><td>Storage conditions</td><td>Keep in the original packaging. Store below 25℃ away from light. It is recommended to use the prepared solution immediately after preparation. It has been proved that the prepared solution can remain stable at 2-8℃ for 24h. Frezzing prohibited.</td></tr><tr><td>Shelf life</td><td>36 months</td></tr><tr><td>Manufacturer</td><td>Boehringer Ingelheim Pharma GmbH&Co.KG</td></tr><tr><td>Supplier</td><td>Guangzhou Recomgen Biotech Co., Ltd.</td></tr></table>
Methods of grouping and administration	Subjects will be randomly assigned to the following groups in a 1:1 ratio: **Treatment group (rhTNK-TPA 0.25mg/kg, 25mg at most) :** RhTNK-TPA 0.25mg/kg: Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the subject, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds. **Control group (rt-PA 0.9mg/kg) :** RT-PA 0.9mg/kg: The product is dissolved in sterile water for injection to obtain a solution with a

	concentration of 1mg/ ml. The amount required for medication is calculated and measured according to the actual body weight of the subject. 10% of the solution is injected by intravenous bolus, and the remaining 90% is injected by intravenous drip within 1h.
Efficacy endpoints	**Primary efficacy endpoint:** The proportion of subjects with 90-day MRS score of 0-1 **Secondary efficacy endpoints:** ⑥ The proportion of subjects with an mRS score of 0-2 at 90±7 days ⑦ MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution) ⑧ The proportion of subjects whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first) ⑨ Health-related quality of life (EQ-5d) at 90±7 day ⑩ The proportion of subjects with a Bathel score ≥ 95 points at 90±7 days
Safety endpoints	⑧ The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASSIII) ⑨ The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASSIII) ⑩ The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS standards) ⑪ The incidence of any intracranial hemorrhage within 90 days ⑫ The incidence of significant hemorrhage events at other sites within 90 days (as defined by GUSTO) ⑬ 90-day overall mortality ⑭ Incidence rates of adverse events/serious adverse events within 90 days
Sample size	In this study, rt-PA active control will be used based on a statistical hypothesis test of non-inferiority, and the proportion of subjects with 90-day MRS score of 0-1 will be used as the primary efficacy endpoint. According to a paper published in the Lancet[7] in 2014 about meta-analysis of the incidence of 90-day MRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the Guidelines for the Design of Non-inferiority Drug Clinical Trials [32], the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high

	priority index. Therefore, based on the lower CI limit of 1.14, together with an efficacy of 50% of the active control, the non-inferior threshold was determined as 0.937. Then assuming α=0.025 using one-sided test, power 1-β=0.85, treatment group (TNK) incidence of 63.64%, control group (rt-PA) incidence of 59.32%, RR= 1.07, the sample size was calculated to be 642 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each group and the total sample size is estimated to be 1430 cases.
Statistical analysis	Efficacy endpoints: Chi-square test or Fisher's exact probability method will be used for qualitative classification, and 95% confidence intervals for incidence differences between groups will be calculated. Qualitative grade data are analyzed by Wilcoxon rank sum test, and quantitative indicators are analyzed by two-sample t-test or rank sum test as appropriate, and 95% confidence intervals for score differences between groups are calculated. Statistical comparison between the primary efficacy indicators analysis groups with CMH chi-square considering the center effect, computational efficient RR OR RD 95% confidence interval, and the center effect, mRS baseline score of logistic regression model analysis, report comparing rhTNK - tPA and rt - PA OR 95% confidence interval, and the primary efficacy endpoint results will also according to whether bridge group subgroup analysis was carried out. Safety endpoints: Chi-square test or Fisher's exact probability method are used for qualitative classification, and 95% confidence intervals for incidence differences between groups are calculated. Qualitative grade data are analyzed by Wilcoxon rank sum test, and quantitative indicators are analyzed by two-sample t-test or rank sum test as appropriate, and 95% confidence intervals for score differences between groups are calculated.

Research Flow Chart

Measures		Screening/Baseline	Treatment period	follow-up			
		4.5 h. (Before thrombolysis)	0h (Thrombolytic Therapy)	24 h + 2 h	24h-36h	7±1 day or before discharge (whichever occurs first)	90±7 days/early end of visit
Signing Informed Consent		X					
Demographic characteristics		X					
Symptoms of this onset		X					
Medical history		X					
Prior medication[1]		X					
Concomitant medication/treatment[9]		X	X	X	X	X	X
Vital signs[2]		X	X	X		X	
mRS		X[3]					X
NIHSS		X		X		X	
BI scale							X
EQ - 5 D scale							X
Blood/urine pregnancy test[4]		X					
Laboratory examination[5]	Routine blood test	X		X		X	
	Blood biochemistry	X		X		X	
	Coagulation function	X		X		X	
	Routine urinalysis			X		X	
Electrocardiogram (ECG)[6]		X				X	
CT or MRI[7]		X			X		
randomization		X					
Thrombolysis information[8]			X				
Adverse Events/Serious Adverse Events			X	X	X	X	X

1. Prior medication is only recorded for drugs that fall into the restrictions in the inclusion/exclusion criteria.
2. The "blood pressure" of screening and enrollment is collected when vital signs are collected."0h" is regarded as the monitoring results of vital signs within 5min before thrombolysis. Vital signs include blood pressure, pulse, body temperature and respiratory

rate;

3. The mRS score at screening include pre-onset score and post-onset pre-thrombolysis score.
4. Blood/urine pregnancy test is applicable to fertile women only;
5. Laboratory examination:
    1) Baseline laboratory tests include routine blood test, blood biochemistry, and coagulation function; Subjects will not need to repeat the test if they have already performed some laboratory tests between the present onset and prior to thrombolysis. For baseline blood lipid tests (total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), triglyceride (TG)), the most recent fasting blood lipid is collected after the onset of the disease. Blood glucose test is performed at baseline. Peripheral blood glucose is measured as one of the inclusion and exclusion criteria, and venous blood is collected for blood glucose test. Blood biochemical results are allowed to be obtained after the study drug has been administered. If abnormal blood biochemical results are obtained, they will be handled by the study physician in accordance with the discipline guidelines and the clinical management pathway of the hospital.
    2) 24h laboratory examination after thrombolysis can accept the test results within 72h after onset (after administration), including routine blood test, blood biochemistry, coagulation function, routine urinalysis;
    3) Routine blood test, blood biochemistry, coagulation function and routine urinalysis 7±1 day or before discharge (according to the first occurrence);
6. If the subject has examined the ECG before thrombolysis after the present onset, no repeat examination is required;
7. Screening/baseline imaging examination is “CT or MRI”, which is used to exclude hemorrhagic stroke; the CT or MRI results generated by other hospitals can also be used as a screening basis, if the study physician deems them acceptable; if a subject has undergone a CT or MRI check post symptom onset before onset of thrombolysis, such examination may be omitted. The imaging examination during the follow-up period is "CT or MRI", which is used to detect hemorrhagic events and should be completed within 24 - 36h.
8. Thrombolysis information includes thrombolysis time of the treatment group and the control group (including intravenous infusion time of the treatment group, intravenous infusion time of the control group, and the start and end time of intravenous infusion), intravenous dose, intravenous infusion dose of the control group, and the occurrence of adverse events.
9. For concomitant medication/treatment, pay attention to collecting information about bridging therapy.

Abbreviations table

ACA	Anterior cerebral artery	HR	Heart rate
ADC	Apparent diffusion coefficient	ICH	Intracranial bleeding
AE	Adverse events	ICU	intensive care unit (ICU)
ASA	aspirin	INR	International standard ratio
BP	Blood pressure	IRB	Ethics committee
CK	Creatine kinase	IVH	Intraventricular hemorrhage
CK-MB	Creatine kinase -MB	IS	Ischemic stroke
COX	Ring oxidase	LDL	Low density lipoprotein
CRP	C-reactive protein	MCA	Middle cerebral artery
CRF	Case report form	MI	Myocardial infarction,
CRO	Contract Research Organization	MoCA	Montreal Cognitive Assessment
CT	Computerized tomography	MRA	Magnetic resonance angiography
CTA	CT angiography	MRI	Magnetic resonance imaging
DRF	Deviation resolution table	mRS	Improved Rankin score
DMC	Data Monitoring Committee	NIHSS	National Institutes of Health Stroke Scale
DWI	Diffusion-weighted imaging	PCA	Posterior cerebral artery
ECG	Electrocardiogram (ECG)	PWI	Perfusion Weighted Imaging
ED-5Q	Quality of Life Scale	RR	Relative risk
EMS	Emergency medical service	RRR	Relative risk reduction rate
ER	The emergency room	SAE	Serious Adverse Events
FLAIR	Liquid attenuation inversion recovery sequence	SAH	Subarachnoid hemorrhage
GCS	Glasgow Coma Score	SFDA	State Food and Drug Administration
GCP	Standardized management of drug clinical trials	sICH	Symptomatic intracranial hemorrhage
GPIIb/IIIa	Glycoprotein IIb/IIIa	TIA	Transient ischemic attack
GUSTO	Global Infarction-related Artery Opening Strategy	TCD	Transcranial Doppler ultrasound
		tPA	Tissue plasminogen activator

1. Research background

1.1 Introduction

The most effective drug treatment for Acute Ischemic Stroke (AIS) is intravenous thrombolysis of recombinant tissue plasminogen activator (rt-PA) for injection at an extremely early stage (< 4.5 h), which has been consistently recommended by domestic and foreign guidelines for cerebrovascular diseases[1-3]. Under the guidance of imaging, the time window of intravenous thrombolysis for partial anterior circulation cerebral infarction or post-awakening ischemic stroke can be extended to less than 9 hours, and rt-PA has good efficacy and safety[4-6]. Therefore, more patients with AIS can benefit from intravenous rt-PA thrombolysis.

However, the proportion of good prognosis of intravenous thrombolysis with rt-PA is relatively low[7], partly because of the second generation of intravenous thrombolysis drug rt - PA vessels take rate for medium and large vascular occlusion take rate lower (MCA telecentric end clogging of intravenous thrombolysis vessels take rate is 38% (95% CI 22-54%), near heart MCA occlusion of blood vessels take rate is 21% (95% CI 15-29%) of the internal carotid artery occlusion intravenous thrombolysis take rate is 4% (95% CI 1-8%), basilar artery occlusion intravenous thrombolysis take rate of 4% (95% CI 0-22%), and significantly related to vascular take rate and prognosis[8]. Five international RCT studies have confirmed that intravenous thrombolytic therapy combined with endovascular therapy is more beneficial than intravenous thrombolytic therapy for patients with great vessel occlusion[9-14]. However, the distribution of medical resources is not balanced, and endovascular surgery cannot meet the needs of AIS emergency treatment in many areas[15]. The time delay caused by referral may reduce the benefit of thrombectomy [16]. Some patients may not be able to tolerate anesthesia or surgery due to comorbidities or may not be able to undergo surgery due to medical costs. Therefore, there is an urgent need for effective treatment options other than endovascular treatment.

Compared with rt-PA, the half-life of the third generation intravenous thrombolytic drug TNK-tPA is 4 times that of rt-PA, the tissue specificity of fibrin is 15 times that of rt-PA, and it is less affected by plasminogen activator inhibitor PAI-1 (PAI-1)[17]. Different studies have shown that TNK-tPA treatment of AIS is superior to

rt-PA in patients with AIS complicated with great vessel occlusion, resulting in higher vascular opening rate or reperfusion and better prognosis[18-22].

In view of the current European and American guidelines and domestic guidelines, intravenous thrombolysis should be given to AIS after general CT examination within 4.5 hours of onset, and imaging examinations such as CTA/CTP/MRI are not recommended to guide intravenous thrombolysis to reduce time delay[1,2]. In the completed AIS study (sample size: 1100 cases) within 4.5 hours of onset, intravenous TNK-tPA or rt-PA thrombolysis nor-test is randomly administered based on general CT examination, and the superior efficacy of TNK-tPA has not been confirmed [23].Meta-analysis showed that the efficacy of TNK-tPA in the treatment of AIS is at least not inferior to rt-PA[24,25]However, there is heterogeneity in the study and there is a lack of direct evidence and Chinese data. Therefore, the hypothesis of this study is that the efficacy of TNK-tPA is at least not inferior to rt-PA in Chinese patients with AIS within 4.5 hours of onset. On the basis of the phase II clinical trial, this study used a national multicenter, prospective, centrally randomized, open label, blind endpoint evaluation, active controlled phase III clinical trial to verify the above hypothesis. On the basis of meeting the non-inferiority test, the superior efficiency will be further tested.

1.2 Background of test drug

Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK - tPA, trade name "MingFuLe"), developed by Guangzhou Recomgen Biotech Co., Ltd. (hereinafter referred to as " Recomgen Biotech"), is a recombinant protein produced using mammalian cells by genetic engineering technology, and on January 14, 2015, got approved by NMPA for marketing as a clinical thrombolytic therapy for acute myocardial infarction. RhTNK-tPA is the third generation of recombinant t-PA product, which is a mutant of alteplase: ① The mutation of T103 site to N103 makes it change from non-glycosylated site to glycosylated site, and prolongs its half-life;(2) The mutation of N117 to Q117 removes the glycosylation function of N103, repairs the adverse effect of N103 mutation on fibrin specificity, and also reduces the clearance rate in plasma. ③ K296, H297, R298 and R299 are mutated into A296, A297, A298 and A299, which enhanced the binding ability of fibrin and reduced the reaction of PAI-1, its inhibitor[26]. RhTNK - tPA, therefore,

compared to the rt - PA, has a longer half-life by four times, improved antagonism ability against the inhibitor PAI – 1 by 80 times, and higher specificity by 14 times, making it the most safe and effective recombinant t - PA thrombolytic drugs to date. Besides, rhTNK - tPA can use a single intravenous bolus injection, easing the trouble of intravenous drip or repeated intravenous push existing in other fibrinolytic drugs, easy to use, more suitable for first aid. RhTNK - tPA has the same pharmacological effects with alteplase, in theory can also be applied to the treatment of acute ischemic stroke, which has been preliminarily confirmed in preclinical animal experiments and the clinical trials before phase 3 for treatment of acute ischemic stroke with good efficacy.

The Phase II clinical study of rhTNK-tPA for acute ischemic stroke is titled "A phase 2, dose-finding, multicenter, prospective, randomized, open label, parallel, active controlled trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) for acute ischemic stroke within 3 hours. "This study was conducted in 22 research centers, and a total of 4 groups were set up, which were the treatment groups, rhTNK-tPA 0.10 mg/kg group, rhTNK-tPA 0.25 mg/kg group, rhTNK-tPA 0.32 mg/kg group and the control group rT-PA group. 60 subjects were included in each group, and 240 subjects were included in total. Drug administration method of rhTNK-tPA: Each vial was dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication was calculated and measured according to the actual body weight of the subject, 25mg at most. The solution was administered via single intravenous bolus injection in 5-10 seconds.. RT-PA administration method: 10% of the total dose (0.9mg/kg) was injected by intravenous bolus within 1 minute, and the remaining 90% was injected by intravenous drip within 1h after mixing well. The results showed that the primary efficacy endpoints, i.e. the proportion of subjects whose NIHSS score was ≤ 1 or was improved compared to the baseline over 4 at 14 days, were 63.33%(38/60), 77.19%(44/57), 66.67%(40/60), and 62.71%(37/59) in the rhTNK-tPA 0.10mg/kg group, the rhTNK-tPA 0.25mg/kg group, the rhTNK-tPA 0.32mg/kg group, and the rT-PA group, respectively, without significant statistical difference ($P > 0.05$). For secondary efficacy endpoints: i.e. the proportion of subjects capable of independent self-care at 90 days (good prognosis at 90 days defined as mRS 0-1), the change of mRS (continuity) at 90 days and the change rate from

baseline, the proportion of subjects with mRS score 0-2, NIHSS (change of NIHSS score at 90-day follow-up), the proportion of patients with a BI score ⩾ 95 at 90 days, the quality of life (EQ - 5 D scale), there was no significant statistical difference between the groups (P > 0.05). For events in the safety endpoints, the proportion of patients with symptomatic intracranial hemorrhage (sICH) at 36h was 5.00%(3/60 cases) in the rhTNK-tPA 0.10mg/kg group, 0.00%(0/57 cases) in the rhTNK-tPA 0.32mg/kg group, and 1.69%(1/59 cases) in the rT-PA group, respectively, without significant statistical difference (P > 0.05). There were no statistically significant differences in the overall mortality at 90 days, the proportion of patients with asymptomatic intracranial hemorrhage at 90 days, the proportion of patients with bleeding events at other sites at 90 days, and the proportion of patients with adverse events/serious adverse events within 90 days (P > 0.05). According to the system organ classification, adverse events with an incidence of ≥5% in each group are shown in the table below. The SAEs that occurred during the study that may be associated with the study drugs are shown in the following table.

AEs with incidences ≥ 5% in phase 2 clinical study

SOC	PT	RhTNK - tPA 0.10 mg/kg Number of cases (incidence)	RhTNK - tPA 0.25 mg/kg Number of cases (incidence)	RhTNK - tPA 0.32 mg/kg Number of cases (incidence)	rt-PA 0.9 mg/kg Number of cases (incidence)
Metabolism and Nutrition Disorders	Hypoproteinemia	6 (10.00%)	1 (1.75%).	5 (8.33%)	3 (5.08%)
	Hypokalemia	6 (10.00%)	2 (3.51%).	2 (3.33%).	3 (5.08%)
	Hyponatremia	2 (3.33%).	1 (1.75%).	3 (5.00%)	0 (0.00%)
	Hyperuricemia	2 (3.33%).	2 (3.51%).	5 (8.33%)	8 (13.56%)
	Hyperhomocysteinemia	12 (20.00%).	9 (15.79%)	4 (6.67%)	6 (10.17%)
	Hyperlipidemia	9 (15.00%)	6 (10.53%)	9 (15.00%)	11 (18.64%).
	Dyslipidemia	2 (3.33%).	3 (5.26%)	3 (5.00%)	0 (0.00%)
Hepatobiliary Disorders	Abnormal liver function	10 (16.67%)	3 (5.26%)	4 (6.67%)	6 (10.17%)
	Liver cyst	1 (1.67%).	1 (1.75%).	0 (0.00%)	4 (6.78%)
	Hepatic steatosis	2 (3.33%).	0 (0.00%)	2 (3.33%).	5 (8.47%)
Infections and Infestations	Lung infection	5 (8.33%)	2 (3.51%).	9 (15.00%)	5 (8.47%)
	Urinary tract infection	1 (1.67%).	2 (3.51%).	4 (6.67%)	3 (5.08%)
Investigations	With blood in his urine	0 (0.00%)	5 (8.77%)	5 (8.33%)	1 (1.69%).
	Potassium lower	1 (1.67%).	3 (5.26%)	2 (3.33%).	2 (3.39%).
	Elevated blood glucose	1 (1.67%).	1 (1.75%).	3 (5.00%)	3 (5.08%)
	Decreased	0 (0.00%)	0 (0.00%)	0 (0.00%)	3 (5.08%)

	fibrinogen				
	High blood pressure	2 (3.33%).	1 (1.75%).	0 (0.00%)	3 (5.08%)
	Abnormal lipoprotein	3 (5.00%)	2 (3.51%).	0 (0.00%)	1 (1.69%).
	Elevated lipid	0 (0.00%)	0 (0.00%)	3 (5.00%)	2 (3.39%).
Nervous System Disorders	Hemorrhagic cerebral infarction	1 (1.67%).	3 (5.26%)	1 (1.67%).	1 (1.69%).
	Cerebral artery stenosis	1 (1.67%).	5 (8.77%)	1 (1.67%).	3 (5.08%)
	Arteriosclerosis cerebri	2 (3.33%).	3 (5.26%)	0 (0.00%)	1 (1.69%).
	Brain infarction	6 (10.00%)	6 (10.53%)	2 (3.33%).	3 (5.08%)
	Carotid stenosis	1 (1.67%).	1 (1.75%).	2 (3.33%).	4 (6.78%)
	Carotid atherosclerosis	2 (3.33%).	0 (0.00%)	3 (5.00%)	4 (6.78%)
	Have a headache	2 (3.33%).	3 (5.26%)	4 (6.67%)	3 (5.08%)
	Dizzy	2 (3.33%).	1 (1.75%).	3 (5.00%)	1 (1.69%).
Injury, Poisoning and Procedural Complications	Cerebral hernia	4 (6.67%)	0 (0.00%)	0 (0.00%)	1 (1.69%).
Respiratory system, chest and mediastinal diseases	Epistaxis	1 (1.67%).	1 (1.75%).	3 (5.00%)	0 (0.00%)
	Lung inflammation	3 (5.00%)	0 (0.00%)	0 (0.00%)	2 (3.39%).
	Pulmonary lumps	0 (0.00%)	1 (1.75%).	4 (6.67%)	2 (3.39%).
	Pneumonia	4 (6.67%)	2 (3.51%).	3 (5.00%)	1 (1.69%).
	Respiratory failure	1 (1.67%).	1 (1.75%).	3 (5.00%)	1 (1.69%).
	Hemoptysis	0 (0.00%)	0 (0.00%)	0 (0.00%)	4 (6.78%)
	Pleural effusion	2 (3.33%).	0 (0.00%)	2 (3.33%).	3 (5.08%)
Psychiatric Disorders	Restlessness	1 (1.67%).	4 (7.02%)	1 (1.67%).	0 (0.00%)
	Insomnia	0 (0.00%)	1 (1.75%).	3 (5.00%)	5 (8.47%)
Skin and Subcutaneous Tissue Disorders	Subcutaneous bleeding	0 (0.00%)	2 (3.51%).	1 (1.67%).	3 (5.08%)
Renal and Urinary Disorders	Blood in the urine	3 (5.00%)	3 (5.26%)	2 (3.33%).	0 (0.00%)
Gastrointestinal Disorders	Constipation	13 (21.67%).	11 (19.30%).	11 (18.33%).	11 (18.64%).
	Abdominal pain	0 (0.00%)	1 (1.75%).	0 (0.00%)	3 (5.08%)
	Diarrhea	1 (1.67%).	1 (1.75%).	6 (10.00%)	2 (3.39%).
	Vomiting	1 (1.67%).	0 (0.00%)	3 (5.00%)	6 (10.17%)
	Gastrointestinal bleeding	0 (0.00%)	0 (0.00%)	3 (5.00%)	0 (0.00%)
	Bleeding gums	6 (10.00%)	12 (21.05%).	9 (15.00%)	9 (15.25%)
	Stress ulcer	4 (6.67%)	1 (1.75%).	3 (5.00%)	1 (1.69%).
Cardiac Disorders	Atrial fibrillation	0 (0.00%)	2 (3.51%).	5 (8.33%)	2 (3.39%).
	Heart failure	2 (3.33%).	1 (1.75%).	4 (6.67%)	5 (8.47%)
Vascular Disorders	Atherosclerosis	3 (5.00%)	5 (8.77%)	9 (15.00%)	4 (6.78%)
	Hypertension	1 (1.67%).	3 (5.26%)	4 (6.67%)	1 (1.69%).
Blood and Lymphatic Disorders	Clotting disorders	3 (5.00%)	0 (0.00%)	1 (1.67%).	2 (3.39%).

	anemia	5 (8.33%)	3 (5.26%)	3 (5.00%)	3 (5.08%)

SAE determined to be possibly related to the study drugs in Phase 2 clinical study

Grouping	Medical terms for SAE (diagnostics)	Severity of SAE	Measures taken on study drugs	SAE outcome	Sequelae	Relationship between SAE and study drugs
RhTNK - tPA 0.10 mg/kg	Right basal ganglia bleeding	Prolonged hospital stay	Not applicable	Symptoms persist		Definitely related
RhTNK - tPA 0.25 mg/kg	Multiple subcutaneous hematomas	Endangering life or death	Not applicable	Resolve	No	Definitely related
RhTNK - tPA 0.32 mg/kg	Cerebral hemorrhage	Endangering life or death	Not applicable	Resolve	Yes	Possibly related
	Gastrointestinal hemorrhage	Endangering life or death	Drug withdrawal	Symptoms persist		Possibly related
	Ischemic optic neuropathy of the left eye	Dysfunction	Drug withdrawal	Symptoms persist		Possibly related
	Hemorrhagic transformation after infarction	Endangering life or death	Not applicable	Death		Definitely related
Rt - PA 0.9 mg/kg	Large cerebral infarction with hemorrhage	Endangering life or death	Not applicable	Death		Possibly related

1.3 Overview of clinical studies at home and abroad

(1) NOR - TEST trial [23]

The purpose of the NOR-test was to compare the safety and efficacy of intravenous thrombolysis with tenecteplase and alteplase. NOR - TEST Trial for phase III, multicenter (13) research center, prospective, randomized, open label, end blinded clinical trial, the target population in super acute phase of ischemic stroke (< 4.5 h) patients or symptoms occur within 4.5 h after waking, suspected of acute ischemic stroke and suitable for thrombolysis or thrombectomy bridge before the treatment, according to the proportion of 1:1 randomly given intravenous injection of tenecteplase at 0.4 mg/kg (maximum 40 mg) or alteplase at 0.9 mg/kg (maximum 90 mg).The primary endpoint was the ratio of good prognosis at 90 days (mRS 0 to 1).A total of 1107 patients were enrolled in the study, 7 patients dropped out of the trial, and 1100 patients were randomly assigned to the tenecteplase group (n=549) or alteplase group (n=551). The median age was 77 years (64-79), and the median NIHSS score was 4 (2-8).A final diagnosis of non-ischemic stroke or non-TIA was

found in 99 (18%) patients in the tenecteplase group and 91 (17%) patients in the alteplase group. A total of 354 patients (64%) in the tenecteplase group and 345 patients (63%) in the alteplase group were included in the final outcome analysis.

The results showed as follows: ① The incidence of primary endpoints in the two groups was 64% and 63%(OR 1.08, 95%CI 0.84 to 1.38;P = 0.52);② The 90-day mortality rates were 5% and 5%(P=0.68), respectively.(3) The incidence of serious adverse events was similar in both groups (26%).

Conclusion: Tenecteplase is not superior to alteplase, and the safety of both is similar. However, in general, the majority of patients in this study were patients with mild stroke, and about 75% of the patients had NIHSS≤7 points before treatment, and the proportion of patients with great artery occlusion was small. The research on patients with severe stroke still needs to be continued.

(2) WAKE UP study[6]

G. Thomalla et al. from the Eibendorf Medical Center, University of Hamburg, Germany, conducted a WAK-UP trial to investigate the efficacy and safety of intravenous thrombolysis therapy with alteplase in patients with acute ischemic stroke with unknown onset time screened based on MRI images.

The WAKE-UP study is a multicenter, randomized, double-blind, placebo-controlled clinical trial. The vast majority of patients with post-awakening stroke have onset a few hours before awakening, and these patients may meet the time window for intravenous thrombolysis. For stroke patients with known onset time, DWI-FLAIR - mismatch on MRI often indicates that the onset of stroke occurs within 4.5 hours before imaging examination. The study was planned to include 800 patients, but 503 patients were included and the trial ended early due to lack of funding. Of these patients, 254 were randomized to alteplase and 249 were randomized to placebo. Patients scheduled for mechanical thrombectomy were excluded from the study. The primary endpoint was good prognosis at 90 days (MRS score of 0-1), and the secondary outcome was MRS score shift analysis.

Results: The ratio of good outcomes at 90 days in the alteplase group and placebo group was 131/246 (53.3%) and 102/244 (41.8%), respectively (adjusted OR, 1.61;95% CI, 1.09 - 2.36;P = 0.02). The median 90-day MRS score was 1 and 2,

respectively (adjusted OR, 1.62;95% CI, 1.17 - 2.23;P = 0.003).The mortality rates were 4.1% and 1.2%, respectively (OR, 3.38;95% CI, 0.92 - 12.52;P = 0.07).The rates of symptomatic intracranial hemorrhage were 2.0% and 0.4% (OR, 4.95;95% CI, 0.57 - 42.87;P = 0.15).

CONCLUSIONS: For stroke patients with unknown onset time and DWI-FLAIR mismatch on MRI, intravenous thrombolytic therapy with alteplase can improve functional outcomes at 90 days, but the incidence of intracranial hemorrhage is higher.

(3) ECASS - III study [27]

Intravenous thrombolysis with alteplase has only been approved for the treatment of acute ischemic stroke patients, but the efficacy and safety of this drug beyond the 3h time window remains unclear. The purpose of this study was to investigate the efficacy and safety of alteplase in patients with 3-4.5h acute ischemic stroke.

Results: A total of 821 patients were enrolled in this study, 418 in the alteplase treatment group and 403 in the placebo control group. The median duration of alteplase administration was 3 hours and 59 minutes. More patients in the treatment group had a good prognosis (52.4% vs. 45.2%, OR, 1.34, 95%CL, 1.02-1.76;P = 0.04).Overall, alteplase improved the prognosis of patients (OR, 1.28, 95%CI, 1.00-1.65, P < 0.05).The rate of intracranial hemorrhage in the alteplase treatment group was higher than that in the control group (all intracranial bleeding, 27.0% vs. 17.6%;P = 0.001;Symptomatic intracranial hemorrhage, 2.4% vs. 0.2%;There was no significant difference in mortality between the two groups (7.7% vs. 8.4%, P=0.68).There was no significant difference in serious adverse reactions between the two groups.

CONCLUSIONS: Intravenous alteplase is effective in patients with acute ischemic stroke 3-4.5h after onset, compared with controls, and significantly improves clinical outcomes, but symptomatic intracranial hemorrhage is common.

(4) TAAIS study[18]

The Australian TNK trial was designed to compare the clinical efficacy and safety of alteplase and tenecteplase. Patients with acute cerebral infarction within 6 hours of onset were enrolled in this Phase IIb randomized trial. Patients were randomly divided into alteplase group (0.9 mg/kg), low-dose tenecteplase group (0.1

mg/kg) and high-dose tenecteplase group (0.25 mg/kg) according to 1:1:1.In order to improve the benefit of thrombolysis, the CTP of all enrolled patients before treatment showed perfusion defect area at least 20% larger than the infarction core, with a volume of at least 20ml, and CTA showed corresponding major artery occlusion (ACA, MCA, and PCA), except ICA and VA occlusion patients. The primary endpoint of the study was the proportion of reperfusion and the rate of clinical symptom improvement (evaluated by NIHSS score) indicated by PVI-DWI after 24h.A total of 75 patients with acute cerebral infarction (ACI) were enrolled, with 25 patients in each group. The baseline mean NIHSS score was 14.4±2.6 and the median time from onset to treatment was 2.9±0.8h.

The results showed as follows: (1) Compared with the alteplase group, the two groups of patients who received tenecteplase after 24h had a higher recirculation rate (P<0.001), and there was no significant difference in the incidence of intracranial hemorrhage and other adverse events between the two groups; ② In all efficacy outcomes, higher doses of tenecteplase and lower doses of tenecteplase and alteplase had more benefits, including 90d non-severe disability rate (72% of tenecteplase vs 40% of alteplase, P=0.02).

Conclusions: Tenecteplase may lead to better vascular recanalization rates and clinical outcomes in patients selected by baseline CTP.

(5) Extend-Ia TNK trial [21-22]

Part 1:

The Extend-Ia TNK trial was also aimed at determining whether tenecteplase had a better reperfusion effect than alteplase prior to thrombectomy. The study was a phase II, multicenter, prospective, randomized, open label, blind endpoint, non-inferior-quality study. This study enrolled patients who received thrombolytic therapy for acute cerebral infarction within 4.5h. CTA indicated great artery occlusion (ICA, BA, MCA-M1, and MCA-M2), and thrombectomy could be performed within 6h of onset. They were randomly divided into intravenous tenecteplase 0.25 mg/kg group or alteplase 0.9 mg/kg group according to 1:1.Multimodal CT was performed before thrombolysis, and perfusion was evaluated by angiography (MTICI grade).The primary endpoint was mTICI 22/3 or the proportion of desirable thrombus

disappearance, the secondary endpoint was a 90-day MRS score, 72hNIHSS score decrease of ≥8 points or a ratio of 0-1, and the safety endpoint was SICH and death.

Results: A total of 202 stroke patients were enrolled in this study, 101 subjects received tenecteplase and 101 subjects received alteplase. Primary endpoint Good perfusion rate (Tenecteplase 22% vs. Alteplase 10%) (Ratio difference 12%, 95%CI,2-21, Ratio 2.2, 95%CI, 1.1-4.4; Non-inferiority P = 0.002;P = 0.03).90-day functional outcome of tenecteplase was better than that of alteplase (median mRS 2 vs 3, OR 1.7, 95%CI, 1.0-2.8; P = 0.04). Symptomatic cerebral hemorrhage occurred in 1% of patients in each group.

Conclusion: Tenecteplase has a better rate of vascular recanalization and reperfusion in patients with aortic occlusion.

Part 2:

The EXTEND - IA a successor Part 2 test is a launched by the investigators, multicenter, prospective, randomized, open label, blind end study, were incorporated into the internal carotid artery, basilar artery or 4.5 hours of stroke onset of middle cerebral artery occlusion for mechanical bolt of ischemic cerebral apoplexy patients, cerebral apoplexy before mRS 3 or less (no age limit) and intravenous (IV) thrombolysis no contraindications. Patients were randomly assigned to IV TNK0.4mg/kg (Max. 40mg) or 0.25mg/kg (Max.25mg) before thrombectomy. The primary clinical outcome was reperfusion of more than 50% of the affected ischemic area before thrombectomy, or an unrecoverable intracranial thrombus. Secondary clinical outcomes included MRS score at 90 days and early neurological improvement (NIHSS score at 3 days: NIHSS score improved by at least 8 points or 0 to 1); The primary safety outcomes were symptomatic intracerebral hemorrhage (SICH) and all-cause death.

Of the 300 patients, 150 were randomized to 0.4mg/kg TNK and 150 to 0.25mg/kg TNK. In the 0.4mg/kg group (29 /150) and the 0.25mg/kg group (29 /150) (19.3%) (unadjusted risk difference, 0.0% [95% CI, −8.9% to −8.9%];Adjusted risk ratio, 1.03[95%CI, 0.66-1.61];P = 0.89).There was no significant difference in any of the four functional outcomes between the 0.4mg/kg group and the 0.25mg/kg group among the six secondary clinical outcomes, with all-cause death (26 [17%] vs 22 [15%];Unadjusted Risk Difference, 2.7% [95%CI, −5.6% to 11.0%]) vs. SICH

(7[4.7%] vs. 2 [1.3%];Unadjusted risk difference, 3.3% [95%CI, −0.5% to 7.2%]) was also not significantly different.

The results of this study suggest that 0.40mg/kg tenecteplase compared with 0.25mg/kg did not significantly improve cerebral reperfusion prior to endovascular thrombectomy in patients with ischemic stroke with intracranial large vessel occlusion. Results showed that 0.40-mg/kg dose of TNK did not have an advantage over 0.25-mg/kg dose in patients with large vessel occlusive ischemic stroke who were scheduled to undergo endovascular thrombectomy.

(6) ATTEST trial [19]

The ATTEST trial was designed to compare the efficacy and safety of tenecteplase and alteplase in patients without baseline imaging selection. This phase II, prospective, randomized, open label, blind outcome trial included patients with acute cerebral infarction within 4.5h after onset, and were randomly assigned to alteplase group (0.9 mg/kg) or tenecteplase group (0.25 mg/kg).CTP and CTA were used to evaluate infarction and perfusion before and after treatment, and the primary endpoint was the ratio of penumbra rescue within 24 to 48 hours.

The results showed as follows: (1) A total of 104 patients were enrolled in the study, and 71 patients reached the primary endpoint. The proportion of penumbra saved in the two groups was basically the same (68% in the tenecteplase group vs. 68% in the alteplase group).② There was no significant difference between the two groups in symptomatic intracranial hemorrhage (1/52 vs 2/51, P=0.55) and total intracranial hemorrhage (3/52 vs 4/51, P=0.59).③ There was no difference in the severity of adverse events between the two groups.

Conclusion: There is no difference between tenecteplase and alteplase in nerve function evaluation and imaging evaluation.

1.4 Experimental design

In conclusion, this study will evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) compared with standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset). This is a multicenter, prospective, centrally randomized, open label, blinded-endpoint, active controlled, non-inferior phase III clinical trial.

The drug administration method of the test drug (rhTNK-TPA) is as follows: according to the body weight of the subjects, the final dose is determined at 0.25mg/kg; Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the subject, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds..

The administration method of active control drug (rt-PA) is as follows: The dose required for medication is calculated and measured according to the actual body weight of the subject based on a standard of 0.9 mg rt-PA/kg. 10% of the dose is injected by intraveno

us bolus, and the remaining 90% is injected by intravenous drip within 1h.

The target population of this study is patients with critical hyperacute ischemic stroke (within 4.5 h of symptom onset), for whom considering the benefit of patients, intravenous thrombolytic therapy should be given as soon as possible after the onset, in order to achieve vascular recanalization or reperfusion and obtain a better prognosis. Due to the different administration methods of test drug and active control drug, and the inconvenience of the double-blind double-dummy design which may delay treatment and is not conducive to the benefit of subjects. Therefore, this study adopts the form of open-label trial. To minimize bias, all endpoints will be evaluated in a blind manner by a qualified study physician.

2. Study objectives

To evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) compared with standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset).

Primary study objectives:

To evaluate the difference in the ratio of 90-day MRS score of 0-1 between rhTNK-tPA (0.25 mg/kg) and standard rT-PA (0.9mg/kg) in the treatment of subjects with hyperacute ischemic stroke (within 4.5 h of symptom onset).

Secondary study objectives:

1. To compare the difference in efficacy between rhTNK-tPA (0.25 mg/kg) and standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset) subjects:

(1) The proportion of subjects with an mRS score of 0-2 at 90±7 days

(2) MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution)

(3) The proportion of subjects whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first)

(4) Health-related quality of life (EQ-5d) at 90±7 day

(5) The proportion of subjects with a Bathel score ≥ 95 points at 90±7 days

2. To compare the difference in safety between rhTNK-tPA (0.25 mg/kg) and standard rT-PA (0.9mg/kg) in the treatment of subjects with hyperacute ischemic stroke (within 4.5 h of symptom onset):

① The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASSIII)

② The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASSIII)

③ The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS standards)

④ The incidence of any intracranial hemorrhage within 90 days

⑤ The incidence of significant hemorrhage events at other sites within 90 days (as defined by GUSTO)

⑥ 90-day overall mortality

⑦ Incidence rates of adverse events/serious adverse events within 90 days

3. Study design

3.1 Overall design

This study is a multicenter, prospective, centrally randomized, open label, blinded-endpoint, parallel active control, and non-inferiority design.

3.2 Grouping

The subjects are randomly divided 1:1 into two groups, the treatment group (rhTNK-tPA 0.25mg/kg) and the control group (rT-PA 0.9mg/kg), and are given treatment within 4.5h after symptom onset.

3.3 Visits

Study visit points: -4.5h-0h (before thrombolysis), 0h (thrombolytic therapy), 24h±2h, 24h-36h, 7±1 day, 90±7 days (end visit).

3.4 Measures to control bias

3.4.1 Randomization

The central randomization method is adopted in this study, and the central randomization system is used to set dynamic block randomization. The proportion of subjects in the treatment group and the control group is 1:1 in each center, and each center assigned random number to each enrolled subject in the way of competitive enrollment.

After the subjects complete all screening tests and the investigators determine that they meet the inclusion criteria or do not meet the exclusion criteria, the staff of the research center is responsible for entering the basic information of the patients (initials, age, sex, identification code, etc.) into the central randomization system based on the network. The system will automatically generate a random number and subject ID according to the above principles and feed back to the central investigator via the network whether the patient is assigned to the trial group (rhTNK-tPA 0.25mg/kg group) or the control group (rT-PA 0.9mg/kg group).Investigators at the research center received the results of randomization and then treated the patients according to the appropriate group.

3.4.2 Blinding

All endpoints in this study will be evaluated in a blind manner by a qualified study physician who is not aware of the treatment grouping. The study physician responsible for blind state evaluation will be authorized by the PI of each center after receiving unified training and passing the examination.

3.5 Academic committee

It consists of the Sponsor, medical experts designated by the Sponsor and key

investigators, and will hold meetings (teleconferences or on-site meetings) as needed to review the progress of the study, and make appropriate guidance and major decisions on the study.

3.6 Data Monitoring Committee

It consists of independent statisticians and neurologists who are not involved in the study execution. The membership of the DMC will be determined by the DMC and the Steering Committee prior to the commencement of the trial, including membership, roles, and responsibilities. The DMC will monitor the progress of this study on a regular basis to ensure that the study meets the highest standards of ethics and subject safety. The DMC will make recommendations on the safety data of the trial: terminate or continue the trial or modify the protocol to continue.

3.7 Clinical event committee

It consists of neurologists, cardiologists, etc., who are not involved in the execution of the study. Membership of the Clinical Event Committee (including qualifications, roles and responsibilities) shall be confirmed by the Academic Committee prior to the commencement of the trial.

Clinical endpoint events include: new vascular events/hemorrhagic stroke (ischemic stroke/mi/cardio-cerebral revascularization (including: carotid intima stripped, intracranial artery interventional therapy, intracranial artery bypass surgery, coronary intervention or bypass surgery)), symptomatic intracranial hemorrhage (ECASSIII definition), PH2 intracranial hemorrhage (SITS), other parts of the significant bleeding (GUSTO hemorrhage definition, severe and moderate bleeding) and death.

All clinical endpoints will be first determined by the investigator and then verified and identified by the Clinical Event Committee based on clinical, laboratory, original images, or documentation. It will be discussed over by the independent Clinical Event Committee.

4. Study population

4.1 Inclusion criteria

(1) Age ≥18 years, male or female;

(2) Time from onset to treatment < 4.5h; the time of symptom onset is defined as "the last time point at which the patient appears normal";
(3) Clinical diagnosis as ischemic stroke (the diagnosis following the *Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018*);
(4) MRS score of 0-1 before onset;
(5) 4 < NIHSS < 26 at baseline;
(6) Subjects or their guardians voluntarily sign the informed consent.

4.2 Exclusion criteria

(1) Patients scheduled to receive endovascular therapy;
(2) NIHSS score 1A > 2;
(3) Known to be allergic to rhTNK-tPA and/or rT-PA;
(4) A history of intracranial hemorrhage;
(5) A history of severe head trauma or stroke within 3 months;
(6) A history of intracranial or spinal surgery within 3 months;
(7) A history of gastrointestinal or urinary tract hemorrhage within 3 weeks;
(8) A history of major surgery within 2 weeks;
(9) A history of arterial puncture at sites difficult for compression hemostasis within 1 week.
(10) Intracranial tumors (except neuroectodermal tumors, such as meningiomas), large intracranial aneurysms;
(11) Intracranial hemorrhage (including parenchymal hemorrhage, intraventricular hemorrhage, subarachnoid hemorrhage, subdural/extradural hematoma, etc.);
(12) Active visceral bleeding;
(13) Aortic arch dissection found.
(14) Uncontrollable hypertension upon active antihypertensive treatment: systolic blood pressure ≥180 mm Hg, or diastolic blood pressure ≥100 mm Hg;
(15) Propensity for acute bleeding, including platelet counts of less than 100×10^9/ L or otherwise;
(16) Blood glucose <2.8 mmol/L or >22.22 mmol/L;
(17) On oral warfarin anticoagulant treatment with INR>1.7 or PT > 15s;
(18) Having received heparin treatment within 24 h;
(19) Having received thrombin inhibitors or factor Xa inhibitors within 48 h;
(20) Head CT or MRI shows a large infarction (infarcted area > 1/3 of the middle

cerebral artery).

(21) Subjects who are unable or unwilling to cooperate due to hemiplegia (Todd's palsy) after epileptic seizure or other neurological/psychiatric disorders;

(22) Pregnant women, lactating women, or subjects who do not agree to use effective contraception during the trial;

(23) Participation in other clinical trials within 3 months prior to screening;

(24) Unsuitability for participation or participation in this study as judged by the investigator may result in exposure to greater risk.

4.3 Withdrawal criteria

4.3.1 Withdrawal decided by the investigator

1) Subjects with poor compliance, failure to take medication as required or participate in study visits, which affects the judgment of efficacy or safety;
2) In the judgment of the Principal Investigator, the subject should withdraw from the study in any case for the benefit/risk of the subject.

4.3.2 Subjects' voluntary withdrawal

According to the provisions of the informed consent, the subject has the right to withdraw from the study midway; In case that the subject has not withdrawn the informed consent, but no longer accepts the medication and testing, it is also considered as withdrawal, also called drop-out. As far as possible, the reasons for their withdrawal should be known and recorded.

If a subject withdraws from the study due to AE or abnormal laboratory test results, this important special event and test results should be recorded in an electronic case report form (eCRF).

For cases that drop out of the trial or are lost to follow-up, investigators should actively take measures to complete the last examination as far as possible in order to analyze the efficacy and safety. Any dropout subject should be documented in detail, safety and efficacy data for the subject should be obtained as far as possible, and an evaluation at the end of all studies should be conducted with subject consent and compliance. All case data should be kept complete for future reference.

Subjects who have signed the informed consent and been randomly grouped may not be replaced.

4.4 Removal criteria

1) Cases who are found not meeting the inclusion criteria or meeting the exclusion criteria after enrollment;
2) Cases to be removed due to violation of the provisions of the clinical trial protocol for concomitant medication and/or treatment, as determined by the Academic Committee;
3) Cases without medication after enrollment;
4) Cases without evaluable records after administration;
5) Cases who fail to take stipulated medication with poor compliance, resulting in impossibility to judge efficacy or incomplete data adversely affecting the judgment of efficacy and safety.

Cases eliminated should be explained, and their data should be kept complete for future reference. If patients have received at least one study drug treatment, ITT analysis could be performed according to the data records. Patients who have received at least one treatment and have a safety record may also be included in the safety analysis as appropriate.

4.5 Discontinuation criteria

Trial discontinuation refers to the discontinuation of all tests in the course of a clinical trial that has not been completed according to the protocol. The purpose of discontinuing the trial is mainly to protect the rights and interests of subjects, ensure the quality of the trial and avoid unnecessary economic losses. If one of the following situations occurs during the trial, the trial should be discontinued.

1) Serious safety problems occur in the trial process;
2) In the course of the trial, the drug efficacy is too poor, being ineffective in 2/3 of the cases;
3) Significant errors in the clinical trial protocol are found, making it difficult to evaluate drug response;
4) Significant deviations occur in the implementation of the trial protocol, making it difficult to evaluate the drug response if it continues;
5) The Sponsor requests the discontinuation or the drug regulatory authority orders the discontinuation of the experimental person for some reason.

The discontinuation of testing can be temporary or permanent. When the trial is discontinued, all trial records should be kept for future reference.

4.6 Termination criteria

1) The Sponsor and/or the investigator deem it inappropriate to proceed with the trial on medical or ethical grounds.

2) The Sponsor and/or the investigator deem it inappropriate to proceed with the study on the basis of scientific reasons or on the basis of factual, accurate or normative considerations for the conduct of the study. For example, major errors in the study protocol (including evaluation criteria, operation, analysis methods, etc.) are found in the trial, so it is difficult to evaluate the drug response; Or a well-designed program, with significant deviations during implementation (including incomplete or unassessable important data), continues, making it difficult to evaluate drug response.

3) Due to various reasons (such as funding reasons, management reasons or changes in medical development policies, etc.), the Sponsor decides to terminate the study under the premise of fully protecting the safety and rights of volunteers;

4) In accordance with the GCP guidelines and local laws and regulations, the National Medical Products Administration, the Ethics Committee, the investigator or the Sponsor, for any reason, should discontinue the study or discontinue the study in which the volunteers participated, taking into account their rights, the safety and health of the volunteers.

5. Study drugs

5.1 Name of study drugs

Test drug:

Drug name	Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-TPA)
Trade name	MingFuLe
Strengths	1.0 x 10^7 IU/16 mg/vial
Storage conditions	Store below 25℃ away from light. This product should be used immediately after dissolution. If not used immediately, it should be stored in cold storage at 2 - 8℃away from light and used within 24 hours.
Shelf life	24 months
Manufacturer	Guangzhou Recomgen Biotech Co., Ltd.
Supplier	Guangzhou Recomgen Biotech Co., Ltd.

Control drug:

Drug name	Alteplase for Injection (rt-PA)

Trade name	Actilyse®
Strengths	50mg/vial, 20mg/vial
Storage conditions	Keep in the original packaging. Store below 25℃ away from light. It is recommended to use the prepared solution immediately after preparation. It has been proved that the prepared solution can remain stable at 2-8℃ for 24h. Frezzing prohibited.
Shelf life	36 months
Manufacturer	Boehringer Ingelheim Pharma GmbH&Co.KG
Supplier	Guangzhou Recomgen Biotech Co., Ltd.

5.2 Labeling of study drugs

The test drug "Recombinant Human TNK Tissue-type Plasminogen Activator for Injection" will be produced and packaged by Guangzhou Recomgen Biotech Co., Ltd. (Sponsor), and the control drug "Alteplase for Injection" will be purchased and provided by Sponsor, and distributed to each clinical research center by the Sponsor.

The outer packing box of the test drug shall be labeled as follows:

For use in TRACE II (0-4.5H) clinical trial only

Please refer to the package insert for using

Protocol No. : MK02-2020-01

Sponsor: Guangzhou Recomgen Biotech Co., Ltd.

The inner packing box of the test drug should be labeled as follows:

Subject random number:	**Product name:** Recombinant Human TNK Tissue-type Plasminogen Activator for Injection
Strength: 1.0 x 10^7IU / 16 mg/vial	**Batch number:**
Production date:	**Expiry date:**

Storage condition: store below 25℃ away from light

Note: For use in TRACE II (0-4.5H) clinical trial only, protocol number: MK02-2020-01

Guangzhou Recomgen Biotech Co., Ltd.

Note: Please complete the subject random number, batch number, production date and expiration date of the test drug according to the actual situation.

The packing box of the control drug should be labeled as follows:

Subject random number:	**Product name:** Alteplase for injection
Strengths: ☐ 20mg/vial /□ 50mg/ vial	**Batch number:**
Production date:	**Expiry date:**

Storage condition: store below 25℃ away from light

Note: For use in TRACE II (0-4.5H) clinical trial only, protocol number: MK02-2020-01

Guangzhou Recomgen Biotech Co., Ltd.

Note: Please select the strength according to the actual dose (20mg/vial or 50mg/vial), and complete the subject random number, batch number, production date and expiration date of the control drug according to the actual situation.

5.3 Method of administration

Treatment group (rhTNK-TPA 0.25mg/kg, 25mg at most):

RhTNK-TPA 0.25mg/kg: Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the

subject, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds.

Control group (rt-PA 0.9mg/kg) :

RT-PA 0.9mg/kg: The product is dissolved in sterile water for injection to obtain a solution with a concentration of 1mg/ ml. The amount required for medication is calculated and measured according to the actual body weight of the subject. 10% of the solution is injected by intravenous bolus, and the remaining 90% is injected by intravenous drip within 1h.

5.4 Drug storage conditions

Study drugs will arrive at the research centers prior to the launch of the center, and then sufficient study drugs will be supplied to the Center on a regular basis based on the actual number of enrolled subjects and the amount of drugs to be used. The study drug will be stored in a secure location under the direct responsibility of the investigator or other authorized person and will be stored in accordance with the conditions described on the label.

5.5 Drug distribution and inventory

The investigator must keep accurate records of the number of boxes/bags of the drug being studied. In this study, the drug is administered intravenously, and the study drug had to be administered by the investigator.

5.6 Drug recovery and destruction

Investigators are required to record information on all study drug dispensations, including date, quantity, drug lot number, and subject number. Used and unused drugs must be recovered and destroyed in accordance with relevant regulations and procedures.

6. Treatment

6.1 Treatment administered

Study drugs should be administered as soon as possible after randomization (less than 4.5h from onset of disease to onset of thrombolysis).

Thrombolysis time (intravenous infusion time in the treatment group,

intravenous infusion time in the control group, and the start and end time of intravenous infusion), administration dose (intravenous infusion dose in the treatment group, intravenous infusion dose in the control group, and intravenous infusion dose), vital signs and the occurrence of adverse events during administration should be recorded.

6.2 Concomitant treatment

All enrolled subjects should be treated in the Acute Stroke Unit or, if needed, in the Intensive Care Unit (ICU). All subjects will be enrolled according to the *Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018*.[28]Subsequent standardized early treatment management and secondary prophylactic medication are performed. Subjects are treated with thrombolysis according to relevant guidelines (*Chinese Guidelines for Endovascular Treatment of Acute Ischemic Stroke 2018*).[29], *Chinese Guidelines for Early Intravascular Interventional Diagnosis and Treatment of Acute Ischemic Stroke 2018*[30]), and it is up to the investigator to make the final decision on whether or not to perform endovascular treatment, and to record the treatment method and drug combination.

Concomitant drugs (including anticoagulants, defibrillators, antiplatelet agents, lipid-lowering agents, antihypertensive agents, hypoglycemic agents, etc.) before and after randomization and during hospitalization and follow-up should be recorded. Non-therapeutic drugs such as contrast agents, pre-flushing tube heparin, etc., are not recorded as concomitant drugs.

(1) If the subject does not receive mechanical thrombotomy, no antiplatelet drugs, anticoagulants, defibrillators, thrombolytic drugs or drugs that may affect platelet and clotting function are allowed to be used within 24 hours after the study drugs is used.

(2) If the subjects have received mechanical thrombotomy but do not receive emergency stent implantation, the investigators will determine whether the concomitant use of antiplatelet and anticoagulant therapy is necessary according to relevant guidelines within 24h after the use of the study drugs, and the concomitant use of drugs will be recorded.

(3) If the subjects have received emergency stent implantation, antiplatelet agents and anticoagulants can be used within 24 hours after the study drugs is used.

(4) Any medication other than those listed above (including blood pressure lowering, blood glucose lowering, lipid-lowering drugs, etc.) is allowed. If necessary,

the investigator should carefully consider administering a relatively stable and safe dose to the subject.

During the study period, any treatment and/or prescriptions issued prior to randomization or any medication changes during the study period should be recorded on an electronic case report form.

6.3 Medication compliance

In the recruitment and screening stage, the purpose of the trial, the basic information of the study drugs, the protocol, the trial process, the administration regimens, clinical observation, the potential risk if participating in the study, compensation, etc., will be introduced in detail, so that subjects could be fully informed and voluntarily participate in the study to improve the compliance of the study. This study uses single dose, to be administered by the study nurse. Before administration, subject number, administration table and dosage should be carefully checked; after administration, the remaining quantity of study drugs, empty packages and administration equipment should be carefully checked.

7. Study process

7.1 Screening/baseline assessment

1) Demographic information: sex, age, ethnicity.

10) The time of present onset as well as the symptoms (the present onset symptoms are mainly reflected in NIHSS score).

11) Risk factors (past medical history and history of present illness) : high blood pressure, diabetes, cerebral infarction, TIA, coronary heart disease, myocardial infarction, arrhythmia, always oral medication (aspirin, clopidogrel, and warfarin, antihypertensive drugs, lipid-lowering drugs, oral glucose-lowering drugs, drug adjust heart rate), dementia, peripheral artery disease, lipid metabolic disorders, liver and kidney function is not complete, and neck intracranial vascular stenosis or deformity, smoking, drinking alcohol.

12) Prior and concomitant medication.

13) Vital signs (blood pressure, pulse, respiratory rate and body temperature).

14) MRS score before onset, MRS score post onset before onset of thrombolysis, NIHSS score post onset before onset of thrombolysis.

15) Electrocardiogram (If the subject has had an electrocardiogram before thrombolysis after the onset of the disease, no repeat examination is required).

16) Imaging (CT or MRI).

Screening/baseline imaging examination is “CT or MRI”, which is used to exclude hemorrhagic stroke; the CT or MRI results generated by other hospitals can also be used as a screening basis, if the study physician deems them acceptable; if a subject has undergone a CT or MRI check post symptom onset before onset of thrombolysis, such examination may be omitted. CT or MRI evaluation should include: cerebral hemorrhage, acute severe symptomatic ischemia in the area of blood supply, or other pathological changes.

17) Laboratory examination:

Routine blood test:

Red blood cell count (RBC), white blood cell count (WBC), neutrophil count (NEU), lymphocyte count (LY), hematocrit (HCT), hemoglobin (Hb), platelet count (PLT);

Blood biochemistry:

Creatinine (GREA), UREA (or blood urea nitrogen BUN), glucose (GLU), uric acid (UA), lactate dehydrogenase (LDH), creatine phosphokinase isoenzyme (CK-MB), alkaline phosphatase (ALP), glutamyltranspeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), triglyceride (TG);

For baseline blood lipid tests (total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), triglyceride (TG)), the most recent fasting blood lipid after the onset of the disease should be measured. Blood glucose test is performed at baseline using peripheral blood glucose measured in venous blood as one of the inclusion and exclusion criteria;

Coagulation function test:

Prothrombin time (PT), activated partial prothrombin time (APTT), thrombin time (TT), fibrinogen (FIB), international standardized ratio (INR);

Blood/urine pregnancy test:

Only for fertile female subjects.

Note: The study population is acute ischemic stroke subjects. For the reason of time, subjects, after signing written informed consent by themselves or their guardians, can be randomly grouped to receive treatment first; but for safety reasons, investigators must learn routine blood test, blood glucose levels (peripheral blood glucose results) and urine pregnancy test (if applicable) results to determine the suitability for participation into the study, if warfarin has been used, including coagulation function results. Blood biochemical and coagulation tests should be completed before administration, and the results can be provided after enrolment. Blood biochemical results are allowed to be obtained after the study drug has been administered. If abnormal blood biochemical results are obtained, they will be handled by the study physician in accordance with the discipline guidelines and the clinical management pathway of the hospital. Subject will not need to repeat the test if they have already performed some laboratory tests between the present onset and prior to thrombolysis.

7.2 Randomization

If the patients agree to participate in the trial, after the patients are screened and agree to be enrolled, the investigator will use the central randomization system to generate randomization numbers and group information for the patients according to the order of their enrollment, and the randomization number will be recorded on the enrolment screening table and the CRF table.

7.3 Treatment

Study drugs should be administered as soon as possible after randomization (less than 4.5h from onset of disease to onset of thrombolysis).

Thrombolysis time (intravenous infusion time in the treatment group, intravenous infusion time in the control group, and the start and end time of intravenous infusion), administration dose (intravenous infusion dose in the treatment group, intravenous infusion dose in the control group, and intravenous infusion dose), vital signs and the occurrence of adverse events during administration should be

recorded.

Vital signs (blood pressure, pulse, body temperature, respiratory rate) should be measured within 5min before thrombolysis.

Concomitant medication and adverse events should be recorded.

7.4 Follow-up

7.4.1 24 + 2 h

During the follow-up:

NIHSS score.

Vital signs (blood pressure, pulse, body temperature, respiratory rate).

Laboratory examination (test results obtained within 72 hours after onset (after thrombolysis) are acceptable):

Routine blood test, blood biochemistry and coagulation tests are to be performed, including the same items as during screening period.

Routine urinalysis: pH value, protein, occult blood, glucose, bilirubin, nitrite, ketone body;

Concomitant medication and adverse events are recorded.

7.4.2 24 - 36 h

During the follow-up:

Imaging: CT or MRI, used to detect hemorrhagic events.

Concomitant medication and adverse events are recorded.

7.4.3 7±1 day or before discharge (whichever occurs first)

During the follow-up:

NIHSS score.

Vital signs (blood pressure, heart rate, body temperature, respiratory rate).

Laboratory examination:

Routine blood test, blood biochemistry and coagulation tests are to be performed, including the same items as during screening period.

Routine urinalysis: pH value, protein, occult blood, glucose, bilirubin,

nitrite, ketone body;

Electrocardiogram.

Concomitant medication and adverse events are recorded.

7.4.4 90 + 7 days

During the follow-up:

MRS score, BI scale and EQ-5D scale are evaluated.

Concomitant medication and adverse events are recorded.

8. Efficacy endpoints

8.1 Primary efficacy endpoints

The proportion of subjects with 90-day MRS score of 0-1: Proportion of subjects with 90-day MRS score of 0-1 to all enrolled subjects. Calculation formula:

The proportion of subjects with 90-day MRS score of 0-1 = the number of subjects with 90-day MRS score of 0-1 / the number of subjects enrolled in this group ×100%

8.2 Secondary efficacy endpoints

6) The proportion of subjects with an mRS score of 0-2 at 90±7 days
7) MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution)
8) The proportion of subjects whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first)
9) Health-related quality of life (EQ-5d) at 90±7 day
10) The proportion of subjects with a Bathel score ≥ 95 points at 90±7 days

8.3 Safety endpoints

8) The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASSIII)
9) The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASSIII)
10) The incidence of PH2 intracranial hemorrhage within 36 hours (according to

SITS standards)

11) The incidence of any intracranial hemorrhage within 90 days

12) The incidence of significant hemorrhage events at other sites within 90 days (as defined by GUSTO)

13) 90-day overall mortality

14) Incidence rates of adverse events/serious adverse events within 90 days;

9. Observation and treatment of adverse events

9.1 Adverse events

9.1.1 Definition of adverse events and adverse reactions

Adverse Event (AE) refers to all adverse medical events that occur after the subject receives the study drugs, which may be manifested as symptoms, signs, diseases or abnormal laboratory tests, but does not necessarily have a causal relationship with the study drugs.

Adverse drug reaction (ADR) refers to any harmful or undesirable reaction that occurs during a clinical trial and may be related to the study drugs with at least a reasonable possibility of a causal relationship between the study drugs and the adverse event, i.e., an association cannot be ruled out.

9.1.2 Recording of adverse events

Adverse event reporting period: adverse event recording starts immediately after the subjects receive the study drug, until the last follow-up after completion of the study. All AE findings must be recorded by the investigator (or designee) in the AE section of the eCRF. The record includes a description of the AE, the time of occurrence and resolution, severity, relationship with the study drugs, measures taken, and outcomes.

9.1.3 Severity assessment

The severity of adverse events should be assessed according to CTCAE version 5.0. In case of adverse events not listed in the table, the following criteria can be used:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate: minimal, local or noninvasive intervention indicated; limiting age- appropriate instrumental Activities of Daily Living.

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

9.1.4 Assessment of association between adverse events and study drugs

All adverse events in the clinical study, including any abnormal symptoms, signs, laboratory tests, or other special tests, should be documented in detail and evaluated for association. The investigator must follow up and obtain sufficient information to determine whether the cause of an adverse event is related to the study drugs. According to the criterion of causality between drugs and adverse events, the correlation between adverse events and the application of the test drug is divided into five grades: definitely related, probably related, possibly related, unlikely related, and definitely not related. Any adverse reactions assessed to be definitely related, probably related, possibly related and unlikely related are listed as adverse drug reactions. The total number of ADR cases is taken as the numerator, and all selected cases for ADR assessment are taken as the denominator to calculate the incidence of ADR.

The judgment criteria are as follows:

Definitely related: consistent with the known type of reaction of the suspected drug, forming a reasonable time sequence between onset and drug administration, reduction or disappearance of the adverse event after dose reduction or withdrawal, and reoccurrence of the adverse event after administration.

Probably related: consistent with the known type of reaction of the suspected drug, forming a reasonable time sequence between onset and drug administration, reduction or disappearance of the adverse event after dose reduction or withdrawal, but may be resulted from the subject's clinical status or other reasons.

Possibly related: consistent with the known type of reaction to the suspected drug, forming a reasonable time sequence between onset and drug administration, reduction or insignificant improvement of the adverse event after dose reduction or withdrawal, but may be explained by the subject's clinical status or other reasons.

Unlikely related: not consistent with the known type of reaction to the suspected drug, not forming a reasonable time sequence between onset and drug administration,

and may be resulted from the subject's clinical status or other reasons.

Definitely not related: not consistent with the known type of reaction to the suspected drug, not forming a reasonable time sequence between onset and drug administration, may be resulted from the subject's clinical status or other reasons, reduction or disappearance of the adverse event after the exclusion of clinical symptoms or other causes.

9.2 Serious adverse events

9.2.1 Definition of serious adverse events

A serious adverse event (SAE) is a medical event that occurs after a subject receives the study drugs, such as death, life-threatening, permanent or severe disability or loss of function, requiring hospitalization or prolonged hospitalization, or leading to a congenital abnormality or birth defect, excluding the following:

Non-therapeutic hospitalization, such as: rehabilitation hospitalization, nursing facility hospitalization, transactional hospitalization, hospitalization for examination only, planned elective hospitalization, etc.

9.2.2 Recording and reporting of serious adverse events

When an SAE occurs during the clinical trial, the investigator should report it in writing to the Sponsor as soon as he becomes aware of it, followed by a detailed, written follow-up report in a timely manner. If a death is involved, the investigator shall immediately submit a written report to the Ethics Committee of the center, as well as provide other necessary information, such as the autopsy report and the final medical report.

Upon receipt of the SAE report, the Sponsor or designee shall immediately analyze, evaluate and promptly report suspected unexpected serious adverse events (SUSAR) to all investigators participating in the clinical trial, the clinical trial facility, the Ethics Committee, the drug regulatory authority and the health authority.

Reporting unit	Contact	Phone	Email
Ethics Committee of Beijing Tiantan Hospital	'	010-59978555	ttyyirb@163.com
Guangzhou Recomgen Biotech Co., Ltd.	School of medicine	18926139511 020-82209991	yangqiao@recomgenbio.com

PV contact	Zhao Qianna	010-51281119	PV@giantcro.com

9.3 Reporting of suspected unexpected serious adverse reaction (SUSAR) [31]

Suspected unexpected serious adverse reactions refer to suspected unexpected serious adverse reactions whose nature and severity of clinical manifestations exceed the available data such as the investigator's brochure of the study drugs, the package insert of a marketed drug, or the summary of product characteristics.

All unexpected serious adverse reactions that are definitely related to or suspected to the study drugs occurring during clinical trials (both within and outside China) should be promptly reported to the national drug evaluation body within the specified time limit. The contents of individual safety reports of unexpected severe adverse events should be reported in accordance with the relevant requirements of ICH E2B (R3): *Clinical Safety Data Management: Data Elements for Transmission of Individual Case Safety Reports*. Relevant terms shall be coded in the ICH M1: *Medical Dictionary for Regulatory Activities* (MEDDRA).

a) Start and end times for expedited reporting of SUSAR

The start time of expedited reporting is the clinical trial approval date/the start date of the implied approval of the national drug evaluation body, and the end time is the end date of the last subject follow-up in China. (Unexpected serious adverse reactions occurring from the end of the clinical trial follow-up to the conclusion of the review and approval should also be subject to expedited reporting.)

b) Time limit for expedited reporting of SUSAR

Fatal or life-threatening: first report within 7 days, followed by follow-up within 8 days.

Non-fatal or life-threatening: first report within 15 days.

c) The electronic transmission mode of SUSAR

Submit through GATEWAY

Submit as an XML file

9.4 Pregnancy events

If a subject becomes pregnant during the study period, the investigator should

report to the relevant authorities (Sponsor, hospital Ethics Committee) within 24h after being informed. Subjects should stop medication immediately after pregnancy is found and continue safety follow-up until the end of pregnancy or termination of pregnancy. Pregnancy events will be reported in accordance with SAE reporting requirements and timelines.

9.5 Possible adverse drug reactions

Adverse events in clinical trials of this product are the same as other thrombolytic drugs. The most common adverse reactions in clinical studies of this product are bleeding, including intracranial hemorrhage and other minor bleeding adverse events. The specific data are shown in the table below.

Hemorrhagic adverse events in clinical trials of this product

	rhTNK-tPA 16mg (n=124)	**rt-PA 50mg (n=127)**
Intracranial bleeding	**1 (0.81%).**	**1 (0.79%).**
Adverse events of minor bleeding:		
Urinary tract hemorrhage	**8 (6.45%)**	**6 (4.72%)**
Bleeding gums	**6 (4.84%)**	**4 (3.15%)**
Subcutaneous ecchymosis	**4 (3.22%)**	**4 (3.15%)**
Gastrointestinal hemorrhage	**3 (2.42%)**	**5 (3.94%)**
RBC indexes such as hemoglobin decreased	**2 (1.61%).**	**3 (2.36%)**
Hematoma at puncture site	**1 (0.81%).**	**4 (3.15%)**
A bleeding point on tongue tip	**0**	**1 (0.79%).**

Thrombolytic therapy should be discontinued when a potential tendency to hemorrhage, especially intracranial hemorrhage, is found.

Allergic reaction: No patients have been found to have allergic reaction after using this product in the clinical trial. Once an allergic reaction occurs, antiallergic treatment is required.

Other adverse events: The following adverse events have been reported in patients who received this product in clinical trials. The effect of this product on the incidence of these adverse events is unclear. These adverse events include cardiogenic shock, heart failure, cardiac rupture and electromechanical separation, ventricular fibrillation, and cardiac rupture (3.23%); increase in aminotransferase, myocardial enzyme, blood lipid and blood glucose, as well as nausea, vomiting, fever and cough,

all mild. The majority of these adverse events are determined not to be related to the test drug, but to underlying and/or comorbidities, as well as concomitant drugs.

Clinical adverse reactions of similar products authorized in the United States and the European Union:

In an international multicenter, double-blind clinical trial (ASSENT-2), TNK-tPA (generic name: Tenecteplase, trade name in the United States: TNKase®, trade name in the European Union: Metalyse®) and rt-PA was compared. The incidence rates of intracranial hemorrhage in patients treated with TNKase® and rt-PA were both 0.9%, and the incidence rates of stroke were 1.8% and 1.7%, respectively. The incidence of stroke in both groups, including intracranial hemorrhage, increased with age. The data of non-intracranial hemorrhagic adverse events in the ASSENT-2 study are shown in the table below.

Non-intracranial hemorrhagic events in the ASSENT-2 study

	TNK-tPA (n=8461)	**rt-PA (n=8488)**	**The relative risk (95% CI)**
Bleeding events			
Severe bleeding event[a]	**4.7%**	**5.9%**	**0.78 (0.69, 0.89)**
Minor bleeding event	**21.8%**	**23.0%**	**0.94 (0.89, 1.00)**
Blood transfusion unit			
all	**4.3%**	**5.5%**	**0.77 (0.67, 0.89)**
1 to 2	**2.6%**	**2.6%**	**-**
> 2	**1.7%**	**2.2%**	**-**

Note A: Severe bleeding events are defined as those that require blood transfusion, or those that cause hemodynamic harm.

Patients treated with TNKase had a lower incidence of non-intracranial bleeding and requiring blood transfusion. The main types of bleeding in 1% or more of patients are hematoma (1.7%) and gastrointestinal bleeding (1%).The main types of bleeding in 1% or less of patients are urinary tract bleeding, bleeding at the site of puncture (including cardiac catheter bleeding), retroperitoneal bleeding, respiratory tract bleeding, and bleeding at unspecified sites. The types of minor bleeding in 1% or more patients are hematoma (12.3%), urinary tract bleeding (3.7%), bleeding at the site of puncture (including cardiac catheter bleeding) (3.6%), pharyngeal bleeding (3.1%), gastrointestinal bleeding (1.9%), nasal bleeding (1.5%), and unspecified site

(1.3%).

Anaphylaxis: Few (< 1%) patients treated with TNKase have reported anaphylaxis (e.g., hypersensitivity, angioedema, laryngeal edema, rash, urticaria). < 0.1% of patients treated with TNKase experienced anaphylaxis, but causality is uncertain. When an allergic reaction occurs, conventional treatment is usually used.

Other adverse events: The following adverse events are observed in patients treated with TNKase in clinical trials. These reactions are often sequelae of the underlying disease, and the effect of TNKase on their incidence is unclear. These adverse events include cardiogenic shock, arrhythmias, atrioventricular block, pulmonary edema, heart failure, cardiac arrest, recurrent myocardial ischemia, recurrent myocardial infarction, cardiac rupture, tamponade, pericarditis, pericardial effusion, mitral insufficiency, thrombosis, embolism, and electromechanical separation of the heart. These events can be life-threatening and may result in death. Nausea and/or vomiting, low blood pressure and fever have also been reported.

9.6 Management and follow-up of adverse events

All adverse events, including laboratory abnormalities, must be followed up to normalization or remission, or return to baseline, or when follow-up is determined by the investigator to be unnecessary, or subjects are lost to follow-up, so as to ensure the safety of the subjects.

If a subject develops any serious adverse event during the clinical trial, the investigator shall immediately take appropriate treatment for the subject, regardless of whether the event is related to the clinical trial or the study drugs. The investigator should immediately make a comprehensive evaluation of the incident and complete the serious adverse event report form and report it to the relevant authorities. Severe adverse events are followed up to normalization or remission, or return to baseline, or when follow-up is determined by the investigator to be unnecessary or subjects are lost to follow-up. A summary and/or follow-up serious adverse event report form is required.

9.7 Emergency response plan for adverse events

1) Bleeding event management plan

In order to prevent the occurrence of bleeding events, the inclusion and exclusion criteria of this study protocol should be strictly followed to exclude the subjects with

potential high blood risk. During thrombolytic therapy, close attention should be paid to the changes of symptoms and signs of subjects so as to detect bleeding events in time. The treatment of bleeding events should follow the clinical routine of the hospital and be based on the clinical judgment of doctors. The following measures can be referred to:

1. If the patient has neurological symptoms and/or abnormal signs within 24 hours after the start of treatment, intracranial hemorrhage should be suspected, and the thrombolytic, antiplatelet and anticoagulant drugs being used should be discontinued, and imaging examination should be performed immediately to confirm the site of bleeding;

2. If intracranial hemorrhage or other clinically visible bleeding (such as hematemesis, hematochezia, hemoptysis, etc.) is confirmed, the following measures are recommended:

(1) Completely discontinue thrombolytic, antiplatelet and anticoagulant drugs;

(2) Invite relevant professional physicians for consultation to jointly determine the symptomatic treatment plan;

(3) Closely monitor the vital signs and timely treat complications;

(4) Fresh frozen plasma infusion;

(5) Use antifibrinolytic drugs such as tranexamic acid within 24 hours after thrombolytic therapy.

(6) If normal heparin therapy is used at the same time, protamine may be used within 4 hours to exert an antagonistic effect of heparin (1mg of protamine to antagonize 100U of normal heparin);

(7) Blood transfusion: Blood transfusion may be required to correct hemodynamic abnormalities (hypotension caused by bleeding) or anemia. The indications for blood transfusion should be mastered according to the clinical judgment of the doctor. While strictly grasping the indications for blood transfusion, attention should be paid to the adverse effects of severe anemia on acute myocardial infarction.

(8) Evaluate the feasibility of surgical intervention when necessary;

3. Minor bleeding, such as skin and mucosal bleeding, nosebleed, gingival bleeding, microscopic hematuria, positive stool occult blood, etc., can be symptomatic in advance,

and thrombolysis, antiplatelet and anticoagulant therapy should be continued according to the bleeding degree.

In particular, symptomatic intracranial hemorrhage (2%-7%).The vast majority of symptomatic intracranial hemorrhage occur within 24 hours of thrombolytic therapy. The symptoms include sudden changes in the state of consciousness, single or multiple location signs of the nervous system, coma, headache, nausea, vomiting and seizures, hypertensive emergency, and rapid death in some cases. When this happens, active measures should be taken:

(1) Stop thrombolysis, antiplatelet and anticoagulant therapy immediately.

(2) Perform imaging examination (emergency CT or MRI) to determine the location, amount and classification of intracranial bleeding.

(3) Measure erythrocyte volume, hemoglobin, prothrombin, activated partial thromboplastin time, platelet count, fibrinogen, D-dimer, blood group and perform a cross-match test.

(4) Reduce intracranial pressure, including proper control of blood pressure, raising the head of the bed by 30 degrees, intravenous mannitol, endotracheal intubation and auxiliary ventilation, surgical hematoma removal, decompression of bone flap and ventricle puncture and drainage when necessary.

(5) If necessary, use reverse thrombolysis, antiplatelet and anticoagulant drugs: 2U of fresh frozen plasma should be given every 6 hours within 24h; it is recommended to neutralize with protamine (1mg protamine to neutralize 100U of normal heparin) for patients who have used normal heparin therapy within 4h. If the bleeding time is abnormal, 6-8U of platelets can be transfused.

(6) Properly control the blood pressure.

2) Allergic reactions

In the clinical trials of this product, no patients have been found to have allergic reaction after using this product. In the event of anaphylaxis, antiallergic treatment is required.

3) Other expected adverse events

The following adverse events have been reported in patients who received this product in clinical trials. The effect of this product on the incidence of these adverse events is unclear. These adverse events include cardiogenic shock, heart failure, cardiac rupture and electromechanical separation, ventricular fibrillation, and cardiac rupture (3.23%); increase in aminotransferase, myocardial enzyme, blood lipid and blood glucose, as well as nausea, vomiting, fever and cough, all mild. The majority of these adverse events are determined not to be related to the test drug, but to underlying and/or comorbidities, as well as concomitant drugs.

The relationship between the above events and TNK-TPA is not clear, and most of them are potential combined diseases. If the above phenomena occur, appropriate treatment should be taken according to the situation.

4) Other adverse reactions

Other adverse reactions should be analyzed by the investigator according to the specific situation, and given symptomatic treatment according to the corresponding treatment guidelines or clinical routine procedures.

10. Data management

10.1 Source data/source files

Source data refers to all information recorded in the original records or copies (certified copies) of the clinical trial, including clinical findings, observations, and other relevant activity records necessary for reconstruction and evaluation of the clinical trial.

Source documents refer to the original medical records, medical documents and data generated during the clinical trial. Source file contains the source data, such as the hospital medical records, medical images, laboratory records, clinical trial related memorandum, clinical trial subjects the diary or assessment form, hair medicine, instruments and automatic recording of data and save the prescription pharmacy, laboratory and the department of medical and related documents and records, including duplicated or copied certified copy thereof. Source files can be paper and/or electronic.

The investigator and the medical institution to which he/she works permit the Sponsor to supervise or audit the clinical trial; The health administration and drug

supervisory and regulatory authorities have the right to inspect clinical trials and have direct access to source data/source documents.

10.2 Database establishment and data entry

(1) Selection of EDC system: In this clinical study, EDC system is used to collect eCRF data.

(2) Database establishment: build eCRF interface according to protocol and CRF table, and set logical review limit conditions; Input the test data to carry on the trial operation of the database, and then establish the special database system for the trial.

(3) Data entry: each user will get an independent user name and password. The designated personnel of the research center will input the original data into the eCRF through the secure network. The system will automatically check the data deviation in the eCRF, generate the corresponding prompt message, and allow the personnel of the research center to modify the input data.

The clinical research associate should confirm that all data in the eCRF is consistent with the original record. Any errors or omissions found will be notified to the staff of the Research Centre in the form of a query sheet requesting modifications to the relevant data in the eCRF. Before the database is locked, the investigators will confirm and electronically sign off to ensure the accuracy of the data recorded.

10.3 Data monitoring

Monitoring of eCRF data and process files:

(1) During the study, the clinical research associate shall come to the research center to check the informed consent and screening and enrollment of the subjects.

(2) Confirm that all eCRFs are filled correctly and consistent with the original data.

(3) Adverse events of each subject shall be confirmed and recorded.

(4) The withdrawal of the subject shall be explained in the eCRF.

(5) Confirm that all AE's have been documented and that SAE has made a report for the record.

(6) Verify whether the study drugs are supplied, stored, distributed and recovered in accordance with relevant regulations, and make corresponding records of the storage conditions, time and records of the remaining study drugs in accordance with relevant requirements.

ECRF data, analytical data and process documents will be stored on paper or electronically. After the database is locked, the locked data will be transferred to the Sponsor.

10.4 Quality assurance of research data

To ensure the accuracy, integrity and reliability of the data, the investigator needs to record all trial data and procedures, including informed consent signing, screening, medication and blood sample collection, and laboratory analysis data. If necessary, the investigator should provide the original data to the Sponsor, the relevant regulatory authorities, and the Ethics Committee.

The Sponsor will designate relevant personnel to complete the following work:

(1) Provide appropriate instructional research documents and materials to the research center.

(2) The Sponsor will organize a kick-off meeting to train the investigators and study coordinators before the study begins. This meeting will explain the study protocol, eCRF filling and study procedure.

(3) Regular follow-up research center for related quality control.

(4) Keep in touch with the Research Centre at all times by telephone, mail and/or fax.

(5) Check and evaluate eCRF data in a timely manner, analyze quality, or use standardized computer editing to detect errors in data collection.

In addition, the Sponsor or designated representative will visit the study center periodically to check that the subject data is consistent with the original records and may audit the study at any time.

10.5 Data review and locking

The data management facility reviews the data recorded in the eCRF in accordance with the standard operating procedures. For any questions in the data, the data management personnel will question the research center personnel in the form of an electronic query sheet, requesting the personnel designated by the research center to answer the questions and make some necessary modifications to the data. All data changes and related operations need to be documented. The data administrator shall write the data audit report according to the final version study protocol, data audit standard and database. The project manager will organize the Sponsor, major

investigators, statisticians and data administrators to hold a data review meeting to review the data, and the representatives of all parties will sign the review resolution. After the parties approve the database locking, the data administrator organizes the database locking work and submits the locked database to the statistician for statistical analysis.

11. Sample size

In this study, rt-PA active control will be used based on a statistical hypothesis test of non-inferiority, and the proportion of subjects with 90-day MRS score of 0-1 will be used as the primary efficacy endpoint.

According to a paper published in the Lancet[7] in 2014 about meta-analysis of the incidence of 90-day MRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the Guidelines for the Design of Non-inferiority Drug Clinical Trials [32], the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high priority index. Therefore, based on the lower CI limit of 1.14, together with an efficacy of 50% of the active control, the non-inferior threshold was determined as 0.937. Then assuming α=0.025 using one-sided test, power 1-β=0.85, treatment group (TNK) incidence of 63.64%, control group (rt-PA) incidence of 59.32%, RR= 1.07, the sample size was calculated to be 642 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each group and the total sample size is estimated to be 1430 cases.

12. Statistical plan

12.1 Statistical analysis

Full analysis set (FAS), per protocol set (PPS) and safety analysis set (SAS) will be established according to the NMPA regulations for data statistical analysis. Strict quality control will be carried out in the whole process of data collection to ensure the authenticity of the evaluation data.

Full analysis set (FAS): a data set consisting of all subjects randomized to receive at least one study drug according to the basic principle of Intention To Treat.

Per protocol set (PPS): PPS includes all subjects who complete the stipulated treatment without missing primary efficacy endpoints or serious violations of the protocol. Serious protocol violation will be finally defined during data review and

may generally include (but not limited to) the following situations: subjects violating inclusion criteria or exclusion criteria, poor compliance in stipulated medication, concomitant medication that affects outcome indicators, and out of visit window.

Safety analysis set (SS): a set of all subjects participating in the trial, receiving at least one study drug, and having at least one safety evaluation.

FAS and PPS are established as sensitive analysis groups; Efficacy analysis is performed on the basis of FAS and PPS. Safety endpoints will be performed on the safety set and safety missing data will not be filled. All baseline demographic data analyses will be performed on the basis of the FAS; illogic data needs to be investigated by the data management facility, investigators or Sponsor together to identify possible reasons, and restrained by the parties in a data review meeting after full discussion: illogic data, if sufficient reasons have been found to indicate its incorrectness, should be removed, or otherwise, should be remained and subjected to analysis and the results obtained without such removed data should be treated as sensitivity analysis; If there is data collected in the study that is not in accordance with the program requirements such as planned external visits, it will be presented in a list, and descriptive or comparative analysis will be decided at the data review meeting depending on the specific situation of the data.

12.2 General principles

All statistical analysis will be conducted by SAS9.4 or higher version statistical software. The content of statistical analysis in the protocol is the general principle of statistical analysis method, and the specific statistical analysis process will be carried out according to the pre-formulated statistical analysis protocol.

Statistical description: quantitative indicators describe mean, standard deviation, minimum, maximum, median, quartile, etc. Qualitative indicators describe the number and percentage of cases in each category or grade.

Statistical inference: the two-sample t-test or non-parametric test is used for the comparison of quantitative indicators between groups depending on the data situation, the non-parametric test is used for the comparison of grade indicators, and the Chi-square or Fisher exact probability method is used for qualitative indicators. Evaluation of efficacy endpoints will be considered for inclusion in baseline or center effect analyses depending on the specific situation.

Statistical hypothesis: Comparison of the primary efficacy endpoint between groups is conducted by a one-sided test, with a significance level of one-sided α=0.025; null hypothesis H0: RR ≤ Δ and alternative hypothesis H1: RR > Δ; RR is the ratio of incidence of the treatment group and the control group; Δ is 0.937 as the non-inferior threshold; when P < 0.025 or lower limit of 95% CI of RR > 0.937 in non-inferiority tests, it indicates that the test drug is not inferior to control drug; if the primary efficacy endpoint meet non-inferiority criteria, the superiority test will be conducted and the test threshold Δ is set to 1; when P<0.025 or lower limit of 95% CI of RR > 1 in superiority tests, it indicates that the test drug is superior to control drug. All other statistical analyses are conducted by two-sided tests, with a significance level of two-sided α=0.05. When P≤0.05, it indicates a statistically significant difference. The confidence interval is 95% on both sides. Primary-efficacy-endpoint hypothesis tests are as follows:

$$H_0: RR_{试验组/对照组} \le \Delta \quad H_0: RR_{试验组/对照组} \le \Delta$$
$$H_1: RR_{试验组/对照组} > \Delta \quad H_1: RR_{试验组/对照组} > \Delta$$

12.3 Analysis content

The actual number of subjects enrolled in each group, completed and dropped out from the study, removed, deviated from or violated the protocol, demographic and other baseline characteristics, compliance, efficacy analysis, and safety analysis. Statistical analysis includes (1) the case number of completed and that of dropped out in each research center, etc.; (2) The demographic and baseline characteristics of each group at the time of enrollment, necessary for comparability analysis between the treatment group and the control group; 3) Efficacy evaluation and safety evaluation, as described below.

12.4 Efficacy evaluation

Primary efficacy endpoints

Description method: Patients with 90-day MRS score of 0-1 among all enrolled patients in the corresponding data set is statistically described in the form of number and percentage respectively.

Analysis methods: in this protocol, the primary endpoint is the proportion of subjects with 90-day mRS score of 0-1; for such endpoint, CMH test with controlled

center effect will be applied to statistically compare the treatment group and control group, and the 95% confidence interval of efficacy, and corresponding RR or RD and corresponding 95% confidence interval will be calculated; Logistic regression model analysis will also be considered to report the 95% confidence intervals of OR for TNK-TPA versus rt-PA. Adjustment for covariates with inter-group differences or center-group interactions will be made as appropriate.

Analysis of secondary efficacy endpoints

Include:

1) The proportion of subjects with an mRS score of 0-2 at 90±7 days
2) MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution)
3) The proportion of subjects whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first)
4) Health-related quality of life (EQ-5d) at 90±7 day
5) The proportion of subjects with a Bathel score ≥ 95 points at 90±7 days

The analysis of secondary endpoints refers to the general principles of statistical analysis. For secondary efficacy outcomes, common odds ratio with its 95% confidence interval was calculated using ordinal logistic regression for the outcome of ordinal 90-day mRS score, and odds ratios with their 95% confidence intervals were calculated using binary logistic regression for other secondary efficacy outcomes.

Subgroup analysis: In this study, bridging therapy is performed within 24h of thrombolysis. Because bridging therapy may be a potential outcome influencing factor, bridging therapy is predesigned as a covariate in the subgroup analysis, and the RR values and 95% confidence intervals of the incidence of the trial and control groups in each subgroup are calculated respectively.

12.5 Safety evaluation

The number and incidence of safety endpoints in each group are statistically described. The number of cases and events, and incidence of adverse events and adverse reactions in each group are statistically described, and the specific list of adverse events is described. Chi-square or Fisher tests are used to compare whether

there are differences between groups in safety endpoints. Laboratory data and other safety data will be descriptive statistics, and the data before and after treatment will be compared by paired t test, mainly to analyze and list the specific situation of normal cases before treatment but abnormal cases after treatment or abnormal cases before treatment but aggravated cases after treatment.

Primary safety evaluation endpoints:

Include:

1) The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASSIII)
2) The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASSIII)
3) The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS standards)
4) The incidence of any intracranial hemorrhage within 90 days
5) The incidence of significant hemorrhage events at other sites within 90 days (as defined by GUSTO)
6) 90-day overall mortality
7) Incidence rates of adverse events/serious adverse events within 90 days;

The analysis of the primary safety endpoints refers to the general statistical analysis principles. The number and incidence of various bleeding events and adverse events in the primary safety endpoints are described. The incidence of events between the two groups is compared using the chi-square test or Fisher's exact probability method, and a 95% confidence interval for the difference in incidence between the groups is calculated.

Other safety endpoints:

① Adverse events

The number of cases and events of AE, adverse reactions, AE causing drop-out and SAE are calculated respectively, and a detailed list is provided. The incidence is calculated using the number of people in each safety dataset as the denominator, and the Chi-square test or Fisher's exact probability method is used for inter-group comparison when necessary.

The number of cases and events of the events mentioned in the above endpoints are counted according to the systematic organ classification and preferred terms encoded by MEDDRA, as detailed in the Statistical Analysis protocol (SAP).

② Evaluation of laboratory test indicators

Include routine blood test, routine urinalysis, blood biochemistry and lipid indexes.

The number of cases is described for each kind of clinical judgment result (normal, abnormal without clinical significance, abnormal with clinical significance and not tested) of laboratory examination results before and after treatment in the form of a cross table.

The patients who are judged to be abnormal after the treatment are described in detail by nature (with clinical significance, without clinical significance, or not tested) in the form of lists.

③ Evaluation of vital signs

The mean, standard deviation, median, minimum and maximum are used to describe the changes of vital signs from each visit to the baseline.

④ Evaluation of physical examination

The physical examination results of each visit are described and the number and percentage of normal and abnormal cases are calculated.

⑤ ECG evaluation

Refer to the evaluation section of laboratory examination indicators.

13. Ethics and regulatory procedures

13.1 Ethical principles

This study protocol has been rigorously reviewed. It contains information that is consistent with current knowledge of the risks and benefits of test drugs and is consistent with the ethical, ethical and scientific principles of clinical trials set out in the *Declaration of Helsinki* 2013, as well as the guidelines of Chinese *Good Clinical Practice*.

13.2 Laws and regulations

The management of this clinical trial will be in accordance with international laws and regulations, and the laws and regulations of China where the trial is conducted, as well as any guidelines on its application.

13.3 Informed consent

Informed consent obtaining is the process by which subjects confirm their consent to voluntarily participate in the clinical trial after being informed of all aspects that may influence their decision to participate in the trial. This process shall be documented by a written, signed and dated informed consent.

To implement informed consent, investigators should abide by the ethical principles of the *Declaration of Helsinki* and meet the following requirements:

(1) The investigator shall use the latest version of the informed consent form approved by the IRB and other information provided to the subject. If necessary, subjects during the clinical trial should re-sign the informed consent.

(2) When the investigator obtains new information that may affect the continued participation of subjects in the study, he/she shall timely inform the subjects or their guardians and make corresponding records.

(3) The investigator shall not influence the subjects to participate in or continue the clinical trial by coercion, inducement for profit or other improper means.

(4) The Investigator or the designated study-related personnel shall fully inform subjects of all matters related to the clinical trial, including written information and the consent of the IRB.

(5) Informed consent form and other oral and written information provided to the subjects shall be easy to understand, so that the subjects or their guardians and witnesses can easily understand.

(6) Before signing the informed consent, the investigator or the designated study-related personnel shall give sufficient time and opportunity for subjects or their guardians to understand the detailed information of the clinical trial and answer questions raised by subjects or their guardians in detail related to the clinical trial.

(7) The subject or his/her guardian, as well as the investigator performing the informed consent shall sign and indicate the date on the informed consent. If not signed by the subject, the relationship shall be indicated.

(8) If the subject or his/her guardian lacks reading ability, an impartial witness should be present to witness the whole informed consent process. The investigator

shall explain in detail the contents of the informed consent and other written materials to the subjects or their guardians and witnesses. Such as participants or their guardians oral agreed to participate in the trial, in the case of ability should sign the informed consent, as witnesses shall also sign the informed consent form and indicate the date, to prove that the participants or their guardians of informed consent and other written materials investigators accurately explain, and understand the relevant content, agreed to participate in clinical trials.

(9) The subject or his/her guardian shall receive the signed and dated original or copy of the informed consent and other written information provided to the subject, including the original or copy of the updated informed consent and the revised version of other written information provided to the subject.

(10) If the subject has no capacity for civil conduct, it shall obtain the written informed consent of its guardian; If the subject is a person with limited capacity for civil conduct, the subject shall obtain the written informed consent of the subject and his/her guardian. When the guardian gives informed consent on behalf of the subjects, the guardian shall inform the subjects of the relevant information of the clinical trial to the extent understandable to the subjects, and try to have the subjects personally sign the informed consent and indicate the date.

(11) Under emergency circumstances, if informed consent of subjects cannot be obtained before participating in the clinical trial, their guardians can give informed consent on behalf of the subjects. If their guardians are not present, the method of enrollment of subjects shall be clearly stated in the trial protocol and other documents, and written consent of the ethics committee shall be obtained; At the same time, informed consent should be obtained as soon as possible for subjects or their guardians to continue to participate in the clinical trial.

(12) The specific time and personnel of informed consent of the subject shall be recorded in the medical history.

(13) Informed consent should be given in the research center to avoid infringement/disclosure of the subject's privacy.

13.4 Review board/independent ethics committee (IRB/IEC)

Before the trial begins, the investigator or Sponsor must submit the clinical trial protocol to the IRB, and the investigator must submit a copy of the IRB's signed written consent to the protocol to the Sponsor.

Clinical trials (study number, study protocol name, version number), reviewed documents (including clinical study protocol, informed consent, investigator operation manual, investigator resume, etc.), voting member qualification and review date should be clearly recorded in the IRB consent form.

The study drugs shall not be released to the study center before the trial has begun, and the clinical study shall not begin until a copy of the written consent received and dated by the Sponsor is obtained.

During the course of a clinical trial, any modification of all protocol should be reported to the IRB and approved before implementation. Any event occurring during the study that may affect the safety of subjects or the continuation of the clinical trial, in particular changes in safety, shall be reported to the ERB. Updates to the Investigator Practice Manual should be submitted to the IRB.

A progress report of the clinical trial and a summary of the clinical results at the end of the clinical trial should be submitted to the IRB annually, if required.

14. Quality control and assurance

The investigator is committed to following the clinical trial protocol and the ICH guidelines for the management of clinical trials of medicinal products, as well as the applicable laws and regulations, when conducting clinical trials.

The investigator must properly handle all data obtained during the course of the clinical study to ensure the rights and privacy of participants in the clinical study. The investigator must consent to access and review of the clinical study data by the clinical research associate/auditor/inspector in order to verify the accuracy of the source data and to understand the progress of the study. If it is not possible to verify the original records, the Investigator agrees to assist the Inspector/Inspector/Inspector in further verifying the quality of the data.

The study hospital site visits will be conducted regularly by the clinical research associate during the clinical trial to ensure that all aspects of the study protocol are strictly followed, and the source data will be checked to ensure that they are consistent with the eCRF. Specific quality control measures shall be included in the design protocol.

Data recorded directly on the EDC (i.e., data without prior written or electronic records) and the identification of data considered as raw data will be specified and specified in the monitoring protocol in advance according to the protocol. Otherwise,

it is deemed to lack raw data.

15. Protocol deviation and violation

All requirements specified in the study protocol must be strictly implemented. Any intentional or unintentional deviation from or violation of the study protocol and the principles of the GCP can be classified as a deviation from or violation of the protocol. In the process of supervision, if the clinical research associate finds any deviation from the program, the investigator or the clinical research associate shall fill in the violation program record, and record in detail the time of discovery, the time and process of occurrence of the event, the reason and the corresponding treatment measures. The investigator shall sign the record and notify the ethics committee. In the data statistics and summary report, investigators analyze and report the impact of the deviation or violation of the protocol on the final data and conclusions.

An assessment should be made when a serious protocol violation occurs. If necessary, the Sponsor may terminate the study in advance.

16. Data and data preservation

The investigator should maintain all study data, including confirmation of all subjects (enabling effective verification of different records, such as study medical records and original hospital records, all original signed informed consents, detailed records of drug distribution, and electronic records of all test indicators (documents requiring traceability). Retain for at least 5 years after the test drug is approved for marketing.

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Appendix 1: Procedures for rhTNK-TPA intravenous thrombolytic therapy

(For reference only)

III. RhTNK -tPA intravenous thrombolytic therapy procedure

1. RhTNK-TPA injection: Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the subject, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds.

2. Monitoring vital signs and changes in neurological function.

- Blood pressure measurement q15min×2h, q60min×22h thereafter (or q30min×6h, q60min×16h thereafter)
- Pulse and respiratory rate are measured q1h×12h, followed by q2h×12h
- The neurological function score is Q1h ×6h and Q3h ×18h
- Neurological examination is performed daily after 24h
- Blood pressure is controlled below 185/110mmHg before thrombolysis and maintained below 185/100 mmHg for at least the first 24 h after rhTNK-tPA administration.

3. Central venipuncture and arterial puncture should be avoided in the first 24 hours after thrombolysis; Try to avoid indwelling catheters during or after thrombolysis for at least 30 minutes. The nasogastric feeding tube should be avoided in the first 24 hours. In patients with thrombolysis, two venous channels should be opened as far as possible.

4. Cranial images are reexamined 24h after rhTNK-TPA injection to guide the use of antiplatelet or anticoagulant agents.

5. Check the tongue and lips 45 minutes after medication to determine if there is vasogenic edema. If vasogenic edema is found, antihistamines and

glucocorticoids should be given.

6. Insulin can be given when blood glucose exceeds 10 mmol/L. Blood glucose monitoring should be strengthened, and hyperglycemia patients can be controlled within 7.8 - 10 mmol/L. When blood glucose is below 3.3mmol /L, 10% - 20% glucose can be given orally or by injection. The goal is to get normal blood glucose.

IV. Treatment of exacerbation after thrombolysis

If, within 24 h after thrombolysis, the symptom is aggravating, imaging shall be conducted first to determine the presence of sICH. In case the imaging shows asymptomatic or hemorrhagic infarction, no special intervention is required but continue with the antiplatelet therapy as stipulated in the *Guidelines*; in case the imaging shows sICH or parenchymal hematoma, suspend or stop the antiplatelet therapy, control the blood pressure, and remove the hematoma through surgery when necessary. For symptoms worsened by non-bleeding causes after thrombolysis, or worsened after improvement, clinical, laboratory and neuroimaging examinations should be conducted to clarify the causes as far as possible, and targeted interventions should be taken. For patients with major artery occlusion or failed intravenous thrombolysis, remedial intra arterial thrombolysis or intravascular therapy can be considered.

Xiong Y, *et al. Stroke Vasc Neurol* 2025; 10:e003048. doi: 10.1136/svn-2023-003048

Appendix 2: Clinical Event Definition

<table>
<tr><td>Symptomatic intracranial hemorrhage</td><td>ECASS-III Criterion: sICH is defined as any apparently extravascular blood in the brain or within the cranium associated with clinical deterioration, defined by an increase of 4 points or more on the NIHSS from the baseline or within 7 days; or that led to death and that was identified as the predominant cause of the neurologic deterioration.</td></tr>
<tr><td>PH2 type intracranial hemorrhage</td><td>Sits Criterion: Within 36 hours after thrombolysis, the NIHSS increases by ⩾4 from the baseline or the lowest NIHSS, and the imaging shows a hematoma occupying 30% or more of the infarcted tissue, with obvious mass effect.

Type — Description
HI-1 — Scattered small petechiae
HI-2 — Confluent petechiae
PH-1 — Hematoma within infarcted tissue, occupying < 30%, no substantive mass effect
PH-2 — Hematoma occupying 30% or more of infarcted tissue, with obvious mass effect</td></tr>
<tr><td>Any intracranial hemorrhage</td><td>All types of intracranial hemorrhage.</td></tr>
<tr><td>Bleeding in other places</td><td>GUSTO bleeding: 1. Severe or life-threatening bleeding: Intracranial bleeding or bleeding that causes substantial hemodynamic compromise requiring treatment
2. Moderate bleeding: Bleeding which needs blood transfusion
3. Minor bleeding: Other bleeding, neither requiring transfusion nor causing hemodynamic compromise</td></tr>
<tr><td>death</td><td>Vasogenic and non-vasogenic deaths.</td></tr>
<tr><td>New vascular events</td><td>Ischemic stroke/hemorrhagic stroke/myocardial infarction/cardio-cerebral revascularization (including: carotid endarterectomy, intracranial and extracranial artery interventional therapy, intracranial and extracranial artery bypass grafting, coronary interventional therapy or bypass grafting)</td></tr>
</table>

Annex 3: Commonly Used Scales

1. Modified Rankin Scale (MRS)

The modified Rankin Scale is used to measure the neurological recovery of patients after stroke. The bold text shows the formal definition of each level, and the italics give further guidance to reduce possible variations between different observers, but do not describe the structure of the interview. Please note that only symptoms that have occurred since stroke are considered. If the patient is able to walk without help from humans or aid tools, he or she is considered able to walk independently.

If both grades seem to be equally applicable to the patient, and further questioning is unlikely to make an absolutely correct choice, the more severe grade should be chosen.

0: No symptoms at all

Although mild symptoms may be present, the patient has no new functional limitations or symptoms since the stroke.

1: No significant disability despite symptoms; able to carry out all usual duties and activities

The patient had some of the symptoms of stroke, both physical and cognitive (e.g., affecting speech, reading, writing; Or physical movement; Or feeling; Or visual; Or swallowing; Or emotional), but may continue to engage in all pre-stroke work, social and leisure activities. The key question to distinguish between levels 1 and 2 (see below) could be, "Are there things you used to do that you couldn't do until after the stroke?". Activities that occur more than once a month are considered regular.

2: Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance

Certain activities that were previously possible after stroke (such as driving, dancing, reading, or working), patients are no longer able to do after stroke, but can

still take care of themselves daily without assistance. Patients are able to dress, walk, eat, go to the bathroom, prepare simple food, shop, and travel locally without assistance. Patients live without supervision. Imagine patients at this level being able to stay at home unattended for a week or more.

3: Moderate disability; requiring some help, but able to walk without assistance

At this grade, patients can walk independently (with walking AIDS), dress themselves, go to the bathroom, eat, etc., but more complex tasks require assistance. For example, the need for someone else to do the shopping, cooking, or cleaning, and visiting the patient more than once a week to ensure that these activities are completed. Help is needed not only to take care of the body, but also to give advice, such as: patients at this level will need supervision or encouragement to manage their finances.

4: Moderately severe disability; unable to walk and attend to bodily needs without assistance

Sufferers need other people to help with daily life, whether it's walking, dressing, going to the bathroom or eating. Patients need to be cared for at least once a day, often twice or more, or must live close to their carers. To distinguish between levels 4 and 5 (see below), consider whether the patient is routinely able to live alone at appropriate times of the day.

5. Severe disability; bedridden, incontinent, and requiring constant nursing care and attention

There is no need for trained nurses, but someone will be required to watch them several times throughout the day and night.

6. Dead

2. National Institutes of Health Stroke Scale (NIHSS)

project	Scoring criteria	score
1a. Level of consciousness (LOC): 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: (It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.) 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.	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 of a partial gaze palsy.	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	
3. Visual fields	0 = no visual loss	

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	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 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using	0 = no drift; limb holds 90 (or 45) degrees for full 10 seconds 1 = drift; limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support 2 = some effort against gravity; limb	

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.	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 9 = 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. Onlyin the case of amputation or joint fusion at the hip, the examiner should record the score as 9, 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 9 = 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 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 9, and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position.	0 = absent 1 = present in one limb 2 = present in two limbs Ataxia present: Left arm 1 = Yes 2 = No 9 = amputation or joint fusion, explain: _____ Right arm 1 = Yes 2 = No 9 = amputation or joint fusion, explain: _____ Left leg 1 = Yes 2 = No 9 = amputation or joint fusion, explain: _____ Right leg 1 = Yes 2 = No 9 = 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	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,	

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 (item 1a=3) are automatically given a 2 on this item.	and leg	
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	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; 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	

should be used only if the patient is mute and follows no one-step commands.		
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 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 9, and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.	0 = normal 1 = mild-to-moderate dysarthria; patient slurs at least some words and, at worst, can be understood with some difficulty 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. 9 = 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 inattentionor 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	
Total		

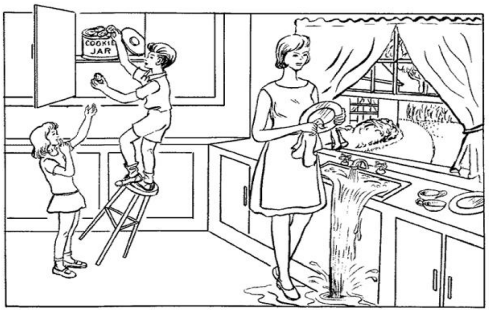
Note:

Grade patients in strict accordance with the above table and record the score. No modification is allowed. The score shall reflect the actual situation of patients, not what the doctor thinks the patient should be. Check patients in the shortest time and record the results. Do not train the patient (e.g., repeatedly asking the patient to perform a certain effort) except give some necessary instructions.

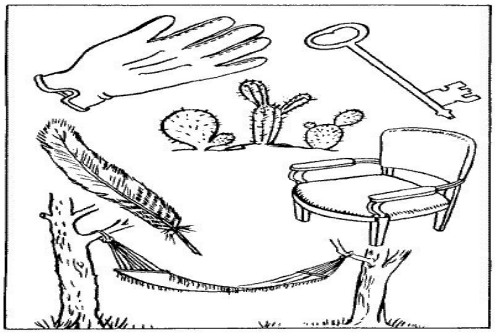
If some scale items are not used, a detailed explanation should be made in the form.

Attached: Pictures for the test of Best language (item 9) and Dysarthria (item 10)

To describe what's happening in Figure 1



To name the items in Figure 2



To read the following sentences in Figure 3

Please read the following sentences:

知道(Zhi Dao; You know how)

下楼梯(Xia Lou Ti; Down the stairs)

回家做饭(Hui Jia Zuo Fan; go home to cook dinner)

在学校复习(Zai Xiao Fu Xi; Review at school)

发表精彩演讲(Fa Biao Jing Cai Yan Jiang; Give a great speech)

To read the following words in Figure 4

Please read out the following words: 妈妈(MAMA) 大地(Da Di; The Earth) 飞机飞机(Fei Ji Fei Ji; The aircraft) 丝绸(Si Chou; silk) 按时开工(An Shi Kai Gong; Start on time) 吃葡萄不吐葡萄皮(Chi Pu Tao Bu Tu Pu Tao Pi; Eat grapes without spitting grape skin)

Basic principles and precautions of NIHSS scoring

Basic principles:

Record the first response of the patient, even though the later ones are better;

Record only what the patient has done, not what you think he can do;

Record while checking, avoid inducing patients as far as possible;

For the untestable items, please record the score as "9", and the computer statistics will automatically process it as a default value;

Principle of "sameness": The same evaluation standard should be applied for multiple follow-up visits for the same patients.

How to score for patients in comma at NIHSS scoring

When the score on item 1a is less than 3, the patient shall go through all the items of NIHSS one by one.

A 3 is scored on item 1a only if the patient makes no movement (other than reflexive posturing) in response to such noxious stimulation as sternum rub and orbital pressure.

If the score = 3 on item 1a, then the score on other items shall be as follows:

On item 1b: 2

On item 1c: 2

On item 2: 1, if the patient can overcome the reflex of the head and eyes; otherwise, 2.

On item 3: depending on the results with visual threat

On item 4: 3

On items 5 and 6: 4 for each

On item 7: Ataxia is scored only if present. The score shall be 0 if the patient fails to finish the finger-nose-finger and heel-shin tests.

On item 8: 2

On item 9: 3

On item 10: 2

On item 11: 2 since a patient in coma has no cognitive competence.

How to calculate the total score of NIHSS?

In calculating the total score, the following items should not be included in the total score:

The score on items 5 and 6 when it is 9 in case of amputation or joint fusion

The score on item 7 in presence of ataxia when "Left arm; 1 = Yes, 2 = No; 9 = amputation or joint fusion, explain: _____" (not required at the time of registration).

3.Barthel Index (BI)

Explanation	project	Scoring criteria	score
Occasional: once a week	Bowels	0 = incontinent 5 = occasional accident 10 = continent	
Occasional: < once per day. A 10 can be scored for a catheterized patient if he/she is continent with the catheter	Bladder	0 = incontinent 5 = occasional accident 10 = continent	
A 5 can be scored even the implements are provided by the carer, for example, prepasted toothburush, water, etc.	Groomi ng	0 = needs to help with personal care 5 = independent face/hair/teeth/shaving	
Toilet use means the patient can walk to a toilet and go back all by himself/herself. A 5 is scored when the patient still need some help	Toilet use	0 = dependent 5 = needs some help, but can do something alone 10 = independent	
Feeding relates to anormal diet (not just soft diet), though the food can be cooked or served by others. A 5 is scored when the patient feed by himself while the dishes and rice are taken by others.	Feeding	0 = unable 5 = needs help taking dishes and rice 10 = independent	
Transfer means transfer from bed to chair and back. A 0 is scored when the patient can sit up only with physical support of two people; a 5 is scored when the patient can stand up with support of one strong/trained people or two untrained people.	Transfer s	0 = unable, no sitting balance 5 = major help (two people, physical), can sit 10 = minor help (one people, verbal or physical) 15 = independent	

Mobility means the (on level surfaces on level surfaces indoor. Aid tools are allowed. If a wheelchair is used, the patients shall be able to corner and go through doors all by himself/herself. A 10 is scored when the patient can walk with the help of the untrained carer or supervisor	Mobility	0 = immobile 5 = independent in wheelchair 10 = walks with help of one person (verbal or physical) 15 = independent (but may use any aid; for example, stick)	
Dressing relates to all kinds of clothes. A 5 is scored when the patients can put on the clothes but needs help with the buttons or zips.	Dressing	0 = dependent 5 = needs help but can do about half unaided 10 = independent (including buttons, zips, laces, etc.)	
A 10 is scored when the patient is able to go upstairs with aid tools	Stairs	0 = unable 5 = needs help (verbal, physical, carrying aid) 10 = independent	
A 5 is scored when the patients can enter and exit the bathroom and scrub his/her body all alone, when the patient can take showers all alone without assistance or supervision	Bathing	0 = dependent 5 = independent (or in shower)	

8. EQ - 5D-3L scale

Under each heading, please tick the ONE box that best describes your health TODAY.

Mobility

☐ I have no problems in walking about

☐ I have some problems in walking about

☐ I'm confined to bed

Self-care

☐ I have no problems with self-care

☐ I have some problems washing or dressing myself

☐ I am unable to wash or dress myself

Usual Activities (e.g., work, study, housework, family or leisure activities)

☐ I have no problems with performing my usual activities

☐ I have some problems with performing my usual activities

☐ I am unable to perform my usual activities

Pain/discomfort

☐ I have no pain or discomfort

☐ I have moderate pain or discomfort

☐ I have extreme pain or discomfort

Anxiety/Depression

☐ I am not anxious or depressed

☐ I am moderately anxious or depressed

☐ I am extremely anxious or depressed

We would like to know how good or bad your health is TODAY. This scale is numbered from 0 to 100. 100 means the best health you can imagine. 0 means the worst health you can imagine. Please mark an X on the scale to indicate how your health is TODAY. Now, write the number you marked on the scale in the box below

The best health you can imagine

The worst health you can imagine

Your health TODAY = ☐

9. sICH definition

ECASS-III Criterion: sICH is defined as any apparently extravascular blood in the brain or within the cranium associated with clinical deterioration, defined by an increase of 4 points or more on the NIHSS from the baseline or within 7 days; or that led to death and that was identified as the predominant cause of the neurologic deterioration.

10. Definition of PH2 intracranial hemorrhage

Sits Criterion: Within 36 hours after thrombolysis, the NIHSS increases by ≥4 from the baseline or the lowest NIHSS, and the imaging shows a hematoma occupying 30% or more of the infarcted tissue, with obvious mass effect.

Type	Description
HI-1	Scattered small petechiae
HI-2	Confluent petechiae
PH-1	Hematoma within infarcted tissue, occupying < 30%, no substantive mass effect
PH-2	Hematoma occupying 30% or more of infarcted tissue, with obvious mass effect

11. Gusto bleeding definition

① Severe or life-threatening bleeding: Intracranial bleeding or bleeding that causes substantial hemodynamic compromise requiring treatment

②Moderate bleeding: Bleeding which needs blood transfusion

③ Minor bleeding: Other bleeding, neither requiring transfusion nor causing hemodynamic compromise

Xiong Y, *et al. Stroke Vasc Neurol* 2025; 10:e003048. doi: 10.1136/svn-2023-003048

Summary of changes to the Protocol of Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II (TRACE II)—A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active-controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours

Project title：A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active-controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours			
Sponsor: Guangzhou Recomgen Biotech Co., Ltd.			
Project Leader: Yongjun Wang			
First revision			
Prior version number / Release date: V1.0/2020.10.13			
Revised version number / Release date: V2.0/2020.12.31			
Prior position	Prior content	Revised content	Notes

<table>
<tr><td>Page header, summary</td><td>rhTNK-tPA phase 3 trial protocol
Version number: V1.0
Release date: Oct. 13th, 2020</td><td>TRACEII trial protocol
Version number: V2.0
Release date: Dec. 31th, 2020</td><td>Revised version, date updated.</td></tr>
<tr><td>Cover</td><td>/</td><td>Addition:
Project abbreviation: TRACE II</td><td>Add project abbreviation to facilitate internal management.</td></tr>
<tr><td>Protocol Revision History</td><td><table><tr><td>Protocol version number</td><td>Release date</td></tr><tr><td>V1.0</td><td>2020.10.13</td></tr></table></td><td><table><tr><td>Protocol version number</td><td>Release date</td></tr><tr><td>V1.0</td><td>2020.10.13</td></tr><tr><td>V2.0</td><td>2020.12.31</td></tr></table></td><td>Add revision records.</td></tr>
<tr><td>Protocol summary</td><td>/</td><td>Addition:
Project abbreviation: TRACEII</td><td>Add project abbreviation to facilitate internal management.</td></tr>
<tr><td>Exclusion</td><td>/</td><td>Addition:</td><td>(1) Exclude</td></tr>
</table>

criteria		(1) Patients scheduled to receive endovascular therapy; (2) NIHSS score 1A > 2.	this group of subjects because endovascular therapy affects efficacy evaluation of the trial; (2) Exclude this group of subjects because they may not be able to complete NIHSS score.

<table>
<tr><td rowspan="4">Research Flow Chart</td><td>Measures</td><td>Screening/Baseline</td><td>Treatment period</td><td colspan="4">Follow-up period</td><td rowspan="4"></td><td>Measures</td><td>Screening/Baseline</td><td>Treatment period</td><td colspan="4">Follow-up period</td><td rowspan="4"></td><td rowspan="4">Delete BI scale and EQ-5D scale on day 7±1 or before discharge because the living conditions of patients cannot be truly reflected during hospitalization.</td></tr>
<tr><td></td><td>-4.5h (Before thrombolysis)</td><td>0h (Thrombolytic Therapy)</td><td>24h±2h</td><td>24h-36h</td><td>day 7±1 or before discharge (whichever occurs first)</td><td>day 90±7 or early end of visit</td><td></td><td>-4.5h (Before thrombolysis)</td><td>0h (Thrombolytic Therapy)</td><td>24h±2h</td><td>24h-36h</td><td>day 7±1 or before discharge (whichever occurs first)</td><td>day 90±7 or early end of visit</td></tr>
<tr><td>BI scale</td><td></td><td></td><td></td><td></td><td>X</td><td>X</td><td>BI scale</td><td></td><td></td><td></td><td></td><td></td><td>X</td></tr>
<tr><td>EQ-5D scale</td><td></td><td></td><td></td><td></td><td>X</td><td>X</td><td>EQ-5D scale</td><td></td><td></td><td></td><td></td><td></td><td>X</td></tr>
</table>

1.4 Experimental design	/	The target population of this study is patients with critical hyperacute ischemic stroke (within 4.5 h of symptom onset), for whom considering the benefit of patients, intravenous thrombolytic therapy should be given as soon as possible after the onset, in order to achieve vascular recanalization or reperfusion and obtain a better prognosis. Due to the different administration methods of test drug and active control drug, and the inconvenience of the double-blind double-dummy design which may delay treatment and is not conducive to the benefit of subjects. Therefore, this study adopts the form of open-label trial. To minimize bias, all endpoints will be evaluated in a blind manner by a qualified study physician.	Add reasons for open-label trial and measures to control bias according to chapter 6, article 61 (3) of *Good Clinical Practice (GCP) 2020*.
7. Study process 7.4 Follow-up 7.4.3 day 7±1 or before discharge (whichever	NIHSS score, BI scale and EQ-5D scale	NIHSS score	Update the content.

occurs first)			
Protocol summary, Methods of grouping and administration	Treatment group（rhTNK-tPA 0.25mg/kg）	Treatment group (rhTNK-tPA 0.25mg/kg, 25mg at most)	Increase the upper limit with reference to trials of similar species.

5.2 Labeling of study drugs	Both the outer and inner packing box of the test drug shall be labeled as follows: **Code:** **Product name:** Recombinant Human TNK Tissue-type Plasminogen Activator for Injection **Strengths:** 1.0×10^7 IU/16mg/vial **Batch number:** **Production date:** **Expiration date:** **Storage condition:** Store below 25℃ away from light **Note:** For use in rhTNK-tPAⅢ clinical trial only. **Guangzhou Recomgen Biotech Co., Ltd.** Note: Please fill in the subject random number at "code", specify the strength (number of vials or dose of single vial) according to different packages, and complete the batch number, production date and expiration date of the test drug according to the actual situation.	The outer packing box of the test drug shall be labeled as follows: **For use in TRACE II (0-4.5H) clinical trial only.** **Please refer to the package insert for using.** **Protocol No. : MK02-2020-01** **Sponsor: Guangzhou Recomgen Biotech Co., Ltd.**	The sponsor adjusted the labeling of study drugs due to internal management.

	The packing box of the control drug should be labeled as follows: **Code:** **Product name:** Alteplase for injection **Strengths:** □20mg/vial / □50mg/vial **Batch number:** **Production date:** **Expiration date:** **Storage condition:** Store below 25℃ away from light **Note:** For use in rhTNK-tPAⅢ clinical trial only. **Guangzhou Recomgen Biotech Co., Ltd.** Note: Please fill in the subject random number at "code", select the strength according to the actual dose (20mg/vial or 50mg/vial), and complete the batch number, production date and expiration date of the control drug according to the actual situation.	The inner packing box of the test drug should be labeled as follows: **Subject random number:** **Product name:** Recombinant Human TNK Tissue-type Plasminogen Activator for Injection **Strength:** 1.0×10^7 IU/16mg/vial **Batch number:** **Production date:** **Expiration date:** **Storage condition:** store below 25℃ away from light **Note:** For use in TRACE II (0-4.5H) clinical trial only, protocol number: MK02-2020-01 **Guangzhou Recomgen Biotech Co., Ltd.** Note: Please complete the subject random number, batch number, production date and expiration date of the test drug according to the actual situation.	

		The packing box of the control drug should be labeled as follows: **Subject random number:** **Product name:** Alteplase for injection **Strengths:** **Batch number:** □20mg/vial / □50mg/vial **Production date:** **Expiration date:** **Storage condition:** store below 25℃ away from light **Note:** For use in TRACE II (0-4.5H) clinical trial only, protocol number: MK02-2020-01 **Guangzhou Recomgen Biotech Co., Ltd.** Note: Please select the strength according to the actual dose	

<table>
<tr><td></td><td></td><td>(20mg/vial or 50mg/vial), and complete the subject random number, batch number, production date and expiration date of the control drug according to the actual situation.</td><td></td></tr>
<tr><td colspan="4">Second revision</td></tr>
<tr><td colspan="4">Prior version number / Release date: V2.0/2020.12.31</td></tr>
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<tr><td>Prior position</td><td>Prior content</td><td>Revised content</td><td>Notes</td></tr>
<tr><td>Page header, summary</td><td>Version number: V2.0
Release date: Dec. 31th, 2020</td><td>Version number: V2.1
Release date: Jan. 22th, 2021</td><td>Revised version with date updated.</td></tr>
<tr><td>Signature Page of Statistical Analysis Facility</td><td>Statistician (name): Chen Yao</td><td>Statistics Director (name): Chen Yao</td><td>Revise statistician information.</td></tr>
<tr><td>Protocol Revision History</td><td><table><tr><td>Protocol version number</td><td>Release date</td></tr><tr><td>V1.0</td><td>2020.10.13</td></tr><tr><td>V2.0</td><td>2020.12.31</td></tr></table></td><td><table><tr><td>Protocol version number</td><td>Release date</td></tr><tr><td>V1.0</td><td>2020.10.13</td></tr><tr><td>V2.0</td><td>2020.12.31</td></tr></table></td><td>Add revision records.</td></tr>
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		V2.1 2021.01.22	
Protocol summary-Sample size, 11. Sample size	According to a paper published in the Lancet[7] in 2014 about meta-analysis of the incidence of 90-day MRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the data under age of 80 was collected with the incidence of rt-PA and Placebo in clinical trials initiated by European researchers, of which the meta-analysis result of RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the Guidelines for the Design of Non-inferiority Drug Clinical Trials [32], the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high priority index. Therefore, based on the lower CI limit of 1.14, together with a efficacy of 25% of the active control, the non-inferior threshold was determined as 0.906. Then assuming α=0.025 using one-sided test, power 1-β=0.8, treatment group (TNK) incidence of 64%, control group (rt-PA) incidence of 63%, real RR= 1.02, the sample	According to a paper published in the Lancet[7] in 2014 about meta-analysis of the incidence of 90-day MRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the Guidelines for the Design of Non-inferiority Drug Clinical Trials [32], the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high priority index. Therefore, based on the lower CI limit of 1.14, together with an efficacy of 25% of the active control, the non-inferior threshold was determined as 0.906. Then assuming α=0.025 using one-sided test, power 1-β=0.8, treatment group (TNK) incidence of 64%, control group (rt-PA) incidence of 63%, real RR= 1.02, the sample size was calculated by PASS software to be 643 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each group, a total of 1430 cases.	Revise sample size description.

	size was calculated by PASS software to be 643 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each group, a total of 1430 cases.		
17. References	/	Addition: [28] Chinese guidelines for the diagnosis and treatment of acute ischemic stroke 2018. Chin J Neurol, 2018, 51 (9): 666-682. Notes: Adjust the marks of references in the text accordingly.	Revise reference.
Third revision			
Prior version number / Release date: V2.1/2021.01.22			
Revised version number / Release date: V2.2/2021.04.20			
Prior position	Prior content	Revised content	Notes
Page header, summary	Version number: V2.1 Release date: Jan. 22th, 2021	Version number: V2.2 Release date: Apr. 20th, 2021	Revised version with date updated.

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<td>A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active-controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours</td>
<td>A phase 3, multicenter, prospective, centrally randomized, open label, blinded-endpoint (PROBE), active-controlled, non-inferiority trial of Recombinant Human TNK Tissue-type Plasminogen Activator for Injection (rhTNK-tPA) versus alteplase for acute ischemic stroke within 4.5 hours</td>
<td>Revise Chinese expression according to medical dictionary for regulatory activities (MedDRA) and experts' recommendation.</td>
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<tr>
<td>Protocol Revision History</td>
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<table>
<tr><th>Protocol version number</th><th>Release date</th></tr>
<tr><td>V1.0</td><td>2020.10.13</td></tr>
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<tr><td>V2.1</td><td>2021.01.22</td></tr>
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<td>Add revision records.</td>
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		V2.2	2021.04.20	
Protocol summary-Study drugs 5.1 Name of study drugs-Manufacturer of control drug	Boehringer Ingelheim Shanghai Pharmaceuticals Co. Ltd.	Boehringer Ingelheim Pharma GmbH&Co.KG		Revise the manufacturer of control drug according to the specification to correct previous clerical errors.
Text	Hyperacute ischemic stroke / acute ischemic stroke / ischemic stroke	Hyperacute ischemic stroke / acute ischemic stroke / ischemic stroke		Revise Chinese expression according to MedDRA and experts'

			recommendation.
Protocol summary-Sample size, 11. Sample size	According to a paper published in the Lancet[7] in 2014 about meta-analysis of the incidence of 90-day MRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the *Guidelines for the Design of Non-inferiority Drug Clinical Trials* [32], the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high priority index. Therefore, based on the lower CI limit of 1.14, together with an efficacy of 25% of the active control, the non-inferior threshold was determined as 0.906. Then assuming α=0.025 using one-sided test, power 1-β=0.8, treatment group (TNK) incidence of 64%, control group (rt-PA) incidence of 63%, real RR= 1.02, the sample size was calculated by PASS software to be 643 cases in each group; considering a	According to a paper published in the Lancet[7] in 2014 about meta-analysis of the incidence of 90-day MRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the Guidelines for the Design of Non-inferiority Drug Clinical Trials [32], the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high priority index. Therefore, based on the lower CI limit of 1.14, together with an efficacy of 50% of the active control, the non-inferior threshold was determined as 0.937. Then assuming α=0.025 using one-sided test, power 1-β=0.85, treatment group (TNK) incidence of 63.64%, control group (rt-PA) incidence of 59.32%, RR= 1.07, the sample size was calculated to be 642 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each	Revise sample size description.

	dropout rate of 10%, the sample size was determined as 715 cases in each group, a total of 1430 cases.	group and the total sample size is estimated to be 1430 cases. *Then assuming α=0.025 using one-sided test, power 1-β=0.85, treatment group (TNK) incidence of 63.64%, control group (rt-PA) incidence of 59.32%, real RR= 1.073, the sample size was calculated by PASS software to be 614 cases in each group; considering a dropout rate of 10%, the sample size was determined as 1364 (682 cases in each group). When the RR value was rounded to 1.07, the sample size was calculated to be 642 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each group and the total sample size is estimated to be 1430 cases.	
12.1 Statistical analysis	FAS and PPS are established as sensitive analysis groups; Efficacy analysis is performed on the basis of FAS and PPS. In efficacy evaluation of FAS, missing data of efficacy endpoints will be carried forward using the last observed data after administration (LOCF). For PPS, missing data of efficacy will not be processed. Safety endpoints will be performed on the safety set and safety missing data will not	FAS and PPS are established as sensitive analysis groups; Efficacy analysis is performed on the basis of FAS and PPS. In efficacy evaluation of FAS, missing data of primary efficacy endpoints will be carried forward using the last observed data after administration (LOCF). Safety endpoints will be performed on the safety set and safety missing data will not be filled.	Revise content to accurately describe the grouping.

	be filled.		
12.2 General principles	Statistical hypothesis: Comparison of the primary efficacy endpoint between groups is conducted by a one-sided test, with a significance level of one-sided α=0.025, null hypothesis H0: RR ≤ Δ and alternative hypothesis H1: RR > - Δ; when P < 0.025 in statistical tests, or lower limit of 95% CI of RR > - Δ, it indicates that the test drug is not inferior to control drug; RR is the ratio of incidence of the treatment group and the control group, Δ is the non-inferior threshold, 0.906 in this trial. All other statistical analyses are conducted by two-sided tests, with a significance level of two-sided α=0.05. When P≤0.05, it indicates a statistically significant difference. The confidence interval is 95% on both sides. Specific non-inferiority hypothesis tests are as follows: H_0: $RR_{试验组/对照组} \leq \Delta$ H_1: $RR_{试验组/对照组} > \Delta$	Statistical hypothesis: Comparison of the primary efficacy endpoint between groups is conducted by a one-sided test, with a significance level of one-sided α=0.025; null hypothesis H0: RR ≤ Δ and alternative hypothesis H1: RR > Δ; RR is the ratio of incidence of the treatment group and the control group; Δ is 0.937 as the non-inferior threshold; when P < 0.025 or lower limit of 95% CI of RR > 0.937 in non-inferiority tests, it indicates that the test drug is not inferior to control drug; if the primary efficacy endpoint meet non-inferiority criteria, the superiority test will be conducted and the test threshold Δ is set to 1; when P<0.025 or lower limit of 95% CI of RR > 1 in superiority tests, it indicates that the test drug is superior to control drug. All other statistical analyses are conducted by two-sided tests, with a significance level of two-sided α=0.05. When P≤0.05, it indicates a statistically significant difference. The confidence interval is 95% on both sides. Primary-efficacy-endpoint hypothesis tests are as follows:	Revise the general principles according to sample size description; Add superiority test description according to the research background and *Guidelines for the Design of*

		H_0: $RR_{试验组/对照组} \leq \Delta$ H_1: $RR_{试验组/对照组} > \Delta$	*Non-inferiority Drug Clinical Trials*.

Original statistical analysis plan

Xiong Y, *et al. Stroke Vasc Neurol* 2025; 10:e003048. doi: 10.1136/svn-2023-003048

Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II (TRACE II)

Statistical Analysis Plan

Principal Investigator

Yongjun Wang, MD

Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Prepared by

Clinical Research Institute of Peking University

Version 1.0

Oct 13, 2020

Xiong Y, *et al. Stroke Vasc Neurol* 2025; 10:e003048. doi: 10.1136/svn-2023-003048

Table of Contents

1. Introduction

This statistical analysis plan (SAP) documents the planned statistical analyses for the TRACE-2 study and is based on the protocol, together with any subsequent amendments.

This SAP is intended for the use of project team members and should be read in conjunction with the aforementioned protocol.

2. Study Objective

To evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) compared with standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset).

Primary study objectives:

To evaluate the difference in the proportion of 90-day mRS score of 0-1 between rhTNK-tPA (0.25 mg/kg) and standard rt-PA (0.9mg/kg) in the treatment of patients with hyperacute ischemic stroke (within 4.5 h of symptom onset).

Secondary study objectives:

(1) To compare the difference in efficacy between rhTNK-tPA (0.25 mg/kg) and standard rt-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset):

1) The proportion of patients with an mRS score of 0-2 at 90±7 days;

2) mRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution);

3) The proportion of patients whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first);

4) Health-related quality of life (EQ-5D) at 90±7 day;

5) The proportion of patients with a Barthel score ≥ 95 points at 90±7 days;

(2) To compare the difference in safety between rhTNK-tPA (0.25 mg/kg) and standard rt-PA (0.9mg/kg) in the treatment of patients with hyperacute ischemic stroke (within 4.5 h of symptom onset):

1) The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASS III criteria);

2) The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASS III criteria);

3) The incidence of PH2 intracranial hemorrhage within 36 days (according to SITS criteria);

4) The incidence of any intracranial hemorrhage within 90 days;

5) The incidence of other significant hemorrhage events within 90 days (as defined by GUSTO criteria);

6) 90-day overall mortality;

7) Incidence rates of adverse events/serious adverse events within 90 days.

3. Study Endpoint(s)

Primary Efficacy Endpoints:

The proportion of patients with mRS score of 0-1 at 90 days.

Calculation formula:

The proportion of patients with 90-day mRS score of 0-1 = the number of patients with 90-day mRS score of 0-1 / the number of patients enrolled in this group ×100%.

Secondary Efficacy Endpoints:

1) The proportion of patients with an mRS score of 0-2 at 90±7 days;

2) mRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution);

3) The proportion of patients whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first);

4) Health-related quality of life (EQ-5D) at 90±7 day;

5) the proportion of patients with a Barthel score ≥ 95 points at 90±7 days;

Safety Endpoint

1) The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASS III criteria).

2) The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASS III criteria);

3) The incidence of PH2 intracranial hemorrhage within 36 days (according to SITS criteria);

4) The incidence of any intracranial hemorrhage within 90 days;

5) The incidence of other significant hemorrhage events within 90 days (as defined by GUSTO criteria);

6) 90-day overall mortality;

7) Incidence rates of adverse events/serious adverse events within 90 days.

4. Statistical Hypotheses

Comparison of the primary efficacy endpoint between groups is conducted by a one-sided test, with a significance level of one-sided α=0.025. Null hypothesis H0: RR $\leqslant$ Δ and alternative hypothesis H1: RR > Δ; when P < 0.025, or lower limit of 95% CI of RR > Δ, it indicates that the test drug is not inferior to control drug; RR is the ratio of incidence of the treatment group and the control group, Δ is the non-inferior threshold, 0.937 in this trial. All other statistical analyses are conducted by two-sided tests, with a significance level of two-sided α =0.05. When P$\leqslant$0.05, it indicates a statistically significant difference. Non-inferiority hypothesis tests are as follows:

$$H_0: RR_{\text{Treatment group/control group}} \leqslant \Delta$$

$$H_1: RR_{\text{Treatment group/control group}} > \Delta$$

5. Design

This study will evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) compared with standard rt-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset). This is a multicenter, prospective, centrally randomized, open label, blinded-endpoint, parallel active controlled, non-inferior phase III clinical trial.

The drug administration method of the test drug (rhTNK-TPA) is as follows: according to the body weight of the patients, the final dose is determined at 0.25mg/kg; Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the patient, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds.

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The administration method of active control drug (rt-PA) is as follows: The dose required for medication is calculated and measured according to the actual body weight of the patient based on a standard of 0.9 mg rt-PA/kg. The 10% of the dose is injected by intravenous bolus, and the remaining 90% is injected by intravenous drip within 1h.

The target population of this study is patients with critical hyperacute ischemic stroke (within 4.5 h of symptom onset), for whom considering the benefit of patients, intravenous thrombolytic therapy should be given as soon as possible after the onset, in order to achieve vascular recanalization or reperfusion and obtain a better prognosis. Due to the different administration methods of test drug and active control drug, and the inconvenience of the double-blind double-dummy design which may delay treatment and is not conducive to the benefit of patients. Therefore, this study adopts the form of open-label trial. To minimize bias, all endpoints will be evaluated in a blind manner by a qualified study physician.

Planned Analyses

The analyses that are detailed in this SAP will be performed only when the database has been locked, all protocol violators identified, and treatment allocations have been unblinded. Membership of the Full Analysis and Per Protocol populations will be determined using the rules set out in this SAP. The treatment allocations will be unblinded and extracted from corresponding system. These will then be merged on to the study database using the treatment number (as allocated to each patient) to match patients with the correct treatment allocation. Membership of the Intention-to-Treat and Per Protocol populations will be determined using the rules set out in this SAP and will be determined prior to unblinding the treatment allocation. At a date to be agreed within the project team, a data look will be performed. This will involve production of all data displays on a subset of the data using dummy treatment codes. These are produced purely as an aide to the pre-programming of the study and no unblinding will occur.

Interim Analyses

No interim analyses are planned for this study. However, a Data and Safety Monitoring Board (DSMB) is in place to ensure the safety of patients in the study. An independent statistician will prepare unblinded summary tables of SAEs, selected demographic data and exposure data and these will be examined by the DSMB. These tables will be provided to the DSMB at regular intervals. If the tables give rise to safety concerns for any treatment, the DSMB may recommend that the trial should be modified or stopped prematurely. The Steering Committee will, in conjunction with the Sponsor, decide whether to act on this recommendation. Further discussion of these safety tabulations is provided in a specific study protocol.

6. Sample size estimates

In this study, rt-PA active control will be used based on a statistical hypothesis test of non-inferiority, and the proportion of patients with 90-day mRS score of 0-1 will be used as the primary efficacy endpoint.

According to a paper published in the Lancet in 2014 about meta-analysis of the incidence of 90-day MRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the data under age of 80 was collected with the incidence of rt-PA and Placebo in clinical trials initiated by European researchers, of which the meta-analysis result of RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the Guidelines for the Design of Non-inferiority Drug Clinical Trials, the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high priority index. Therefore, based on the lower CI limit of 1.14, together with a efficacy of 25% of the active control, the non-inferior threshold was determined as 0.906. Then assuming α=0.025 using one-sided test, power 1-β=0.8, treatment group (TNK) incidence of 64%, control group (rt-PA) incidence of 63%, real RR= 1.02, the sample size was calculated by PASS software to be 643 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each group, a total of 1430 cases.

7. Analysis populations

According to National Medical Products Administration (NMPA), statistical analysis of data was established including Full Analysis Set (FAS), Per Protocol Set (PPS) and Safety Set (SS). The entire process of statistical analysis of data will be received strict control of quality in order to ensure facticity of data.

Full Analysis Set (FAS)：

According to the basic principle of intention-to-treat (ITT), all patients who were enrolled, randomized and had the record of at least one-day treatment of study drugs will be included. Patients missing outcome data will be censored at the last follow‐up assessment time (end of study or last visit preceding loss to follow up).

Per Protocol Set (PPS)

Per Protocol Set (PPS) is a subset of FAS. All patients with finishing the treatment without violating the trail program seriously or missing data for primary efficacy endpoints are included in PPS. The exact definition of a serious violation will be finalized at the time of data review and may generally include (but is not limited to) the following criteria: failure to meet the main inclusion criteria, concomitant treatment that seriously interferes with the evaluation of the efficacy of study drugs after randomization, poor compliance, and exceed the time window of follow-up seriously and so on. A partial protocol violator will be included in the Per Protocol Population up to the time of their violation. For the Per Protocol Population, participants will be analyzed according to the treatment received, providing the same treatment was taken for the duration of the study. If

study medication was changed then the participant will be considered a partial protocol violator (from the point of change onwards).

Safety Set (SS)

All patients who received at least 1-time of study drug according to the study protocol and safety assessment available will be included in the safety population. Throughout the safety results sections, erroneously treated patients (eg, those randomized to rhTNK-tPA group but actually given rhTNK-tPA) will be accounted for in the actual treatment group. This population will be used for safety analyses.

8. Treatment comparisons

The treatment comparison of interest in this study is to evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) versus standard rt-PA (0.9mg/kg) in Chinese patients in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset).

9. General considerations for data analyses

All programming will be performed using SAS Version 9.4 or superior. The content of statistical analysis is the general principle of statistical analysis method, and the detail process will refer to protocol. All analysis output will use the following treatment group naming conventions and treatment order:

Treatment group(rhTNK-TPA): RhTNK-TPA 0.25mg/kg: Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the patient, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds.

Control group(rt-PA): rt-PA 0.9mg/kg: The product is dissolved in sterile water for injection to obtain a solution with a concentration of 1mg/ ml. The amount required for medication is calculated and measured according to the actual body weight of the patient. The 10% of the solution is injected by intravenous bolus, and the remaining 90% is injected by intravenous drip within 1h.

Multicenter Studies

In multicenter randomized controlled clinical study, there were some differences affect in

different center due to different baseline, clinical practice or other factor, therefore, center effect analysis was required. Stratified analysis was used to exclude the mixed effect of results caused by center effect: each center was served as a stratum, calculating the OR by logistic regression model.

Examination of Subgroups

In this study, bridging therapy may be performed within 24h of thrombolysis. Because bridging therapy may be a potential outcome influencing factor, bridging therapy is prespecified as a covariate in the subgroup analysis, and the RR values and 95% confidence intervals of the proportion of the trial and control groups in each subgroup are calculated respectively.

Multiple Comparisons and Multiplicity

A single primary efficacy variable has been defined for this study, with all other efficacy variables were identified as secondary or other. Similarly, only one treatment comparison is of interest in the study and therefore there are no requirements to adjust for multiple comparisons or multiple endpoints within this study.

10. Data handling conventions

Premature Withdrawal and Missing Data

If any subject withdraws prematurely from the study (prior to the final visit D90±7 days assessment), they are required to complete the withdrawal visit in the CRF. The reasons for withdrawal will be presented in a summary table. For the purposes of summaries and analysis of clinic visit data, this visit will be assigned to the next scheduled clinic visit for that patient, regardless of whether the date falls within the next visit window.

Patients who withdraw before the end of the study, but who do provide at least one post-baseline measure for a particular endpoint, will be included in the analysis.

Patients who do not attend any visits after randomization will be excluded from analysis of efficacy outcomes and safety outcomes, as no post-baseline data will be available.

In efficacy evaluation using the FAS population, missing data of efficacy endpoints will be carried forward (LOCF) using the last observed data after administration. Safety missing data will not be filled. Illogic data needs to be investigated by the data management facility, investigators or Sponsor together to identify possible reasons, and restrained by the parties in a data review meeting after full discussion: illogic data, if sufficient reasons have been found to indicate its

incorrectness, should be removed, or otherwise, should be remained and subjected to analysis and the results obtained without such removed data should be treated as sensitivity analysis; If there is data collected in the study that is not in accordance with the program requirements such as planned external visits, it will be presented in a list, and descriptive or comparative analysis will be decided at the data review meeting depending on the specific situation of the data.

Event Rates

The number of people of events should be recorded in detail and showing the event rate in 90 days of each treatment group in summary statement.

The event rate for each treatment group will be calculated as: the sum of number of event for all the patients / the number of patients enrolled in this group.

11. Study Population

Disposition of Participants

The number of patients in each analysis population will be presented, patients to be excluded from the Per Protocol population will be listed, and the total number of patients attending each clinic visit will also be summarized by treatment group.

The number of patients randomized, completed and prematurely withdrawn from the study will be presented for each treatment group. The primary reasons for withdrawal both prior to and post randomization will also be presented.

A data display listing and summary of deviations from the inclusion/exclusion criteria will be presented for all patients who were either entered or randomized into the trial.

Protocol Deviations

Participant data will be examined for evidence of protocol violators in order to assess how well the protocol was followed. Inclusion and exclusion criteria are detailed in the study protocol.

Participants who commit protocol violations will be included in the FAS Population but excluded from the Per Protocol Population. These protocol violations will be shown in a listing. Patients can either be full or partial protocol violators. A full protocol violator is completely

excluded from the Per Protocol Population. A partial protocol violator has only some data excluded. For those who violated the protocol during the treatment period due to unpermitted changes in the medication or prohibited concurrent medication, the analysis will only use data recorded prior to the violation. For all violations which reference the treatment period, the treatment start date will be used as the reference date.

A listing of all possible protocol violators will be produced for clinical review. The final list of patients who are protocol violators and are therefore excluded from the Per-Protocol population will be agreed by the study team.

Demographic and Baseline Characteristics

Demographic, medical histories and baseline characteristics information will be listed and summarized for patients in each treatment group based on the FAS population.

The continuous data followed normal distribution will be presented as mean and standard deviation, and the continuous data followed skewness distribution will be presented as median and interquartile range; categorical data will be presented as n(%). T-test or Wilcoxon rank sum test will be used for comparison between two continuous data, and Chi-squared tests or Fisher exact test will be used for comparison between two categorical data.

12. Efficacy Analyses

Primary efficacy analysis will be performed using FAS and PPS populations. The analyses for the primary efficacy endpoint is conducted by one-sided test with a significance level of one-sided α=0.025. All other statistical analyses will use a 2-sided test at the 5% level of significance.

Primary Efficacy Analysis

The primary endpoint is the proportion of patients with 90-day mRS score of 0-1.

Main Model

The proportion of patients with 90-day mRS score of 0-1 between treatment groups will be compared by CMH Chi-squared tests with the adjustment of center effect. Effect rate with 95% CI in each treatment group and the RR or RD of effect rates between the two groups and corresponding 95% CI will be reported. This will also be tested by logistic regression model with the adjustment of center effect and mRS scores at baseline. The odds ratio with 95% CI will be

reported. According to the protocol, superiority of the treatment group will be tested if the non-inferiority is met.

Interactions with Subgroups

In this study, bridging therapy is prespecified as a covariate in the subgroup analysis. The RR values and 95% confidence intervals of the incidence of the trial and control groups in each subgroup are calculated respectively. Summary tables will be produced for the predefined subgroups and interactions between treatment and these subgroups will be investigated, using a logistic regression model. A separate model will be used for each interaction to determine its significance.

Secondary Efficacy Analyses

The proportion of patients with an mRS score of 0-2 at 90±7 days

The proportion of patients with 90-day mRS score of 0-2 in treatment groups will be compared by Chi-squared tests. This will also be tested by logistic regression model. The odds ratio with 95% CI will be reported.

MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution)

Differences in mRS level distribution at 90 days between the two treatment groups will be tested by Wilcoxon rank sum test. This will also be tested by ordinal logistic regression model. The common odds ratio with 95% CI will be reported.

The proportion of patients whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first)

The proportion of patients whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first) in treatment groups will be compared by Chi-squared tests. This will also be tested by logistic regression model. The odds ratio with 95% CI will be reported.

Health-related quality of life (EQ-5D) at 90±7 day

Health related quality of life will be measured using EuroQol EQ-5D scale among the survivors. Treatment differences will be tested using student t-test or Wilcoxon rank sum test as appropriate. The difference of the scores between two groups with 95% CI will be reported.

The proportion of patients with a Bathel score ≥ 95 points at 90±7 days

The proportion of patients with a Bathel score ≥ 95 points at 90 days in treatment groups will be compared by Chi-squared tests. This will also be tested by logistic regression model. The odds ratio with 95% CI will be reported.

13. Safety Analyses

All analyses of safety data will be carried out using the safety set (SS) population.

Bleeding Events

The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASS III criteria);

The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASS III criteria);

The incidence of PH2 intracranial hemorrhage within 90 days (according to SITS criteria);

The incidence of any intracranial hemorrhage within 90 days;

The incidence of other significant hemorrhage events within 90 days (as defined by GUSTO criteria).

For most bleeding events, the number of bleeding events occurring over the treatment period will be summarized and Chi-squared test or Fisher's Exact test will be used to compare the number of bleeding events between treatment groups.

Adverse Events

Adverse events (AEs) will be coded using the MedDRA coding dictionary (Version 6.0 or a later release) and grouped by system organ class (as detailed in the study protocol). Separate data display listings and summaries will be presented for adverse events that start prior to first dose of study medication (pre-treatment), whilst on study medication (during treatment) and after the last dose of study medication (post-treatment).

Within each treatment group, the number and percentage of patients experiencing an AE will be summarized by system organ class and preferred term and Chi-squared test or Fisher's Exact test will be used to compare the number of each grouped AE event between treatment groups. In addition, a separate summary will be provided for AEs experienced by more than 5% of patients in either of the treatment groups.

Deaths and Serious Adverse Events

Summary tables and data displays will be provided for serious adverse events (as detailed in the study protocol). In addition, all deaths and serious AE's will be documented in a case narrative format in the clinical study report.

The number of deaths occurring over the treatment period will be summarized and Chi-squared test or Fisher's Exact test will be used to compare the number of deaths between treatment groups.

14. References

1. Jonathan Emberson, Kennedy R Lees, Patrick Lyden,et al. Effects of Treatment Delay, Age, and Stroke Severity on the Effects of Acute Ischaemic Stroke with Alteplase: A New Type of Acute Ischaemic Stroke A meta-analysis of individual patient data from randomised trials. Lancet. 2014 NOV 29;384 (9958) : 1929-35.
2. CDE. Guidelines for the design of non-inferiority drug clinical trials.2020,07

Final statistical analysis plan

Xiong Y, *et al. Stroke Vasc Neurol* 2025; 10:e003048. doi: 10.1136/svn-2023-003048

Tenecteplase Reperfusion Therapy in Acute ischemic Cerebrovascular Events-II (TRACE II)

Statistical Analysis Plan

Principal Investigator

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Prepared by

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Version 1.3

April 20, 2021

Table of Contents

1. Introduction

This statistical analysis plan (SAP) documents the planned statistical analyses for the TRACE-2 study and is based on the protocol, together with any subsequent amendments.

This SAP is intended for the use of project team members and should be read in conjunction with the aforementioned protocol.

2. Study Objective

To evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) compared with standard rT-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset).

Primary study objectives:

To evaluate the difference in the proportion of 90-day mRS score of 0-1 between rhTNK-tPA (0.25 mg/kg) and standard rt-PA (0.9mg/kg) in the treatment of patients with hyperacute ischemic stroke (within 4.5 h of symptom onset).

Secondary study objectives:

(1) To compare the difference in efficacy between rhTNK-tPA (0.25 mg/kg) and standard rt-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset):

6) The proportion of patients with an mRS score of 0-2 at 90±7 days;

7) MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution);

8) The proportion of patients whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first);

9) Health-related quality of life (EQ-5D) at 90±7 day;

10) The proportion of patients with a Bathel score ≥ 95 points at 90±7 days;

(2) To compare the difference in safety between rhTNK-tPA (0.25 mg/kg) and standard rt-PA (0.9mg/kg) in the treatment of patients with hyperacute ischemic stroke (within 4.5 h of symptom onset):

1) The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASS III criteria);

2) The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASS III criteria);

3) The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS criteria);

4) The incidence of any intracranial hemorrhage within 90 days;

5) The incidence of other significant hemorrhage events within 90 days (as defined by GUSTO criteria);

6) 90-day overall mortality;

7) Incidence rates of adverse events/serious adverse events within 90 days.

3. Study Endpoint(s)

Primary Efficacy Endpoints:

The proportion of patients with mRS score of 0-1 at 90 days.

Calculation formula:

The proportion of patients with 90-day mRS score of 0-1 = the number of patients with 90-day mRS score of 0-1 / the number of patients enrolled in this group ×100%.

Secondary Efficacy Endpoints:

6) The proportion of patients with an mRS score of 0-2 at 90±7 days;

7) MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution);

8) The proportion of patients whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first);

9) Health-related quality of life (EQ-5D) at 90±7 day;

10) the proportion of patients with a Bathel score ≥ 95 points at 90±7 days;

Safety Endpoint

8) The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASS III criteria).

9) The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASS III criteria);

10) The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS criteria);

11) The incidence of any intracranial hemorrhage within 90 days;

12) The incidence of other significant hemorrhage events within 90 days (as defined by GUSTO criteria);

13) 90-day overall mortality;

14) Incidence rates of adverse events/serious adverse events within 90 days.

4. Statistical Hypotheses

Comparison of the primary efficacy endpoint between groups is conducted by a one-sided test, with a significance level of one-sided α=0.025. Null hypothesis H0: RR $\leqslant$ Δ and alternative hypothesis H1: RR > Δ; when P < 0.025, or lower limit of 95% CI of RR > Δ, it indicates that the test drug is not inferior to control drug; RR is the ratio of incidence of the treatment group and the control group, Δ is the non-inferior margin, 0.937 in this trial. All other statistical analyses are conducted by two-sided tests, with a significance level of two-sided α =0.05. When P$\leqslant$0.05, it indicates a statistically significant difference. Non-inferiority hypothesis tests are as follows:

$$H_0: RR_{\text{Treatment group/control group}} \leqslant \Delta$$

$$H_1: RR_{\text{Treatment group/control group}} > \Delta$$

5. Design

This study will evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) compared with standard rt-PA (0.9mg/kg) in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset). This is a multicenter, prospective, centrally randomized, open label, blinded-endpoint, parallel active controlled, non-inferior phase III clinical trial.

The drug administration method of the test drug (rhTNK-TPA) is as follows: according to the

body weight of the patients, the final dose is determined at 0.25mg/kg; Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the patient, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds.

The administration method of active control drug (rt-PA) is as follows: The dose required for medication is calculated and measured according to the actual body weight of the patient based on a standard of 0.9 mg rt-PA/kg. The 10% of the dose is injected by intravenous bolus, and the remaining 90% is injected by intravenous drip within 1h.

The target population of this study is patients with critical hyperacute ischemic stroke (within 4.5 h of symptom onset), for whom considering the benefit of patients, intravenous thrombolytic therapy should be given as soon as possible after the onset, in order to achieve vascular recanalization or reperfusion and obtain a better prognosis. Due to the different administration methods of test drug and active control drug, and the inconvenience of the double-blind double-dummy design which may delay treatment and is not conducive to the benefit of patients. Therefore, this study adopts the form of open-label trial. To minimize bias, all endpoints will be evaluated in a blind manner by a qualified study physician.

Planned Analyses

The analyses that are detailed in this SAP will be performed only when the database has been locked, all protocol violators identified, and treatment allocations have been unblinded. Membership of the Full Analysis and Per Protocol populations will be determined using the rules set out in this SAP. The treatment allocations will be unblinded and extracted from corresponding system. These will then be merged on to the study database using the treatment number (as allocated to each patient) to match patients with the correct treatment allocation. Membership of the modified Intention-to-Treat and Per Protocol populations will be determined using the rules set out in this SAP and will be determined prior to unblinding the treatment allocation. At a date to be agreed within the project team, a data look will be performed. This will involve production of all data displays on a subset of the data using dummy treatment codes. These are produced purely as an aide to the pre-programming of the study and no unblinding will occur.

Interim Analyses

No interim analyses are planned for this study. However, a Data and Safety Monitoring Board (DSMB) is in place to ensure the safety of patients in the study. An independent statistician will prepare unblinded summary tables of SAEs, selected demographic data and exposure data and these will be examined by the DSMB. These tables will be provided to the DSMB at regular intervals. If the tables give rise to safety concerns for any treatment, the DSMB may recommend that the trial should be modified or stopped prematurely. The Steering Committee will, in conjunction with the Sponsor, decide whether to act on this recommendation. Further discussion of these safety tabulations is provided in a specific study protocol.

6. Sample size estimates

In this study, rt-PA active control will be used based on a statistical hypothesis test of non-inferiority, and the proportion of patients with 90-day mRS score of 0-1 will be used as the primary efficacy endpoint.

According to a paper published in the Lancet [1] in 2014 about meta-analysis of the proportion of 90-day mRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the *Guidelines for the Design of Non-inferiority Drug Clinical Trials* [2], the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high priority index. Therefore, based on the lower CI limit of 1.14, together with an efficacy of 50% of the active control, the non-inferior threshold was determined as 0.937. Then assuming α=0.025 using one-sided test, power 1-β=0.85, treatment group (TNK) incidence of 63.64%, control group (rt-PA) incidence of 59.32%, RR= 1.07, the sample size was calculated to be 642 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each group and the total sample size is estimated to be 1430 cases.

7. Analysis populations

According to National Medical Products Administration (NMPA), statistical analysis of data was established including Full Analysis Set (FAS), Per Protocol Set (PPS) and Safety Set (SS). The entire process of statistical analysis of data will be received strict control of quality in order to ensure facticity of data.

Full Analysis Set (FAS)：

According to the basic principle of intention-to-treat (ITT), all patients who were enrolled, randomized and had the record of at least one-day treatment of study drugs will be included. This population was also referred as modified ITT population.

Per Protocol Set (PPS)

Per Protocol Set (PPS) is a subset of FAS. All patients with finishing the treatment without violating the trail program seriously or missing data for primary efficacy endpoints are included in PPS. The exact definition of a serious violation will be finalized at the time of data review and may generally include (but is not limited to) the following criteria: failure to meet the main inclusion

criteria, concomitant treatment that seriously interferes with the evaluation of the efficacy of study drugs after randomization, poor compliance, and exceed the time window of follow-up seriously and so on. A partial protocol violator will be included in the Per Protocol Population up to the time of their violation. For the Per Protocol Population, participants will be analyzed according to the treatment received, providing the same treatment was taken for the duration of the study. If study medication was changed then the participant will be considered a partial protocol violator (from the point of change onwards).

Safety Set (SS)

All patients who received at least 1-time of study drug according to the study protocol and safety assessment available will be included in the safety population. Throughout the safety results sections, erroneously treated patients (eg, those randomized to rhTNK-tPA group but actually given rhTNK-tPA) will be accounted for in the actual treatment group. This population will be used for safety analyses.

8. Treatment comparisons

The treatment comparison of interest in this study is to evaluate the efficacy and safety of rhTNK-tPA (0.25 mg/kg) versus standard rt-PA (0.9mg/kg) in Chinese patients in the treatment of hyperacute ischemic stroke (within 4.5 h of symptom onset).

9. General considerations for data analyses

All programming will be performed using SAS Version 9.4 or superior. The content of statistical analysis is the general principle of statistical analysis method, and the detail process will refer to protocol. All analysis output will use the following treatment group naming conventions and treatment order:

Treatment group(rhTNK-TPA): RhTNK-TPA 0.25mg/kg: Each vial is dissolved in 3ml of sterile water for injection to obtain a solution with a concentration of 5.33mg/ml. The amount required for medication is calculated and measured according to the actual body weight of the patient, 25mg at most. The solution is administered via single intravenous bolus injection in 5-10 seconds.

Control group(rt-PA): rt-PA 0.9mg/kg: The product is dissolved in sterile water for injection to obtain a solution with a concentration of 1mg/ ml. The amount required for medication is calculated and measured according to the actual body weight of the patient. The 10% of the solution is injected by intravenous bolus, and the remaining 90% is injected by intravenous drip

within 1h.

Multicenter Studies

In multicenter randomized controlled clinical study, there were some differences affect in different center due to different baseline, clinical practice or other factor, therefore, center effect analysis was required. Analysis will be performed to exclude the center effect.

Examination of Subgroups

In this study, bridging therapy may be performed within 24h of thrombolysis. Because bridging therapy may be a potential outcome influencing factor, bridging therapy is prespecified as a covariate in the subgroup analysis, and the RR values and 95% confidence intervals of the proportion of the trial and control groups in each subgroup are calculated respectively.

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A single primary efficacy variable has been defined for this study, with all other efficacy variables were identified as secondary or other. Similarly, only one treatment comparison is of interest in the study and therefore there are no requirements to adjust for multiple comparisons or multiple endpoints within this study.

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Premature Withdrawal and Missing Data

If any subject withdraws prematurely from the study (prior to the final visit D90±7 days assessment), they are required to complete the withdrawal visit in the CRF. The reasons for withdrawal will be presented in a summary table. For the purposes of summaries and analysis of clinic visit data, this visit will be assigned to the next scheduled clinic visit for that patient, regardless of whether the date falls within the next visit window.

Patients who withdraw before the end of the study, but who do provide at least one post-baseline measure for a particular endpoint, will be included in the analysis.

Patients who do not attend any visits after randomization will be excluded from analysis of efficacy outcomes and safety outcomes, as no post-baseline data will be available.

Missing data will not be imputed in the main analysis. Illogic data needs to be investigated by the data management facility, investigators or Sponsor together to identify possible reasons, and restrained by the parties in a data review meeting after full discussion: illogic data, if sufficient

reasons have been found to indicate its incorrectness, should be removed, or otherwise, should be remained and subjected to analysis and the results obtained without such removed data should be treated as sensitivity analysis; If there is data collected in the study that is not in accordance with the program requirements such as planned external visits, it will be presented in a list, and descriptive or comparative analysis will be decided at the data review meeting depending on the specific situation of the data. In sensitivity analysis, multiple imputation was performed to impute the missing data of the primary efficacy outcome.

Event Rates

The number of people of events should be recorded in detail and showing the event rate in 90 days of each treatment group in summary statement.

The event rate for each treatment group will be calculated as: the sum of number of event for all the patients / the number of patients enrolled in this group.

11. Study Population

Disposition of Participants

The number of patients in each analysis population will be presented, patients to be excluded from the Per Protocol population will be listed, and the total number of patients attending each clinic visit will also be summarized by treatment group.

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A data display listing and summary of deviations from the inclusion/exclusion criteria will be presented for all patients who were either entered or randomized into the trial.

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Participant data will be examined for evidence of protocol violators in order to assess how well the protocol was followed. Inclusion and exclusion criteria are detailed in the study protocol.

Participants who commit protocol violations will be included in the FAS Population but

excluded from the Per Protocol Population. These protocol violations will be shown in a listing. Patients can either be full or partial protocol violators. A full protocol violator is completely excluded from the Per Protocol Population. A partial protocol violator has only some data excluded. For those who violated the protocol during the treatment period due to unpermitted changes in the medication or prohibited concurrent medication, the analysis will only use data recorded prior to the violation. For all violations which reference the treatment period, the treatment start date will be used as the reference date.

A listing of all possible protocol violators will be produced for clinical review. The final list of patients who are protocol violators and are therefore excluded from the Per-Protocol population will be agreed by the study team.

Demographic and Baseline Characteristics

Demographic, medical histories and baseline characteristics information will be listed and summarized for patients in each treatment group based on the FAS population.

The continuous data followed normal distribution will be presented as mean and standard deviation, and the continuous data followed skewness distribution will be presented as median and interquartile range; categorical data will be presented as n(%). T-test or Wilcoxon rank sum test will be used for comparison between two continuous data, and Chi-squared tests or Fisher exact test will be used for comparison between two categorical data.

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Primary efficacy analysis will be performed using FAS and PPS populations. The analyses for the primary efficacy endpoint will be conducted by one-sided test with a significance level of one-sided α=0.025. All other statistical analyses will use a 2-sided test at the 5% level of significance.

Primary Efficacy Analysis

The primary endpoint is the proportion of patients with 90-day mRS score of 0-1.

Main Model

The proportion of patients with 90-day mRS score of 0-1 between treatment groups will be

compared by Cochran-Mantel-Haenszel Chi-squared tests with the adjustment of center effect. Effect rate with 95% CI in each treatment group and the RR or RD of effect rates between the two groups and corresponding 95% CI will be reported. This will also be tested by logistic regression model with the adjustment of center effect and mRS scores at baseline. The odds ratio with 95% CI will be reported. According to the protocol, superiority of the treatment group will be tested if the non-inferiority is met.

Interactions with Subgroups

In this study, bridging therapy is prespecified as a covariate in the subgroup analysis. The RR values and 95% confidence intervals of the incidence of the trial and control groups in each subgroup are calculated respectively. Summary tables will be produced for the predefined subgroups and interactions between treatment and these subgroups will be investigated, using a logistic regression model. A separate model will be used for each interaction to determine its significance.

Secondary Efficacy Analyses

The proportion of patients with an mRS score of 0-2 at 90±7 days

The proportion of patients with 90-day mRS score of 0-2 in treatment groups will be compared by Chi-squared tests. This will also be tested by logistic regression model. The odds ratio with 95% CI will be reported.

MRS level distribution at 90±7 days (Shift Analysis /Ordinal Distribution)

Differences in mRS level distribution at 90 days between the two treatment groups will be tested by Wilcoxon rank sum test. This will also be tested by ordinal logistic regression model. The common odds ratio with 95% CI will be reported.

The proportion of patients whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ≥ 4 at 24±2h, 7±1d or before discharge (whichever comes first)

The proportion of patients whose NIHSS score is 0-1 or is improved compared to the baseline with ΔNIHSS ⩾ 4 at 24 ± 2h, 7 ± 1d or before discharge (whichever comes first) in treatment groups will be compared by Chi-squared tests. This will also be tested by logistic regression model. The odds ratio with 95% CI will be reported.

Health-related quality of life (EQ-5D) at 90±7 day

Health related quality of life will be measured using EuroQol EQ-5D scale among the survivors. Treatment differences will be tested using student t-test or Wilcoxon rank sum test as appropriate. The difference of the scores between two groups with 95% CI will be reported.

The proportion of patients with a Bathel score ≥ 95 points at 90±7 days

The proportion of patients with a Bathel score ⩾ 95 points at 90 days in treatment groups will be compared by Chi-squared tests. This will also be tested by logistic regression model. The odds

ratio with 95% CI will be reported.

13. Safety Analyses

All analyses of safety data will be carried out using the safety set (SS) population.

Bleeding Events

The incidence of symptomatic intracranial hemorrhage within 36 hours (as defined by ECASS III criteria);

The incidence of symptomatic intracranial hemorrhage within 90 days (as defined by ECASS III criteria);

The incidence of PH2 intracranial hemorrhage within 36 hours (according to SITS criteria);

The incidence of any intracranial hemorrhage within 90 days;

The incidence of other significant hemorrhage events within 90 days (as defined by GUSTO criteria).

For most bleeding events, the number of bleeding events occurring over the treatment period will be summarized and Chi-squared test or Fisher's Exact test will be used to compare the number of bleeding events between treatment groups. This will also be tested by logistic regression model. The odds ratio with 95% CI will be reported.

Adverse Events

Adverse events (AEs) will be coded using the MedDRA coding dictionary (Version 6.0 or a later release) and grouped by system organ class (as detailed in the study protocol). Separate data display listings and summaries will be presented for adverse events that start prior to first dose of study medication (pre-treatment), whilst on study medication (during treatment) and after the last dose of study medication (post-treatment).

Within each treatment group, the number and percentage of patients experiencing an AE will be summarized by system organ class and preferred term and Chi-squared test or Fisher's Exact test will be used to compare the number of each grouped AE event between treatment groups. In addition, a separate summary will be provided for AEs experienced by more than 5% of patients in either of the treatment groups.

Deaths and Serious Adverse Events

Summary tables and data displays will be provided for serious adverse events (as detailed in the study protocol). In addition, all deaths and serious AE's will be documented in a case narrative format in the clinical study report.

The number of deaths occurring over the treatment period will be summarized and Chi-squared test or Fisher's Exact test will be used to compare the number of deaths between treatment groups.

14. References

3. Jonathan Emberson, Kennedy R Lees, Patrick Lyden,et al. Effects of Treatment Delay, Age, and Stroke Severity on the Effects of Acute Ischaemic Stroke with Alteplase: A New Type of Acute Ischaemic Stroke A meta-analysis of individual patient data from randomised trials. Lancet. 2014 NOV 29; 384 (9958) : 1929-35.

4. CDE. Guidelines for the design of non-inferiority drug clinical trials.2020,07

Changes in the statistical analysis plan

SAP version 1.0	Changes in SAP version 1.3
6. Sample size estimates According to a paper published in the Lancet in 2014 about meta-analysis of the incidence of 90-day MRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the data under age of 80 was collected with the incidence of rt-PA and Placebo in clinical trials initiated by European researchers, of which the meta-analysis result of RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the Guidelines for the Design of Non-inferiority Drug Clinical Trials, the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high priority index. Therefore, based on the lower CI limit of 1.14, together with a efficacy of 25% of the active control, the non-inferior threshold was determined as 0.906. Then assuming α=0.025 using one-sided test, power 1-β=0.8, treatment group (TNK) incidence of 64%, control group (rt-PA) incidence of 63%, real RR= 1.02, the sample size was calculated by PASS software to be 643 cases in each group;	6. Sample size estimates According to a paper published in the Lancet [1] in 2014 about meta-analysis of the proportion of 90-day mRS score of 0-1 (treatment within 4.5h) regarding rt-PA versus Placebo, the RR and 95% confidence interval were 1.24 and 1.14-1.36, respectively. According to the Guidelines for the Design of Non-inferiority Drug Clinical Trials [2], the lower 95% confidence interval of the Meta analysis should be used to calculate the non-inferiority threshold given that the primary endpoint is a high priority index. Therefore, based on the lower CI limit of 1.14, together with an efficacy of 50% of the active control, the non-inferior threshold was determined as 0.937. Then assuming α=0.025 using one-sided test, power 1-β=0.85, treatment group (TNK) incidence of 63.64%, control group (rt-PA) incidence of 59.32%, RR= 1.07, the sample size was calculated to be 642 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each group and the total sample size is estimated to be 1430 cases. *Then assuming α=0.025 using one-sided test, power 1-β=0.85, treatment group (TNK) incidence of

considering a dropout rate of 10%, the sample size was determined as 715 cases in each group, a total of 1430 cases.	63.64%, control group (rt-PA) incidence of 59.32%, real RR= 1.073, the sample size was calculated by PASS software to be 614 cases in each group; considering a dropout rate of 10%, the sample size was determined as 1364 (682 cases in each group). When the RR value was rounded to 1.07, the sample size was calculated to be 642 cases in each group; considering a dropout rate of 10%, the sample size was determined as 715 cases in each group and the total sample size is estimated to be 1430 cases.
10. Premature Withdrawal and Missing Data In efficacy evaluation using the FAS population, missing data of efficacy endpoints will be carried forward (LOCF) using the last observed data after administration. Safety missing data will not be filled.	10. Premature Withdrawal and Missing Data Missing data will not be imputed in the main analysis. … In sensitivity analysis, multiple imputation was performed to impute the missing data of the primary efficacy outcome.
13. Safety Analyses For most bleeding events, the number of bleeding events occurring over the treatment period will be summarized and Chi-squared test or Fisher's Exact test will be used to compare the number of bleeding events between treatment groups.	13. Safety Analyses For most bleeding events, the number of bleeding events occurring over the treatment period will be summarized and Chi-squared test or Fisher's Exact test will be used to compare the number of bleeding events between treatment groups. This will also be tested by logistic regression model. The odds ratio with 95% CI will be reported.