





Safety and efficacy of tenecteplase versus alteplase in patients with acute ischaemic stroke (TRACE): a multicentre, randomised, open label, blinded-endpoint (PROBE) controlled phase II study

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ABSTRACT

Background Tenecteplase (TNK) possesses several pharmacological characteristics superior to conventional alteplase (rt-PA), with well-established safety and efficacy profile in Caucasians. There exists controversy over the optimal dose of intravenous rt-PA for East Asians with acute ischaemic stroke (AIS). Current study aimed to determine the safety dose range of recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) for patients with AIS in China.

Methods This multicentre, prospective, randomised, open-label, blinded end-point, phase II study compared three tiers of 0.1, 0.25, 0.32 mg/kg rhTNK-tPA (to a maximum of 40 mg) with standard 0.9 mg/kg rt-PA (to a maximum of 90 mg) in patients who were eligible for intravenous thrombolysis. The safety outcome were symptomatic intracranial haemorrhage (sICH) within 36 hours.

Results Between May 2018 and February 2020, 240 patients were randomly assigned to four group, 4 of whom did not receive study treatment. The intention-to-treat analysis included 236 patients. There was no difference in the improvement on National Institutes of Health Stroke Scale at day 14 in the 3 tiers and control group (63.3%, 77.2%, 66.7% vs 62.7%). The number of sICH was 3 of 60 (5.0%) in the 0.1 mg/kg group, none in the 0.25 mg/kg group, 2 of 60 (3.3%) in the 0.32 mg/kg group and 1 (1.7%) of 59 in the rt-PA group. There were no significant between-group differences in severe adverse events.

Conclusions Similar to the Caucasians, rhTNK-tPA was well tolerated in Chinese patients with AIS at all doses administered within 3 hours of symptom onset. The dose-efficacy profile of rhTNK-tPA needs to be established with future investigations.

Trial registration number NCT04676659.

INTRODUCTION

Intravenous thrombolysis with alteplase (rt-PA) is recommended as the standard treatment for eligible patients with acute ischaemic stroke (AIS) in guidelines.^{1 2} The

short half-life of rt-PA limits its use, especially in patients who need endovascular thrombectomy. Tenecteplase (TNK) is a genetically modified form of human tissue plasminogen activator (tPA) that has advantages over rt-PA on three aspects: (1) higher fibrin specificity that make it possible to reduce haemorrhagic complications, (2) greater resistance to plasminogen activator inhibitor-1 that may possess higher efficacy in clot lysis and (3) longer serum half-life that allows for single-bolus administration.^{3 4}

TNK has been used for treatment of Caucasians patients with AIS at doses of 0.1, 0.25, 0.4 or 0.5 mg/kg.^{5–8} Different doses were chosen in two phase III studies. The EXTEND-IA TNK study came to the conclusion that TNK usage before thrombectomy was associated with a higher rate of reperfusion and better functional outcome than rt-PA in patients with large vessel occlusion (LVO) AIS treated within 4.5 hours after symptom onset.⁹ TNK (0.4 mg/kg) did not confer better outcome over the 0.25 mg/kg dose.¹⁰ The Norwegian tenecteplase stroke trial (NOR-TEST) showed a similar safety profile between the TNK (0.4 mg/kg) and rt-PA in patients with mild AIS.^{11 12} There is a lack of consensus on the safe and effective dose of TNK in patients with AIS.

Moreover, the optimal dosage of rt-PA for East Asians is still in debate.^{13–16} As a potential competitor of rt-PA, it is necessary to determine the dose range of optimal safety in Chinese patients with AIS.

This study, Tenecteplase Reperfusion therapy in Acute ischemic Cerebrovascular Events (TRACE), compared three different

doses of recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) with standard rt-PA treatment in patients with AIS within 3 hours from symptom onset. Patients were chosen randomly to receive either rhTNK-tPA or rt-PA. rhTNK-tPA, approved for treating acute myocardial infarction in China, had the same terminal amino acid sequence and different production process to the TNK made by Boehringer (Metalyse) and Genentech (TNKase) which was used in previous studies (online supplemental tables 1 and 2).

METHODS

Study design

The TRACE was a multicentre, prospective, randomised, open-label, blinded end-point, phase II study done in 22 stroke units in China (online supplemental table 3) between May 2018 and February 2020. The protocol was approved by the local institutional review board and all patients or legal representatives had given informed consent.

Patients

Patients were eligible if they were 18 years of age or older, were diagnosed of AIS with measurable deficits on the National Institutes of Health Stroke Scale (NIHSS) 4–25 (both included), haemorrhage ruled out by non-contrast computer tomography (NCCT) scan, were admitted

within 3 hours of symptom onset which refers to ‘last known to be well’, living independently (prestroke modified Rankin Scale (mRS) ≤ 2 or without history of stroke) and had no contraindications to intravenous thrombolysis (online supplemental table 4).

Randomisation and masking

Patients were randomly assigned (1:1:1:1) to rhTNK-tPA 0.1, 0.25, 0.32 mg/kg (to a maximum of 40 mg) or rt-PA 0.9 mg/kg (to a maximum of 90 mg) using a strict central block randomisation system (over the internet). A total of 4 lots of rhTNK-tPA (Guangzhou Recomgen Biotech) and 25 lots of rt-PA (Boehringer Ingelheim) were provided free of charge to patients and used within the validity period. The treatment was conducted openly. Outcomes were assessed by an independent investigators who were blinded during all procedures and well trained with NIHSS and mRS evaluation.

Procedures

Eligibility for intravenous thrombolysis was assessed at the emergency department and a baseline NIHSS examination was performed. The planned number of patients to be enrolled in each dosage tier was 60. rhTNK-tPA (Guangzhou Recomgen Biotech, China; 1.0×10^7 IU/16 mg per vial) was given as a single, intravenous bolus (over 5–10 s) immediately after randomisation. Maximum

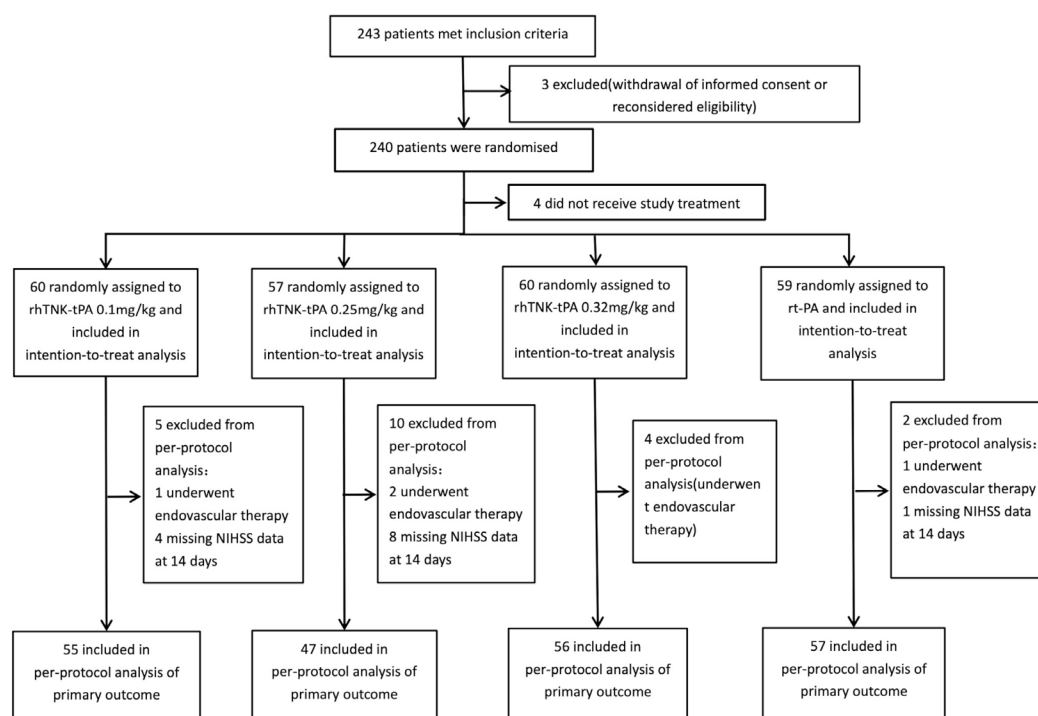


Figure 1 Flowchart.

Table 1 Characteristics of patients at baseline

Characteristic	rhTNK-tPA			rt-PA
	0.1 mg/kg (N=60)	0.25 mg/kg (N=57)	0.32 mg/kg (N=60)	0.9 mg/kg (N=59)
Age (years)				
Mean (SD)	62.4 (11.1)	64.3 (12.8)	64.8 (12.1)	66.5 (12.6)
Age group (n)				
<60	28 (46.7)	21 (36.8)	15 (25.0)	17 (28.8)
60–80	31 (51.7)	28 (49.1)	40 (66.7)	36 (61.0)
>80	1 (1.7)	8 (14.0)	5 (8.3)	6 (10.2)
Sex (n)				
Women	12 (20.0)	15 (26.3)	18 (30.0)	21 (35.6)
BMI (kg/m²)				
Mean (SD)	24.52 (3.3)	25.00 (3.8)	24.64 (4.4)	23.77 (3.4)
Hypertension (n, %)				
Diabetes mellitus (n, %)	14 (23.3)	9 (15.8)	15 (25.0)	11 (18.6)
Hyperlipidaemia (n, %)	17 (28.3)	13 (22.8)	10 (16.7)	11 (18.6)
Atrial fibrillation (n, %)	8 (13.3)	4 (7.0)	14 (23.3)	6 (10.2)
Current smoking (n, %)	25 (41.7)	25 (43.9)	21 (35.0)	24 (40.7)
Medical history (n, %)				
Antiplatelet	11 (18.3)	6 (10.5)	13 (21.7)	13 (22.0)
Anticoagulant	2 (3.3)	0 (0.0)	0 (0.0)	2 (3.4)
NIHSS score				
Median (IQR)	7.0 (5.0–10.0)	8.0 (5.0–12.0)	7.5 (6.0–12.0)	8.0 (5.0–12.0)
Premorbid mRS score (n, %)				
0	55 (91.7)	54 (94.7)	47 (78.3)	50 (84.8)
1	5 (8.3)	3 (5.3)	11 (18.3)	5 (8.5)
2	0 (0.0)	0 (0.0)	2 (3.3)	4 (6.8)
Onset-to-needle time (min)				
Median (IQR)	154 (56–195)	149 (80–179)	147 (69–220)	153 (18–187)
Door-to-needle time (min)				
Median (IQR)	71 (28–149)	60 (5–180)	69 (10–134)	71 (10–146)

BMI, body mass index; mRS, modified Rankin Scale; rhTNK-tPA, recombinant human tenecteplase tissue-type plasminogen activator.

dose was 40 mg. 10% dose of rt-PA (Actilyse; Boehringer Ingelheim, Germany; 50 mg, 20 mg per vial) of the active control group was given as bolus and the remainder over 1 hour (maximum dose was 90 mg). Other treatments were carried out adhering to established clinical principles and medical practice guidelines. Bridging thrombolysis plus endovascular thrombectomy was allowed but was excluded from per protocol analysis.

Outcomes

Safety outcomes were incidences of symptomatic intracranial haemorrhage (sICH) within 36 hours (defined as European Cooperative Acute Stroke Study, ECASS III),¹⁷ asymptomatic intracranial haemorrhage identified by routine repeat brain imaging within 90 days, systematic bleeding at 90 days, deaths from any cause and adverse events/serious adverse events (SAEs) within 90 days. The primary efficacy outcome was defined as the proportion

of subjects with improvement on NIHSS of ≥ 4 points or a score ≤ 1 at day 14.^{18,19} The secondary efficacy outcomes included (1) the proportion of subjects with mRS ≤ 1 point at 90 days; (2) the proportion of subjects with mRS ≤ 2 point at 90 days and (3) ordinal distribution of mRS at 90 days. An independent data monitoring committee (iDMC) maintained surveillance of patient safety. An iDMC meeting was held to decide whether to discontinue the study if three cases of sICH occurred in any group. The clinical events committee which was not involved in the execution of the study verified and adjudicated all clinical endpoints based on clinical symptoms, laboratory tests and imaging data.

Statistical analysis

All patients randomised to receive study treatment will be included in the final analysis for safety and clinical outcome (intention-to-treat analysis (ITT)). The

Table 2 Prespecified safety outcomes and other serious adverse events

	rhTNK-tPA			rt-PA	P value
	0.1 mg/kg (N=60)	0.25 mg/kg (N=57)	0.32 mg/kg (N=60)	0.9 mg/kg (N=59)	
Symptomatic ICH at 36 hours (ECASS III)	3 (5.0)	0 (0.0)	2 (3.3)	1 (1.7)	0.52
Death	6 (10.0)	1 (1.8)	5 (8.3)	6 (10.2)	0.22
Asymptomatic intracranial haemorrhage	4 (6.7)	3 (5.3)	5 (8.3)	2 (3.4)	0.75
Other extracranial bleeding	18 (30.0)	18 (31.6)	22 (36.7)	14 (23.7)	0.49
Serious adverse events	12 (20.0)	7 (12.3)	11 (18.3)	14 (23.7)	0.46

ECASS, European Cooperative Acute Stroke Study; ICH, intracranial haemorrhage; rhTNK-tPA, recombinant human tenecteplase tissue-type plasminogen activator.

per-protocol (PP) population was defined as all patients who received any dose of study drug and met all the inclusion and exclusion criteria. The last observation carried forward was used for patients who withdrew from the study and counted as lack of data. Proportions were used for categorical variables, and means with SD or median with IQRs were used for continuous variables. Differences in efficacy outcomes among four groups were analysed. The proportion of NIHSS ≥ 4 points or a score ≤ 1 at day 14 were compared by logistic regression. OR and the 95% CI were used as significance measures. Ordinal distribution of mRS at 3 months was analysed. All tests were two-sided, and a p value of 0.05 or less was considered statistically significant. All statistical analyses were performed using SAS V.9.4 software.

RESULTS

Baseline characteristics

Between May 2018 and February 2020, 243 patients with AIS were enrolled at 22 clinical sites. Three patients were excluded because informed consents were withdrawn after enrolment. Out of 240 patients randomised, 4 did not receive study treatment. Sixty patients were assigned to receive 0.1 mg/kg of rhTNK-tPA, 57 were assigned to receive 0.25 mg/kg of rhTNK-tPA, 60 were assigned to receive 0.32 mg/kg of rhTNK-tPA and 59 to receive 0.9 mg/kg of rt-PA. Patients (21) missing NIHSS data at 14 days or undergoing endovascular therapy were excluded from the PP population (figure 1). The four groups were well balanced regarding baseline characteristics (table 1). The mean ages were 62.4 (11.1), 64.3 (12.8) and 64.8 (12.1) for rhTNK-tPA groups vs 66.5 (12.6) of the rt-PA group and the median NIHSS were 7.0 (5.0–10.0), 8.0 (5.0–12.0) and 7.5 (6.0–12.0) for rh-TNK-tPA group vs 8.0 (5.0–12.0) of the rt-PA group.

Safety outcomes

Table 2 summarised the haemorrhagic complications, death and SAE encountered in the trial. No datum was missing in safety outcomes. The sICH events were observed in 3 (5%), 0, 2 (3.3%) and 1 (1.7%) patients in the three-tier rhTNK-tPA groups and the rt-PA group, respectively. The incidence of fatality with 0.1 mg/kg

of rhTNK-tPA was 6 (10%), 0.25 mg/kg was 1 (1.8%), 0.32 mg/kg was 5 (8.3%) and 0.9 mg/kg of rt-PA was 6 (10.2%). SAEs (life-threatening, resulting in prolonged or recurrent hospitalisation, disability, dysfunction, lead to abnormalities) were observed in 12 (20%), 7 (12.3%), 11 (18.3%) and 14 (23.7%) patients in the 0.1 mg/kg, 0.25 mg/kg 0.32 mg/kg of rhTNK-tPA group and the rt-PA group, respectively. SAEs classified by system organ were listed in online supplemental table 7. No significant difference was found in all safety outcomes.

Efficacy outcomes

Table 3 displayed the efficacy outcomes of NIHSS at 14 days and mRS at 3 months. Data of improvement on NIHSS at 14 days were missing in 20 patients: 11 patients for death within 14 days and 9 patients for telephone follow-up (online supplemental table 5). In the ITT analysis, the proportion of primary efficacy outcome in the 0.1 mg/kg, 0.25 mg/kg and 0.32 mg/kg of rhTNK-tPA group and the rt-PA group was 63.3%, 77.2%, 66.7% and 62.7%, respectively. The mRS distribution is shown in figure 2. No significant difference of any efficacy outcomes was observed between the three-tier rhTNK-tPA groups and the rt-PA group (online supplemental table 6). The data from PP population were consistent with the ITT population.

DISCUSSION

TNK was indicated non-inferiority to rt-PA based on meta-analysis.^{20 21} On the other hand, the optimal dose has varied between 0.25 mg/kg and 0.40 mg/kg. rhTNK-tPA used in TRACE study has the same terminal amino acid sequence and different production process to TNK used in previous studies. In this study, patients with AIS treated with 3-tiers of rhTNK-tPA showed similar rates of improvements on neurological deficits and sICH, compared with patients treated with rt-PA. The dose tier of 0.25 mg/kg indicated more proportion of improvement on neurological deficit, lower proportion of death and no sICH event.

A pilot dose-escalation study for the safety of TNK in AIS provided the initial experience with dose tiers of TNK and discarded 0.5 mg/kg.⁵ The 0.4 mg/kg tier was considered unsafe in a prematurely terminated randomised

Table 3 Primary efficacy outcome and secondary efficacy outcome in the intention-to-treat and per-protocol populations at 90 days

	rhTNK-tPA			rt-PA
	0.1 mg/kg	0.25 mg/kg	0.32 mg/kg	0.9 mg/kg
<i>Intention-to-treat analysis</i>	N=60	N=57	N=60	N=59
Primary efficacy outcome				
Improvement on NIHSS at 14 days				
n (%)	38 (63.3)	44 (77.2)	40 (66.7)	37 (62.7)
OR (95% CI)	1.03 (0.49 to 2.16)	2.01 (0.89 to 4.54)	1.19 (0.56 to 2.52)	ref.
Secondary efficacy outcomes				
mRS score ≤1 point at 3 months				
n.(%)	33 (55.0)	35 (63.6)	36 (62.1)	35 (59.3)
OR (95% CI)	0.84 (0.41 to 1.73)	1.20 (0.56 to 2.56)	1.12 (0.53 to 2.36)	ref.
mRS score ≤2 point at 3 months				
n.(%)	41 (68.3)	42 (76.7)	41 (70.7)	43 (72.9)
OR (95% CI)	0.80 (0.36 to 1.77)	1.20 (0.52 to 2.80)	0.90 (0.40–2.01)	ref.
Ordinal distribution of mRS at 3 months				
OR (95% CI)	0.91 (0.47 to 1.73)	1.12 (0.57 to 2.17)	1.00 (0.52 to 1.92)	ref.
<i>Per-protocol analysis</i>	N=55	N=47	N=56	N=57
Primary efficacy outcome				
Improvement on NIHSS at 14 days				
n (%)	37 (67.3)	37 (78.7)	39 (69.6)	37 (64.9)
OR (95% CI)	1.11 (0.51 to 2.43)	2.00 (0.83 to 4.85)	1.24 (0.56 to 2.73)	ref.
Secondary efficacy outcomes				
mRS score ≤1 point at 3 months				
n (%)	32 (58.2)	29 (63.0)	35 (63.6)	34 (59.7)
OR (95% CI)	0.94 (0.44 to 2.00)	1.15 (0.52 to 2.57)	1.18 (0.55 to 2.54)	ref.
mRS score ≤2 point at 3 months				
n (%)	38 (69.1)	35 (76.1)	40 (72.7)	42 (73.7)
OR (95% CI)	0.80 (0.35 to 1.82)	1.14 (0.46 to 2.79)	0.95 (0.41 to 2.20)	ref.
Ordinal distribution of mRS at 3 months				
OR (95% CI)	0.95 (0.49 to 1.86)	1.17 (0.58 to 2.37)	1.03 (0.53 to 2.02)	ref.

mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; rhTNK-tPA, recombinant human tenecteplase tissue-type plasminogen activator.

clinical trial as 3 (16%) of 19 patients suffered sICH.⁶ However, the NOR-TEST showed relatively low rates (9%, 47 of 549) of sICH in patients receiving 0.4 mg/kg of TNK.¹¹ The EXTENT-IA TNK part 2 used the dose of 0.4 mg/kg in patients with large-vessel occlusion and the rates of sICH was 4.7% (7 of 150).¹⁰ In TRACE study, 3.3% patients suffered sICH in the 0.32 mg/kg dose tier, and no sICH in the 0.25 mg/kg dose tier was the same as in the rt-PA group (1.7%). The low rates of sICH in our study might be related to the baseline characteristics of patients and the improved quality of medical practice. The mean age was younger and the median NIHSS was lower in our study than most previous studies. The study population of NOR-TEST was the closest to the population of our study, which used 0.4 mg/kg of TNK in patients with AIS within 4.5 hours. The mean age of TNK group in NOR-TEST

was 70.8 (14.4), and the median NIHSS score was 4 (2-7). No new safety concern of rhTNK-tPA was observed in this study.

The intrinsic characteristics of the drug recipient and extrinsic characteristics associated with environment and culture that could affect the results of clinical studies carried out in regions. The low-dose (0.6 mg/kg) rt-PA strategy to treat patients with AIS in East Asian countries derived from the traditional concept that East Asians had a higher prevalence of sICH than Caucasians patients.²² Despite the Enhanced Control of Hypertension and Thrombolysis Stroke Study (ENCHANTED) did not support low-dose rt-PA to standard-dose rt-PA in predominantly Asian patients (63.2%)¹³ or in Chinese population,²³ small sample data of East Asian countries, as represented by China, Japan and South Korea, did not

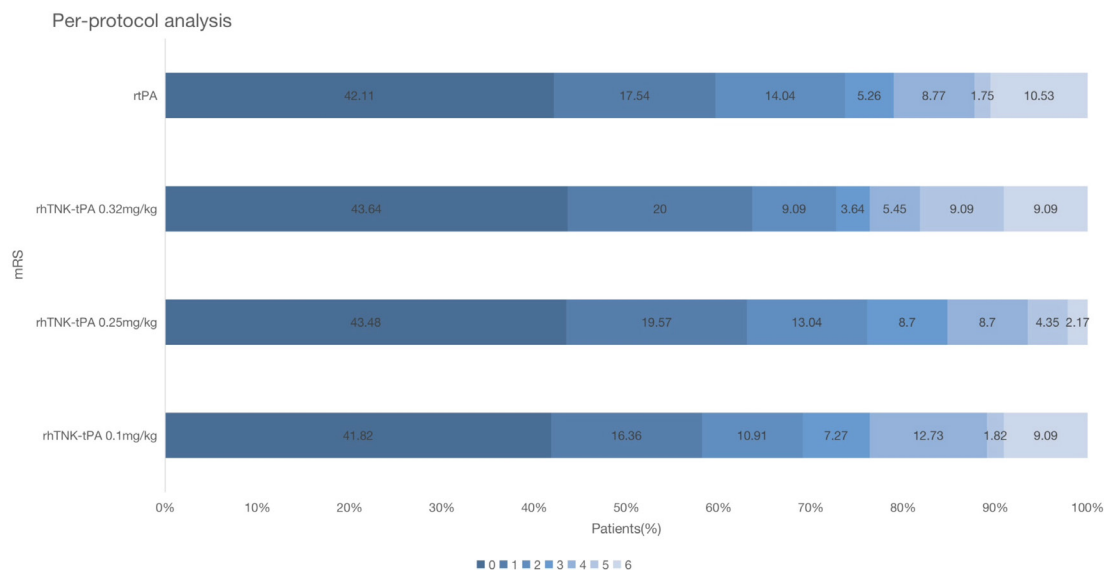


Figure 2 mRS at 90 days in the per-protocol population. mRS, modified Rankin Scale.

coincide with each other.^{14–16} A recently pool analysis of two observational registries demonstrated that low-dose rt-PA was associated with significant reduction of sICH and non-inferior performance in efficacy for moderate stroke patients in China.²⁴ As a target-modified variant of rt-PA, TNK would face the same problem. The data from local population may be required to determine whether TNK used in East Asians is as effective and safe as in Caucasians. The results of current study of rhTNK-tPA in China were comparable to the study of TNK in Caucasians. The 0.25 mg/kg dose tier performed better on improvement of neurological deficits (77.2% vs 66.7% and 62.7%) and excellent functional outcomes (mRS score ≤ 1 , 63.6% vs 62.1% and 59.3%) than other groups, which shall be selected as the study dose in future trials of greater sample sizes. In our study, 3 hours within stroke attack was chosen because rt-PA was not yet approved to use in the 3–4.5 hours window after stroke attack, when our study was conceptualised. During the recruitment stage of our study, the results of the phase III trial of rt-PA used in 3–4.5 hours after AIS were reported and rt-PA received the label for use in 4.5 hours within stroke attack.²⁵ A prospective TRACE II study will investigate the efficacy and safety of rhTNK-tPA for AIS within 4.5 hours.

TNK is a promising agent for AIS treatment.^{26 27} TRACE study gave us the confidence to carry out further investigations of rhTNK-tPA. Two phase III trials (TRACE II and TRACE III) of rhTNK-tPA for injection versus standard medical treatment for AIS within 4.5 hours and for AIS due to LVO with perfusion mismatch up to 24 hours of symptom onset will be launched in the coming year. Other ongoing trials of TNK whose results are highly anticipated include NOR-TEST2 (NCT03854500), TASTE (ACTRN12613000243718), ATTEST2 (NCT02814409), TWIST (NCT03181360) and AcT (NCT03889249).

Limitations

This study had several limitations, such as small sample size, an open-label design and a maximum dose lower than 0.4 mg/kg. Due to the limitation of sample size, the evidence collected from this study was still inadequate for an unambiguous recommendation of optimal dose. Although the maximum dose of rhTNK-tPA is 0.32 mg/kg in our study, the efficacy and safety of rhTNK-tPA seem to be either comparable if not superior to published studies. In addition, NCCT is effective before intravenous thrombolysis for patients with AIS within 4.5 hours of onset according to the guidelines. We did not use advanced imaging to identify LVO at baseline. The upcoming TRACE III trial will assess the effect of rh-TNK for patients with AIS with LVO in the late time window (4.5–24 hours after stroke onset).

CONCLUSION

Our results showed that intravenous rhTNK-tPA, given within 3 hours of symptom onset, is a well-tolerated option in patients with AIS in China. A dose of 0.25 mg/kg may be suggested for future efficacy studies in Caucasians patients with AIS based on the results of contemporary clinical studies. The efficacy dose of rhTNK-tPA in East Asians needs to be investigated with further investigation.

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Competing interests None declared.

Patient consent for publication Not required.

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Data availability statement Data are available on reasonable request.

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Supplementary material

Table 1. Result of quality indicators tested between rhTNK- tPA and Melalyse inside Guangzhou Recolgen Biotech Co.,Ltd

Item	Specification	rhTNK-tPA2017110402	rhTNK-tPA2018120102	rhTNK-tPA2019050102	Metalyse 804823	Metalyse 902235
Potency	Indicated amount%	91	95	97	106	101
Protein content	Indicated amount%	101	99	101	100	96
Purity (electrophoresis)	Monomer content%	95.6	96.0	95.7	①	
Purity (HPLC)	Monomer content%	98.4	98.2	98.5	98.4	99.0
	Aggregates content%	1.5	1.6	1.3	0.9	0.6
	Two-chain content%	4.6	8.8	6.5	41.6	34.5
Molecular size	KD Single-chain	69.2	68.6	67.9	68.2	70.7
	KD Two-chain	35.4	33.8	33.5	32.2	33.7
Type I/ Type II content	Type I%	29.0	29.6	30.3	29.1	29.4

Item	Specification	rhTNK-tPA2017110402	rhTNK-tPA2018120102	rhTNK-tPA2019050102	Metalyse 804823	Metalyse 902235
(Home made)	Type II%	71.0	70.4	69.7	70.9	70.6
Type I/ Type II content(commercial)	Type I%	40.9	41.0	41.2	44.1	42.2
	Type II%	59.1	59.0	58.8	55.9	57.8
Host Cell Protein	%	0.006	0.006	0.008	0.002	0.003
Isoelectric point	/	5.9-6.9	5.8-6.9	6.0-6.9	5.8-7.0	5.9-7.0
Tryptic-peptide HapMap	/	Met specification	Met specification	Met specification	Same with rhTNK-tPA	Same with rhTNK-tPA
PeptideHapMap	/	Met specification	Met specification	Met specification	Same with rhTNK-tPA	Same with rhTNK-tPA
Bacterial endotoxin	IU/mg	<1	<1	<1	<1	<1
Sialic acid	%	98	105	100	82	91
Exogenous DNAresidues	<100pg/vial	Met specification	Met specification	Met specification	Same with rhTNK-tPA	Same with rhTNK-tPA

Item	Specification	rhTNK-tPA201711040 2	rhTNK-tPA2018120 102	rhTNK-tPA201905010 2	Metalyse 804823	Metalyse 902235
Remark:						
①Purity of Metalyse and rhTNK-TPA were tested with electrophoresis method, HapMap of Metalyse is a little bit different from rhTNK-TPA, rhTNK-TPA HapMap has Monomer, aggregates and fragment stripe.						

Table 2. Result of quality indicators between rhTNK- tPA and Melalyse outsourced to external company

Item	Specification	rhTNK-tPA20171104 02	rhTNK-tPA2018120 102	rhTNK-tPA201905010 2	Metalyse 804823	Metalyse 902235
Relative molecular mass (De sugar reduction)	Da	58924.29	58924.39	58924.39	58925.17	58924.78
N-terminal sequence	/	Consistent theoretical sequence (NH ₂ -Ser-Tyr-Gln-Val-Ile-Cys-Arg-Asp-Glu-Lys-Thr-Gln-Met-Ile-Tyr)				
C-terminal sequence	/	Consistent theoretical sequence (VTNYLDWIRDNMRP)				
Post-translational modifications	/	Site of oxidative modification: M13/M207/M455/M490/M525 Site of deamidization: N37/N140/N205/N370/N454/Q475				
Free sulfhydryl	Mol S-H/Mol	0.033	0.019	0.029	0.213	0.222
Glycosylation	N103	100.0%	100.0%	100.0%	100.0%	100.0%

Item	Specification	rhTNK-tPA20171104 02	rhTNK-tPA2018120 102	rhTNK-tPA201905010 2	Metalyse 804823	Metalyse 902235
	N184	45.1%	45.1%	44.2%	51.6%	48.8%
	N448	100.0%	99.9%	99.9%	100.0%	100.0%
	T61 (O suger)	83.2%	83.1%	83.3%	84.5%	84.9%
Monosaccharide (pmol/pmol Protein)	Glucosamine	7.63	7.65	8.70	8.63	7.52
	Galactosamine	<0.5	<0.5	<0.5	<0.5	<0.5
	Galactose	4.86	4.60	4.38	4.73	5.32
	Mannose	5.65	5.32	5.08	5.52	5.36
	Glucose	<0.5	<0.5	<0.5	<0.5	<0.5
	Fucose	2.78	2.57	2.46	3.28	3.01
Circular dichroism (CD)	/	Consistent with Circular dichroism (CD) of Far and near ultraviolet				
	Helix	5.9%	6.1%	6.0%	5.7%	5.9%
	Antiparallel β -pleated sheet	43.1%	43.4%	43.4%	42.1%	42.0%
	Parallel β -pleated sheet	3.4%	3.4%	3.4%	3.3%	3.3%
	Turn	18.9%	19.0%	18.9%	18.8%	19.0%

Item	Specification	rhTNK-tPA20171104 02	rhTNK-tPA2018120 102	rhTNK-tPA201905010 2	Metalyse 804823	Metalyse 902235
	Irregular crimp	31.1%	30.8%	30.9%	31.7%	31.6%
Thermostability	(°C) Hot melt temperature	70.13	70.01	69.88	65.84	65.62
Aggregates	Peak 1	1.65%	1.68%	1.59%	1.48%	0.95%
	Peak 2	98.35%	98.32%	98.41%	98.52%	99.15%
	Peak 3	-	-	-	-	-
	KDPeak 1 molecular size	124.2 (±25.8%)	112.8 (±80.7%)	132.8 (±57.4%)	118.6 (±22.5%)	120.6 (±40.6%)
	KDPeak 2 molecular size	62.3 (±2.3%)	60.5 (±2.1%)	59.8 (±1.8%)	61.2 (±1.7%)	60.8 (±1.2%)
	/	It is supposed that Peak 1 is an aggregate peak of conjugate of monomer and fractured fragment, and Peak 2 is monomer peak.				

Table 3. The participating sites and number of patients enrolled in each site

	Sites	N
1	Linyi People's Hospital	64
2	Baogang Hospital of Inner Mongolia	21
3	General Hospital of Northern Theater Command	20
4	Yantai Yuhuanding Hospital	18

5	The First People's Hospital of Shenyang	18
6	Mei He Kou Central Hospital	15
7	Beijing Tiantan Hospital,Capital,Medical University	13
8	Zhongshan Hospital of Fudan University	12
9	The First Bethune Hospital of JILIN University	12
10	Tangshan Gongren Hospital	8
11	The Third Hospital of HEBEI Medical University	7
12	Huai'an Second People's Hospital	6
13	The First Affiliated Hospital of Zhengzhou University	5
14	Huashan Hospital of Fudan University	4
15	The First Hospital of QIQIHAR	4
16	The First Affiliated Hospital of Jinan University	2
17	The Affiliated Hospital of Guizhou Medical University	2
18	West China Hospital,Sichuan University	2
19	The Ninth People's Hospital of Chongqing	2
20	Hainan General Hospital	2
21	Baotou City Central Hospital	2
22	Luo Yang First People's Hospital	1
Total number		240

Table 4. Inclusion and exclusion criteria

Inclusion criteria
Age is ≥ 18 years
Acute ischemic stroke symptom onset < 3 hours; onset time refers to the time the patient was last known to be well

Ischemic stroke is defined as sudden acute focal neurological impairment with suspected cerebral ischemia, hemorrhage ruled out by NCCT scan
Pre-stroke mRS score ≤ 2 or without history of stroke
Baseline National Institutes of Health Stroke Scale (NIHSS) score between 4 and 26
Signed informed consent
Exclusion Criteria
Absolute contraindications:
● History of severe head trauma or stroke within 3 months
● Suspected subarachnoid hemorrhage
● Arterial puncture at a non-compressible site within the previous 1 week
● History of intracranial hemorrhage
● Intracranial tumor, vascular malformation, or arterial aneurysm
● Recent intracranial or intraspinal surgery
● Systolic blood pressure ≥ 180 mm Hg, or diastolic blood pressure ≥ 100 mm Hg; Increased blood pressure
● Active internal bleeding
● Acute bleeding tendency, including platelet count below $100 \times 10^9/L$ or otherwise
● Heparin treatment was performed within 48 h (APTT exceeded the upper limit of normal range)

<ul style="list-style-type: none"> ● Warfarin has been taken orally , and international normalized ratio (INR) is > 1.7 or prothrombin time (PT) > 15 s
<ul style="list-style-type: none"> ● Anticoagulant drugs such as thrombin inhibitor or Xa factor inhibitor, argatroban (including new anticoagulants with unclear mechanism) are currently being used , and various sensitive laboratory tests are abnormal (such as live) APTT , INR , Platelet count , Serpentine ECT of pulse enzyme setting time ; thrombin time TT or appropriate determination of Xa factor activity)
<ul style="list-style-type: none"> ● Blood glucose < 2.7 mmol/L
<ul style="list-style-type: none"> ● CT showed multilobular infarction (low density > 1 / 3 cerebral hemisphere)
<p>Relative contraindications: The risks and benefits of thrombolysis should be carefully considered and weighed in the following cases (that is, although there is one or more relative contraindications, it is not absolutely impossible to thrombolysis)</p>
<ul style="list-style-type: none"> ● Mild stroke or stroke with rapid improvement of symptoms
<ul style="list-style-type: none"> ● Women in pregnancy
<ul style="list-style-type: none"> ● Symptoms of neurological impairment after seizures
<ul style="list-style-type: none"> ● There have been major surgical operations or serious injuries in the last 2 weeks
<ul style="list-style-type: none"> ● There were gastrointestinal or urinary system bleeding in recent 3 weeks
<ul style="list-style-type: none"> ● History of myocardial infarction within 3 months
<p>Participated in other clinical trials within 30 days before randomization or currently involved in other clinical trials</p>
<p>Pregnant women, nursing mothers , or reluctanc to take effective contraceptive measures during the trial</p>

Known allergy to rhTNK-tPA and/or rt-PA or relevant excipients
Any condition that, in the judgment of the investigator could impose hazards to the patient if study therapy is initiated or affect the participation of the patient in the study
Can not comply with the test program or follow-up requirements

Table 5. Missing data of primary efficacy outcomes

	0.1mg/kg(N=60)	0.25mg/kg(N=57)	0.32mg/kg(N=60)	rt-PA(N=59)
Missing data n(%)	6 (10.00%)	6 (10.53%)	4 (6.67%)	4 (6.78%)
Death	4 (6.67%)	0 (0.00%)	4 (6.67%)	3 (5.08%)
Telephone follow-up	2 (3.33%)	6 (10.53%)	0 (0.00%)	1 (1.69%)

Table 6. Efficacy outcome comparison of TNK groups

	rhTNK-tPA			p value
	0.1mg/kg	0.25mg/kg	0.32mg/kg	
Intention-to-treat analysis				
Improvement on NIHSS at 14 days	38(63.3)	44(77.2)	40(66.7)	0.24

mRS score \leq 1 point at 3 months	33(55.0)	35(63.6)	36(62.1)	0.60
mRS score \leq 2 point at 3 months	41(68.3)	42(76.7)	41(70.7)	0.62
Ordinal distribution of mRS at 3 months	/	/	/	0.71

Per-protocol analysis

Improvement on NIHSS at 14 days	37(67.3)	37(78.7)	39(69.6)	0.41
mRS score \leq 1 point at 3 months	32(58.2)	29(63.0)	35(63.6)	0.81
mRS score \leq 2 point at 3 months	38(69.1)	35(76.1)	40(72.7)	0.73
Ordinal distribution of mRS at 3 months	/	/	/	0.75

Table 7. SAEs within 90 days by system organ class

	rhTNK-tPA			rt-PA
	0.1mg/kg (N=60)	0.25mg/kg (N=57)	0.32mg/kg (N=60)	0.9mg/kg (N=59)
Metabolic and nutritional diseases	1	0	0	0
Infections and infestations	2	1	2	1
Nervous system disorders	3	3	5	5
Injury, poisoning and procedural complications	6	1	0	2
Respiratory, thoracic and mediastinal disorders	1	1	2	2

Systemic disease and administration site reaction	1	1	0	1
Gastrointestinal disorders	1	1	1	0
Cardiac disorders	0	0	1	3
Eye disorders	0	0	1	0
