Supplementary material

Table 1. Result of quality indicators tested between rhTNK- tPA and Melalyse inside Guangzhou Recomgen 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-tPA2017110402	rhTNK-tPA2018120102	rhTNK-tPA2019050102	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-tPA2017110402	rhTNK-tPA2018120102	rhTNK-tPA2019050102	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（NH2-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-tPA2017110402	rhTNK-tPA2018120102	rhTNK-tPA2019050102	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-tPA2017110402	rhTNK-tPA2018120102	rhTNK-tPA2019050102	Metalyse 804823	Metalyse 902235
	Irregular crimp	31.1%	30.8%	30.9%	31.7%	31.6%
Thermostability	（℃）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 Monglia	21
3	General Hospital of Northern Theater Command	20
4	Yantai Yuhuanding Hospitai	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 100×109/L or otherwise
● Heparin treatment was performed within 48 h (APTT exceeded the upper limit of normal range)

● Warfarin has been taken orally , and international normalized ratio (INR) is > 1.7 or prothrombin time (PT) > 15 s
● 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)
● Blood glucose < 2.7 mmol/L
● CT showed multilobular infarction (low density > 1 / 3 cerebral hemisphere)
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)
● Mild stroke or stroke with rapid improvement of symptoms
● Women in pregnancy
● Symptoms of neurological impairment after seizures
● There have been major surgical operations or serious injuries in the last 2 weeks
● There were gastrointestinal or urinary system bleeding in recent 3 weeks
● History of myocardial infarction within 3 months
Participated in other clinical trials within 30 days before randomization or currently involved in other clinical trials
Pregnant women, nursing mothers , or reluctanc to take effective contraceptive measures during the trial

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 ≤ 1 point at 3 months	33(55.0)	35(63.6)	36(62.1)	0.60
mRS score ≤ 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 ≤ 1 point at 3 months	32(58.2)	29(63.0)	35(63.6)	0.81
mRS score ≤ 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 symtem 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