

Supplementary Material

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Supplementary Tables

eTable 1 Reported serious adverse events including deaths

0.25mg/kg Tenecteplase						0.32mg/kg Tenecteplase					
SAE #	Age	Sex	Event description	Category	Causality	SAE #	Age	Sex	Event description	Category	Causality
1	73	Male	Intracranial hemorrhage transformation	Prolongation of hospitalization	Possibly not related	16	76	Male	Left hemiparalysis	Significant disability	Not related
2	84	Male	Death	Death	Possibly not related	17	75	Male	Aspiration pneumonia	Prolongation of hospitalization	Possibly not related
3	57	Female	Left hemiparalysis	Significant disability	Not related	18	68	Male	Right hemiparalysis	Significant disability	Not related
4	76	Male	Intracranial hemorrhage transformation	Death	Related	19	69	Male	Left hemiparalysis	Significant disability	Possibly not related
5	74	Female	Hemorrhagic transformation	Prolongation of hospitalization	Possibly not related	20	73	Female	Right hemiparalysis	Significant disability	Not related

6	74	Female	Pleural Effusion	Prolongation of hospitalization	Possibly not related	21	66	Female	Right hemiparalysis	Significant disability	Possibly not related
7	86	Male	Respiratory Failure	Death	Possibly not related	22	75	Male	Septic shock	Death	Possibly not related
8	68	Female	Left hemiparalysis	Significant disability	Possibly not related	23	80	Female	Venous thrombus embolism in left lower limb	Prolongation of hospitalization	Not related
9	66	Male	Left hemiparalysis	Significant disability	Not related	24	85	Male	Parenchymal hematoma with intraventricular extension	Death	Related
10	75	Female	Left hemiparalysis	Significant disability	Not related	26	76	Male	Parenchymal hematoma with intraventricular extension	Prolongation of hospitalization	Related
11	61	Male	Right hemiparalysis	Significant disability	Not related	26	75	Female	Hemorrhagic transformation	Prolongation of hospitalization	Related
12	58	Female	Large infarct volume with brain edema		Not related	27	76	Male	Venous thrombus embolism in left lower limb	Prolongation of hospitalization	Not related
13	73	Male	Atrial fibrillation	Prolongation of hospitalization	Not related						

14	66	Male	Right hemiparalysis	Significant disability	Not related
15	66	Male	Respiratory Failure	Death	Possibly not related

Abbreviations: SAE Serious adverse events

eTable2 Baseline characteristics of patients with complete artery occlusion

	Tenecteplase 0.25mg/kg (n=35)	Tenecteplase 0.32mg/kg (n=38)
Age , mean (SD), y	67.5 (13.8)	66.3 (11.4)
Male sex , No. (%)	19 (54.3%)	29 (76.3%)
NIHSS score at randomization , median (IQR)	12.0 (10.0, 16.0)	9.0 (5.8, 13.0)
Stroke etiology , No. (%)		
Cardio-embolism	13 (37.1%)	6 (15.8%)
Large artery atherosclerosis	15 (42.9%)	25 (65.8%)
Undetermined etiology	7 (20.0%)	7 (18.4%)
Medical history , No. (%)		
Atrial fibrillation	12 (34.3%)	3 (7.9%)
Hypertension	21 (60.0%)	24 (63.2%)
Diabetes	10 (28.6%)	13 (34.2%)
Smoking	12 (34.3%)	23 (60.5%)
Ischemic stroke or transient ischemic attack	6 (17.1%)	5 (13.2%)
TLSW to randomization , No. (%)		
4.5-12h	22 (62.9%)	24 (63.2%)
12- 24h	13 (37.1%)	14 (36.8%)
Witnessed stroke , No. (%)	17 (48.6%)	25 (65.8%)
Transferred to catheter room , No. (%)	16 (45.7%)	16 (42.1%)
Underwent endovascular treatment , No. (%)	16 (45.7%)	15 (39.5%)
TLSW to hospital arrival , median (IQR), min	447.0 (273.0, 764.0)	513.5 (381.0, 636.8)
TLSW to initiation of intravenous therapy , median (IQR), min	627.0 (470.0, 888.0)	667.5 (546.0, 791.0)
Time from hospital arrival to initiation of intravenous therapy , median (IQR), min	123.0 (93.0, 156.0)	138.0 (109.8, 190.5)

Time from initiation of intravenous thrombolysis to initial angiographic assessment , median (IQR), min ^a	65.0 (45.0, 80.8)	59.5 (41.3, 74.5)
Site of artery occlusion , No. (%)		
Extracranial segment of intracranial carotid artery	3 (8.6%)	6 (15.8%)
Intracranial segment of intracranial carotid artery	3 (8.6%)	3 (7.9%)
First segment of middle cerebral artery	20 (57.1%)	14 (36.8%)
Second segment of middle cerebral artery	6 (17.1%)	8 (21.1%)
Anterior cerebral artery	3 (8.6%)	6 (15.8%)
Tandem occlusion	0 (0.0%)	1 (2.6%)
Hypoperfusion lesion volume at baseline , median (IQR), ml	79.0 (48.0, 104.0)	82.0 (49.8, 121.0)
Ischemic core volume at baseline , median (IQR), ml	9.0 (5.0, 16.0)	8.5 (5.8, 20.5)

^a Tenecteplase 0.25mg/kg: n=16; Tenecteplase 0.32mg/kg: n=16

Abbreviations: SD=standard deviation; IQR=interquartile range; TLSW=time from last-seen-well

eTable 3 Exploratory analysis of primary and secondary efficacy and safety outcomes in patients with complete artery occlusion

	Tenecteplase 0.25mg/kg (n=35)	Tenecteplase 0.32mg/kg (n=38)
Primary outcome	9 (25.7%)	9 (23.7%)
Secondary Outcome		
Efficacy		
Recanalization^a	12 (36.4%)	15 (41.7%)
Infarct growth at 3-5 days, mL^b	25.9 (11.4, 68.7)	23.7 (7.3, 91.1)
Major Neurological Improvement at 24-48 hours^c	4 (12.1%)	7 (18.4%)
Change in NIHSS score at 24-48 hours compared with baseline^c	0.0 (-6.5, 2.5)	0.0 (-3.0, 3.3)
mRS score 0-1 at 90 days	8 (22.9%)	17 (44.7%)
mRS score 0-2 at 90 days	14 (40.0%)	22 (57.9%)
mRS score at 90 days		
0	4 (11.4%)	7 (18.4%)
1	4 (11.4%)	10 (26.3%)
2	6 (17.1%)	5 (13.2%)
3	4 (11.4%)	3 (7.9%)
4	6 (17.1%)	6 (15.8%)
5	5 (14.3%)	5 (13.2%)
6	6 (17.1%)	2 (5.3%)
Safety		
Symptomatic intracranial haemorrhage	4 (11.4%)	4 (10.5%)
Any intracranial haemorrhage	18 (51.4%)	12 (31.6%)
Parenchymal haemorrhage type 2	5 (14.3%)	1 (2.6%)
mRS score 5-6 at 90 days	11 (31.4%)	7 (18.4%)

Systematic Haemorrhage	3 (8.6%)	1 (2.6%)
Barthel Index at 90 days^d	100.0 (50.0, 100.0)	95 (52.5, 100.0)

Data are n (%), median (IQR).

^a tenecteplase 0.25mg/kg: n=33; tenecteplase 0.32mg/kg: n=36

^b tenecteplase 0.25mg/kg: n=29; tenecteplase 0.32mg/kg: n=36

^c tenecteplase 0.25mg/kg: n=33

^d tenecteplase 0.25mg/kg: n=25; tenecteplase 0.32mg/kg: n=36

eTable4 Baseline characteristics of patients without bridging endovascular treatment

	Tenecteplase 0.25mg/kg (n=26)	Tenecteplase 0.32mg/kg (n=26)
Age , mean (SD), y	70.4 (11.0)	67.0 (13.0)
Male sex , No. (%)	15 (57.7%)	19 (73.1%)
NIHSS score at randomization , median (IQR)	10.5 (7.8, 16.0)	8.0 (4.0, 10.8)
Stroke etiology , No. (%)		
Cardio-embolism	10 (38.5%)	5 (19.2%)
Large artery atherosclerosis	12 (46.2%)	15 (57.7%)
Undetermined etiology	4 (15.4%)	6 (23.1%)
Medical history , No. (%)		
Atrial fibrillation	10 (38.5%)	3 (11.5%)
Hypertension	17 (65.4%)	18 (69.2%)
Diabetes	9 (34.6%)	9 (34.6%)
Smoking	9 (34.6%)	15 (57.7%)
Ischemic stroke or transient ischemic attack	4 (15.4%)	3 (11.5%)
TLSW to randomization , No. (%)		
4.5-12h	15 (57.7%)	14 (53.9%)
12- 24h	11 (42.3%)	12 (46.2%)
Witnessed stroke , No. (%)	11 (42.3%)	16 (61.5%)
Transferred to catheter room , No. (%)	0 (0.0%)	1 (3.9%)
TLSW to hospital arrival , median (IQR), min	491.0 (272.8, 796.3)	528.0 (403.5, 637.5)
TLSW to initiation of intravenous therapy , median (IQR), min	652.5 (459.0, 979.8)	730.0 (596.8, 817.0)
Time from hospital arrival to initiation of intravenous therapy , median (IQR), min	153.5 (109.5, 189.8)	153.0 (117.8, 238.3)
Site of artery occlusion , No. (%)		
Extracranial segment of intracranial carotid artery	2 (7.7%)	5 (19.2%)

Intracranial segment of intracranial carotid artery	1 (3.9%)	1 (3.9%)
First segment of middle cerebral artery	12 (46.2%)	6 (23.1%)
Second segment of middle cerebral artery	8 (30.8%)	7 (26.9%)
Anterior cerebral artery	3 (11.5%)	6 (23.1%)
Tandem occlusion	0 (0.0%)	1 (3.9%)
Hypoperfusion lesion volume at baseline , median (IQR), ml	60.5 (46.0, 106.5)	64.0 (41.0, 112.5)
Ischemic core volume at baseline , median (IQR), ml	7.5 (4.0, 16.0)	7.5 (3.5, 16.3)

Abbreviations: SD=standard deviation; IQR=interquartile range; TLSW=time from last-seen-well

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**CHinese Acute tissue-Based imaging selection for
Lysis In Stroke -Tenecteplase
(CHABLIS-T)**

Protocol

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Protocol Version: 1.0

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Protocol Summary

Protocol Title	CHABLIS-T: CHinese Acute tissue-Based imaging selection for Lysis In Stroke -Tenecteplase
Protocol Number	KY2019-474
Development Phase	Phase 2a
Study Population	Acute ischemic stroke patients with large/medium vessel occlusion or severe stenosis presented within 4.5 to 24 hours from last known well, with penumbral mismatch identified by multimodal CT.
Study Objective	To explore the promise of efficacy and safety of different doses of tenecteplase (0.25mg/kg vs. 0.32mg/kg) in Chinese acute ischemic stroke patients with large/medium vessel occlusion or severe stenosis in an extended time window.
Trial Design	<p>The CHABLIS-T trial is a phase IIa, randomized, multicenter, open-label, blinded-endpoint, Simon's two-stage, umbrella design study.</p> <p>Eligible patients will be enrolled and 1:1 randomized into 2 dose group (0.25mg/kg and 0.32mg/kg) of tenecteplase (Guangzhou Recomgen Biotech Co., Ltd). An interim analysis will be conducted when each treatment arm recruits 18 patients. If three or more patients achieve the primary endpoint in either arm, then the study would continue to the 2nd stage, which would enroll an addition of 25 patients in that tenecteplase dose group (resulting in a total of 43 patients in that dose group), otherwise the dose group would be stopped. The intervention dose would be deemed to be of sufficient promise if 8 or more out of 43 patients achieved the primary outcome.</p>
Inclusion criteria	<ol style="list-style-type: none"> 1. Patients presenting with acute ischemic stroke within 4.5-24 hours from time last known well 2. Patients' age \geq 18 years 3. Premorbid modified Rankin Scale 0-2 4. Clinically significant acute neurologic deficit (with no lower or upper limit of the National Institutes of Health Stroke Scale [NIHSS] score) at the discretion of the investigator 5. Anterior large/medium vessel occlusion or severe stenosis ($>70\%$ of the vessel caliber), including extracranial or intracranial ICA, M1 or M2 segment of MCA, and A1 or A2 segment of ACA, identified on head and neck CTA 6. Penumbral mismatch on CTP: Hypoperfusion lesion volume (Delay time[DT]$>$ 3 s) to infarct core volume ratio (relative cerebral blood flow [rCBF]$<$30%) $>$ 1.2, an absolute difference of volume $>$ 10 ml, and an ischemic core volume $<$ 70ml 7. Patient/Legally Authorized Representative has signed the Informed Consent form.

Exclusion criteria	<ol style="list-style-type: none"> 1. Extensive hypoattenuation region (more than 1/3 of the MCA territory) identified on baseline non-contrast CT (NCCT) 2. Any sign of an acute intracranial hemorrhage or subarachnoid hemorrhage identified on baseline NCCT 3. Previous ischemic stroke within 3 months 4. Past history of intracranial hemorrhage 5. Acute head trauma at presentation or recent major head trauma within 3 months 6. Recent history of intracranial/intraspinal surgery within 3 months 7. Gastrointestinal malignancy or gastrointestinal bleed within 21 days 8. Known bleeding diatheses: platelets count <100 000/mm³, INR >1.7, APTT >40 s, or PT >15 s 9. Use of a full dosage of low-molecular weighted heparin treatment within the previous 24 hours 10. Use of direct thrombin inhibitors or direct factor Xa inhibitors within the previous 48 hours unless the laboratory test of coagulation function is normal 11. Symptoms consistent with infective endocarditis 12. Known or suspected with aortic arch dissection 13. Presence of an intra-axial intracranial neoplasm <p style="text-align: center;">In addition to:</p> <ol style="list-style-type: none"> 1. Rapidly improving symptoms at the discretion of the investigator, which may indicate spontaneous recanalization 2. Contraindications for CT contrast precluding a CT angiography or perfusion study. 3. Pregnancy or breastfeeding 4. Recent Participation in another investigational drug or device study or registry in the past 30 days 5. Allergy to the test drug and its ingredients 6. Any terminal illness such that the patient would not be expected to survive more than three months 7. Other conditions in which investigators believe that participating in this study may be harmful to the patient
Investigational drug	Intravenous tenecteplase
Interventions	<p>Patients were randomized in a 1:1 ratio into 2 tenecteplase dose groups, 0.25mg/kg and 0.32 mg/kg. Randomization was performed using permuted blocks through a centralized website. Patients were stratified according to time of randomization after last known well (4.5-12 hours, 12-24 hours) and site of occlusion/severe stenosis (ICA-IC and MCA-M1; ICA-EC, MCA-M2 and ACA).</p> <p>Patients received either dose of intravenous tenecteplase (0.25mg/kg, maximum 25 mg, or 0.32mg/kg, maximum 40mg) as a bolus over 5-10s and a following 2 mL bolus of saline for injection.</p>
Study outcome	<p>Primary outcome</p> <p>The primary outcome is a binary composite of efficacy and safety, i.e. major reperfusion and absence of sICH at 24-48 hours after intravenous tenecteplase.</p> <p>Secondary outcomes</p>

	<ol style="list-style-type: none"> 1. Secondary radiological efficacy outcomes: <ol style="list-style-type: none"> 1) Recanalization defined by the Thrombolysis in Myocardial Infarction (TIMI) scale of 2/3 at the 4- to 6- hour CTA (reconstructed from CTP) for patients not transferred to the catheter room, or at the first digital subtraction angiography run prior to thrombectomy for patients transferred to the catheter room after thrombolysis 2. Secondary clinical efficacy outcomes <ol style="list-style-type: none"> 1) Excellent functional outcome (mRS 0-1) at 90 days 2) Good functional outcome (mRS 0-2) at 90 days 3) mRS distribution at 90 days 4) Neurological improvement at 24-48 hours (NIHSS 0-1 or a NIHSS-improvement of ≥ 8) 5) Change in NIHSS as a continuous variable at 24-48 hours 3. Secondary radiological safety outcomes <ol style="list-style-type: none"> 1) Type 2 parenchymal hematoma at 24-48 hours 2) sICH according to the ECASS II criteria at 24-48 hours 3) Any intracranial hemorrhagic transformation at 24-48 hours post-treatment 4. Secondary clinical safety outcomes <ol style="list-style-type: none"> 1) Poor functional outcome (mRS 5-6) at 90 days 2) Systemic bleeding defined by The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries before discharge 5. Others: <ul style="list-style-type: none"> Barthel Index at 90 days
Primary analysis	<p>For the analysis of the primary endpoint, if more than 2 out of the 18 patients in the first stage of either dosage group reach the primary endpoint, then another additive 25 patients will be enrolled in that group. If more than 7 out of the 43 patients reach the primary endpoint after the second stage, then the tenecteplase dosage could be considered as a qualified candidate dosage for a phase IIb trial.</p> <p>Secondary outcomes will be described using percentages, mean and standard deviation, median and interquartile range (IQR) as appropriate. Normality will be tested using the Shapiro-Wilk test.</p>

1. Introduction

Intravenous thrombolysis using alteplase is recommended as the first-line therapy for acute ischemic stroke (AIS) patients presented within 4.5 hours after stroke onset¹. However, only a very limited number of patients are able to receive the treatment due to its short treatment time window. A comparison study based on the data of China National Stroke Registry (CNSR) and the American Heart Association Get With The Guidelines Stroke Program (GWTG-Stroke Program) has shown that only 2.5% patients in CNSR and 8.1% patients in the GWTG-Stroke Program received intravenous thrombolytic therapy². Therefore, extending the treatment time window for AIS patients has become a hot research topic of clinical trials recently. Additionally, because alteplase acquires 1-hour continuous infusion after bolus and its efficacy can be undermined by any infusion delay and interruption, the necessity for alteplase in patients with large vessel occlusion has been questioned^{3,4} since the effect of endovascular treatment has been validated and acknowledged⁵.

Existence of penumbral mismatch (substantial volume of tissue to salvage) is of great importance to the effect of reperfusion therapy⁶. Recent randomized controlled trials (RCTs) using perfusion imaging to select patients with penumbral mismatch have demonstrated that intravenous thrombolysis may also be beneficial for AIS patients in an extend time window beyond 4.5 hours^{7,8}.

Tenecteplase (TNK) is a mutant variant of alteplase with a longer half-time life and higher fibrin specificity. TNK currently is recommended as the first-line intravenous medication for myocardial infarction with a dose of 0.5mg/kg, and its safety and efficacy in acute ischemic stroke patients have long been studied. The meta-analysis of the 5 RCTs (Haley et al.⁹, Australian TNK¹⁰, ATTEST¹¹, NOR-TEST¹², EXTEND-IA TNK¹³) comparing the effect of TNK and alteplase reveals that though the imaging selection criteria and treatment time window for each trial differ, the dose of 0.25mg/kg and 0.4 mg/kg TNK are non-inferior to alteplase in AIS patients (the noninferiority test of 0.1mg/kg TNK was underpowered due to limited sample size)¹⁴. Furthermore, pooled analyses using data of Australian TNK and ATTEST show that TNK may have superior recanalization efficacy in patients with complete large vessel occlusion to alteplase¹⁵, especially in patients with penumbral mismatch on perfusion imaging¹⁶. EXTEND-IA TNK has proven that for anterior circulation stroke patients with large vessel occlusion and perfusion mismatch, TNK could lead to a significantly higher rate of major reperfusion preceding endovascular treatment and better functional outcome, compared with alteplase in the 4.5-hour time window (rate of major of reperfusion: TNK 22% vs. alteplase 10%; median [IQR] mRS at 90 days: TNK 2[0-3] vs. alteplase 3[1-4]). These previous results implicate that based on the noninferiority of TNK to alteplase in AIS patients without advanced imaging selection, the superiority of TNK to alteplase may emerge in large vessel occlusion patients with perfusion mismatch.

Endovascular treatment has become a routine practice for the treatment of AIS patients with large vessel occlusion¹, and its time window has been extended to 24 hours after last known normal through advanced imaging selection^{17,18}. However, Chinese LVO patients have been faced with the challenge of a longer reperfusion time compared with the Western population¹⁹⁻²³. One of the main reasons may relate to the high prevalence of in-situ thrombosis due to intracranial atherosclerotic disease (ICAD) in Asian patients with acute LVO²⁴, which is refractory to the

current stent retrievers or aspiration systems²⁵. Additionally, the number of comprehensive stroke centers with capability of endovascular treatment is still insufficient given the broad landscape of China, and the drip-and-ship transfer system is still under-developed. Therefore, TNK can be a great candidate for reperfusion therapy considering its performance in large/medium vessel occlusion patients to extend the time window of intravenous thrombolysis, improving the clinical outcome of Chinese AIS patients with large/medium vessel occlusion.

The doses of TNK tested in the previous RCTs in acute ischemic stroke patients are generally 0.1mg/kg, 0.25mg/kg and 0.4mg/kg. Tenecteplase has been approved for the intravenous thrombolysis in myocardial infarction by China Food and Drug Administration (CFDA). The tenecteplase dose proven to be safe during the dose escalation study in Chinese myocardial infarction patients ranges from 0.08mg/kg to 0.32mg/kg. However, the optimal dose of tenecteplase for Chinese acute ischemic stroke patients with large/medium vessel occlusion still remains undetermined. Additionally, Australia TNK¹⁰ demonstrated that 0.25mg/kg TNK was superior to 0.1mg/kg TNK in reperfusion efficacy. Therefore, the doses of 0.25mg/kg and 0.32mg/kg tenecteplase are chosen as candidates of this Phase IIa trial to select an optimal dose of Chinese AIS patients with large/medium vessel occlusion.

Umbrella design is a trial design allowing evaluation of multiple therapies of a single disease without a control group in a minimal sample size, and is frequently applied in trials of oncology therapies²⁶. Based on the umbrella design, Simon's two-stage design is further acknowledged as a simpler and more effective dose selection study method²⁷, especially in oncology trials²⁸⁻³⁰. In order to evaluate the promise of efficacy and safety of 0.25mg/kg and 0.32 mg/kg tenecteplase in AIS patients with large/medium vessel occlusion with a minimal sample size, we therefore adopted the Simon's two stage umbrella design in the CHABLIS-T trial and designed it as a phase IIa, randomized, multicenter, open-label, blinded-endpoint study.

2. Study Objective

To explore the promise of efficacy and safety of different doses of tenecteplase (0.25mg/kg vs. 0.32mg/kg) in Chinese acute ischemic stroke patients with large/medium vessel occlusion in an extended time window.

3. Study Design

3.1 Overall design

The CHABLIS-T trial is a phase IIa, randomized, multicenter, open-label, blinded-endpoint, Simon's two-stage, umbrella design study.

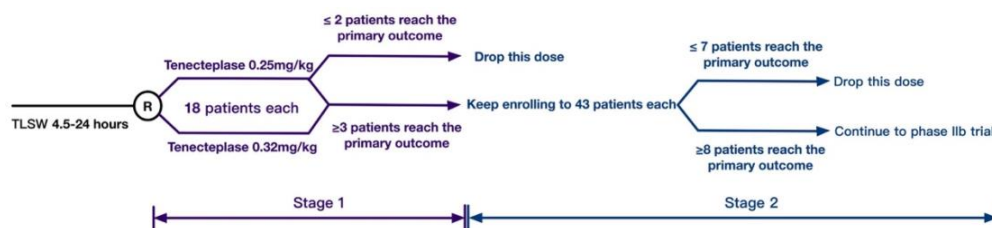
3.2 Investigational Drug

Tenecteplase (Guangzhou Recomgen Biotech Co., Ltd)

3.3 Treatment allocation

Eligible patients will be enrolled and 1:1 randomized into 2 dose group (0.25mg/kg and 0.32mg/kg) of tenecteplase (Guangzhou Recomgen Biotech Co., Ltd). An interim analysis will be conducted when each treatment arm recruits 18 patients. If three or more patients achieve the primary endpoint in either arm, then the study would continue to the second stage, which would enroll an addition of 25 patients in that tenecteplase dose group (resulting in a total of 43 patients

in that dose group), otherwise the dose group would be stopped. The intervention dose would be deemed to be of sufficient promise if 8 or more out of 43 patients achieved the primary outcome.



4. Patient Population

4.1 Inclusion criteria

- 1) Patients presenting with acute ischemic stroke within 4.5-24 hours from time last known well
- 2) Patients' age \geq 18 years
- 3) Premorbid modified Rankin Scale 0-2
- 4) Clinically significant acute neurologic deficit (with no lower or upper limit of the National Institutes of Health Stroke Scale [NIHSS] score) at the discretion of the investigator
- 5) Anterior large/medium vessel occlusion or severe stenosis ($>70\%$ of the vessel caliber), including extracranial or intracranial ICA, M1 or M2 segment of MCA, and A1 or A2 segment of ACA, identified on head and neck CTA
- 6) Penumbra mismatch on CTP: Hypoperfusion lesion volume (Delay time[DT] $>$ 3 s) to infarct core volume ratio (relative cerebral blood flow [rCBF] $<$ 30%) $>$ 1.2, an absolute difference of volume $>$ 10 ml, and an ischemic core volume $<$ 70ml
- 7) Patient/Legally Authorized Representative has signed the Informed Consent form.

4.2 Exclusion criteria

Patients are not allowed to participate if they are presented with any of the following standard intravenous thrombolysis exclusion criteria:

- 1) Extensive hypoattenuation region (more than 1/3 of the MCA territory) identified on baseline non-contrast CT (NCCT)
- 2) Any sign of an acute intracranial hemorrhage or subarachnoid hemorrhage identified on baseline NCCT
- 3) Previous ischemic stroke within 3 months
- 4) Past history of intracranial hemorrhage
- 5) Acute head trauma at presentation or recent major head trauma within 3 months
- 6) Recent history of intracranial/intraspinal surgery within 3 months
- 7) Gastrointestinal malignancy or gastrointestinal bleeding within 21 days
- 8) Known bleeding diatheses: platelets count $<$ 100 000/mm³, INR $>$ 1.7, APTT $>$ 40 s, or PT $>$ 15 s
- 9) Use of a full dosage of low-molecular weighted heparin treatment within the previous 24 hours
- 10) Use of direct thrombin inhibitors or direct factor Xa inhibitors within the previous 48 hours unless the laboratory test of coagulation function is normal

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- 11) Symptoms consistent with infective endocarditis
 - 12) Known or suspected aortic arch dissection
 - 13) Presence with an intra-axial intracranial neoplasm

In addition to:

- 1) Rapidly improving symptoms at the discretion of the investigator, which may indicate spontaneous recanalization
- 2) Contraindications for CT contrast precluding a CT angiography or perfusion study
- 3) Pregnancy or breastfeeding
- 4) Recent participation in another investigational drug or device study or registry in the past 30 days
- 5) Allergy to the test drug and its ingredients
- 6) Any terminal illness such that the patient would not be expected to survive more than three months
- 7) Other conditions in which investigators believe that participating in this study may be harmful to the patient

5. Subject withdrawal, removal and study termination

5.1 Subject withdrawal

Subjects who meet the inclusion criteria but fail to complete the study for some reasons are considered as withdrawers. Subject withdrawal includes two situations where subject withdraws by himself/herself and subjects withdraws by the discretion of investigators.

Subjects have the right to withdraw at any time during the study and are subjected to withdraw with occurrence of any of the following situations:

- 1) Poor study compliance: subjects do not take medication as prescribed or participate in study visits in time, affecting the analysis of efficacy and safety outcomes;
- 2) Suffering from adverse events or serious adverse events and are considered unsuitable to continue participating in the study by the judgement of the investigators;
- 3) Other situations that an investigator decides the subject's participation should be ceased;
- 4) Subjects require to withdraw from the study.

Subjects who withdraw from the study should attend a final withdrawal visit. The date and reason for patient withdrawal should be recorded.

5.2 Subject removal

Subjects should be removed from the study if one of the following situations occurs.

- 1) Subject do not meet one of the inclusion criteria or meet one of the exclusion criteria after enrollment;
- 2) Violation of the principles of concomitant medication according to study protocol;
- 3) Subjects do not receive allocated treatment after enrollment;
- 4) Subjects do not complete any study visits after treatment;
- 5) Subjects with poor compliance that cannot guarantee to follow the prescribed treatment, therefore, affecting the analysis of efficacy and safety outcomes.

5.3 Study early termination

The study may be terminated prematurely by the principal investigator. Reasons include but are not limited to:

- 1) The number and/or severity of adverse events justifies discontinuation of the study
- 2) The number and/or severity of protocol violation justifies discontinuation of the study
- 3) New data become available which raise concern about the safety of the investigational product, so that continuation might cause unacceptable risks to subjects.

After such a decision, the Investigator must contact all participating subjects within two weeks, and written notification must be sent to the Ethics Committee.

6. Sample size calculation

The sample size is calculated based on the results derived from the EXTEND-IA TNK trial: 22% patients of the intravenous tenecteplase group and 10% patients of the intravenous alteplase group reached major reperfusion, and 1% patients in both groups respectively were found to have sICH. To adopt a conservative approach, for each dose stratum within the umbrella design, the null hypothesis was that not more than 10% of patients would achieve a positive primary outcome following the respective dose of tenecteplase, while the alternative hypothesis was that, not less than 25% of patients would achieve a positive primary outcome following the respective dose of tenecteplase. Sample size estimation using Simon's two-stage design was conducted for each dose stratum, according to which the null hypothesis that the true response rate was 10% was tested against a one-sided alternative. In the first stage, 18 patients were to be accrued. If there were 2 or fewer positive responses in these 18 patients, meaning that the null hypothesis was not rejected and the dose stratum was to be stopped. Otherwise, 25 additional patients were to be accrued for a total of 43 patients for each dose stratum. The null hypothesis for each stratum was to be rejected if 8 or more positive responses were observed in 43 patients. This design was to yield a type I error rate of 0.05 and a power of 0.8 when the true response rate was 25%. Overall, at least 36 patients were to be enrolled with a maximum enrollment of 86 patients equally distributed between the 2 dose strata. This design would yield a one-sided type I error of 0.5 and a power of 80% to detect the presumed significant difference. In all, at least 36 patients would be enrolled with a maximum enrollment of 86 patients in sum of the 2 dose strata.

7. Treatment intervention

7.1 Investigational Product

- 1) Investigational product:
Tenecteplase (Guangzhou Recomgen Biotech Co., Ltd)
- 2) Product labelling

Commercial packages of tenecteplase (Guangzhou Recomgen Biotech Co., Ltd) are labelled by the manufacturer, Guangzhou Recomgen Biotech Co., Ltd, with storage conditions (store below 25°C), batch number and expiry date. The study sponsor shall provide supplementary labels which include protocol name and identification number, coordinating Principal Investigator details, sponsor contact details, and the words 'for clinical trial use only'.

3) Administration

Patients will receive either dose of intravenous tenecteplase (0.25mg/kg, maximum 25 mg, or 0.32mg/kg, maximum 32mg) as a bolus over 5-10s and a following 2 mL bolus of saline for injection. Bridging endovascular treatment is optional.

4) Transportation and storage

Investigational products are delivered by the manufacturer Guangzhou Recomgen Biotech Co., Ltd with temperature controlled below 25°C through the whole process of transportation.

Investigational product will be stored below 25°C until use in accordance with manufacturer's instructions.

The investigator or his/her designee must maintain an adequate record regarding the administration of all investigational product within the trial. Used vials of investigational product must be labelled and retained for accountability purposes.

7.2 Randomization and blinding

Randomization is allowed when all the inclusion criteria are confirmed to be met (including signed informed consent by the patient or the proxies) and all the exclusion criteria have been ruled out,. Randomization is performed using permuted blocks through a centralized website named Easy Random Trial (ERT) designed by Chuang Da medical science and Technology (Shanghai) Co., Ltd. by a team of professional and independent statisticians. Patients are stratified according to the time of randomization after last known well (4.5-12 hours, 12-24 hours) and site of occlusion/severe stenosis (intracranial segment of internal carotid artery and M1-segment of middle cerebral artery; extracranial segment of internal carotid artery, M2-segment of middle cerebral artery and anterior cerebral artery). The purpose of randomization in the Phase IIa umbrella-designed trial with multiple strata is not to enable subsequent comparisons between dose strata, but to ensure that participants in both strata are equally representative of the patient population. The dosage of tenecteplase is open-label while the raters involved in the subsequent radiological and clinical evaluation are blinded to the allocation. The independent data safety monitoring board (DSMB) has the access to all the unblinded data.

7.3 Endovascular treatment

If endovascular treatment is consented by the patient or the proxies and is considered feasible at the discretion of the neuro-interventionalists, the patient would be transferred to the interventional catheter suite to receive bridging endovascular treatment after thrombolysis. Thrombectomy will not be considered if hemorrhagic transformation or complete recanalization occurred before any operation of thrombectomy or angioplasty. Before any thrombectomy operation, catheter angiography should be performed to confirm the occlusion site and primary score on the modified Treatment In Cerebral Infarction (mTICI) scale. Maximally 3 passes of thrombectomy operation are allowed for each occluded artery. Digital subtraction angioplasty (DSA) should be performed following each pass and at the completion of the thrombectomy operation. Type of anesthesia should be recorded. Angioplasty and/or stenting are allowed if necessary.

7.4 Concomitant medication

If endovascular treatment is not performed, any drugs that can affect coagulation is prohibited within 24 hours after tenecteplase thrombolysis, including antiplatelet agents, anticoagulation agents, defibrinated agents, thrombolytic agents, any drug that can affect the function of platelets, and any traditional Chinese medicine agents that can affect coagulation.

If only thrombectomy operation is performed during endovascular treatment, any drugs that can affect coagulation is prohibited within 24 hours after tenecteplase thrombolysis, including antiplatelet agents, anticoagulation agents, defibrinated agents, thrombolytic agents, any drug that can affect the function of platelets, and any traditional Chinese medicine agents that can affect coagulation.

If angioplasty and/or stenting is performed during endovascular treatment, antiplatelets and/or anticoagulants are allowed if necessary, within 24 hours after tenecteplase thrombolysis.

Any concomitant treatment is allowed (including antihypertensive treatment) except for the medications mentioned above.

Any prescription and treatment before randomization and during the study should be recorded in the case report form (CRF).

8. Radiological and clinical assessment

8.1 Radiological assessment

Standard multimodal CT (non-contrast head CT, CTP, head and neck CTA) imaging of each potentially eligible patient is acquired before enrollment. Perfusion imaging is real-time processed using fully-automated MISTar (Apollo Medical Imaging Technology, Melbourne, Victoria, Australia) with single value deconvolution with delay and dispersion correction to calculate volumes of hypoperfusion lesion (delay time [Delay Time]>3s) and infarct core (relative cerebral blood flow [rCBF] <30% within areas of DT>3s) at each site.

For patients not transferred to the catheter room after thrombolysis, repeat CT perfusion imaging (and neck CTA for patients with ICA-EC occlusion/severe stenosis) is performed at 4-6 hours after thrombolysis to assess reperfusion. For patients transferred to the catheter room, reperfusion is evaluated as a modified Treatment In Cerebral Ischemia (mTICI) score on the initial catheter angiography. CT angiography is centrally reconstructed from repeat CTP imaging also using MISTar to evaluate recanalization status at 4-6 hours after treatment for patients without catheter angiography. For patients transferred to the catheter room, recanalization status prior to thrombectomy/angioplasty is evaluated also using the first-run imaging of digital subtraction angiography (DSA).

A non-contrast head CT scan is performed at 24-48 hours after thrombolysis to check the occurrence of intracranial hemorrhagic transformation. Diffusion-weighted imaging (DWI) or non-contrast head CT for magnetic resonance (MR)-incompatible patients is performed at 3-5 days to calculate volumes of final infarct core using MISTar.

All of the imaging protocols of multimodal CT at each center will be centrally standardized through careful quality control. All of the imaging were centrally analyzed in a core lab. Baseline multimodal CT imaging will be re-analyzed to make sure that the entry criteria are met. The radiological outcome measurements will be evaluated by two independent and neuroradiologists, and a third independent rater will be consulted in cases of disagreement, who are all blinded to the treatment allocation.

8.2 Clinical assessments

Neurological defects and functional scores are measured by one certified investigator blinded to treatment allocation in each participating center.

- 1) NIHSS is evaluated before randomization, 4-6 hours and 24-48 hours after tenecteplase treatment, and repeated at 3-5 days, 7±1 days (or at discharge), 30±7 days and 90±7 days post treatment.
- 2) Modified Rankin Scale is assessed at 7±1 days (or at discharge), 30±7 days and 90±7 days post treatment through in-person visit or standardized telephone follow-up.
- 3) Barthel Index (BI) is assessed at 7±1 days (or at discharge), 30±7 days and 90±7 days post treatment through in-person visit or standardized telephone follow-up.
- 4) Cognitive evaluation is optional 90±7 days post treatment using MoCA and MMSE through in-person visit.

9. Adverse Events (AEs) and Severe Adverse Events (SAEs)

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or a serious adverse event (SAE) as provided in this protocol. During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs, as detailed in this section of the protocol.

9.1 Definitions of AE and SAE

An Adverse Event (AE) is any untoward medical occurrence in a patient temporarily associated with the use of an investigational product, whether or not considered related to the investigational product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the product, whether or not considered related to the product.

Events belonging to the normal procedure of disease diagnosis and treatment (e.g. rehospitalization for second-stage stenting) are not reported as AEs.

A Serious Adverse Event (SAE) is any untoward medical occurrence that, at any dose, meeting at least one of the following criteria

- 1) Results in death;
- 2) Is life threatening;

Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death if it were more severe.

- 3) Requires hospitalization or prolongation of an existing hospitalization;
- 4) Results in permanent or severe disability;
- 5) Is a congenital abnormality/birth defect;
- 6) Is a significant medical event

Medical and scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. SAEs require immediate report to the ethics committee within 24 hours.

9.2 Recording of AEs/SAEs

- 1) AE

When an AE occurs, the investigator should record all relevant information regarding an AE in to the CRF regardless of its severity and causality with the investigational product, from the moment when the participant signs the informed consent or from the first study visit to the last study visit. The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In the absence of a diagnosis, the individual signs/symptoms should be documented. For each adverse event, start and stop dates, action taken, outcome, intensity and relationship to study product (causality) must be documented.

2) SAEs

Serious Adverse Events require immediate action.

Once an investigator becomes aware that an SAE has occurred, he/she should immediately notify the principal investigator, and fax the AE page in the CRF with all available details of the event and any non-serious AEs related to the SAE, to the principal investigator and the ethics committee, within 24 hours.

9.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to actively follow each subject and provide further information to the Steering Committee on the subject's condition.

All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits/contacts.

All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up. Once resolved, the appropriate AE/SAE CRF page(s) will be updated. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, or consultation with other health care professionals.

New or updated information will be recorded on the originally completed SAE form, with all changes signed and dated by the investigator.

10. Study Plan

Standard multi-modal CT are performed to assess the eligibility of patients, including non-contrast head CT, CTP, head and neck CTA. CTP imaging will be real-time processed by MISTar (Apollo Medical Imaging Technology, Melbourne, Victoria, Australia).

Neurological impairment and functional scores (NIHSS, pre-stroke mRS) will be assessed by a trained stroke neurologist.

Bloods for standard care diagnostic evaluations will be collected and analyzed. Patients will be assessed for trial eligibility according to the Inclusion/Exclusion criteria including a reprocessing of the baseline imaging data for assessment of the occlusion site by the core imaging lab.

An informed consent will be obtained from patients or their authorized representative prior to enrollment.

10.1 Baseline assessments

- 1) Demographic Information: Name, sex, age, ethnics, telephone number, type of health insurance
- 2) Time of last known well, time of symptom recognition and time of hospital arrival

- 3) Risk factor (medical history): hypertension, diabetes mellitus, stroke, transient ischemic attack, coronary heart disease, myocardial infarction, arrhythmia, history of medication (aspirin, clopidogrel, warfarin, antihypertensive drugs, lipid-lowering drugs, antidiabetic drugs, heart rate control drugs), dementia, peripheral artery disease, dyslipidemia, hepatic and /or renal dysfunction, cervical and/intracranial artery stenosis or malformation, smoking, drinking
- 4) Clinical assessments: vital signs (blood pressure, heart rate, respiratory rate and body temperature), NIHSS, and pre-stroke mRS
- 5) Imaging: Patients will have standardized multimodal CT (non-contrast head CT, CTP, head and neck CTA) prior to treatment, as previously described (9.1 Radiological assessments).
- 6) ECG
- 7) Laboratory test (including HCG test for women of reproductive age, routine hematology, biochemistry and coagulation screening tests)
Routine hematology: red blood cell count, mean corpuscular volume, white blood cell count, neutrophil (count, percent), eosinophil (count, percent), basophils (count, percent), lymphocyte (count, percent), monocyte (count, percent), hematocrit, hemoglobin, mean corpuscular hemoglobin, platelet count
Biochemistry: Renal function (creatinine, uric acid, urea nitrogen or urea), hepatic function (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, glutamyl transpeptidase, total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin), glucose
Cardiac markers and pro-BNP: Troponin-T, Myoglobin, CK-MB, NT-pro BNP
Coagulation: PT, APTT, TT, FIB, INR, D-Dimer, fibrinogen degradation products (FDP)
HCG test

10.2 Randomization

Randomization is allowed when all the inclusion criteria are confirmed to be met (including signed informed consent by the patient or the proxies) and all the exclusion criteria have been ruled out. Randomization is performed using permuted blocks through a centralized website named Easy Random Trial (ERT) designed by Chuang Da medical science and Technology (Shanghai) Co., Ltd. by a team of professional and independent statisticians. Patients are stratified according to the time of randomization after last known well (4.5-12 hours, 12-24 hours) and site of occlusion/severe stenosis (intracranial segment of internal carotid artery and M1-segment of middle cerebral artery; extracranial segment of internal carotid artery, M2-segment of middle cerebral artery and anterior cerebral artery). The purpose of randomization in the Phase IIa umbrella-designed trial with multiple strata is not to enable subsequent comparisons between dose strata, but to ensure that participants in both strata are equally representative of the patient population. The dosage of tenecteplase is open-label while the raters involved in the subsequent radiological and clinical evaluation are blinded to the allocation.

10.3 Treatment administration

- 1) Intravenous thrombolysis: Patients will receive intravenous tenecteplase, either 0.25mg/kg (maximum 25mg) or 0.32mg/kg (maximum 32mg), administered as a bolus over 5~10 seconds. Vital signs will be recorded during and after the period of infusion as per standard

care. The time of infusion commencement, completion and the dose administered is recorded.

- 2) Endovascular treatment: Endovascular treatment is optional: If endovascular treatment is consented by the patient or the proxies and is considered feasible at the discretion of the neuro-interventionalists, the patient will be transferred to the interventional catheter suite to receive bridging endovascular treatment after thrombolysis. Thrombectomy will not be considered if hemorrhagic transformation or complete recanalization occurred before any operation of thrombectomy or angioplasty. Before any thrombectomy operation, catheter angiography should be performed to confirm the occlusion site and primary score on the modified Treatment In Cerebral Infarction (mTICI) scale. Maximally 3 passes of thrombectomy operation are allowed for each occluded artery. Digital subtraction angioplasty (DSA) should be performed following each pass and at the completion of the thrombectomy operation. Type of anesthesia should be recorded. Angioplasty and/or stenting are allowed if necessary and may require the use of antiplatelets. Attention should be paid to stabilize blood pressure and minimize delays in starting the procedure. The initial and final angiograms will be centrally graded for angiographic reperfusion using the mTICI classification.

Vital signs and any occurrence of AEs or SAEs during treatment should be documented. Close neurological observation will be conducted primarily during the first 48 hours after treatment administration according to local clinical practice.

10.4 Study visits (Table 1)

- 1) 4-6 hours
 - Vital signs, concomitant medication, NIHSS, new-onset cardio-cerebral vascular events, AEs/SAEs
 - For patients transferred to the catheter room: time of arterial puncture, time of first angiographic run, time of recanalization
 - Imaging:
For patients not transferred to the catheter room: CTP (+neck CTA if stroke is primarily due to severe stenosis/occlusion at ICA-EC)
For patients transferred to the catheter room: DSA
- 2) 24-48 hours
 - Vital signs, concomitant medication, NIHSS, new-onset cardio-cerebral vascular events, AEs/SAEs
 - Imaging: Non-contrast head CT
 - Laboratory test: routine hematology, renal and hepatic function, cardiac markers, cardiac enzymes, pro-BNP, blood lipid, routine urine analysis, coagulation
 - Symptomatic intracranial hemorrhage (ECASS II criteria)
 - Systematic hemorrhage (GUSTO criteria)
- 3) 3-5 days
 - Vital signs, concomitant medication, NIHSS, new-onset cardio-cerebral vascular events, AEs/SAEs

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- Imaging: head MRI (including DWI), head TOF-MRA (+neck MRA if stroke is primarily due to severe stenosis/occlusion at ICA-EC) SWI (optional).
 - Symptomatic intracranial hemorrhage (ECASS II criteria)
 - Systematic hemorrhage (GUSTO criteria)
- 4) 7±1 days (or at discharge)
- Vital signs, concomitant medication, NIHSS, mRS, BI, new-onset cardio-cerebral vascular events, AEs/SAEs
 - Laboratory test: routine hematology, renal and hepatic function, cardiac markers, cardiac enzymes, pro-BNP, blood lipid, routine urine analysis, coagulation
- 5) 30±7 days
- Vital signs, concomitant medication, NIHSS, mRS, BI, new-onset cardio-cerebral vascular events, AEs/SAEs
 - Cognitive tests (optional): MoCA, MMSE
- 6) 90±7 days
- Vital signs, concomitant medication, NIHSS, mRS, BI, new-onset cardio-cerebral vascular events, AEs/SAEs
 - Cognitive tests (optional): MoCA, MMSE

10.5 Study outcomes

1) Primary outcome

The primary outcome is a binary composite of efficacy and safety, i.e. major reperfusion and absence of symptomatic intracranial hemorrhagic transformation (ICH) at 24-48 hours after intravenous tenecteplase. Major reperfusion was considered as the restoration of blood flow of greater than 50% of the involved territory.

- For patients not transferred to the catheter room after thrombolysis, major reperfusion was assessed as reperfusion greater than 50% of the involved baseline hypoperfusion lesion volume (delay time > 3s) on repeat CT perfusion imaging at 4-6 hours after thrombolysis.
- For patients transferred to the catheter room, major reperfusion was evaluated as a modified Treatment In Cerebral Ischemia (mTICI) score 2b/3 at the initial catheter angiography.

Symptomatic ICH was defined according to the European Co-operative Acute Stroke Study-II (ECASS II) criteria, where the patients presented any type of intracranial hemorrhage with no less than 4 point-increase in National Institutes of Health Stroke Scale (NIHSS) from baseline or from the lowest NIHSS between baseline at 24-48 hours, or leading to death.

2) Secondary outcomes

- Secondary radiological efficacy outcomes
 - ✓ Recanalization defined by the Thrombolysis in Myocardial Infarction (TIMI) scale of 2/3 at the 4- to 6- hour CTA (reconstructed from CTP) for patients not transferred to the catheter room, or at digital subtraction angiography imaging prior to thrombectomy for patients transferred to the catheter room after thrombolysis;
 - ✓ Infarct growth on diffusion-weighted imaging (DWI) or non-contrast head CT at 3-5 days.

- Secondary clinical efficacy outcomes
 - ✓ Excellent functional outcome (disability-free, defined as modified Rankin Scale [mRS] 0-1) at 90 days;
 - ✓ Good functional outcome (functional independence, defined as mRS 0-2) at 90 days; mRS distribution at 90 days;
 - ✓ Major neurological improvement at 24-48 hours (NIHSS reduction greater than 7 points or NIHSS 0-1);
 - ✓ Change in NIHSS as a continuous variable at 24-48 hours.
- Secondary radiological safety outcomes
 - ✓ Type 2 parenchymal hematoma (PH2);
 - ✓ Symptomatic ICH;
 - ✓ Any ICH at 24-48 hours post treatment.
- Secondary clinical safety outcomes
 - ✓ Poor functional outcome (severe disability or death, defined as mRS 5-6) at 90 days;
 - ✓ Systemic bleeding defined by The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) before discharge.
- Others:
 - ✓ Barthel index at 90 days.

All of these clinical assessments will be conducted through on-site personnel who are blinded to the treatment allocation.

10.6 Protocol compliance

Study visits before discharge (4-6 hours, 24-48 hours, 3-5 days, 7±1 days or at discharge) will be completed together by a fellow doctor in charge of the treatment patients in hospital and one of the investigators both of whom are blinded to the treatment allocation.

Study visits after discharge (30±7 days, 90±7 days) will be completed through stroke clinic by one of the investigators who is blinded to the treatment allocation. Transportation fee of the participants will be covered by the trial. If the participant is incapable of arrival to the clinic in person due to disability, these visits will be completed through standardized telephone follow-up by a trained investigator who is blinded to the treatment allocation.

11. Data analysis and statistical consideration

11.1 Sample size calculation

The sample size is calculated based on the results derived from the EXTEND-IA TNK trial: 22% patients of the intravenous tenecteplase group and 10% patients of the intravenous alteplase group reached major reperfusion, and 1% patients in both groups respectively were found to have sICH. To adopt a conservative approach, for each dose stratum within the umbrella design, the null hypothesis was that not more than 10% of patients would achieve a positive primary outcome following the respective dose of tenecteplase, while the alternative hypothesis was that, not less than 25% of patients would achieve a positive primary outcome following the respective dose of tenecteplase. Sample size estimation using Simon's two-stage design was conducted for each dose stratum, according to which the null hypothesis that the true response rate was 10% was tested against a one-sided alternative. In the first stage, 18 patients were to be accrued. If

there were 2 or fewer positive responses in these 18 patients, meaning that the null hypothesis was not rejected and the dose stratum was to be stopped. Otherwise, 25 additional patients were to be accrued for a total of 43 patients for each dose stratum. The null hypothesis for each stratum was to be rejected if 8 or more positive responses were observed in 43 patients. This design was to yield a type I error rate of 0.05 and a power of 0.8 when the true response rate was 25%. Overall, at least 36 patients were to be enrolled with a maximum enrollment of 86 patients equally distributed between the 2 dose strata. This design would yield a one-sided type I error of 0.5 and a power of 80% to detect the presumed significant difference. In all, at least 36 patients would be enrolled with a maximum enrollment of 86 patients in sum of the 2 dose strata.

11.2 General principles of data analysis

All of the statistical analysis will be performed on STATA v15.1 (StataCorp, Ltd, College Station, Texas).

11.3 Analysis of primary outcome

For the analysis of primary endpoint, as described above, if 3 or more of the 18 patients in the first stage of either dose stratum reach the primary endpoint, then additional 25 patients would be enrolled in that dose stratum. If 8 or more out of the 43 patients reach the primary endpoint within a given stratum after the second stage, then the corresponding tenecteplase dose could be considered to be of sufficient promise in terms of efficacy and safety and could be a qualified candidate dose for the subsequent phase IIb trial.

11.4 Analysis of secondary outcome

Since the design of this study is not aimed to compare the safety and efficacy of the two tenecteplase doses within the umbrella design, the analysis of secondary outcomes only involved data description without comparative analysis. Secondary outcomes were described using percentages, mean and standard deviation, median and interquartile range (IQR) as appropriate. Normality will be tested using Shapiro-Wilk test.

11.5 Sensitivity analysis

Preplanned sensitivity analysis is conducted by removing patients with severe stenosis and analyzing the primary and secondary outcomes of patients with complete artery occlusion in the two tenecteplase dose groups.

In case of any post-hoc subgroup analysis, they will be justified and identified as data-driven and, they will follow the principles and regulatory recommendations.

12. Ethical and regulatory standards

12.1 Ethical principles

This study will be conducted in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and all applicable amendments laid down by the World Medical Assemblies and the ICH guidelines for Good Clinical Practice.

12.2 Laws and regulations

This study will be conducted in compliance with all international laws and regulations, and Chinese laws and regulations in which the Clinical Trial is performed, as well as any applicable guidelines.

12.3 Informed Consent

The investigator (according to applicable regulatory requirements), or a person designated by the investigator and under the investigator's responsibility, should fully inform the patient of all pertinent aspects of the Clinical Trial including the written information giving approval/favorable opinion by the Ethics Committee (IRB/IEC). All participants should be informed to the fullest extent possible about the study, in language and terms they are able to understand.

Prior to a patient's participation in the CHABLIS-T trial, the written informed consent form should be signed, name filled in and personally dated by the patient or by the patient's legally acceptable representative, and by the person who conducted the informed consent discussion. A copy of the signed and dated written Informed Consent Form will be provided to the patient.

The informed consent form used by the investigator for obtaining the patient's informed consent must be reviewed and approved by the sponsor prior to submission to the appropriate Ethics Committee (IRB/IEC) for approval/favorable opinion.

The protocol will be submitted for approval to the appropriate local institutional ethics committee, and written approval obtained, before participants are recruited and enrolled. The Investigators will receive all the documentation needed for submitting the present protocol to the ethics committee. A copy of the respective approval letters will be transmitted to the Study Monitor before starting the study. The compliance/composition of the Ethics Committee will also be provided to the Study Monitor. If approval is suspended or terminated by the ethics committee, the investigator will notify the Study Monitor immediately.

It is the responsibility of the Investigator to report study progress to the ethics committee as required, or at intervals not greater than one year.

The principal investigator, or his/her nominee, will be responsible for reporting any SAEs to the ethics committee as soon as possible, and in accordance with the guidelines of the Ethics Committee.

12.4 Institutional Review Board/ Independent Ethics Committee (IRB/IEC)

The investigator or the sponsor must submit this protocol to the appropriate ethics committee (IRB/IEC), and is required to forward to the Sponsor a copy of the written and dated approval/favorable opinion signed by the chairman with ethics committee (IRB/IEC) composition.

The clinical trial (study number, Clinical Trial Protocol title and version number), the documents reviewed (clinical trial protocol, informed consent form, investigator's brochure, investigator's CV, etc.), the list of voting members along with their qualification and the date of the review should be clearly stated on the written (IRB/IEC) approval/favorable opinion.

Investigational product will not be released at the study site and the trial will not start until a copy of this written and dated approval/favorable opinion has been received by the Sponsor.

During the study, any amendment or modification to the protocol should be submitted to the Ethics Committee (IRB/IEC). It should also be informed of any event likely to affect the safety of patients or the continued conduct of this trial, in particular any change in safety. All updates to the investigator's brochure will be sent to the ethics committee (IRB/IEC).

If requested, a progress report will be sent to the ethics committee (IRB/IEC) annually and a summary of the trial's outcome at the end of this trial.

13. Study monitoring

13.1 Responsibilities of the investigators

The Investigator(s) undertake(s) to perform the clinical trial in accordance with this protocol, ICH guidelines for Good Clinical Practice and the applicable regulatory requirements.

The investigator is required to ensure compliance with all procedures required by the protocol and with all study procedures provided by the Sponsor (including security rules). The investigator agrees to provide reliable data and all information requested by the Clinical

Trial protocol (with the help of the Case Report Form [CRF], Discrepancy Resolution Form [DRF] or other appropriate instrument) in an accurate and legible manner according to the instructions provided and to ensure direct access to source documents by Sponsor representatives.

The investigator may appoint such other individuals as he/she may deem appropriate as sub-investigators to assist in the conduct of the trial in accordance with the protocol. All sub-investigators shall be appointed and listed in a timely manner. The sub-investigators will be supervised by and under the responsibility of the Investigator. The investigator will provide them with a copy of this protocol and all necessary information.

The Sponsor of this Clinical Trial is responsible to Health Authorities for taking all reasonable steps to ensure the proper conduct of the clinical trial protocol as regards ethics, clinical trial protocol compliance, and integrity and validity of the data recorded on the case report forms. Thus, the main duty of the monitoring team is to help the Investigator and the Sponsor maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the trial.

At regular intervals during the clinical trial, the site will be contacted, through monitoring visits, letters or telephone calls, by a representative of the Monitoring Team to review study progress, Investigator and patient compliance with the protocol requirements and any emergent problems. During these monitoring visits, the following, but not exhaustive, points will be scrutinized with the Investigator: patient informed consent, patient recruitment and follow-up, SAE documentation and reporting, investigational product allocation, patient compliance with the investigational product regimen, investigational product accountability, concomitant therapy use and quality of data.

13.2 Source document requirements

According to the ICH guidelines for Good Clinical Practice, the monitoring team must check the case report form entries against the source documents, except for the pre-identified source data directly recorded in the case report form. The informed consent form will include a statement by which the patient allows the sponsor's duly authorized personnel, the ethics committee (IRS/IEC), and the regulatory authorities to have direct access to source data which support the data on the case report forms (e.g., patient's medical file, appointment books, original laboratory records, etc.). Such personnel, bound by professional secrecy, must keep confidential all personal identity or personal medical information (according to confidentiality rules).

13.3 Data management

Paper-based case report form (CRF) and electronic data capture (EDC) system will be used for data collection and entry. All the content required by the protocol in the system must be provided, the unfilled content should be explained, and the reason needs to be filled in the remarks under each form of the EDC system.

Site investigators should use black or blue-black recording pens to fill out the paper-based CRF neatly and clearly to ensure that the data is clear and readable. If the paper-based CRF information needs to be modified, it should not be altered or overwritten. The correct information should be written next to the original information, signed and dated by the person who modified it. The

clinical research monitor (CRA) will review the completeness and accuracy of the case report form and guide the investigator to make necessary corrections and supplements. After the paper-based CRF is completed, the research coordinator (CRC) will input the content of the paper CRF into the EDC system. The paper-based CRF is submitted after the investigator has approved it. After the data is submitted, all data revisions and feedback are carried out through the EDC system. If the EDC system has submitted a form that needs to be modified, you need to contact the CRA of this center. After the CRA opens the form, the investigator can guide the CRC to modify the EDC system data. Data will then be monitored by the CRA according to a data monitoring plan in a regular basis. After the last study visit of the last participant is finished, the data from the EDC will be exported to a database (in excel sheets), then it will be proofread by the data administrator. Obvious errors will be corrected by the data administrator. Other errors or missing values will be filled in the data query form and returned to the participating center for solution through email, express, telephone and WeChat.

The participating centers are responsible for correcting the data in the EDC system after verifying the original data and related information. Site investigators must answer these queries by verifying or modifying relevant information or data.

13.4 Confidentiality

All of the personal data of research participants (subjects) must be kept strictly confidential. Information and data of the study participants (subjects) are identified only by subject numbers rather than names. Identifiable information of participants (subjects) will not be disclosed to members outside the investigator teams, unless permission is obtained from the research participants (subjects). All of the investigators are required to keep the identities of study participants (subjects) confidential. The files of study participants (subjects) will be kept in locked filing cabinets and will only be accessible to authorized investigators. In order to ensure that the study is carried out in accordance with laws and regulations, government authorities or members of the ethics committee can access to the personal data of the research participants (subjects) according to regulations when necessary. No personal information of the research participants (subjects) will be disclosed at the time of publication of this research.

The research data are also confidential. All of the investigators are required to keep confidential to the research data. They shall not disclose the research data without the permission of the principal investigator to anyone who is not the member of the research team, and shall not transfer the research data to other institute without the permission of the hospital. The research data are not allowed to be disclosed to foreign institutes, or domestic institutes with foreign capital, without the permission of Human Genetic Resource Administration Office of China, except for the publication of research results that meet regulatory requirements under normal circumstances.

14. Funding

The study is sponsored by the National Key Research and Development Program of China (2017YFC1308201), Science and Technology Ministry of China. Guangzhou Recomgen Biotech Co., Ltd. supplied the investigative drug and covered the trial insurance, but had no role in study design and execution.

15. Study Organization

15.1 Principle investigator

Qiang Dong, Huashan Hospital, Fudan University, Shanghai, China

15.2 Trial steering committee members

Qiang Dong (Principal Investigator), Mark Parsons, Xin Cheng, Leonid Churilov, Yilong Wang

- ✓ The steering committee will provide scientific and strategic direction for the trial and will have overall responsibility for its design, execution, and publication.
- ✓ The steering committee will also be responsible for ensuring that study execution and management are of the highest quality.
- ✓ It will approve the protocol and the operational guidelines of the trial prior to its commencement.
- ✓ The steering committee will convene regularly by teleconference or face-to-face meetings to discuss and report on the progress of the study.

15.3 Data safety and monitoring board

The DSMB will meet regularly and monitor the progress of the CHABLIS-T study to ensure that the study meets the highest standards of ethics and patient safety. It is composed of Academic Members, including an independent statistician, who are not otherwise participating in the trial.

Written recommendations and their rationale will be provided to the Chairs of the Steering Committee immediately after each DSMB meeting.

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Table 1 Study Visits in CHABLIS-T

Assessment	Screening and Randomization	Thrombolysis Commencement	Visits					
	-24h~4.5h	0h	4-6h	24-48h	3-5 days	7±1 days or Discharge	30±7 days	90±7days
Demographic	X							
Previous medical	X							
Concomitant	X		X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X
Physical examinations	X							
Pregnancy test ¹	X							
Blood lab tests	X			X				
Electrocardiography	X							
Inclusion and exclusion criteria	X							
Randomization	X							
Imaging (CT/MRI/DSA)	X		X	X	X			
Symptomatic intracranial				X	X			
Modified Rankin Scale	X					X	X	X
NIH Stroke Scale	X		X	X	X	X	X	X
Cognitive evaluations							X	X
Adverse events		X	X	X	X	X	X	X
Barthel Index						X	X	X

1. Only for female patients at reproductive age

**CHinese Acute tissue-Based imaging selection for
Lysis In Stroke -Tenecteplase
(CHABLIS-T)**

Protocol

Research Team: Department of Neurology, Huashan Hospital,
Fudan University, Shanghai, China

Principle Investigator: Qiang Dong, MD, PhD, Professor of
Neurology

Protocol Version: 2.0

Nov. 2019

Catalogue

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Protocol Summary

Protocol Title	CHABLIS-T: CHinese Acute tissue-Based imaging selection for Lysis In Stroke -Tenecteplase
Protocol Number	KY2019-474
Development Phase	Phase 2a
Study Population	Acute ischemic stroke patients with large/medium vessel occlusion or severe stenosis presented within 4.5 to 24 hours from last known well, with penumbral mismatch identified by multimodal CT.
Study Objective	To explore the promise of efficacy and safety of different doses of tenecteplase (0.25mg/kg vs. 0.32mg/kg) in Chinese acute ischemic stroke patients with large/medium vessel occlusion or severe stenosis in an extended time window.
Trial Design	<p>The CHABLIS-T trial is a phase IIa, randomized, multicenter, open-label, blinded-endpoint, Simon's two-stage, umbrella design study.</p> <p>Eligible patients will be enrolled and 1:1 randomized into 2 dose group (0.25mg/kg and 0.32mg/kg) of tenecteplase (Guangzhou Recomen Biotech Co., Ltd). An interim analysis will be conducted when each treatment arm recruits 18 patients. If three or more patients achieve the primary endpoint in either arm, then the study would continue to the 2nd stage, which would enroll an addition of 25 patients in that tenecteplase dose group (resulting in a total of 43 patients in that dose group), otherwise the dose group would be stopped. The intervention dose would be deemed to be of sufficient promise if 8 or more out of 43 patients achieved the primary outcome.</p>
Inclusion criteria	<ol style="list-style-type: none"> 1. Patients presenting with acute ischemic stroke within 4.5-24 hours from time last known well. 2. Patients' age \geq 18 years 3. Premorbid modified Rankin Scale 0-2 4. Clinically significant acute neurologic deficit (with no lower or upper limit of the National Institutes of Health Stroke Scale [NIHSS] score) at the discretion of the investigator 5. Anterior large/medium vessel occlusion or severe stenosis ($>70\%$ of the vessel caliber), including extracranial or intracranial ICA, M1 or M2 segment of MCA, and A1 or A2 segment of ACA, identified on head and neck CTA

	<p>6. Penumbra mismatch on CTP: Hypoperfusion lesion volume (Delay time[DT]> 3 s) to infarct core volume ratio (relative cerebral blood flow [rCBF]<30%) > 1.2, an absolute difference of volume > 10 ml, and an ischemic core volume < 70ml</p> <p>7. Patient/Legally Authorized Representative has signed the Informed Consent form.</p>
Exclusion criteria	<p>14. Extensive hypoattenuation region (more than 1/3 of the MCA territory) identified on baseline non-contrast CT (NCCT)</p> <p>15. Any sign of an acute intracranial hemorrhage or subarachnoid hemorrhage identified on baseline NCCT</p> <p>16. Previous ischemic stroke within 3 months</p> <p>17. Past history of intracranial hemorrhage</p> <p>18. Acute head trauma at presentation or recent major head trauma within 3 months</p> <p>19. Recent history of intracranial/intraspinal surgery within 3 months</p> <p>20. Gastrointestinal malignancy or gastrointestinal bleed within 21 days</p> <p>21. Known bleeding diatheses: platelets count <100 000/mm³, INR >1.7, APTT >40 s, or PT >15 s</p> <p>22. Use of a full dosage of low-molecular weighted heparin treatment within the previous 24 hours</p> <p>23. Use of direct thrombin inhibitors or direct factor Xa inhibitors within the previous 48 hours unless the laboratory test of coagulation function is normal</p> <p>24. Symptoms consistent with infective endocarditis</p> <p>25. Known or suspected with aortic arch dissection</p> <p>26. Presence of an intra-axial intracranial neoplasm</p> <p>In addition to:</p> <p>1. Rapidly improving symptoms at the discretion of the investigator, which may indicate spontaneous recanalization</p> <p>2. Contraindications for CT contrast precluding a CT angiography or perfusion study.</p> <p>3. Pregnancy or breastfeeding</p> <p>4. Recent Participation in another investigational drug or device study or registry in the past 30 days</p> <p>5. Allergy to the test drug and its ingredients</p> <p>6. Any terminal illness such that the patient would not be expected to survive more than three months</p> <p>7. Other conditions in which investigators believe that participating in this study may be harmful to the patient</p>
Investigational drug	Intravenous Tenecteplase
Interventions	<p>Patients were randomized in a 1:1 ratio into 2 tenecteplase dose groups, 0.25mg/kg and 0.32 mg/kg. Randomization was performed using permuted blocks through a centralized website. Patients were stratified according to time of randomization after last known well (4.5-12 hours, 12-24 hours) and site of occlusion/severe stenosis (ICA-IC and MCA-M1; ICA-EC, MCA-M2 and ACA).</p> <p>Patients received either dose of intravenous tenecteplase (0.25mg/kg, maximum 25 mg, or 0.32mg/kg, maximum 40mg) as a bolus over 5-10s and a following 2 mL bolus of saline for injection.</p>

<p>Study outcome</p>	<p>Primary outcome</p> <p>The primary outcome is a binary composite of efficacy and safety, i.e. major reperfusion and absence of sICH at 24-48 hours after intravenous tenecteplase.</p> <p>Secondary outcomes</p> <ol style="list-style-type: none"> 1. Secondary radiological efficacy outcomes: 2) Recanalization defined by the Thrombolysis in Myocardial Infarction (TIMI) scale of 2/3 at the 4- to 6- hour CTA (reconstructed from CTP) for patients not transferred to the catheter room, or at the first digital subtraction angiography run prior to thrombectomy for patients transferred to the catheter room after thrombolysis 2. Secondary clinical efficacy outcomes <ol style="list-style-type: none"> 1) Excellent functional outcome (mRS 0-1) at 90 days 2) Good functional outcome (mRS 0-2) at 90 days 3) mRS distribution at 90 days 4) Neurological improvement at 24-48 hours (NIHSS 0-1 or a NIHSS-improvement of ≥ 8) 5) Change in NIHSS as a continuous variable at 24-48 hours 3. Secondary radiological safety outcomes <ol style="list-style-type: none"> 1) Type 2 parenchymal hematoma at 24-48 hours 2) sICH according to the ECASS II criteria at 24-48 hours 3) Any intracranial hemorrhagic transformation at 24-48 hours post-treatment 4. Secondary clinical safety outcomes <ol style="list-style-type: none"> 1) Poor functional outcome (mRS 5-6) at 90 days 2) Systemic bleeding defined by The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries before discharge 5. Others: <p>Barthel Index at 90 days</p>
<p>Primary analysis</p>	<p>For the analysis of the primary endpoint, if more than 2 out of the 18 patients in the first stage of either dosage group reach the primary endpoint, then another additive 25 patients will be enrolled in that group. If more than 7 out of the 43 patients reach the primary endpoint after the second stage, then the tenecteplase dosage could be considered as a qualified candidate dosage for a phase IIb trial.</p> <p>Secondary outcomes will be described using percentages, mean and standard deviation, median and interquartile range (IQR) as appropriate. Normality will be tested using the Shapiro-Wilk test</p>

1. Introduction

Intravenous thrombolysis using alteplase is recommended as the first-line therapy for acute ischemic stroke (AIS) patients presented within 4.5 hours after stroke onset¹. However, only a very limited number of patients are able to receive the treatment due to its short treatment time window. A comparison study based on the data of China National Stroke Registry (CNSR) and the American Heart Association Get With The Guidelines Stroke Program (GWTG-Stroke Program) has shown that only 2.5% patients in CNSR and 8.1% patients in the GWTG-Stroke Program received intravenous thrombolytic therapy². Therefore, extending the treatment time window for AIS patients has become a hot research topic of clinical trials recently. Additionally, because alteplase acquires 1-hour continuous infusion after bolus and its efficacy can be undermined by any infusion delay and interruption, the necessity for alteplase in patients with large vessel occlusion has been questioned^{3,4} since the effect of endovascular treatment has been validated and acknowledged⁵.

Existence of penumbral mismatch (substantial volume of tissue to salvage) is of great importance to the effect of reperfusion therapy⁶. Recent randomized controlled trials (RCTs) using perfusion imaging to select patients with penumbral mismatch have demonstrated that intravenous thrombolysis may also be beneficial for AIS patients in an extend time window beyond 4.5 hours^{7,8}.

Tenecteplase (TNK) is a mutant variant of alteplase with a longer half-time life and higher fibrin specificity. TNK currently is recommended as the first-line intravenous medication for myocardial infarction with a dose of 0.5mg/kg, and its safety and efficacy in acute ischemic stroke patients have long been studied. The meta-analysis of the 5 RCTs (Haley et al.⁹, Australian TNK¹⁰, ATTEST¹¹, NOR-TEST¹², EXTEND-IA TNK¹³) comparing the effect of TNK and alteplase reveals that though the imaging selection criteria and treatment time window for each trial differ, the dose of 0.25mg/kg and 0.4 mg/kg TNK are non-inferior to alteplase in AIS patients (the noninferiority test of 0.1mg/kg TNK was underpowered due to limited sample size)¹⁴. Furthermore, pooled analyses using data of Australian TNK and ATTEST show that TNK may have superior recanalization efficacy in patients with complete large vessel occlusion to alteplase¹⁵, especially in patients with penumbral mismatch on perfusion imaging¹⁶. EXTEND-IA TNK has proven that for anterior circulation stroke patients with large vessel occlusion and perfusion mismatch, TNK could lead to a significantly higher rate of major reperfusion preceding endovascular treatment and better functional outcome, compared with alteplase in the 4.5-hour time window (rate of major reperfusion: TNK 22% vs. alteplase 10%; median [IQR] mRS at 90 days: TNK 2[0-3] vs. alteplase 3[1-4]). These previous results implicate that based on the noninferiority of TNK to alteplase in AIS patients without advanced imaging selection, the superiority of TNK to alteplase may emerge in large vessel occlusion patients with perfusion mismatch.

Endovascular treatment has become a routine practice for the treatment of AIS patients with large vessel occlusion¹, and its time window has been extended to 24 hours after last known normal through advanced imaging selection^{17,18}. However, Chinese LVO patients have been faced with the challenge of a longer reperfusion time compared with the Western population¹⁹⁻²³. One of the main reasons may relate to the high prevalence of in-situ thrombosis due to intracranial atherosclerotic disease (ICAD) in Asian patients with acute LVO²⁴, which is refractory to the

current stent retrievers or aspiration systems²⁵. Additionally, the number of comprehensive stroke centers with capability of endovascular treatment is still insufficient given the broad landscape of China, and the drip-and-ship transfer system is still under-developed. Therefore, TNK can be a great candidate for reperfusion therapy considering its performance in large/medium vessel occlusion patients to extend the time window of intravenous thrombolysis, improving the clinical outcome of Chinese AIS patients with large/medium vessel occlusion.

The doses of TNK tested in the previous RCTs in acute ischemic stroke patients are generally 0.1mg/kg, 0.25mg/kg and 0.4mg/kg. Tenecteplase has been approved for the intravenous thrombolysis in myocardial infarction by China Food and Drug Administration (CFDA). The tenecteplase dose proven to be safe during the dose escalation study in Chinese myocardial infarction patients ranges from 0.08mg/kg to 0.32mg/kg. However, the optimal dose of tenecteplase for Chinese acute ischemic stroke patients with large/medium vessel occlusion still remains undetermined. Additionally, Australia TNK¹⁰ demonstrated that 0.25mg/kg TNK was superior to 0.1mg/kg TNK in reperfusion efficacy. Therefore, the doses of 0.25mg/kg and 0.32mg/kg tenecteplase are chosen as candidates of this Phase IIa trial to select an optimal dose of Chinese AIS patients with large/medium vessel occlusion.

Umbrella design is a trial design allowing evaluation of multiple therapies of a single disease without a control group in a minimal sample size, and is frequently applied in trials of oncology therapies²⁶. Based on the umbrella design, Simon's two-stage design is further acknowledged as a simpler and more effective dose selection study method²⁷, especially in oncology trials²⁸⁻³⁰. In order to evaluate the promise of efficacy and safety of 0.25mg/kg and 0.32 mg/kg tenecteplase in AIS patients with large/medium vessel occlusion with a minimal sample size, we therefore adopted the Simon's two stage umbrella design in the CHABLIS-T trial and designed it as a phase IIa, randomized, multicenter, open-label, blinded-endpoint study.

2. Study Objective

To explore the promise of efficacy and safety of different doses of tenecteplase (0.25mg/kg vs. 0.32mg/kg) in Chinese acute ischemic stroke patients with large/medium vessel occlusion or severe stenosis in an extended time window.

3. Study Design

3.1 Overall design

The CHABLIS-T trial is a phase IIa, randomized, multicenter, open-label, blinded-endpoint, Simon's two-stage, umbrella design study.

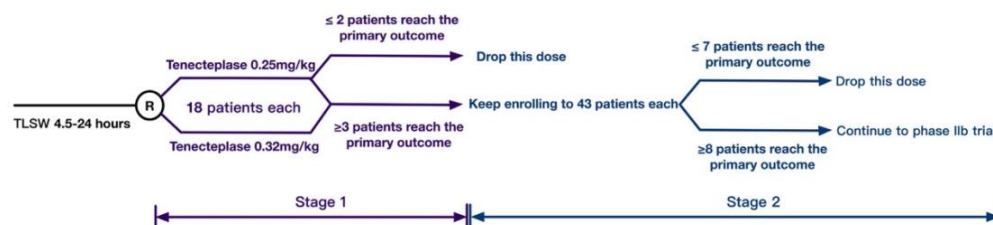
3.2 Investigational Drug

Tenecteplase (Guangzhou Recomgen Biotech Co., Ltd)

3.3 Treatment allocation

Eligible patients will be enrolled and 1:1 randomized into 2 dose group (0.25mg/kg and 0.32mg/kg) of tenecteplase (Guangzhou Recomgen Biotech Co., Ltd). An interim analysis will be conducted when each treatment arm recruits 18 patients. If three or more patients achieve the primary endpoint in either arm, then the study would continue to the second stage, which would enroll an addition of 25 patients in that tenecteplase dose group (resulting in a total of 43 patients

in that dose group), otherwise the dose group would be stopped. The intervention dose would be deemed to be of sufficient promise if 8 or more out of 43 patients achieved the primary outcome.



4. Patient Population

4.1 Inclusion criteria

- 1) Patients presenting with acute ischemic stroke within 4.5-24 hours from time last known well
- 2) Patients' age \geq 18 years
- 3) Premorbid modified Rankin Scale 0-2
- 4) Clinically significant acute neurologic deficit (with no lower or upper limit of the National Institutes of Health Stroke Scale [NIHSS] score) at the discretion of the investigator
- 5) Anterior large/medium vessel occlusion or severe stenosis ($>70\%$ of the vessel caliber), including extracranial or intracranial ICA, M1 or M2 segment of MCA, and A1 or A2 segment of ACA, identified on head and neck CTA
- 6) Penumbra mismatch on CTP: Hypoperfusion lesion volume (Delay time[DT] $>$ 3 s) to infarct core volume ratio (relative cerebral blood flow [rCBF] $<$ 30%) $>$ 1.2, an absolute difference of volume $>$ 10 ml, and an ischemic core volume $<$ 70ml
- 7) Patient/Legally Authorized Representative has signed the Informed Consent form.

4.2 Exclusion criteria

Patients are not allowed to participate if they are presented with any of the following standard intravenous thrombolysis exclusion criteria:

- 1) Extensive hypoattenuation region (more than 1/3 of the MCA territory) identified on baseline non-contrast CT (NCCT)
- 2) Any sign of an acute intracranial hemorrhage or subarachnoid hemorrhage identified on baseline NCCT
- 3) Previous ischemic stroke within 3 months
- 4) Past history of intracranial hemorrhage
- 5) Acute head trauma at presentation or recent major head trauma within 3 months
- 6) Recent history of intracranial/intraspinal surgery within 3 months
- 7) Gastrointestinal malignancy or gastrointestinal bleeding within 21 days
- 8) Known bleeding diatheses: platelets count $<$ 100 000/mm³, INR $>$ 1.7, APTT $>$ 40 s, or PT $>$ 15 s
- 9) Use of a full dosage of low-molecular weighted heparin treatment within the previous 24 hours

-
- 10) Use of direct thrombin inhibitors or direct factor Xa inhibitors within the previous 48 hours unless the laboratory test of coagulation function is normal
 - 11) Symptoms consistent with infective endocarditis
 - 12) Known or suspected aortic arch dissection
 - 13) Presence with an intra-axial intracranial neoplasm

In addition to:

- 1) Rapidly improving symptoms at the discretion of the investigator, which may indicate spontaneous recanalization
- 2) Contraindications for CT contrast precluding a CT angiography or perfusion study
- 3) Pregnancy or breastfeeding
- 4) Recent participation in another investigational drug or device study or registry in the past 30 days
- 5) Allergy to the test drug and its ingredients
- 6) Any terminal illness such that the patient would not be expected to survive more than three months
- 7) Other conditions in which investigators believe that participating in this study may be harmful to the patient

5. Subject withdrawal, removal and study termination

5.1 Subject withdrawal

Subjects who meet the inclusion criteria but fail to complete the study for some reasons are considered as withdrawers. Subject withdrawal includes two situations where subject withdraws by himself/herself and subjects withdraws by the discretion of investigators.

Subjects have the right to withdraw at any time during the study and are subjected to withdraw with occurrence of any of the following situations:

- 1) Poor study compliance: subjects do not take medication as prescribed or participate in study visits in time, affecting the analysis of efficacy and safety outcomes;
- 2) Suffering from adverse events or serious adverse events and are considered unsuitable to continue participating in the study by the judgement of the investigators;
- 3) Other situations that an investigator decides the subject's participation should be ceased;
- 4) Subjects require to withdraw from the study.

Subjects who withdraw from the study should attend a final withdrawal visit. The date and reason for patient withdrawal should be recorded.

5.2 Subject removal

Subjects should be removed from the study if one of the following situations occurs.

- 1) Subject do not meet one of the inclusion criteria or meet one of the exclusion criteria after enrollment;
- 2) Violation of the principles of concomitant medication according to study protocol;
- 3) Subjects do not receive allocated treatment after enrollment;
- 4) Subjects do not complete any study visits after treatment;

- 5) Subjects with poor compliance that cannot guarantee to follow the prescribed treatment, therefore, affecting the analysis of efficacy and safety outcomes.

5.3 Study early termination

The study may be terminated prematurely by the principal investigator. Reasons include but are not limited to:

- 1) The number and/or severity of adverse events justifies discontinuation of the study
- 2) The number and/or severity of protocol violation justifies discontinuation of the study
- 3) New data become available which raise concern about the safety of the investigational product, so that continuation might cause unacceptable risks to subjects.

After such a decision, the Investigator must contact all participating subjects within two weeks, and written notification must be sent to the Ethics Committee.

6. Sample size calculation

The sample size is calculated based on the results derived from the EXTEND-IA TNK trial: 22% patients of the intravenous tenecteplase group and 10% patients of the intravenous alteplase group reached major reperfusion, and 1% patients in both groups respectively were found to have sICH. To adopt a conservative approach, for each dose stratum within the umbrella design, the null hypothesis was that not more than 10% of patients would achieve a positive primary outcome following the respective dose of tenecteplase, while the alternative hypothesis was that, not less than 25% of patients would achieve a positive primary outcome following the respective dose of tenecteplase. Sample size estimation using Simon's two-stage design was conducted for each dose stratum, according to which the null hypothesis that the true response rate was 10% was tested against a one-sided alternative. In the first stage, 18 patients were to be accrued. If there were 2 or fewer positive responses in these 18 patients, meaning that the null hypothesis was not rejected and the dose stratum was to be stopped. Otherwise, 25 additional patients were to be accrued for a total of 43 patients for each dose stratum. The null hypothesis for each stratum was to be rejected if 8 or more positive responses were observed in 43 patients. This design was to yield a type I error rate of 0.05 and a power of 0.8 when the true response rate was 25%. Overall, at least 36 patients were to be enrolled with a maximum enrollment of 86 patients equally distributed between the 2 dose strata. This design would yield a one-sided type I error of 0.5 and a power of 80% to detect the presumed significant difference. In all, at least 36 patients would be enrolled with a maximum enrollment of 86 patients in sum of the 2 dose strata.

7. Treatment intervention

7.1 Investigational Product

- 1) Investigational product:

Tenecteplase (Guangzhou Recomgen Biotech Co., Ltd)

- 2) Product labelling

Commercial packages of tenecteplase (Guangzhou Recomgen Biotech Co., Ltd) are labelled by the manufacturer, Guangzhou Recomgen Biotech Co., Ltd, with storage conditions (store below 25°C), batch number and expiry date. The study sponsor shall provide supplementary labels which include protocol name and identification number, coordinating

Principal Investigator details, sponsor contact details, and the words ‘for clinical trial use only’.

3) Administration

Patients will receive either dose of intravenous tenecteplase (0.25mg/kg, maximum 25 mg, or 0.32mg/kg, maximum 32mg) as a bolus over 5-10s and a following 2 mL bolus of saline for injection. Bridging endovascular treatment is optional.

4) Transportation and storage

Investigational products are delivered by the manufacturer Guangzhou Recolgen Biotech Co., Ltd with temperature controlled below 25°C through the whole process of transportation.

Investigational product will be stored below 25°C until use in accordance with manufacturer’s instructions.

The investigator or his/her designee must maintain an adequate record regarding the administration of all investigational product within the trial. Used vials of investigational product must be labelled and retained for accountability purposes.

7.2 Randomization and blinding

Randomization is allowed when all the inclusion criteria are confirmed to be met (including signed informed consent by the patient or the proxies) and all the exclusion criteria have been ruled out. Randomization is performed using permuted blocks through a centralized website named Easy Random Trial (ERT) designed by Chuang Da medical science and Technology (Shanghai) Co., Ltd. by a team of professional and independent statisticians. Patients are stratified according to the time of randomization after last known well (4.5-12 hours, 12-24 hours) and site of occlusion/severe stenosis (intracranial segment of internal carotid artery and M1-segment of middle cerebral artery; extracranial segment of internal carotid artery, M2-segment of middle cerebral artery and anterior cerebral artery). The purpose of randomization in the Phase IIa umbrella-designed trial with multiple strata is not to enable subsequent comparisons between dose strata, but to ensure that participants in both strata are equally representative of the patient population. The dosage of tenecteplase is open-label while the raters involved in the subsequent radiological and clinical evaluation are blinded to the allocation. The independent data safety monitoring board (DSMB) has the access to all the unblinded data.

7.3 Endovascular treatment

If endovascular treatment is consented by the patient or the proxies and is considered feasible at the discretion of the neuro-interventionalists, the patient would be transferred to the interventional catheter suite to receive bridging endovascular treatment after thrombolysis. Thrombectomy will not be considered if hemorrhagic transformation or complete recanalization occurred before any operation of thrombectomy or angioplasty. Before any thrombectomy operation, catheter angiography should be performed to confirm the occlusion site and primary score on the modified Treatment In Cerebral Infarction (mTICI) scale. Maximally 3 passes of thrombectomy operation are allowed for each occluded artery. Digital subtraction angioplasty (DSA) should be performed following each pass and at the completion of the thrombectomy operation. Type of anesthesia should be recorded. Angioplasty and/or stenting are allowed if necessary.

7.4 Concomitant medication

If endovascular treatment is not performed, any drugs that can affect coagulation is prohibited within 24 hours after tenecteplase thrombolysis, including antiplatelet agents, anticoagulation

agents, defibrinated agents, thrombolytic agents, any drug that can affect the function of platelets, and any traditional Chinese medicine agents that can affect coagulation.

If only thrombectomy operation is performed during endovascular treatment, any drugs that can affect coagulation is prohibited within 24 hours after tenecteplase thrombolysis, including antiplatelet agents, anticoagulation agents, defibrinated agents, thrombolytic agents, any drug that can affect the function of platelets, and any traditional Chinese medicine agents that can affect coagulation.

If angioplasty and/or stenting is performed during endovascular treatment, antiplatelets and/or anticoagulants are allowed if necessary, within 24 hours after tenecteplase thrombolysis.

Any concomitant treatment is allowed (including antihypertensive treatment) except for the medications mentioned above.

Any prescription and treatment before randomization and during the study should be recorded in the case report form (CRF).

8. Radiological and clinical assessment

8.1 Radiological assessment

Standard multimodal CT (non-contrast head CT, CTP, head and neck CTA) imaging of each potentially eligible patient is acquired before enrollment. Perfusion imaging is real-time processed using fully-automated MISar (Apollo Medical Imaging Technology, Melbourne, Victoria, Australia) with single value deconvolution with delay and dispersion correction to calculate volumes of hypoperfusion lesion (delay time [Delay Time] >3 s) and infarct core (relative cerebral blood flow [rCBF] $<30\%$ within areas of DT >3 s) at each site.

For patients not transferred to the catheter room after thrombolysis, repeat CT perfusion imaging (and neck CTA for patients with ICA-EC occlusion/severe stenosis) is performed at 4-6 hours after thrombolysis to assess reperfusion. For patients transferred to the catheter room, reperfusion is evaluated as a modified Treatment In Cerebral Ischemia (mTICI) score on the initial catheter angiography. CT angiography is centrally reconstructed from repeat CTP imaging also using MISar to evaluate recanalization status at 4-6 hours after treatment for patients without catheter angiography. For patients transferred to the catheter room, recanalization status prior to thrombectomy/angioplasty is evaluated also using the first-run imaging of digital subtraction angiography (DSA).

A non-contrast head CT scan is performed at 24-48 hours after thrombolysis to check the occurrence of intracranial hemorrhagic transformation. Diffusion-weighted imaging (DWI) or non-contrast head CT for magnetic resonance (MR)-incompatible patients is performed at 3-5 days to calculate volumes of final infarct core using MISar.

All of the imaging protocols of multimodal CT at each center will be centrally standardized through careful quality control. All of the imaging were centrally analyzed in a core lab. Baseline multimodal CT imaging will be re-analyzed to make sure that the entry criteria are met. The radiological outcome measurements will be evaluated by two independent and neuroradiologists, and a third independent rater will be consulted in cases of disagreement, who are all blinded to the treatment allocation.

8.2 Clinical assessments

Neurological defects and functional scores are measured by one certified investigator blinded to treatment allocation in each participating center.

- 1) NIHSS is evaluated before randomization, 4-6 hours and 24-48 hours after tenecteplase treatment, and repeated at 3-5 days, 7±1 days (or at discharge), 30±7 days and 90±7 days post treatment.
- 2) Modified Rankin Scale is assessed at 7±1 days (or at discharge), 30±7 days and 90±7 days post treatment through in-person visit or standardized telephone follow-up.
- 3) Barthel Index (BI) is assessed at 7±1 days (or at discharge), 30±7 days and 90±7 days post treatment through in-person visit or standardized telephone follow-up.
- 4) Cognitive evaluation is optional 90±7 days post treatment using MoCA and MMSE through in-person visit.

9. Adverse Events (AEs) and Severe Adverse Events (SAEs)

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or a serious adverse event (SAE) as provided in this protocol. During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs, as detailed in this section of the protocol.

9.1 Definitions of AE and SAE

An Adverse Event (AE) is any untoward medical occurrence in a patient temporarily associated with the use of an investigational product, whether or not considered related to the investigational product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the product, whether or not considered related to the product.

Events belonging to the normal procedure of disease diagnosis and treatment (e.g. rehospitalization for second-stage stenting) are not reported as AEs.

A Serious Adverse Event (SAE) is any untoward medical occurrence that, at any dose, meeting at least one of the following criteria

- 1) Results in death;
- 2) Is life threatening;

Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death if it were more severe.

- 3) Requires hospitalization or prolongation of an existing hospitalization;
- 4) Results in permanent or severe disability;
- 5) Is a congenital abnormality/birth defect;
- 6) Is a significant medical event

Medical and scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. SAEs require immediate report to the ethics committee within 24 hours.

9.2 Recording of AEs/SAEs

1) AE

When an AE occurs, the investigator should record all relevant information regarding an AE in to the CRF regardless of its severity and causality with the investigational product, from the moment when the participant signs the informed consent or from the first study visit to the last study visit. The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In the absence of a diagnosis, the individual signs/symptoms should be documented. For each adverse event, start and stop dates, action taken, outcome, intensity and relationship to study product (causality) must be documented.

2) SAEs

Serious Adverse Events require immediate action.

Once an investigator becomes aware that an SAE has occurred, he/she should immediately notify the principal investigator, and fax the AE page in the CRF with all available details of the event and any non-serious AEs related to the SAE, to the principal investigator and the ethics committee, within 24 hours.

9.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to actively follow each subject and provide further information to the Steering Committee on the subject's condition.

All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits/contacts.

All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up. Once resolved, the appropriate AE/SAE CRF page(s) will be updated. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, or consultation with other health care professionals.

New or updated information will be recorded on the originally completed SAE form, with all changes signed and dated by the investigator.

10. Study Plan

Standard multi-modal CT are performed to assess the eligibility of patients, including non-contrast head CT, CTP, head and neck CTA. CTP imaging will be real-time processed by MISTar (Apollo Medical Imaging Technology, Melbourne, Victoria, Australia).

Neurological impairment and functional scores (NIHSS, pre-stroke mRS) will be assessed by a trained stroke neurologist.

Bloods for standard care diagnostic evaluations will be collected and analyzed. Patients will be assessed for trial eligibility according to the Inclusion/Exclusion criteria including including a reprocessing of the baseline imaging data for assessment of the occlusion site by the core imaging lab.

An informed consent will be obtained from patients or their authorized representative prior to enrollment.

10.1 Baseline assessments

- 1) Demographic Information: Name, sex, age, ethnics, telephone number, type of health insurance
- 2) Time of last known well, time of symptom recognition and time of hospital arrival
- 3) Risk factor (medical history): hypertension, diabetes mellitus, stroke, transient ischemic attack, coronary heart disease, myocardial infarction, arrhythmia, history of medication (aspirin, clopidogrel, warfarin, antihypertensive drugs, lipid-lowering drugs, antidiabetic drugs, heart rate control drugs), dementia, peripheral artery disease, dyslipidemia, hepatic and /or renal dysfunction, cervical and/intracranial artery stenosis or malformation, smoking, drinking
- 4) Clinical assessments: vital signs (blood pressure, heart rate, respiratory rate and body temperature), NIHSS, and pre-stroke mRS
- 5) Imaging: Patients will have standardized multimodal CT (non-contrast head CT, CTP, head and neck CTA) prior to treatment, as previously described (9.1 Radiological assessments).
- 6) ECG
- 7) Laboratory test (including HCG test for women of reproductive age, routine hematology, biochemistry and coagulation screening tests)
Routine hematology: red blood cell count, mean corpuscular volume, white blood cell count, neutrophil (count, percent), eosinophil (count, percent), basophils (count, percent), lymphocyte (count, percent), monocyte (count, percent), hematocrit, hemoglobin, mean corpuscular hemoglobin, platelet count
Biochemistry: Renal function (creatinine, uric acid, urea nitrogen or urea), hepatic function (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, glutamyl transpeptidase, total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin), glucose
Cardiac markers and pro-BNP: Troponin-T, Myoglobin, CK-MB, NT-pro BNP
Coagulation: PT, APTT, TT, FIB, INR, D-Dimer, fibrinogen degradation products (FDP)
HCG test

10.2 Randomization

Randomization is allowed when all the inclusion criteria are confirmed to be met (including signed informed consent by the patient or the proxies) and all the exclusion criteria have been ruled out. Randomization is performed using permuted blocks through a centralized website named Easy Random Trial (ERT) designed by Chuang Da medical science and Technology (Shanghai) Co., Ltd. by a team of professional and independent statisticians. Patients are stratified according to the time of randomization after last known well (4.5-12 hours, 12-24 hours) and site of occlusion/severe stenosis (intracranial segment of internal carotid artery and M1-segment of middle cerebral artery; extracranial segment of internal carotid artery, M2-segment of middle cerebral artery and anterior cerebral artery). The purpose of randomization in the Phase IIa umbrella-designed trial with multiple strata is not to enable subsequent comparisons between dose strata, but to ensure that participants in both strata are equally representative of the patient population. The dosage of tenecteplase is open-label while the raters involved in the subsequent radiological and clinical evaluation are blinded to the allocation.

10.3 Treatment administration

- 1) Intravenous thrombolysis: Patients will receive intravenous tenecteplase, either 0.25mg/kg (maximum 25mg) or 0.32mg/kg (maximum 32mg), administered as a bolus over 5~10 seconds. Vital signs will be recorded during and after the period of infusion as per standard care. The time of infusion commencement, completion and the dose administered is recorded.
- 2) Endovascular treatment: Endovascular treatment is optional: If endovascular treatment is consented by the patient or the proxies and is considered feasible at the discretion of the neuro-interventionalists, the patient will be transferred to the interventional catheter suite to receive bridging endovascular treatment after thrombolysis. Thrombectomy will not be considered if hemorrhagic transformation or complete recanalization occurred before any operation of thrombectomy or angioplasty. Before any thrombectomy operation, catheter angiography should be performed to confirm the occlusion site and primary modified Treatment In Cerebral Infarction (mTICI) scale. Only at most 3 passes of thrombectomy operation are allowed for each occluded artery. Digital subtraction angioplasty (DSA) should be performed following each pass and at the completion of the thrombectomy operation. Type of anesthesia should be recorded. Angioplasty and/or stenting are allowed if necessary and may require the use of antiplatelets Attention should be paid to maintaining stable blood pressure and minimizing delays in starting the procedure. The initial and final angiograms will be centrally graded for angiographic reperfusion using the mTICI classification.

Vital signs and any occurrence of AEs or SAEs during treatment should be documented. Close neurological observation will be conducted primarily during the first 48 hours after treatment administration according to local clinical practice.

10.4 Study visits (Table 1)

- 1) 4-6 hours
 - Vital signs, concomitant medication, NIHSS, new-onset cardio-cerebral vascular events, AEs/SAEs
 - For patients transferred to the catheter room: time of arterial puncture, time of first angiographic run, time of recanalization
 - Imaging:
For patients not transferred to the catheter room: CTP (+neck CTA if patients are primarily with ICA-EC severe stenosis/occlusion)
For patients transferred to the catheter room: DSA
- 2) 24-48 hours
 - Vital signs, concomitant medication, NIHSS, new-onset cardio-cerebral vascular events, AEs/SAEs
 - Imaging: Non-contrast head CT
 - Laboratory test: routine hematology, renal and hepatic function, cardiac markers, cardiac enzymes, pro-BNP, blood lipid, routine urine analysis, coagulation
 - Symptomatic intracranial hemorrhage (ECASS II criteria)
 - Systematic hemorrhage (GUSTO criteria)
- 3) 3-5 days

- Vital signs, concomitant medication, NIHSS, new-onset cardio-cerebral vascular events, AEs/SAEs
 - Imaging: head MRI (including DWI), head TOF-MRA (,+neck MRA if patients are primarily with ICA-EC severe stenosis/occlusion) SWI (optional).
 - Symptomatic intracranial hemorrhage (ECASS II criteria)
 - Systematic hemorrhage (GUSTO criteria)
- 4) 7±1 days (or at discharge)
- Vital signs, concomitant medication, NIHSS, mRS, BI, TOAST classification, new-onset cardio-cerebral vascular events, AEs/SAEs, holter monitoring results
 - Laboratory test: routine hematology, renal and hepatic function, cardiac markers, cardiac enzymes, pro-BNP, blood lipid, routine urine analysis, coagulation
- 5) 30±7 days
- Vital signs, concomitant medication, NIHSS, mRS, BI, TOAST classification, new-onset cardio-cerebral vascular events, AEs/SAEs, holter monitoring results
 - Cognitive tests (optional): MoCA, MMSE
- 6) 90±7 days
- Vital signs, concomitant medication, NIHSS, mRS, BI, TOAST classification, new-onset cardio-cerebral vascular events, AEs/SAEs, holter monitoring results
 - Cognitive tests (optional): MoCA, MMSE

10.5 Study outcomes

1) Primary outcome

The primary outcome is a binary composite of efficacy and safety, i.e., major reperfusion and absence of symptomatic intracranial hemorrhagic transformation (ICH) at 24-48 hours after intravenous tenecteplase. Major reperfusion was considered as the restoration of blood flow of greater than 50% of the involved territory.

- For patients not transferred to the catheter room after thrombolysis, major reperfusion was assessed as reperfusion greater than 50% of the involved baseline hypoperfusion lesion volume (delay time > 3s) on repeat CT perfusion imaging at 4-6 hours after thrombolysis.
- For patients transferred to the catheter room, major reperfusion was evaluated as a modified Treatment In Cerebral Ischemia (mTICI) score 2b/3 at the initial catheter angiography.

Symptomatic ICH was defined according to the European Co-operative Acute Stroke Study-II (ECASS II) criteria, where the patients presented any type of intracranial hemorrhage with no less than 4 point-increase in National Institutes of Health Stroke Scale (NIHSS) from baseline or from the lowest NIHSS between baseline at 24-48 hours, or leading to death.

2) Secondary outcomes

- Secondary radiological efficacy outcomes
 - ✓ Recanalization defined by the Thrombolysis in Myocardial Infarction (TIMI) scale of 2/3 at the 4- to 6- hour CTA (reconstructed from CTP) for patients not transferred to the catheter room, or at digital subtraction angiography imaging prior to thrombectomy for patients transferred to the catheter room after thrombolysis;

- ✓ Infarct growth on diffusion-weighted imaging (DWI) or non-contrast head CT at 3-5 days.
- Secondary clinical efficacy outcomes
 - ✓ Excellent functional outcome (disability-free, defined as modified Rankin Scale [mRS] 0-1) at 90 days;
 - ✓ Good functional outcome (functional independence, defined as mRS 0-2) at 90 days; mRS distribution at 90 days;
 - ✓ Major neurological improvement at 24-48 hours (NIHSS reduction greater than 7 points or NIHSS 0-1);
 - ✓ Change in NIHSS as a continuous variable at 24-48 hours.
- Secondary radiological safety outcomes
 - ✓ Type 2 parenchymal hematoma (PH2);
 - ✓ Symptomatic ICH;
 - ✓ Any ICH at 24-48 hours post treatment.
- Secondary clinical safety outcomes
 - ✓ Poor functional outcome (severe disability or death, defined as mRS 5-6) at 90 days;
 - ✓ Systemic bleeding defined by The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) before discharge.
- Others:
 - ✓ Barthel index at 90 days.

All of these clinical assessments will be conducted through on-site personnel who are blinded to the treatment allocation.

10.6 Protocol compliance

Study visits before discharge (4-6 hours, 24-48 hours, 3-5 days, 7±1 days or at discharge) will be completed together by a fellow doctor in charge of the treatment patients in hospital and one of the investigators both of whom are blinded to the treatment allocation.

Study visits after discharge (30±7 days, 90±7 days) will be completed through stroke clinic by one of the investigators who is blinded to the treatment allocation. Transportation fee of the participants will be covered by the trial. If the participant is incapable of arrival to the clinic in-person due to disability, these visits will be completed through standardized telephone follow-up by a trained investigator who is blinded to the treatment allocation.

11. Data analysis and statistical consideration

11.1 Sample size calculation

The sample size is calculated based on the results derived from the EXTEND-IA TNK trial: 22% patients of the intravenous tenecteplase group and 10% patients of the intravenous alteplase group reached major reperfusion, and 1% patients in both groups respectively were found to have sICH. To adopt a conservative approach, for each dose stratum within the umbrella design, the null hypothesis was that not more than 10% of patients would achieve a positive primary outcome following the respective dose of tenecteplase, while the alternative hypothesis was that, not less than 25% of patients would achieve a positive primary outcome following the respective dose of tenecteplase. Sample size estimation using Simon's two-stage design was conducted for each dose

stratum, according to which the null hypothesis that the true response rate was 10% was tested against a one-sided alternative. In the first stage, 18 patients were to be accrued. If there were 2 or fewer positive responses in these 18 patients, meaning that the null hypothesis was not rejected and the dose stratum was to be stopped. Otherwise, 25 additional patients were to be accrued for a total of 43 patients for each dose stratum. The null hypothesis for each stratum was to be rejected if 8 or more positive responses were observed in 43 patients. This design was to yield a type I error rate of 0.05 and a power of 0.8 when the true response rate was 25%. Overall, at least 36 patients were to be enrolled with a maximum enrollment of 86 patients equally distributed between the 2 dose strata. This design would yield a one-sided type I error of 0.5 and a power of 80% to detect the presumed significant difference. In all, at least 36 patients would be enrolled with a maximum enrollment of 86 patients in sum of the 2 dose strata.

11.2 General principles of data analysis

All of the statistical analysis will be performed on STATA v15.1 (StataCorp, Ltd, College Station, Texas).

11.3 Analysis of primary outcome

For the analysis of primary endpoint, as described above, if 3 or more of the 18 patients in the first stage of either dose stratum reach the primary endpoint, then additional 25 patients would be enrolled in that dose stratum. If 8 or more out of the 43 patients reach the primary endpoint within a given stratum after the second stage, then the corresponding tenecteplase dose could be considered to be of sufficient promise in terms of efficacy and safety and could be a qualified candidate dose for the subsequent phase IIb trial.

11.4 Analysis of secondary outcome

Since the design of this study is not aimed to compare the safety and efficacy of the two tenecteplase doses within the umbrella design, the analysis of secondary outcomes only involved data description without comparative analysis. Secondary outcomes were described using percentages, mean and standard deviation, median and interquartile range (IQR) as appropriate. Normality will be tested using Shapiro-Wilk test.

11.5 Sensitivity analysis

Preplanned sensitivity analysis is conducted by removing patients with severe stenosis and analyzing the primary and secondary outcomes of patients with complete artery occlusion in the two tenecteplase dose groups.

In case of any post-hoc subgroup analysis, they will be justified and identified as data-driven and, they will follow the principles and regulatory recommendations.

12. Ethical and regulatory standards

12.1 Ethical principles

This study will be conducted in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and all applicable amendments laid down by the World Medical Assemblies and the ICH guidelines for Good Clinical Practice.

12.2 Laws and regulations

This study will be conducted in compliance with all international laws and regulations, and Chinese laws and regulations in which the Clinical Trial is performed, as well as any applicable guidelines.

12.3 Informed Consent

The investigator (according to applicable regulatory requirements), or a person designated by the investigator and under the investigator's responsibility, should fully inform the patient of all pertinent aspects of the Clinical Trial including the written information giving approval/favorable opinion by the Ethics Committee (IRB/IEC). All participants should be informed to the fullest extent possible about the study, in language and terms they are able to understand.

Prior to a patient's participation in the CHABLIS-T trial, the written informed consent form should be signed, name filled in and personally dated by the patient or by the patient's legally acceptable representative, and by the person who conducted the informed consent discussion. A copy of the signed and dated written Informed Consent Form will be provided to the patient.

The informed consent form used by the investigator for obtaining the patient's informed consent must be reviewed and approved by the sponsor prior to submission to the appropriate Ethics Committee (IRB/IEC) for approval/favorable opinion.

The protocol will be submitted for approval to the appropriate local institutional ethics committee, and written approval obtained, before participants are recruited and enrolled. The Investigators will receive all the documentation needed for submitting the present protocol to the ethics committee. A copy of the respective approval letters will be transmitted to the Study Monitor before starting the study. The compliance/composition of the Ethics Committee will also be provided to the Study Monitor. If approval is suspended or terminated by the ethics committee, the investigator will notify the Study Monitor immediately.

It is the responsibility of the Investigator to report study progress to the ethics committee as required, or at intervals not greater than one year.

The principal investigator, or his/her nominee, will be responsible for reporting any SAEs to the ethics committee as soon as possible, and in accordance with the guidelines of the Ethics Committee.

12.4 Institutional Review Board/ Independent Ethics Committee (IRB/IEC)

The investigator or the sponsor must submit this protocol to the appropriate ethics committee (IRB/IEC), and is required to forward to the Sponsor a copy of the written and dated approval/favorable opinion signed by the chairman with ethics committee (IRB/IEC) composition.

The clinical trial (study number, Clinical Trial Protocol title and version number), the documents reviewed (clinical trial protocol, informed consent form, investigator's brochure, investigator's CV, etc.), the list of voting members along with their qualification and the date of the review should be clearly stated on the written (IRB/IEC) approval/favorable opinion.

Investigational product will not be released at the study site and the trial will not start until a copy of this written and dated approval/favorable opinion has been received by the Sponsor.

During the study, any amendment or modification to the protocol should be submitted to the Ethics Committee (IRB/IEC). It should also be informed of any event likely to affect the safety of patients or the continued conduct of this trial, in particular any change in safety. All updates to the investigator's brochure will be sent to the ethics committee (IRB/IEC).

If requested, a progress report will be sent to the ethics committee (IRB/IEC) annually and a summary of the trial's outcome at the end of this trial.

13. Study monitoring

13.1 Responsibilities of the investigators

The Investigator(s) undertake(s) to perform the clinical trial in accordance with this protocol, ICH guidelines for Good Clinical Practice and the applicable regulatory requirements.

The investigator is required to ensure compliance with all procedures required by the protocol and with all study procedures provided by the Sponsor (including security rules). The investigator agrees to provide reliable data and all information requested by the Clinical

Trial protocol (with the help of the Case Report Form [CRF], Discrepancy Resolution Form [DRF] or other appropriate instrument) in an accurate and legible manner according to the instructions provided and to ensure direct access to source documents by Sponsor representatives.

The investigator may appoint such other individuals as he/she may deem appropriate as sub-investigators to assist in the conduct of the trial in accordance with the protocol. All sub-investigators shall be appointed and listed in a timely manner. The sub-investigators will be supervised by and under the responsibility of the Investigator. The investigator will provide them with a copy of this protocol and all necessary information.

The Sponsor of this Clinical Trial is responsible to Health Authorities for taking all reasonable steps to ensure the proper conduct of the clinical trial protocol as regards ethics, clinical trial protocol compliance, and integrity and validity of the data recorded on the case report forms. Thus, the main duty of the monitoring team is to help the Investigator and the Sponsor maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the trial.

At regular intervals during the clinical trial, the site will be contacted, through monitoring visits, letters or telephone calls, by a representative of the Monitoring Team to review study progress, Investigator and patient compliance with the protocol requirements and any emergent problems. During these monitoring visits, the following, but not exhaustive, points will be scrutinized with the Investigator: patient informed consent, patient recruitment and follow-up, SAE documentation and reporting, investigational product allocation, patient compliance with the investigational product regimen, investigational product accountability, concomitant therapy use and quality of data.

13.2 Source document requirements

According to the ICH guidelines for Good Clinical Practice, the monitoring team must check the case report form entries against the source documents, except for the pre-identified source data directly recorded in the case report form. The informed consent form will include a statement by which the patient allows the sponsor's duly authorized personnel, the ethics committee (IRS/IEC), and the regulatory authorities to have direct access to source data which support the data on the case report forms (e.g., patient's medical file, appointment books, original laboratory records, etc.). Such personnel, bound by professional secrecy, must keep confidential all personal identity or personal medical information (according to confidentiality rules).

13.3 Data management

Paper-based case report form (CRF) and electronic data capture (EDC) system will be used for data collection and entry. All the content required by the protocol in the system must be provided, the unfilled content should be explained, and the reason needs to be filled in the remarks under each form of the EDC system.

Site investigators should use black or blue-black recording pens to fill out the paper-based CRF neatly and clearly to ensure that the data is clear and readable. If the paper-based CRF information needs to be modified, it should not be altered or overwritten. The correct information should be written next to the original information, signed and dated by the person who modified it. The clinical research monitor (CRA) will review the completeness and accuracy of the case report form and guide the investigator to make necessary corrections and supplements. After the paper-based CRF is completed, the research coordinator (CRC) will input the content of the paper CRF into the EDC system. The paper-based CRF is submitted after the investigator has approved it. After the data is submitted, all data revisions and feedback are carried out through the EDC system. If the EDC system has submitted a form that needs to be modified, you need to contact the CRA of this center. After the CRA opens the form, the investigator can guide the CRC to modify the EDC system data. Data will then be monitored by the CRA according to a data monitoring plan in a regular basis. After the last study visit of the last participant is finished, the data from the EDC will be exported to a database (in excel sheets), then it will be proofread by the data administrator. Obvious errors will be corrected by the data administrator. Other errors or missing values will be filled in the data query form and returned to the participating center for solution through email, express, telephone and WeChat.

The participating centers are responsible for correcting the data in the EDC system after verifying the original data and related information. Site investigators must answer these queries by verifying or modifying relevant information or data.

13.4 Confidentiality

All of the personal data of research participants (subjects) must be kept strictly confidential. Information and data of the study participants (subjects) are identified only by subject numbers rather than names. Identifiable information of participants (subjects) will not be disclosed to members outside the investigator teams, unless permission is obtained from the research participants (subjects). All of the investigators are required to keep the identities of study participants (subjects) confidential. The files of study participants (subjects) will be kept in locked filing cabinets and will only be accessible to authorized investigators. In order to ensure that the study is carried out in accordance with laws and regulations, government authorities or members of the ethics committee can access to the personal data of the research participants (subjects) according to regulations when necessary. No personal information of the research participants (subjects) will be disclosed at the time of publication of this research.

The research data are also confidential. All of the investigators are required to keep confidential to the research data. They shall not disclose the research data without the permission of the principal investigator to anyone who is not the member of the research team, and shall not transfer the research data to other institute without the permission of the hospital. The research data are not allowed to be disclosed to foreign institutes, or domestic institutes with foreign capital, without the permission of Human Genetic Resource Administration Office of China, except for the publication of research results that meet regulatory requirements under normal circumstances.

14. Funding

The study is sponsored by the National Key Research and Development Program of China (2017YFC1308201), Science and Technology Ministry of China. Guangzhou Recomgen Biotech

Co., Ltd. supplied the investigative drug and covered the trial insurance, but had no role in study design and execution.

15. Study Organization

15.1 Principle investigator

Qiang Dong, Huashan Hospital, Fudan University, Shanghai, China

15.2 Trial steering committee members

Qiang Dong (Principal Investigator), Mark Parsons, Xin Cheng, Leonid Churilov, Yilong Wang

- ✓ The steering committee will provide scientific and strategic direction for the trial and will have overall responsibility for its design, execution, and publication.
- ✓ The steering committee will also be responsible for ensuring that study execution and management are of the highest quality.
- ✓ It will approve the protocol and the operational guidelines of the trial prior to its commencement.
- ✓ The steering committee will convene regularly by teleconference or face-to-face meetings to discuss and report on the progress of the study.

15.3 Data safety and monitoring board

The DSMB will meet regularly and monitor the progress of the CHABLIS-T study to ensure that the study meets the highest standards of ethics and patient safety. It is composed of Academic Members, including an independent statistician, who are not otherwise participating in the trial.

Written recommendations and their rationale will be provided to the Chairs of the Steering Committee immediately after each DSMB meeting.

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Table 1 Study Visits in CHABLIS-T

Assessment	Screening and Randomization	Thrombolysis Commencement	Visits					
	-24h~4.5h	0h	4-6h	24- 48h	3-5 days	7±1 days or Discharge	30±7 days	90±7days
Demographic	X							
Previous medical	X							
Concomitant	X		X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X
Physical examinations	X							
Pregnancy test ¹	X							
Blood lab tests	X			X				
Electrocardiography	X							
Inclusion and exclusion criteria	X							
Randomization	X							
Imaging (CT/MRI/DSA)	X		X	X	X			
Symptomatic intracranial				X	X			
Modified Rankin Scale	X					X	X	X
NIH Stroke Scale	X		X	X	X	X	X	X
TOAST Classification						X		
Cognitive evaluations							X	X
Adverse events		X	X	X	X	X	X	X
Barthel Index						X	X	X

1. Only for female patients at reproductive age

2. 24-hour Holter monitoring

**CHinese Acute tissue-Based imaging selection for
Lysis In Stroke -Tenecteplase
(CHABLIS-T)**

Protocol

Research Team: Department of Neurology, Huashan Hospital,
Fudan University, Shanghai, China

Principle Investigator: Qiang Dong, MD, PhD, Professor of
Neurology

Protocol Version: 3.0

Nov. 2020

Catalogue

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Protocol Summary

Protocol Title	CHABLIS-T: CHinese Acute tissue-Based imaging selection for Lysis In Stroke -Tenecteplase
Protocol Number	KY2019-474
Development Phase	Phase 2a
Study Population	Acute ischemic stroke patients with large/medium vessel occlusion or severe stenosis presented within 4.5 to 24 hours from last known well, with penumbral mismatch identified by multimodal CT.
Study Objective	To explore the promise of efficacy and safety of different doses of tenecteplase (0.25mg/kg vs. 0.32mg/kg) in Chinese acute ischemic stroke patients with large/medium vessel occlusion or severe stenosis in an extended time window.
Trial Design	<p>The CHABLIS-T trial is a phase IIa, randomized, multicenter, open-label, blinded-endpoint, Simon's two-stage, umbrella design study.</p> <p>Eligible patients will be enrolled and 1:1 randomized into 2 dose group (0.25mg/kg and 0.32mg/kg) of tenecteplase (Guangzhou Recomgen Biotech Co., Ltd). An interim analysis will be conducted when each treatment arm recruits 18 patients. If three or more patients achieve the primary endpoint in either arm, then the study would continue to the 2nd stage, which would enroll an addition of 25 patients in that tenecteplase dose group (resulting in a total of 43 patients in that dose group), otherwise the dose group would be stopped. The intervention dose would be deemed to be of sufficient promise if 8 or more out of 43 patients achieved the primary outcome.</p>
Inclusion criteria	<ol style="list-style-type: none"> 1. Patients presenting with acute ischemic stroke within 4.5-24 hours from time last known well 2. Patients' age \geq 18 years 3. Premorbid modified Rankin Scale 0-2 4. Clinically significant acute neurologic deficit (with no lower or upper limit of the National Institutes of Health Stroke Scale [NIHSS] score) at the discretion of the investigator 5. Anterior large/medium vessel occlusion or severe stenosis ($>70\%$ of the vessel caliber), including extracranial or intracranial ICA, M1 or M2 segment of MCA, and A1 or A2 segment of ACA, identified on head and neck CTA 6. Penumbral mismatch on CTP: Hypoperfusion lesion volume (Delay time[DT]$>$ 3 s) to infarct core volume ratio (relative cerebral blood flow [rCBF]$<$30%) $>$ 1.2, an absolute difference of volume $>$ 10 ml, and an ischemic core volume $<$ 70ml 7. Patient/Legally Authorized Representative has signed the Informed Consent form.

Exclusion criteria	<ol style="list-style-type: none"> 1. Extensive hypoattenuation region (more than 1/3 of the MCA territory) identified on baseline non-contrast CT (NCCT) 2. Any sign of an acute intracranial hemorrhage or subarachnoid hemorrhage identified on baseline NCCT 3. Previous ischemic stroke within 3 months 4. Past history of intracranial hemorrhage 5. Acute head trauma at presentation or recent major head trauma within 3 months 6. Recent history of intracranial/intraspinal surgery within 3 months 7. Gastrointestinal malignancy or gastrointestinal bleed within 21 days 8. Known bleeding diatheses: platelets count <100 000/mm³, INR >1.7, APTT >40 s, or PT >15 s 9. Use of a full dosage of low-molecular weighted heparin treatment within the previous 24 hours 10. Use of direct thrombin inhibitors or direct factor Xa inhibitors within the previous 48 hours unless the laboratory test of coagulation function is normal 11. Symptoms consistent with infective endocarditis 12. Known or suspected with aortic arch dissection 13. Presence of an intra-axial intracranial neoplasm <p style="text-align: center;">In addition to:</p> <ol style="list-style-type: none"> 1. Rapidly improving symptoms at the discretion of the investigator, which may indicate spontaneous recanalization 2. Contraindications for CT contrast precluding a CT angiography or perfusion study. 3. Pregnancy or breastfeeding 4. Recent Participation in another investigational drug or device study or registry in the past 30 days 5. Allergy to the test drug and its ingredients 6. Any terminal illness such that the patient would not be expected to survive more than three months 7. Other conditions in which investigators believe that participating in this study may be harmful to the patient
Investigational drug	Intravenous Tenecteplase
Interventions	<p>Patients were randomized in a 1:1 ratio into 2 tenecteplase dose groups, 0.25mg/kg and 0.32 mg/kg. Randomization was performed using permuted blocks through a centralized website. Patients were stratified according to time of randomization after last known well (4.5-12 hours, 12-24 hours) and site of occlusion/severe stenosis (ICA-IC and MCA-M1; ICA-EC, MCA-M2 and ACA).</p> <p>Patients received either dose of intravenous tenecteplase (0.25mg/kg, maximum 25 mg, or 0.32mg/kg, maximum 40mg) as a bolus over 5-10s and a following 2 mL bolus of saline for injection.</p>
Study outcome	<p>Primary outcome</p> <p>The primary outcome is a binary composite of efficacy and safety, i.e. major reperfusion and absence of sICH at 24-48 hours after intravenous tenecteplase.</p> <p>Secondary outcomes</p> <ol style="list-style-type: none"> 1. Secondary radiological efficacy outcomes:

	<ol style="list-style-type: none"> 1) Recanalization defined by the Thrombolysis in Myocardial Infarction (TIMI) scale of 2/3 at the 4- to 6- hour CTA (reconstructed from CTP) for patients not transferred to the catheter room, or at the first digital subtraction angiography run prior to thrombectomy for patients transferred to the catheter room after thrombolysis 2. Secondary clinical efficacy outcomes 6) Excellent functional outcome (mRS 0-1) at 90 days 7) Good functional outcome (mRS 0-2) at 90 days 8) mRS distribution at 90 days 9) Neurological improvement at 24-48 hours (NIHSS 0-1 or a NIHSS-improvement of ≥ 8) 10) Change in NIHSS as a continuous variable at 24-48 hours 3. Secondary radiological safety outcomes 4) Type 2 parenchymal hematoma at 24-48 hours 5) sICH according to the ECASS II criteria at 24-48 hours 6) Any intracranial hemorrhagic transformation at 24-48 hours post-treatment 4. Secondary clinical safety outcomes 3) Poor functional outcome (mRS 5-6) at 90 days 4) Systemic bleeding defined by The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries before discharge 5. Others: Barthel Index at 90 days
Primary analysis	<p>For the analysis of the primary endpoint, if more than 2 out of the 18 patients in the first stage of either dosage group reach the primary endpoint, then another additive 25 patients will be enrolled in that group. If more than 7 out of the 43 patients reach the primary endpoint after the second stage, then the tenecteplase dosage could be considered as a qualified candidate dosage for a phase IIb trial.</p> <p>Secondary outcomes will be described using percentages, mean and standard deviation, median and interquartile range (IQR) as appropriate. Normality will be tested using the Shapiro-Wilk test.</p>

1. Introduction

Intravenous thrombolysis using alteplase is recommended as the first-line therapy for acute ischemic stroke (AIS) patients presented within 4.5 hours after stroke onset¹. However, only a very limited number of patients are able to receive the treatment due to its short treatment time window. A comparison study based on the data of China National Stroke Registry (CNSR) and the American Heart Association Get With The Guidelines Stroke Program (GWTG-Stroke Program) has shown that only 2.5% patients in CNSR and 8.1% patients in the GWTG-Stroke Program received intravenous thrombolytic therapy². Therefore, extending the treatment time window for AIS patients has become a hot research topic of clinical trials recently. Additionally, because alteplase acquires 1-hour continuous infusion after bolus and its efficacy can be undermined by any infusion delay and interruption, the necessity for alteplase in patients with large vessel occlusion has been questioned^{3, 4} since the effect of endovascular treatment has been validated and acknowledged⁵.

Existence of penumbral mismatch (substantial volume of tissue to salvage) is of great importance to the effect of reperfusion therapy⁶. Recent randomized controlled trials (RCTs) using perfusion imaging to select patients with penumbral mismatch have demonstrated that intravenous thrombolysis may also be beneficial for AIS patients in an extend time window beyond 4.5 hours^{7, 8}.

Tenecteplase (TNK) is a mutant variant of alteplase with a longer half-time life and higher fibrin specificity. TNK currently is recommended as the first-line intravenous medication for myocardial infarction with a dose of 0.5mg/kg, and its safety and efficacy in acute ischemic stroke patients have long been studied. The meta-analysis of the 5 RCTs (Haley et al.⁹, Australian TNK¹⁰, ATTEST¹¹, NOR-TEST¹², EXTEND-IA TNK¹³) comparing the effect of TNK and alteplase reveals that though the imaging selection criteria and treatment time window for each trial differ, the dose of 0.25mg/kg and 0.4 mg/kg TNK are non-inferior to alteplase in AIS patients (the noninferiority test of 0.1mg/kg TNK was underpowered due to limited sample size)¹⁴. Furthermore, pooled analyses using data of Australian TNK and ATTEST show that TNK may have superior recanalization efficacy in patients with complete large vessel occlusion to alteplase¹⁵, especially in patients with penumbral mismatch on perfusion imaging¹⁶. EXTEND-IA TNK has proven that for anterior circulation stroke patients with large vessel occlusion and perfusion mismatch, TNK could lead to a significantly higher rate of major reperfusion preceding endovascular treatment and better functional outcome, compared with alteplase in the 4.5-hour time window (rate of major reperfusion: TNK 22% vs. alteplase 10%; median [IQR] mRS at 90 days: TNK 2[0-3] vs. alteplase 3[1-4]). These previous results implicate that based on the noninferiority of TNK to alteplase in AIS patients without advanced imaging selection, the superiority of TNK to alteplase may emerge in large vessel occlusion patients with perfusion mismatch.

Endovascular treatment has become a routine practice for the treatment of AIS patients with large/medium vessel occlusion¹, and its time window has been extended to 24 hours after last known normal through advanced imaging selection^{17, 18}. However, Chinese LVO patients have been faced with the challenge of a longer reperfusion time compared with the Western population¹⁹⁻²³. One of the main reasons may relate to the high prevalence of in-situ thrombosis due to intracranial atherosclerotic disease (ICAD) in Asian patients with acute LVO²⁴, which is refractory to the current stent retrievers or aspiration systems²⁵. Additionally, the number of comprehensive stroke centers with capability of endovascular treatment is still insufficient given the broad landscape of China, and the drip-and-ship transfer system is still under-developed. Therefore, TNK can be a great candidate for reperfusion therapy considering its performance in large/medium vessel occlusion patients to extend the time

window of intravenous thrombolysis, improving the clinical outcome of Chinese AIS patients with large/medium vessel occlusion.

The doses of TNK tested in the previous RCTs in acute ischemic stroke patients are generally 0.1mg/kg, 0.25mg/kg and 0.4mg/kg. Tenecteplase has been approved for the intravenous thrombolysis in myocardial infarction by China Food and Drug Administration (CFDA). The tenecteplase dose proven to be safe during the dose escalation study in Chinese myocardial infarction patients ranges from 0.08mg/kg to 0.32mg/kg. However, the optimal dose of tenecteplase for Chinese acute ischemic stroke patients with large/medium vessel occlusion still remains undetermined. Additionally, Australia TNK¹⁰ demonstrated that 0.25mg/kg TNK was superior to 0.1mg/kg TNK in reperfusion efficacy. Therefore, the doses of 0.25mg/kg and 0.32mg/kg tenecteplase are chosen as candidates of this Phase IIa trial to select an optimal dose of Chinese AIS patients with large/medium vessel occlusion.

Umbrella design is a trial design allowing evaluation of multiple therapies of a single disease without a control group in a minimal sample size, and is frequently applied in trials of oncology therapies²⁶. Based on the umbrella design, Simon's two-stage design is further acknowledged as a simpler and more effective dose selection study method²⁷, especially in oncology trials²⁸⁻³⁰. In order to evaluate the promise of efficacy and safety of 0.25mg/kg and 0.32 mg/kg tenecteplase in AIS patients with large/medium vessel occlusion with a minimal sample size, we therefore adopted the Simon's two stage umbrella design in the CHABLIS-T trial and designed it as a phase IIa, randomized, multicenter, open-label, blinded-endpoint study.

2. Study Objective

To explore the promise of efficacy and safety of different doses of tenecteplase (0.25mg/kg vs. 0.32mg/kg) in Chinese acute ischemic stroke patients with large/medium vessel occlusion or severe stenosis in an extended time window.

3. Study Design

3.1 Overall design

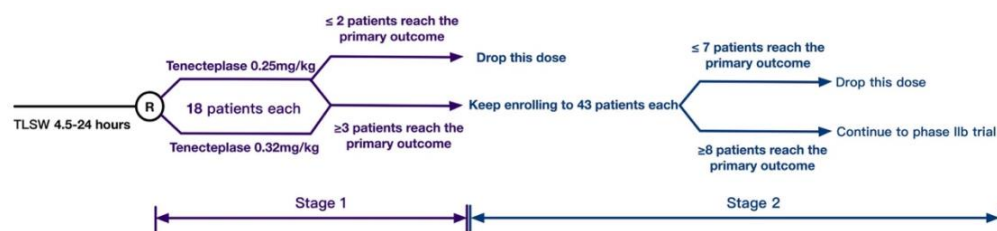
The CHALIS-T trial is a phase IIa, randomized, multicenter, open-label, blinded-endpoint, Simon's two-stage, umbrella design study.

3.2 Investigational Drug

Tenecteplase (Guangzhou Recomgen Biotech Co., Ltd)

3.3 Treatment allocation

Eligible patients will be enrolled and 1:1 randomized into 2 dose group (0.25mg/kg and 0.32mg/kg) of tenecteplase (Guangzhou Recomgen Biotech Co., Ltd). An interim analysis will be conducted when each treatment arm recruits 18 patients. If three or more patients achieve the primary endpoint in either arm, then the study would continue to the second stage, which would enroll an addition of 25 patients in that tenecteplase dose group (resulting in a total of 43 patients in that dose group), otherwise the dose group would be stopped. The intervention dose would be deemed to be of sufficient promise if 8 or more out of 43 patients achieved the primary outcome.



4. Patient Population

4.1 Inclusion criteria

- 1) Patients presenting with acute ischemic stroke within 4.5-24 hours from time last known well
- 2) Patients' age \geq 18 years
- 3) Premorbid modified Rankin Scale 0-2
- 4) Clinically significant acute neurologic deficit (with no lower or upper limit of the National Institutes of Health Stroke Scale [NIHSS] score) at the discretion of the investigator
- 5) Anterior large/medium vessel occlusion or severe stenosis ($>70\%$ of the vessel caliber), including extracranial or intracranial ICA, M1 or M2 segment of MCA, and A1 or A2 segment of ACA, identified on head and neck CTA
- 6) Penumbra mismatch on CTP: Hypoperfusion lesion volume (Delay time[DT] $>$ 3 s) to infarct core volume ratio (relative cerebral blood flow [rCBF] $<$ 30%) $>$ 1.2, an absolute difference of volume $>$ 10 ml, and an ischemic core volume $<$ 70ml
- 7) Patient/Legally Authorized Representative has signed the Informed Consent form.

4.2 Exclusion criteria

Patients are not allowed to participate if they are presented with any of the following standard intravenous thrombolysis exclusion criteria:

- 1) Extensive hypoattenuation region (more than 1/3 of the MCA territory) identified on baseline non-contrast CT (NCCT)
- 2) Any sign of an acute intracranial hemorrhage or subarachnoid hemorrhage identified on baseline NCCT
- 3) Previous ischemic stroke within 3 months
- 4) Past history of intracranial hemorrhage
- 5) Acute head trauma at presentation or recent major head trauma within 3 months
- 6) Recent history of intracranial/intraspinal surgery within 3 months
- 7) Gastrointestinal malignancy or gastrointestinal bleeding within 21 days
- 8) Known bleeding diatheses: platelets count $<$ 100 000/mm³, INR $>$ 1.7, APTT $>$ 40 s, or PT $>$ 15 s
- 9) Use of a full dosage of low-molecular weighted heparin treatment within the previous 24 hours
- 10) Use of direct thrombin inhibitors or direct factor Xa inhibitors within the previous 48 hours unless the laboratory test of coagulation function is normal
- 11) Symptoms consistent with infective endocarditis

-
- 12) Known or suspected aortic arch dissection
 - 13) Presence with an intra-axial intracranial neoplasm

In addition to:

- 1) Rapidly improving symptoms at the discretion of the investigator, which may indicate spontaneous recanalization
- 2) Contraindications for CT contrast precluding a CT angiography or perfusion study
- 3) Pregnancy or breastfeeding
- 4) Recent participation in another investigational drug or device study or registry in the past 30 days
- 5) Allergy to the test drug and its ingredients
- 6) Any terminal illness such that the patient would not be expected to survive more than three months
- 7) Other conditions in which investigators believe that participating in this study may be harmful to the patient

5. Subject withdrawal, removal and study termination

5.1 Subject withdrawal

Subjects who meet the inclusion criteria but fail to complete the study for some reasons are considered as withdrawers. Subject withdrawal includes two situations where subject withdraws by himself/herself and subjects withdraws by the discretion of investigators.

Subjects have the right to withdraw at any time during the study and are subjected to withdraw with occurrence of any of the following situations:

- 5) Poor study compliance: subjects do not take medication as prescribed or participate in study visits in time, affecting the analysis of efficacy and safety outcomes;
- 6) Suffering from adverse events or serious adverse events and are considered unsuitable to continue participating in the study by the judgement of the investigators;
- 7) Other situations that an investigator decides the subject's participation should be ceased;
- 8) Subjects require to withdraw from the study.

Subjects who withdraw from the study should attend a final withdrawal visit. The date and reason for patient withdrawal should be recorded.

5.2 Subject removal

Subjects should be removed from the study if one of the following situations occurs.

- 1) Subject do not meet one of the inclusion criteria or meet one of the exclusion criteria after enrollment;
- 2) Violation of the principles of concomitant medication according to study protocol;
- 3) Subjects do not receive allocated treatment after enrollment;
- 4) Subjects do not complete any study visits after treatment;
- 5) Subjects with poor compliance that cannot guarantee to follow the prescribed treatment, therefore, affecting the analysis of efficacy and safety outcomes.

5.3 Study early termination

The study may be terminated prematurely by the principal investigator. Reasons include but are not limited to:

- 1) The number and/or severity of adverse events justifies discontinuation of the study
- 2) The number and/or severity of protocol violation justifies discontinuation of the study
- 3) New data become available which raise concern about the safety of the investigational product, so that continuation might cause unacceptable risks to subjects.

After such a decision, the Investigator must contact all participating subjects within two weeks, and written notification must be sent to the Ethics Committee.

6. Sample size calculation

The sample size is calculated based on the results derived from the EXTEND-IA TNK trial: 22% patients of the intravenous tenecteplase group and 10% patients of the intravenous alteplase group reached major reperfusion, and 1% patients in both groups respectively were found to have sICH. To adopt a conservative approach, for each dose stratum within the umbrella design, the null hypothesis was that not more than 10% of patients would achieve a positive primary outcome following the respective dose of tenecteplase, while the alternative hypothesis was that, not less than 25% of patients would achieve a positive primary outcome following the respective dose of tenecteplase. Sample size estimation using Simon's two-stage design was conducted for each dose stratum, according to which the null hypothesis that the true response rate was 10% was tested against a one-sided alternative. In the first stage, 18 patients were to be accrued. If there were 2 or fewer positive responses in these 18 patients, meaning that the null hypothesis was not rejected and the dose stratum was to be stopped. Otherwise, 25 additional patients were to be accrued for a total of 43 patients for each dose stratum. The null hypothesis for each stratum was to be rejected if 8 or more positive responses were observed in 43 patients. This design was to yield a type I error rate of 0.05 and a power of 0.8 when the true response rate was 25%. Overall, at least 36 patients were to be enrolled with a maximum enrollment of 86 patients equally distributed between the 2 dose strata. This design would yield a one-sided type I error of 0.5 and a power of 80% to detect the presumed significant difference. In all, at least 36 patients would be enrolled with a maximum enrollment of 86 patients in sum of the 2 dose strata.

7. Treatment intervention

7.1 Investigational Product

- 1) Investigational product:

Tenecteplase (Guangzhou Recomgen Biotech Co., Ltd)

- 2) Product labelling

Commercial packages of tenecteplase(Guangzhou Recomgen Biotech Co., Ltd) are labelled by the manufacturer, Guangzhou Recomgen Biotech Co., Ltd, with storage conditions (store below 25°C), batch number and expiry date. The study sponsor shall provide supplementary labels which include protocol name and identification number, coordinating Principal Investigator details, sponsor contact details, and the words 'for clinical trial use only'.

- 3) Administration

Patients will receive either dose of intravenous tenecteplase (0.25mg/kg, maximum 25 mg, or 0.32mg/kg, maximum 32mg) as a bolus over 5-10s and a following 2 mL bolus of saline for injection. Bridging endovascular treatment is optional.

4) Transportation and storage

Investigational products are delivered by the manufacturer Guangzhou Recomgen Biotech Co., Ltd with temperature controlled below 25°C through the whole process of transportation.

Investigational product will be stored below 25°C until use in accordance with manufacturer's instructions.

The investigator or his/her designee must maintain an adequate record regarding the administration of all investigational product within the trial. Used vials of investigational product must be labelled and retained for accountability purposes.

7.2 Randomization and blinding

Randomization is allowed when all the inclusion criteria are confirmed to be met (including signed informed consent by the patient or the proxies) and all the exclusion criteria have been ruled out. Randomization is performed using permuted blocks through a centralized website named Easy Random Trial (ERT) designed by Chuang Da medical science and Technology (Shanghai) Co., Ltd. by a team of professional and independent statisticians. Patients are stratified according to the time of randomization after last known well (4.5-12 hours, 12-24 hours) and site of occlusion/severe stenosis (intracranial segment of internal carotid artery and M1-segment of middle cerebral artery; extracranial segment of internal carotid artery, M2-segment of middle cerebral artery and anterior cerebral artery). The purpose of randomization in the Phase IIa umbrella-designed trial with multiple strata is not to enable subsequent comparisons between dose strata, but to ensure that participants in both strata are equally representative of the patient population. The dosage of tenecteplase is open-label while the raters involved in the subsequent radiological and clinical evaluation are blinded to the allocation. The independent data safety monitoring board (DSMB) has the access to all the unblinded data.

7.3 Endovascular treatment

If endovascular treatment is consented by the patient or the proxies and is considered feasible at the discretion of the neuro-interventionalists, the patient would be transferred to the interventional catheter suite to receive bridging endovascular treatment after thrombolysis. Thrombectomy will not be considered if hemorrhagic transformation or complete recanalization occurred before any operation of thrombectomy or angioplasty. Before any thrombectomy operation, catheter angiography should be performed to confirm the occlusion site and primary score on the modified Treatment In Cerebral Infarction (mTICI) scale. Maximally 3 passes of thrombectomy operation are allowed for each occluded artery. Digital subtraction angioplasty (DSA) should be performed following each pass and at the completion of the thrombectomy operation. Type of anesthesia should be recorded. Angioplasty and/or stenting are allowed if necessary.

7.4 Concomitant medication

If endovascular treatment is not performed, any drugs that can affect coagulation is prohibited within 24 hours after tenecteplase thrombolysis, including antiplatelet agents, anticoagulation agents, defibrinated agents, thrombolytic agents, any drug that can affect the function of platelets, and any traditional Chinese medicine agents that can affect coagulation.

If only thrombectomy operation is performed during endovascular treatment, any drugs that can affect coagulation is prohibited within 24 hours after tenecteplase thrombolysis, including antiplatelet

agents, anticoagulation agents, defibrinated agents, thrombolytic agents, any drug that can affect the function of platelets, and any traditional Chinese medicine agents that can affect coagulation.

If angioplasty and/or stenting is performed during endovascular treatment, antiplatelets and/or anticoagulants are allowed if necessary, within 24 hours after tenecteplase thrombolysis.

Any concomitant treatment is allowed (including antihypertensive treatment) except for the medications mentioned above.

Any prescription and treatment before randomization and during the study should be recorded in the case report form (CRF).

8. Radiological and clinical assessment

8.1 Radiological assessment

Standard multimodal CT (non-contrast head CT, CTP, head and neck CTA) imaging of each potentially eligible patient is acquired before enrollment. Perfusion imaging is real-time processed using fully-automated MISTar (Apollo Medical Imaging Technology, Melbourne, Victoria, Australia) with single value deconvolution with delay and dispersion correction to calculate volumes of hypoperfusion lesion (delay time [Delay Time]>3s) and infarct core (relative cerebral blood flow [rCBF] <30% within areas of DT>3s) at each site.

For patients not transferred to the catheter room after thrombolysis, repeat CT perfusion imaging (and neck CTA for patients with ICA-EC occlusion/severe stenosis) is performed at 4-6 hours after thrombolysis to assess reperfusion. For patients transferred to the catheter room, reperfusion is evaluated as a modified Treatment In Cerebral Ischemia (mTICI) score on the initial catheter angiography. CT angiography is centrally reconstructed from repeat CTP imaging also using MISTar to evaluate recanalization status at 4-6 hours after treatment for patients without catheter angiography. For patients transferred to the catheter room, recanalization status prior to thrombectomy/angioplasty is evaluated also using the first-run imaging of digital subtraction angiography (DSA).

A non-contrast head CT scan is performed at 24-48 hours after thrombolysis to check the occurrence of intracranial hemorrhagic transformation. Diffusion-weighted imaging (DWI) or non-contrast head CT for magnetic resonance (MR)-incompatible patients is performed at 3-5 days to calculate volumes of final infarct core using MISTar.

All of the imaging protocols of multimodal CT at each center will be centrally standardized through careful quality control. All of the imaging were centrally analyzed in a core lab. Baseline multimodal CT imaging will be re-analyzed to make sure that the entry criteria are met. The radiological outcome measurements will be evaluated by two independent and neuroradiologists, and a third independent rater will be consulted in cases of disagreement, who are all blinded to the treatment allocation.

8.2 Clinical assessments

Neurological defects and functional scores are measured by one certified investigator blinded to treatment allocation in each participating center.

- 1) NIHSS is evaluated before randomization, 4-6 hours and 24-48 hours after tenecteplase treatment, and repeated at 3-5 days, 7±1 days (or at discharge), 30±7 days and 90±7 days post treatment.

- 2) Modified Rankin Scale is assessed at 7±1 days (or at discharge), 30±7 days and 90±7 days post treatment through in-person visit or standardized telephone follow-up.
- 3) Barthel Index (BI) is assessed at 7±1 days (or at discharge), 30±7 days and 90±7 days post treatment through in-person visit or standardized telephone follow-up.
- 4) Cognitive evaluation is optional 90±7 days post treatment using MoCA and MMSE through an in-person visit.

9. Adverse Events (AEs) and Severe Adverse Events (SAEs)

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or a serious adverse event (SAE) as provided in this protocol. During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs, as detailed in this section of the protocol.

9.1 Definitions of AE and SAE

An Adverse Event (AE) is any untoward medical occurrence in a patient temporarily associated with the use of an investigational product, whether or not considered related to the investigational product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the product, whether or not considered related to the product.

Events belonging to the normal procedure of disease diagnosis and treatment (e.g. rehospitalization for second-stage stenting) are not reported as AEs.

A Serious Adverse Event (SAE) is any untoward medical occurrence that, at any dose, meeting at least one of the following criteria

- 1) Results in death;
- 2) Is life threatening;

Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death if it were more severe.

- 3) Requires hospitalization or prolongation of an existing hospitalization;
- 4) Results in permanent or severe disability;
- 5) Is a congenital abnormality/birth defect;
- 6) Is a significant medical event

Medical and scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. SAEs require immediate report to the ethics committee within 24 hours.

9.2 Recording of AEs/SAEs

- 1) AE

When an AE occurs, the investigator should record all relevant information regarding an AE in to the CRF regardless of its severity and causality with the investigational product, from the moment when the participant signs the informed consent or from the first study visit to the last study visit. The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or

other clinical information. In the absence of a diagnosis, the individual signs/symptoms should be documented. For each adverse event, start and stop dates, action taken, outcome, intensity and relationship to study product (causality) must be documented.

2) SAEs

Serious Adverse Events require immediate action.

Once an investigator becomes aware that an SAE has occurred, he/she should immediately notify the principal investigator, and fax the AE page in the CRF with all available details of the event and any non-serious AEs related to the SAE, to the principal investigator and the ethics committee, within 24 hours.

9.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to actively follow each subject and provide further information to the Steering Committee on the subject's condition on the subject's condition.

All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits/contacts.

All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up. Once resolved, the appropriate AE/SAE CRF page(s) will be updated. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, or consultation with other health care professionals.

New or updated information will be recorded on the originally completed SAE form, with all changes signed and dated by the investigator.

10. Study Plan

Standard multi-modal CT are performed to assess the eligibility of patients, including non-contrast head CT, CTP, head and neck CTA. CTP imaging will be real-time processed by MISTar (Apollo Medical Imaging Technology, Melbourne, Victoria, Australia).

Neurological impairment and functional scores (NIHSS, pre-stroke mRS) will be assessed by a trained stroke neurologist.

Bloods for standard care diagnostic evaluations will be collected and analyzed. Patients will be assessed for trial eligibility according to the Inclusion/Exclusion criteria including a reprocessing of the baseline imaging data for assessment of the occlusion site by the core imaging lab.

An informed consent will be obtained from patients or their authorized representative prior to enrollment.

10.1 Baseline assessments

- 1) Demographic Information: Name, sex, age, ethnics, telephone number, type of health insurance
- 2) Time of last known well, time of symptom recognition and time of hospital arrival
- 3) Risk factor (medical history): hypertension, diabetes mellitus, stroke, transient ischemic attack, coronary heart disease, myocardial infarction, arrhythmia, history of medication (aspirin, clopidogrel, warfarin, antihypertensive drugs, lipid-lowering drugs, antidiabetic drugs, heart

rate control drugs), dementia, peripheral artery disease, dyslipidemia, hepatic and /or renal dysfunction, cervical and/intracranial artery stenosis or malformation, smoking, drinking

- 4) Clinical assessments: vital signs (blood pressure, heart rate, respiratory rate and body temperature), NIHSS, and pre-stroke mRS
- 5) Imaging: Patients will have standardized multimodal CT (non-contrast head CT, CTP, head and neck CTA) prior to treatment, as previously described (9.1 Radiological assessments).
- 6) ECG
- 7) Laboratory test (including HCG test for women of reproductive age, routine hematology, biochemistry and coagulation screening tests)
Routine hematology: red blood cell count, mean corpuscular volume, white blood cell count, neutrophil (count, percent), eosinophil (count, percent), basophils (count, percent), lymphocyte (count, percent), monocyte (count, percent), hematocrit, hemoglobin, mean corpuscular hemoglobin, platelet count
Biochemistry: Renal function (creatinine, uric acid, urea nitrogen or urea), hepatic function (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, glutamyl transpeptidase, total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin), glucose
Cardiac markers and pro-BNP: Troponin-T, Myoglobin, CK-MB, NT-pro BNP
Coagulation: PT, APTT, TT, FIB, INR, D-Dimer, fibrinogen degradation products (FDP)
HCG test

10.2 Randomization

Randomization is allowed when all the inclusion criteria are confirmed to be met (including signed informed consent by the patient or the proxies) and all the exclusion criteria have been ruled out. Randomization is performed using permuted blocks through a centralized website named Easy Random Trial (ERT) designed by Chuang Da medical science and Technology (Shanghai) Co., Ltd. by a team of professional and independent statisticians. Patients are stratified according to the time of randomization after last known well (4.5-12 hours, 12-24 hours) and site of occlusion/severe stenosis (intracranial segment of internal carotid artery and M1-segment of middle cerebral artery; extracranial segment of internal carotid artery, M2-segment of middle cerebral artery and anterior cerebral artery). The purpose of randomization in the Phase IIa umbrella-designed trial with multiple strata is not to enable subsequent comparisons between dose strata, but to ensure that participants in both strata are equally representative of the patient population. The dosage of tenecteplase is open-label while the raters involved in the subsequent radiological and clinical evaluation are blinded to the allocation.

10.3 Treatment administration

- 1) Intravenous thrombolysis: Patients will receive intravenous tenecteplase, either 0.25mg/kg (maximum 25mg) or 0.32mg/kg (maximum 32mg), administered as a bolus over 5-10 seconds. Vital signs will be recorded during and after the period of infusion as per standard care. The time of infusion commencement, completion and the dose administered is recorded.
- 2) Endovascular treatment: Endovascular treatment is optional: If endovascular treatment is consented by the patient or the proxies and is considered feasible at the discretion of the neuro-interventionalists, the patient will be transferred to the interventional catheter suite to receive bridging endovascular treatment after thrombolysis. Thrombectomy will not be considered if

hemorrhagic transformation or complete recanalization occurred before any operation of thrombectomy or angioplasty. Before any thrombectomy operation, catheter angiography should be performed to confirm the occlusion site and primary score on the modified Treatment In Cerebral Infarction (mTICI) scale. Maximally 3 passes of thrombectomy operation are allowed for each occluded artery. Digital subtraction angioplasty (DSA) should be performed following each pass and at the completion of the thrombectomy operation. Type of anesthesia should be recorded. Angioplasty and/or stenting are allowed if necessary and may require the use of antiplatelets. Attention should be paid to maintaining stable blood pressure and minimizing delays in starting the procedure. The initial and final angiograms will be centrally graded for angiographic reperfusion using the mTICI classification.

Vital signs and any occurrence of AEs or SAEs during treatment should be documented. Close neurological observation will be conducted primarily during the first 48 hours after treatment administration according to local clinical practice.

10.4 Study visits (Table 1)

1) 4-6 hours

- Vital signs, concomitant medication, NIHSS, new-onset cardio-cerebral vascular events, AEs/SAEs
- For patients transferred to the catheter room: time of arterial puncture, time of first angiographic run, time of recanalization
- Imaging:
For patients not transferred to the catheter room: CTP (+neck CTA if patients are primarily with ICA-EC severe stenosis/occlusion)
For patients transferred to the catheter room: DSA

2) 24-48 hours

- Vital signs, concomitant medication, NIHSS, new-onset cardio-cerebral vascular events, AEs/SAEs
- Imaging: Non-contrast head CT
- Laboratory test: routine hematology, renal and hepatic function, cardiac markers, cardiac enzymes, pro-BNP, blood lipid, routine urine analysis, coagulation
- Symptomatic intracranial hemorrhage (ECASS II criteria)
- Systematic hemorrhage (GUSTO criteria)

3) 3-5 days

- Vital signs, concomitant medication, NIHSS, new-onset cardio-cerebral vascular events, AEs/SAEs
- Imaging: head MRI (including DWI), head TOF-MRA (+neck MRA if patients are primarily with ICA-EC severe stenosis/occlusion) SWI (optional). Head non-contrast CT, head CTA (+neck CTA if patients are primarily with ICA-EC severe stenosis/occlusion), If patients are incompatible with MRI (e.g., mental device implantation).
- Symptomatic intracranial hemorrhage (ECASS II criteria)
- Systematic hemorrhage (GUSTO criteria)

4) 7±1 days (or at discharge)

-
- Vital signs, concomitant medication, NIHSS, mRS, BI, TOAST classification, new-onset cardio-cerebral vascular events, AEs/SAEs, holter monitoring results
 - Laboratory test: routine hematology, renal and hepatic function, cardiac markers, cardiac enzymes, pro-BNP, blood lipid, routine urine analysis, coagulation
- 5) 30±7 days
- Vital signs, concomitant medication, NIHSS, mRS, BI, TOAST classification, new-onset cardio-cerebral vascular events, AEs/SAEs, holter monitoring results
 - Cognitive tests (optional): MoCA, MMSE
- 6) 90±7 days
- Vital signs, concomitant medication, NIHSS, mRS, BI, TOAST classification, new-onset cardio-cerebral vascular events, AEs/SAEs, holter monitoring results
 - Cognitive tests (optional): MoCA, MMSE

10.5 Study outcomes

1) Primary outcome

The primary outcome is a binary composite of efficacy and safety, i.e., major reperfusion and absence of symptomatic intracranial hemorrhagic transformation (ICH) at 24-48 hours after intravenous tenecteplase. Major reperfusion was considered as the restoration of blood flow of greater than 50% of the involved territory.

- For patients not transferred to the catheter room after thrombolysis, major reperfusion was assessed as reperfusion greater than 50% of the involved hypoperfusion lesion volume (delay time > 3s) at baseline repeated CT perfusion imaging at 4-6 hours after thrombolysis.
- For patients transferred to the catheter room, major reperfusion was evaluated as a modified Treatment In Cerebral Ischemia (mTICI) score 2b/3 at the initial catheter angiography.

Symptomatic ICH was defined according to the European Co-operative Acute Stroke Study-II (ECASS II) criteria, where the patients presented any type of intracranial hemorrhage with no less than 4 point-increase in National Institutes of Health Stroke Scale (NIHSS) from baseline or from the lowest NIHSS between baseline at 24-48 hours, or leading to death.

2) Secondary outcomes

- Secondary radiological efficacy outcomes
 - ✓ Recanalization defined by the Thrombolysis in Myocardial Infarction (TIMI) scale of 2/3 at the 4- to 6- hour CTA (reconstructed from CTP) for patients not transferred to the catheter room, or at digital subtraction angiography imaging prior to thrombectomy for patients transferred to the catheter room after thrombolysis;
 - ✓ Infarct growth on diffusion-weighted imaging (DWI) or non-contrast head CT at 3-5 days.
- Secondary clinical efficacy outcomes
 - ✓ Excellent functional outcome (disability-free, defined as modified Rankin Scale [mRS] 0-1) at 90 days;
 - ✓ Good functional outcome (functional independence, defined as mRS 0-2) at 90 days; mRS distribution at 90 days;
 - ✓ Major neurological improvement at 24-48 hours (NIHSS reduction greater than 7 points or NIHSS 0-1);

- ✓ Change in NIHSS as a continuous variable at 24-48 hours.
- Secondary radiological safety outcomes
 - ✓ Type 2 parenchymal hematoma (PH2);
 - ✓ Symptomatic ICH;
 - ✓ Any ICH at 24-48 hours post treatment.
- Secondary clinical safety outcomes
 - ✓ Poor functional outcome (severe disability or death, defined as mRS 5-6) at 90 days;
 - ✓ Systemic bleeding defined by The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) before discharge.
- Others:
 - ✓ Barthel index at 90 days.

All of these clinical assessments will be conducted through on-site personnel who are blinded to the treatment allocation.

10.6 Protocol compliance

Study visits before discharge (4-6 hours, 24-48 hours, 3-5 days, 7±1 days or at discharge) will be completed together by a fellow doctor in charge of the treatment patients in hospital and one of the investigators both of whom are blinded to the treatment allocation.

Study visits after discharge (30±7 days, 90±7 days) will be completed through stroke clinic by one of the investigators who is blinded to the treatment allocation. Transportation fee of the participants will be covered by the trial. If the participant is incapable of arrival to the clinic in-person due to disability, these visits will be completed through standardized telephone follow-up by a trained investigator who is blinded to the treatment allocation.

11. Data analysis and statistical consideration

11.1 Sample size calculation

The sample size is calculated based on the results derived from the EXTEND-IA TNK trial: 22% patients of the intravenous tenecteplase group and 10% patients of the intravenous alteplase group reached major reperfusion, and 1% patients in both groups respectively were found to have sICH. To adopt a conservative approach, for each dose stratum within the umbrella design, the null hypothesis was that not more than 10% of patients would achieve a positive primary outcome following the respective dose of tenecteplase, while the alternative hypothesis was that, not less than 25% of patients would achieve a positive primary outcome following the respective dose of tenecteplase. Sample size estimation using Simon's two-stage design was conducted for each dose stratum, according to which the null hypothesis that the true response rate was 10% was tested against a one-sided alternative. In the first stage, 18 patients were to be accrued. If there were 2 or fewer positive responses in these 18 patients, meaning that the null hypothesis was not rejected and the dose stratum was to be stopped. Otherwise, 25 additional patients were to be accrued for a total of 43 patients for each dose stratum. The null hypothesis for each stratum was to be rejected if 8 or more positive responses were observed in 43 patients. This design was to yield a type I error rate of 0.05 and a power of 0.8 when the true response rate was 25%. Overall, at least 36 patients were to be enrolled with a maximum enrollment of 86 patients equally distributed between the 2 dose strata. This design would yield a one-sided type I error of 0.5 and a power of 80% to detect the presumed significant difference. In all, at least 36 patients would be enrolled with a maximum enrollment of 86 patients in sum of the 2 dose strata.

11.2 General principles of data analysis

All of the statistical analysis will be performed on STATA v15.1 (StataCorp, Ltd, College Station, Texas).

11.3 Analysis of primary outcome

For the analysis of primary endpoint, as described above, if 3 or more of the 18 patients in the first stage of either dose stratum reach the primary endpoint, then additional 25 patients would be enrolled in that dose stratum. If 8 or more out of the 43 patients reach the primary endpoint within a given stratum after the second stage, then the corresponding tenecteplase dose could be considered to be of sufficient promise in terms of efficacy and safety and could be a qualified candidate dose for the subsequent phase IIb trial.

11.4 Analysis of secondary outcome

Since the design of this study is not aimed to compare the safety and efficacy of the two tenecteplase doses within the umbrella design, the analysis of secondary outcomes only involved data description without comparative analysis. Secondary outcomes were described using percentages, mean and standard deviation, median and interquartile range (IQR) as appropriate. Normality will be tested using Shapiro-Wilk test.

11.5 Sensitivity analysis

Preplanned sensitivity analysis is conducted by removing patients with severe stenosis and analyzing the primary and secondary outcomes of patients with complete artery occlusion in the two tenecteplase dose groups.

In case of any post-hoc subgroup analysis, they will be justified and identified as data-driven and, they will follow the principles and regulatory recommendations.

12. Ethical and regulatory standards

12.1 Ethical principles

This study will be conducted in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and all applicable amendments laid down by the World Medical Assemblies and the ICH guidelines for Good Clinical Practice.

12.2 Laws and regulations

This study will be conducted in compliance with all international laws and regulations, and Chinese laws and regulations in which the Clinical Trial is performed, as well as any applicable guidelines.

12.3 Informed Consent

The investigator (according to applicable regulatory requirements), or a person designated by the investigator and under the investigator's responsibility, should fully inform the patient of all pertinent aspects of the Clinical Trial including the written information giving approval/favorable opinion by the Ethics Committee (IRB/IEC). All participants should be informed to the fullest extent possible about the study, in language and terms they are able to understand.

Prior to a patient's participation in the CHABLIS-T trial, the written informed consent form should be signed, name filled in and personally dated by the patient or by the patient's legally acceptable representative, and by the person who conducted the informed consent discussion. A copy of the signed and dated written Informed Consent Form will be provided to the patient.

The informed consent form used by the investigator for obtaining the patient's informed consent must be reviewed and approved by the sponsor prior to submission to the appropriate Ethics Committee (IRB/IEC) for approval/favorable opinion.

The protocol will be submitted for approval to the appropriate local institutional ethics committee, and written approval obtained, before participants are recruited and enrolled. The Investigators will receive all the documentation needed for submitting the present protocol to the ethics committee. A copy of the respective approval letters will be transmitted to the Study Monitor before starting the study. The compliance/composition of the Ethics Committee will also be provided to the Study Monitor. If approval is suspended or terminated by the ethics committee, the investigator will notify the Study Monitor immediately.

It is the responsibility of the Investigator to report study progress to the ethics committee as required, or at intervals not greater than one year.

The principal investigator, or his/her nominee, will be responsible for reporting any SAEs to the ethics committee as soon as possible, and in accordance with the guidelines of the Ethics Committee.

12.4 Institutional Review Board/ Independent Ethics Committee (IRB/IEC)

The investigator or the sponsor must submit this protocol to the appropriate ethics committee (IRB/IEC), and is required to forward to the Sponsor a copy of the written and dated approval/favorable opinion signed by the chairman with ethics committee (IRB/IEC) composition.

The clinical trial (study number, Clinical Trial Protocol title and version number), the documents reviewed (clinical trial protocol, informed consent form, investigator's brochure, investigator's CV, etc.), the list of voting members along with their qualification and the date of the review should be clearly stated on the written (IRB/IEC) approval/favorable opinion.

Investigational product will not be released at the study site and the trial will not start until a copy of this written and dated approval/favorable opinion has been received by the Sponsor.

During the study, any amendment or modification to the protocol should be submitted to the Ethics Committee (IRB/IEC). It should also be informed of any event likely to affect the safety of patients or the continued conduct of this trial, in particular any change in safety. All updates to the investigator's brochure will be sent to the ethics committee (IRB/IEC).

If requested, a progress report will be sent to the ethics committee (IRB/IEC) annually and a summary of the trial's outcome at the end of this trial.

13. Study monitoring

13.1 Responsibilities of the investigators

The Investigator(s) undertake(s) to perform the clinical trial in accordance with this protocol, ICH guidelines for Good Clinical Practice and the applicable regulatory requirements.

The investigator is required to ensure compliance with all procedures required by the protocol and with all study procedures provided by the Sponsor (including security rules). The investigator agrees to provide reliable data and all information requested by the Clinical

Trial protocol (with the help of the Case Report Form [CRF], Discrepancy Resolution Form [DRF] or other appropriate instrument) in an accurate and legible manner according to the instructions provided and to ensure direct access to source documents by Sponsor representatives.

The investigator may appoint such other individuals as he/she may deem appropriate as sub-investigators to assist in the conduct of the trial in accordance with the protocol. All sub-investigators

shall be appointed and listed in a timely manner. The sub-investigators will be supervised by and under the responsibility of the Investigator. The investigator will provide them with a copy of this protocol and all necessary information.

The Sponsor of this Clinical Trial is responsible to Health Authorities for taking all reasonable steps to ensure the proper conduct of the clinical trial protocol as regards ethics, clinical trial protocol compliance, and integrity and validity of the data recorded on the case report forms. Thus, the main duty of the monitoring team is to help the Investigator and the Sponsor maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the trial.

At regular intervals during the clinical trial, the site will be contacted, through monitoring visits, letters or telephone calls, by a representative of the Monitoring Team to review study progress, Investigator and patient compliance with the protocol requirements and any emergent problems. During these monitoring visits, the following, but not exhaustive, points will be scrutinized with the Investigator: patient informed consent, patient recruitment and follow-up, SAE documentation and reporting, investigational product allocation, patient compliance with the investigational product regimen, investigational product accountability, concomitant therapy use and quality of data.

13.2 Source document requirements

According to the ICH guidelines for Good Clinical Practice, the monitoring team must check the case report form entries against the source documents, except for the pre-identified source data directly recorded in the case report form. The informed consent form will include a statement by which the patient allows the sponsor's duly authorized personnel, the ethics committee (IRB/IEC), and the regulatory authorities to have direct access to source data which support the data on the case report forms (e.g., patient's medical file, appointment books, original laboratory records, etc.). Such personnel, bound by professional secrecy, must keep confidential all personal identity or personal medical information (according to confidentiality rules).

13.3 Data management

Paper-based case report form (CRF) and electronic data capture (EDC) system will be used for data collection and entry. All the content required by the protocol in the system must be provided, the unfilled content should be explained, and the reason needs to be filled in the remarks under each form of the EDC system.

Site investigators should use black or blue-black recording pens to fill out the paper-based CRF neatly and clearly to ensure that the data is clear and readable. If the paper-based CRF information needs to be modified, it should not be altered or overwritten. The correct information should be written next to the original information, signed and dated by the person who modified it. The clinical research monitor (CRA) will review the completeness and accuracy of the case report form and guide the investigator to make necessary corrections and supplements. After the paper-based CRF is completed, the research coordinator (CRC) will input the content of the paper CRF into the EDC system. The paper-based CRF is submitted after the investigator has approved it. After the data is submitted, all data revisions and feedback are carried out through the EDC system. If the EDC system has submitted a form that needs to be modified, you need to contact the CRA of this center. After the CRA opens the form, the investigator can guide the CRC to modify the EDC system data. Data will then be monitored by the CRA according to a data monitoring plan in a regular basis. After the last study visit of the last participant is finished, the data from the EDC will be exported to a database (in excel sheets), then it will be proofread by the data administrator. Obvious errors will be corrected by the data administrator.

Other errors or missing values will be filled in the data query form and returned to the participating center for solution through email, express, telephone and WeChat.

The participating centers are responsible for correcting the data in the EDC system after verifying the original data and related information. Site investigators must answer these queries by verifying or modifying relevant information or data.

13.4 Confidentiality

All of the personal data of research participants (subjects) must be kept strictly confidential. Information and data of the study participants (subjects) are identified only by subject numbers rather than names. Identifiable information of participants (subjects) will not be disclosed to members outside the investigator teams, unless permission is obtained from the research participants (subjects). All of the investigators are required to keep the identities of study participants (subjects) confidential. The files of study participants (subjects) will be kept in locked filing cabinets and will only be accessible to authorized investigators. In order to ensure that the study is carried out in accordance with laws and regulations, government authorities or members of the ethics committee can access to the personal data of the research participants (subjects) according to regulations when necessary. No personal information of the research participants (subjects) will be disclosed at the time of publication of this research.

The research data are also confidential. All of the investigators are required to keep confidential to the research data. They shall not disclose the research data without the permission of the principal investigator to anyone who is not the member of the research team, and shall not transfer the research data to other institute without the permission of the hospital. The research data are not allowed to be disclosed to foreign institutes, or domestic institutes with foreign capital, without the permission of Human Genetic Resource Administration Office of China, except for the publication of research results that meet regulatory requirements under normal circumstances.

14. Funding

The study is sponsored by the National Key Research and Development Program of China (2017YFC1308201), Science and Technology Ministry of China. Guangzhou Recolgen Biotech Co., Ltd. supplied the investigative drug and covered the trial insurance, but had no role in study design and execution.

15. Study Organization

15.1 Principle investigator

Qiang Dong, Huashan Hospital, Fudan University, Shanghai, China

15.2 Trial steering committee members

Qiang Dong (Principal Investigator), Mark Parsons, Xin Cheng, Leonid Churilov, Yilong Wang

- ✓ The steering committee will provide scientific and strategic direction for the trial and will have overall responsibility for its design, execution, and publication.
- ✓ The steering committee will also be responsible for ensuring that study execution and management are of the highest quality.

- ✓ It will approve the protocol and the operational guidelines of the trial prior to its commencement.
- ✓ The steering committee will convene regularly by teleconference or face-to-face meetings to discuss and report on the progress of the study.

15.3 Data safety and monitoring board

The DSMB will meet regularly and monitor the progress of the CHABLIS-T study to ensure that the study meets the highest standards of ethics and patient safety. It is composed of Academic Members, including an independent statistician, who are not otherwise participating in the trial.

Written recommendations and their rationale will be provided to the Chairs of the Steering Committee immediately after each DSMB meeting.

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Table 1 Study Visits in CHABLIS-T

Assessment	Screening and Randomization	Thrombolysis Commencement	Visits					
	-24h~4.5h	0h	4-6h	24- 48h	3-5 days	7±1 days or Discharge	30±7 days	90±7days
Demographic information	X							
Previous medical history	X							
Concomitant medications	X		X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X
Physical examinations	X							
Pregnancy test ¹	X							
Blood lab tests ²	X			X				
Electrocardiography	X							
Inclusion and exclusion criteria confirmation	X							
Randomization	X							
Imaging (CT/MRI/DSA)	X		X	X	X			
Symptomatic intracranial hemorrhage				X	X			
Modified Rankin Scale	X					X	X	X
NIH Stroke Scale	X		X	X	X	X	X	X
TOAST Classification						X		
Cognitive evaluations							X	X
Adverse events		X	X	X	X	X	X	X
Barthel Index						X	X	X

1. Only for female patients at reproductive age

2. 24-hour Holter monitoring

Summary of protocol amendments

Protocol changes version 1.0 (Sept. 2019) to 2.0 (Nov. 2019)

Protocol 1.0	Protocol 2.0
<p>7) 7±1 days (or at discharge)</p> <ul style="list-style-type: none"> ● Vital signs, concomitant medication, NIHSS, mRS, BI, new-onset cardio-cerebral vascular events, AEs/SAEs ● Laboratory test: routine hematology, renal and hepatic function, cardiac markers, cardiac enzymes, pro-BNP, blood lipid, routine urine analysis, coagulation <p>8) 30±7 days</p> <ul style="list-style-type: none"> ● Vital signs, concomitant medication, NIHSS, mRS, BI, new-onset cardio-cerebral vascular events, AEs/SAEs, ● Cognitive tests (optional): MoCA, MMSE <p>9) 90±7 days</p> <ul style="list-style-type: none"> ● Vital signs, concomitant medication, NIHSS, mRS, BI, new-onset cardio-cerebral vascular events, AEs/SAEs ● Cognitive tests (optional): MoCA, MMSE 	<p>4) 7±1 days (or at discharge)</p> <ul style="list-style-type: none"> ● Vital signs, concomitant medication, NIHSS, mRS, BI, TOAST classification, new-onset cardio-cerebral vascular events, AEs/SAEs, holter monitoring results ● Laboratory test: routine hematology, renal and hepatic function, cardiac markers, cardiac enzymes, pro-BNP, blood lipid, routine urine analysis, coagulation <p>5) 30±7 days</p> <ul style="list-style-type: none"> ● Vital signs, concomitant medication, NIHSS, mRS, BI, TOAST classification, new-onset cardio-cerebral vascular events, AEs/SAEs, holter monitoring results ● Cognitive tests (optional): MoCA, MMSE <p>6) 90±7 days</p> <ul style="list-style-type: none"> ● Vital signs, concomitant medication, NIHSS, mRS, BI, TOAST classification, new-onset cardio-cerebral vascular events, AEs/SAEs, holter monitoring results ● Cognitive tests (optional): MoCA, MMSE

Protocol changes Version 2.0 (Nov. 2019) to 3.0 (Nov. 2020)

Protocol 2.0	Protocol 3.0
<p>3) 3-5 days</p> <ul style="list-style-type: none"> ● Vital signs, concomitant medication, NIHSS, new-onset cardio-cerebral vascular events, AEs/SAEs ● Imaging: head MRI (including DWI), head TOF-MRA (+neck MRA if patients are primarily with ICA-EC severe stenosis/occlusion) SWI (optional). ● Symptomatic intracranial hemorrhage (ECASS II criteria) 	<p>3) 3-5 days</p> <ul style="list-style-type: none"> ● Vital signs, concomitant medication, NIHSS, new-onset cardio-cerebral vascular events, AEs/SAEs ● Imaging: head MRI (including DWI), head TOF-MRA (+neck MRA if patients are primarily with ICA-EC severe stenosis/occlusion) SWI (optional). Head non-contrast CT, head CTA (+neck CTA if patients are primarily with ICA-EC severe

<ul style="list-style-type: none">● Systematic hemorrhage (GUSTO criteria)	<p>stenosis/occlusion), If patients are incompatible with MRI (e.g., mental device implantation).</p> <ul style="list-style-type: none">● Symptomatic intracranial hemorrhage (ECASS II criteria)● Systematic hemorrhage (GUSTO criteria)
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**CHinese Acute tissue-Based imaging selection for
Lysis In Stroke -Tenecteplase
(CHABLIS-T)**

Statistical Analysis Plan

Research Team: Department of Neurology, Huashan Hospital,
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1. Study Overview

1.1 Study design

The CHALIS-T trial is a phase IIa, randomized, multicenter, open-label, blinded-endpoint, Simon's two-stage, umbrella design study.

1.2 Study aim

To explore the promise of efficacy and safety of different doses of tenecteplase (0.25mg/kg vs. 0.32mg/kg) in Chinese acute ischemic stroke patients with large/medium vessel occlusion or severe stenosis in an extended time window.

1.3 Patient population

1.3.1 Inclusion criteria

- 1) Patients presenting with acute ischemic stroke within 4.5-24 hours from time last known well.
- 2) Patient's age \geq 18 years
- 3) Premorbid modified Rankin Scale 0-2
- 4) Clinically significant acute neurologic deficit (with no lower or upper limit of the National Institutes of Health Stroke Scale [NIHSS] score) at the discretion of the investigator.
- 5) Anterior large/medium vessel occlusion or severe stenosis (>70% of the vessel caliber), including extracranial or intracranial ICA, M1 or M2 segment of MCA, and A1 or A2 segment of ACA, identified on head and neck CTA
- 6) Penumbra mismatch on CTP: Hypoperfusion lesion volume (Delay time[DT]> 3 s) to infarct core volume ratio (relative cerebral blood flow [rCBF]<30%) > 1.2, an absolute difference of volume > 10 ml, and an ischemic core volume < 70ml.
- 7) Patient/Legally Authorized Representative has signed the Informed Consent form.

1.3.2 Exclusion criteria

Patients are not allowed to participate if they are presented with any of the following standard intravenous thrombolysis exclusion criteria:

- 1) Extensive hypoattenuation region (more than 1/3 of the MCA territory) identified on baseline non-contrast CT (NCCT).
- 2) Any sign of an acute intracranial hemorrhage or subarachnoid hemorrhage identified on baseline NCCT.
- 3) Previous ischemic stroke within 3 months
- 4) Past history of intracranial hemorrhage.
- 5) Acute head trauma at presentation or recent major head trauma within 3 months
- 6) Recent history of intracranial/intraspinal surgery within 3 months
- 7) Gastrointestinal malignancy or gastrointestinal bleeding within 21 days
- 8) Known bleeding diatheses: platelets count <100 000/mm³, INR >1.7, APTT >40 s, or PT >15 s
- 9) Use of a full dosage of low-molecular weighted heparin treatment within the previous 24 hours
- 10) Use of direct thrombin inhibitors or direct factor Xa inhibitors within the previous 48 hours

unless the laboratory test of coagulation function is normal

- 11) Symptoms consistent with infective endocarditis
- 12) Known or suspected aortic arch dissection
- 13) Presence with an intra-axial intracranial neoplasm

In addition to:

- 14) Rapidly improving symptoms at the discretion of the investigator, which may indicate spontaneous recanalization
- 15) Contraindications for CT contrast precluding a CT angiography or perfusion study
- 16) Pregnancy or breastfeeding
- 17) Recent participation in another investigational drug or device study or registry in the past 30 days
- 18) Allergy to the test drug and its ingredients
- 19) Any terminal illness such that the patient would not be expected to survive more than three months
- 20) Other conditions in which investigators believe that participating in this study may be harmful to the patient

1.4 Randomization procedure

Randomization is allowed when all the inclusion criteria are confirmed to be met (including signed informed consent by the patient or the proxies) and all the exclusion criteria have been ruled out, as well as informed consent is signed by the patient or the proxies. Randomization is performed using permuted blocks through a centralized website named Easy Random Trial (ERT) designed by Chuang Da medical science and Technology (Shanghai) Co., Ltd. by a team of professional and independent statisticians. Patients are stratified according to the time of randomization after last known well (4.5-12 hours, 12-24 hours) and site of occlusion/severe stenosis (intracranial segment of internal carotid artery and M1-segment of middle cerebral artery; extracranial segment of internal carotid artery, M2-segment of middle cerebral artery and anterior cerebral artery). The dosage of tenecteplase is open-label while the raters involved in the subsequent radiological and clinical evaluation are blinded to the allocation. The independent data safety monitoring board (DSMB) has the access to all the unblinded data.

1.5 Baseline and follow-up assessments

All responsible investigators receive training in the systems for data collection and data entry and training in Good Clinical Practice (GCP). All investigators responsible for patient assessment participate in training in critical assessment items (NIHSS, mRS).

The assessment schedule for this trial is found in the protocol. Briefly, once eligibility is confirmed and informed consent is obtained, the responsible investigator is able to randomize the patient through the Easy Random Trial (ERT) webpage. The baseline assessments include NIHSS, pre-morbid mRS, demographic data, past medical history, previous stroke history and cerebral imaging information.

All patients are followed up at 4-6 hours, 24-48 hours, 3-5 days, 7±1 days or discharge, 30±7days, 90±7days, unless death occurs. The 3-month assessments are conducted by telephone, by an assessor at each site trained in study procedures who is blinded to treatment allocation.

The trial management team are responsible for ensuring that all data are completed in a timely manner. Institutions receive small reimbursements for their staff time involved in data collection and in the provision of the intervention for this trial. Patients do not receive payment for participation.

1.6 Sample size justification

The sample size is calculated based on the results derived from the EXTEND-IA TNK trial: 22% patients of the intravenous tenecteplase group and 10% patients of the intravenous alteplase group reached major reperfusion, and 1% patients in both groups respectively were found to have sICH. To adopt a conservative approach, for each dose stratum within the umbrella design, the null hypothesis was that not more than 10% of patients would achieve a positive primary outcome following the respective dose of tenecteplase, while the alternative hypothesis was that, not less than 25% of patients would achieve a positive primary outcome following the respective dose of tenecteplase. Sample size estimation using Simon's two-stage design was conducted for each dose stratum, according to which the null hypothesis that the true response rate was 10% was tested against a one-sided alternative. In the first stage, 18 patients were to be accrued. If there were 2 or fewer positive responses in these 18 patients, meaning that the null hypothesis was not rejected and the dose stratum was to be stopped. Otherwise, 25 additional patients were to be accrued for a total of 43 patients for each dose stratum. The null hypothesis for each stratum was to be rejected if 8 or more positive responses were observed in 43 patients. This design was to yield a type I error rate of 0.05 and a power of 0.8 when the true response rate was 25%. Overall, at least 36 patients were to be enrolled with a maximum enrollment of 86 patients equally distributed between the 2 dose strata. This design would yield a one-sided type I error of 0.5 and a power of 80% to detect the presumed significant difference. In all, at least 36 patients would be enrolled with a maximum enrollment of 86 patients in sum of the 2 dose strata.

1.7 Unblinding

Only the Data and Safety Monitoring Board (DSMB) have access to interim data and results. The DSMB Chair is Professor Anding Xu, Department of Neurology and Stroke Center, The First Affiliated Hospital, Jinan University, Guangzhou, China. The DSMB review unblinded data in accordance with the DSMB Charter. Treatment allocations are securely stored and separated from the outcome assessors. Statisticians not involved in the DSMB will remain blinded and work on dummy datasets until the computer codes for statistical analysis are validated.

1.8 Definition of outcomes

1.8.1 Primary outcome

The primary outcome is a binary composite of efficacy and safety, i.e., major reperfusion and absence of symptomatic intracranial hemorrhagic transformation (ICH) at 24-48 hours after intravenous tenecteplase. Major reperfusion was considered as the restoration of blood flow of greater than 50% of the involved territory.

- For patients not transferred to the catheter room after thrombolysis, major reperfusion was assessed as reperfusion greater than 50% of the involved hypoperfusion lesion volume (delay time >3s) at baseline repeated CT perfusion imaging at 4-6 hours after thrombolysis.
- For patients transferred to the catheter room, major reperfusion was evaluated as a modified Treatment In Cerebral Ischemia (mTICI) score 2b/3 at the initial catheter angiography.

Symptomatic ICH was defined according to the European Co-operative Acute Stroke Study-II (ECASS II) criteria, where the patients presented any type of intracranial hemorrhage with no less than 4 or more point-increase in National Institutes of Health Stroke Scale (NIHSS) from baseline or from the lowest NIHSS between baseline at 24-48 hours, or leading to death.

1.8.2 Secondary outcomes

- Secondary radiological efficacy outcomes
 - ✓ Recanalization defined by the Thrombolysis in Myocardial Infarction (TIMI) scale of 2/3 at the 4- to 6- hour CTA (reconstructed from CTP) for patients not transferred to the catheter room, or at digital subtraction angiography imaging prior to thrombectomy for patients transferred to the catheter room after thrombolysis;
 - ✓ Infarct growth on diffusion-weighted imaging (DWI) or non-contrast head CT at 3-5 days.
- Secondary clinical efficacy outcomes
 - ✓ Excellent functional outcome (disability-free, defined as modified Rankin Scale [mRS] 0-1) at 90 days;
 - ✓ Good functional outcome (functional independence, defined as mRS 0-2) at 90 days; mRS distribution at 90 days;
 - ✓ Major neurological improvement at 24-48 hours (NIHSS reduction greater than 7 points or NIHSS 0-1);
 - ✓ Change in NIHSS as a continuous variable at 24-48 hours.
- Secondary radiological safety outcomes
 - ✓ Type 2 parenchymal hematoma (PH2);
 - ✓ Symptomatic ICH;
 - ✓ Any ICH at 24-48 hours post treatment.
- Secondary clinical safety outcomes
 - ✓ Poor functional outcome (severe disability or death, defined as mRS 5-6) at 90 days;
 - ✓ systemic bleeding defined by The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) before discharge.
- Others:
 - ✓ Barthel index at 90 days

All of these clinical assessments will be conducted through on-site personnel who were blinded to the treatment allocation.

2. Funding

This study is supported by National Key R&D Program of China (2017YFC1308201), Clinical Research Plan of SHDC (SHDC2020CR4016).

3. Statistical analysis

3.1 Descriptive analysis

- 3.1.1 Continuous variables: Continuous variables will be described using mean and standard deviation (SD) if normally distributed, or median and interquartile range (IQR) if skewedly distributed. Normality will be tested using Shapiro-Wilk test.
- 3.1.2 Categorical variables: Categorical variables will be described using the exact number in each category and percentage.

3.2 Analysis of primary outcome

For the analysis of primary endpoint, as described above, if 3 or more of the 18 patients in the first stage of either dose stratum reach the primary endpoint, then additional 25 patients will be enrolled in that dose stratum. If 8 or more out of the 43 patients reach the primary endpoint within a given stratum after the second stage, then the corresponding tenecteplase dose can be considered to be of sufficient promise in terms of efficacy and safety and could be a qualified candidate dose for the subsequent phase IIb trial.

3.3 Analysis of secondary outcome

Since the design of this study is not aimed to compare the safety and efficacy of the two tenecteplase doses within the umbrella design, the analysis of secondary outcomes only involved data description without comparative analysis. Secondary outcomes were described using percentages, mean and standard deviation, median and interquartile range (IQR) as appropriate. Normality will be tested using Shapiro-Wilk test.

- 3.3.1 Secondary radiological efficacy outcomes
 - ***Recanalization defined by the Thrombolysis in Myocardial Infarction (TIMI) scale of 2/3 at the 4- to 6- hour CTA (reconstructed from CTP) for patients not transferred to the catheter room, or at digital subtraction angiography imaging prior to thrombectomy for patients transferred to the catheter room after thrombolysis;***
This outcome will be presented using the exact number and percentage of patients reaching recanalization at 4-6 hours in each dose stratum.
 - ***Infarct growth on diffusion-weighted imaging (DWI) or non-contrast head CT at 3-5 days.***
This outcome will be presented using mean and standard deviation (SD) if infarct growth at 3-5 days is normally distributed, or median and interquartile range (IQR) if skewedly distributed in each dose stratum. Normality will be tested using Shapiro-Wilk test.
- 3.3.2 Secondary clinical efficacy outcomes
 - ***Excellent functional outcome (disability-free, defined as modified Rankin Scale [mRS] 0-1) at 90 days;***
This outcome will be presented using the exact number and percentage of patients reaching excellent functional outcome at 90 days in each dose stratum.
 - ***Good functional outcome (functional independence, defined as mRS 0-2) at 90 days; mRS distribution at 90 days;***

This outcome will be presented using the exact number and percentage of patients reaching good functional outcome at 90 days in each dose stratum.

- **Major neurological improvement at 24-48 hours (NIHSS reduction greater than 7 points or NIHSS 0-1);**

This outcome will be presented using the exact number and percentage of patients reaching major neurological improvement at 24-48 hours in each dose stratum.

- **Change in NIHSS as a continuous variable at 24-48 hours.**

This outcome will be presented using mean and standard deviation (SD) if change in NIHSS at 24-48 hours is normally distributed, or median and interquartile range (IQR) if skewedly distributed in each dose stratum. Normality will be tested using Shapiro-Wilk test.

3.3.3 Secondary radiological safety outcomes

- **Type 2 parenchymal hematoma (PH2) at 24-48 hours post treatment;**

This outcome will be presented using the exact number and percentage of patients demonstrating PH2 at 24-48 hours in each dose stratum.

- **Symptomatic ICH at 24-48 hours post treatment;**

This outcome will be presented using the exact number and percentage of patients demonstrating symptomatic ICH at 24-48 hours in each dose stratum.

- **Any ICH at 24-48 hours post treatment.**

This outcome will be presented using the exact number and percentage of patients demonstrating any ICH at 24-48 hours in each dose stratum.

3.3.4 Secondary clinical safety outcomes

- **Poor functional outcome (severe disability or death, defined as mRS 5-6) at 90 days;**

This outcome will be presented using the exact number and percentage of patients suffering from poor functional outcome at 90 days at 24-48 hours in each dose stratum.

- **Systemic bleeding defined by The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) before discharge.**

This outcome will be presented using the exact number and percentage of patients suffering from systemic bleeding before discharge in each dose stratum.

3.3.5 Others:

- **Barthel index at 90 days.**

This outcome will be presented using mean and standard deviation (SD) if Barthel index at 90 days is normally distributed, or median and interquartile range (IQR) if skewedly distributed in each dose stratum. Normality will be tested using Shapiro-Wilk test.

3.4 Interim analysis

Interim analysis will be conducted when 18 patients have been enrolled in each dose stratum. If more than 2 out of the 18 patients in either dose stratum reach the primary endpoint, then another additive 25 patients will be enrolled in that group, otherwise the dose strata will be dropped.

3.5 Sensitivity analysis

3.5.1 Preplanned sensitivity analysis

Preplanned sensitivity analysis is conducted removing patients with severe stenosis, analyzing the primary and second outcomes of patients with complete artery occlusion in the two tenecteplase dose groups.

3.5.2 Post-hoc sensitivity analysis

In case of any post-hoc subgroup analysis, they will be justified and identified as data-driven and, they will follow the principles and regulatory recommendations.

3.6 Handling of missing data

If missing data were observed, multiple imputations would be performed for the primary outcome. However, we did not plan any imputations for secondary outcomes, and frequencies with percentages of missing data on secondary outcomes would be reported.

3.7 Interim analysis

An interim analysis will be conducted when each treatment arm has recruited 18 patients. If three or more patients achieve the positive primary endpoint in either arm, then the study will continue to the 2nd stage, which will enroll an addition of 25 patients in that tenecteplase dose group (43 patients in total in each dose group), otherwise the dose group will be stopped. The intervention dose will be deemed to be of sufficient promise if 8 or more out of 43 patients achieve positive primary outcome.

3.8 Data blind review

The Data Blind Review (DBR) will be performed before lock of database. Data will be examined for compliance with the trial protocol by the monitor and the data manager. Criteria for deviations will be sent to the project statistician to plan listings for the Data Blind Review (DBR). The objective is to carry out the population selection and definition of the final study populations as well as a preliminary assessment of the quality of the trial data and the applicability of some statistical procedures such as the handling of missing data.

During the DBR, missing data and intercurrent events will be classified according to the plan described in section 3.6. Changes from that plan to adapt to new/unexpected intercurrent events during the blinded review are permitted but they should be traced and justified in the statistical report.

3.9 Data safety monitoring board

An independent Data Safety Monitoring Board (DSMB) will be established. The purpose of the DSMB is to review, on a regular basis, accumulating data from the ongoing trial. The DSMB will be composed of two stroke neurologists and a statistician who are not participating in the study and are not affiliated with the sponsor. The role of the DSMB will be to: 1) Review the occurrence of AEs and SAEs and 2) Make recommendations to the Executive Committee regarding safety of the

study. A strict control of predefined AEs and SAEs will be ensured through monitoring by the CRO.

A DSMB will follow up the safety of the study. DSMB will review the data in a blinded manner so that the study will maintain the integrity and will avoid any operational bias. Any potential analysis amendment will be traced and justified, if applicable. The study followed the regulatory recommendations regarding the functions and procedures of these committees.