Chinese Acute Tissue-Based Imaging Selection for Lysis In Stroke Tenecteplase II (CHABLIS-T II): rationale and design

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ABSTRACT

Background and purpose Tenecteplase (TNK) has demonstrated non-inferiority to alteplase in patients who had an acute ischaemic stroke presenting within 4.5 hours from symptom onset. The trial is aimed to explore the efficacy and safety of TNK in Chinese patients who had an acute ischaemic stroke with large/medium vessel occlusion in an extended time window.

Methods and design Chinese Acute Tissue-Based Imaging Selection for Lysis In Stroke Tenecteplase II (CHABLIS-T II) is a multicentre, prospective, block-randomised, open-label, blinded-endpoint, phase IIb study. Eligible patients are 1:1 randomised into two groups: 0.25 mg/kg TNK versus best medical management (excluding TNK). The safety and efficacy of 0.25 mg/kg TNK are assessed through reperfusion status and presence of symptomatic intracranial haemorrhage (sICH).

Study outcomes The primary outcome is major reperfusion without sICH at 24–48 hours after randomisation. Major reperfusion is defined as restoration of blood flow to greater than 50% of the involved ischaemic territory assessed by catheter angiography or repeated perfusion imaging. Secondary outcomes include post-thrombolytic recanalisation, neurological improvements, change in the National Institutes of Health Stroke Scale score, haemorrhagic transformation at 24–48 hours, systematic bleeding at discharge, modified Rankin Scale (mRS) 0–1, mRS 0–2, mRS 5–6, mRS distribution and Barthel index at 90 days.

Discussion CHABLIS-T II will provide important evidence of intravenous thrombolysis with TNK for patients who had an acute stroke in an extended time window.

INTRODUCTION

Emerging evidence has proven the non-inferiority of intravenous thrombolysis using tenecteplase (TNK) to alteplase in patients who had an acute ischaemic stroke (AIS) presenting within 4.5 hours after stroke onset. However, only a very limited number of patients are able to receive intravenous thrombolytic treatment due to its short treatment time window. Therefore, efforts have been made to extend the treatment time window of intravenous thrombolysis using alteplase beyond 4.5 hours.

Previous randomised controlled trials showed that TNK may have superior recanalisation efficacy in patients with complete large vessel occlusion to alteplase, especially in patients with penumbral mismatch on perfusion imaging. These results implicate the superiority of TNK to alteplase may emerge in patients with large vessel occlusion and favourable perfusion profile.

The TNK produced by Guangzhou Recomgen Biotech Co in China was firstly demonstrated by Tenecteplase Reperfusion therapy in Acute ischemic Cerebrovascular Events (TRACE) investigators to be non-inferior to alteplase in patients who had an AIS presenting within the 4.5-hour time window at 0.25 mg/kg. The lately-completed Chinese Acute Tissue-Based Imaging Selection for Lysis In Stroke Tenecteplase (CHABLIS-T) trial, exploring the optimal dose of Chinese patients who had an AIS with large/medium vessel occlusion or severe stenosis, demonstrated that among patients with significant
penumbral mismatch presenting within 4.5–24 hours from time last known well, both TNK 0.25 mg/kg and 0.32 mg/kg were effective and safe to achieve substantial reperfusion without symptomatic intracerebral haemorrhage (NCT04086147). Based on the prior findings, the CHABLIS-T II trial is conducted to further explore the efficacy and safety of TNK at the dose of 0.25 mg/kg in Chinese patients who had an AIS with large/medium vessel occlusion in an extended time window.

**METHODS**

**Design**

The CHABLIS-T II trial is a multicentre, prospective, block-randomised, open-label, blinded-endpoint, phase IIb study. The study is aimed to explore the efficacy and safety of TNK at the dose of 0.25 mg/kg in Chinese patients who had an AIS with large/medium vessel occlusion in an extended time window. Eligible patients are enrolled and 1:1 randomised to receive either 0.25 mg/kg TNK or best medical management (excluding intravenous TNK). The safety and efficacy of 0.25 mg/kg TNK are assessed through reperfusion status and presence of symptomatic intracranial haemorrhage (sICH).

**Patient population**

**Inclusion criteria**

1. AIS within 4.5–24 hours from time last known well.
2. Age within 18–80 years.
3. Pre-stroke modified Rankin Scale (mRS) 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, including extracranial or intracranial segment of the internal carotid artery (ICA), M1 or M2 segment of the middle cerebral artery (MCA), and A1 or A2 segment of the anterior cerebral artery (ACA), identified on baseline CT angiography (CTA) of head and neck.
6. Penumbral mismatch on baseline CT perfusion (CTP) imaging: a greater-than-1.2 mismatch ratio of hypoperfusion lesion volume (delay time (DT)> 3 s) to ischaemic core volume (relative cerebral blood flow (rCBF)<30%), an absolute difference in volume>10 mL, and an ischaemic core volume<70 mL.
7. Written informed consent from the participant or their legally authorised representative.

**Exclusion criteria**

Patients are not allowed to participate if they meet any of the following standard intravenous thrombolysis contraindications:

1. Extensive hypoattenuation region (greater than 1/3 of the MCA territory) identified on baseline non-contrast CT (NCCT).
2. Any sign of an acute intracranial haemorrhage or subarachnoid haemorrhage identified on baseline NCCT.
3. History of ischaemic stroke within the last 3 months.
4. Any intracranial haemorrhage in the past.
5. Acute head trauma at presentation or with recent major head trauma within 3 months.
6. History of intracranial/intraspinal surgery during the last 3 months.
7. Gastrointestinal malignancy or gastrointestinal bleeding within 21 days.
8. Known bleeding diatheses: platelets count<100 000/\(\text{mm}^3\), international normalised ratio>1.7, prothrombin time>15 s, or activated partial thromboplastin clotting time>40 s.
9. Treatment with a full dosage of low-molecular-weight heparin in the last 24 hours.
10. Treatment with 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 of an intra-axial intracranial neoplasm. In addition to:
   1. Rapidly improving symptoms at the discretion of the investigator, which may indicate spontaneous recanalisation.
   2. Clinical presentation or imaging profile consistent with moyamoya disease/syndrome.
   3. Contraindications for CT contrast precluding a CTA or perfusion study.
   4. Pregnancy or breast feeding.
   5. Recent participation in any other interventional study or registry in the past 30 days before enrolment.
   6. Allergy to the test drug and its ingredients.
   7. Any terminal illness such that the patient would not be expected to survive more than 3 months.
   8. Any condition in which investigators believe that participating in this study may be harmful to the patient.

**Treatment intervention**

**Treatment allocation**

Eligible subjects will be enrolled and randomised in a 1:1 ratio into two groups:

1. 0.25 mg/kg TNK (maximum 25 mg, Guangzhou Recomgen Biotech Co.) as a bolus over 5–10 s and a following 2 mL bolus of saline for injection.
2. Best medical treatment (excluding TNK). If the patients are presented within 4.5–9 hours from the onset of stroke, or 9 hours from the midpoint of sleep for wake-up strokes, alteplase is allowed to use at the discretion of local stroke neurologists.

Bridging endovascular treatment is optional for both groups if the patients meet the criteria of endovascular treatment of the latest Chinese Guidelines on Early Management of Acute Ischemic Stroke.

**Endovascular treatment**

If endovascular treatment is consented by the patient or the proxies and is considered feasible at the discretion
of the neurointerventionalists, the patient will be transferred to the angio-suite. Thrombectomy will be averted if haemorrhagic transformation or complete recanalisation occurred before any operation of thrombectomy or angioplasty. Before any thrombectomy operation, intracranial angiography should be performed to confirm the occlusion site and to evaluate the primary score on the modified Treatment In Cerebral Infarction (mTICI) scale.11

**Radiological and clinical assessment**

**Radiological assessment**

Standard multimodal CT (head NCCT, CTP, head and neck CTA) imaging of each potentially eligible patient will be acquired before enrolment. Perfusion imaging will be real-time processed using full-automated MIStar (Apollo Medical Imaging Technology, Melbourne, Victoria, Australia). Based on single value deconvolution with delay and dispersion correction, the software is able to quantify volumes of hypoperfusion lesion (DT > 3s) and infarct core (rCBF < 30% within areas of DT>3s) at each site.12

For patients not transferred to angio-suite after thrombolysis, repeated CTP imaging (and neck CTA for patients with extracranial segment of ICA occlusion) will be performed at 4–6 hours after thrombolysis to assess reperfusion. For patients transferred to angio-suite, reperfusion will be evaluated by mTICI score at the initial angiogram. For patients transferred to angio-suite, recanalisation status prior to thrombectomy/angioplasty will also be evaluated using the first-running imaging of digital subtraction angiography.

A head NCCT scan will be scheduled at 24–48 hours after thrombolysis to assess if there is any intracranial haemorrhagic transformation. Diffusion-weighted imaging (DWI) or head NCCT for magnetic resonance-incompatible patients will be performed at 3–5 days to evaluate final infarct volume using MIStar.

Centrally standardised imaging protocols of multimodal CT will be implemented at each centre through discreet quality control. All of the imaging will be centrally analysed in a core lab. The baseline multimodal CT imaging of each subject will be reanalysed to make sure that the entry criteria are met. The radiological outcome will be evaluated by two independent neuroradiologists, and a third independent rater will be consulted for cases with disagreement. All three raters will be blinded to treatment allocation.

**Clinical assessments**

Neurological deficits and functional scores will be assessed by certified investigators blinded to treatment allocation in each participating centre.

1. NIHSS will be evaluated before randomisation, at 4–6 hours and 24–48 hours after randomisation and repeatedly evaluated at 3–5 days, 7±1 days (or at discharge), 30±7 days and 90±7 days after randomisation.
2. mRS will be assessed at 7±1 days (or at discharge), 30±7 days and 90±7 days after randomisation via in-person visit or standardised telephone interview. The evaluation of mRS at 90±7 days will be audio recorded and reviewed again by a second blinded evaluator. In case of any disagreement, a second blinded evaluator will be consulted.

3. Barthel Index (BI) will be assessed at 7±1 days (or at discharge), 30±7 days and 90±7 days after randomisation via in-person visit or standardised telephone interview.

4. Cognitive evaluation will be optionally measured at 90±7 days post randomisation by Montreal Cognitive Assessment and Mini-Mental State Examination through in-person visits.

**Outcomes and follow-up visits**

**Primary outcome**

The primary outcome is a binary composite of efficacy and safety, that is, major reperfusion and the absence of sICH at 24–48 hours after randomisation. Major reperfusion is defined as ≥50% restoration of blood flow to the involved ischaemic territory.

1. For patients not transferred to angio-suite after treatment, major reperfusion is achieved when there is at least 50% of reperfusion of the involved baseline hypoperfusion lesion volume (DT>3s) on 4-to-6-hour repeat CTP imaging.

2. For patients transferred to angio-suite, major reperfusion is achieved when mTICI score is at 2b or 3 on the initial angiogram.

SICH is defined as any type of intracranial haemorrhage with at least four points increase in the NIHSS score from baseline.13

**Secondary outcomes**

1. Secondary radiological efficacy outcomes
   - Recanalisation defined as a score≥2 on the Thrombolysis in Myocardial Infarction scale of at the 4-hour to 6-hour CTA (reconstructed from CTP) for patients not transferred to angio-suite, or at first angiographic acquisition prior to thrombectomy for patients transferred to angio-suite
   - Infarct growth defined by the volumetric difference between the infarct volume on DWI/NCCT at 3–5 days and the ischaemic core volume at baseline.

2. Secondary clinical efficacy outcomes
   - Excellent functional outcome (free of disability, mRS 0–1) at 90 days.
   - Good functional outcome (functional independence, mRS 0–2) at 90 days.
   - mRS distribution at 90 days.
   - Major neurological improvement within 24–48 hours (reduction of greater than 7 points on NIHSS or a NIHSS score of 0–1).
   - Change in NIHSS as a continuous variable at 24–48 hours.

3. Secondary radiological safety outcomes
   - Type 2 parenchymal haematoma at 24–48 hours after treatment;

4. Secondary clinical safety outcomes
– Poor functional outcome (severe disability or death, defined as mRS 5–6 at 90 days).
– Systemic bleeding before discharge (according to The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries criteria).

5. Others
– BI at 90±7 days.
All of these clinical assessments will be conducted through on-site personnel who are blinded to the treatment allocation.

Follow-up visits
Baseline screening tests and follow-up visits are listed in Table 1.

Randomisation and blinding
Randomisation is allowed when all the inclusion criteria are confirmed to be met, all the exclusion criteria have been ruled out, and that written informed consent is obtained from the patient or the proxies. Randomisation is performed using permuted blocks through a centralised website named Easy Random Trial designed by Trial Data Pharmaceutical Technology (Shanghai) Co., by a team of professional and independent statisticians. Since the most updated evidence for thrombolysis in an extended time window under the guidance of perfusion imaging is from the Thrombolysis Guided by Perfusion Imaging up to 9 Hour after Onset of Stroke (EXTEND) study, patients in CHABLIS-T II are stratified according to the time since last known well at randomisation (4.5–9 hours vs 9–24 hours). Treatment allocation is open label while the raters involved in subsequent radiological and clinical evaluation are blinded. The data safety monitoring board (DSMB), independent from the trial committee, has access to all the unblinded grouped data.

Sample size and statistical analysis
Sample size calculation
The sample size is calculated based on the results derived from the EXTEND-IA TNK trial. In the EXTEND-IA TNK trial, 22% of patients from the intravenous TNK group and 10% of patients from the intravenous alteplase group reached major reperfusion, and 1% of patients in
both groups, respectively, were found to develop sICH. We hypothesise that in our study, there will be 21% and 7% of patients in the TNK and best medical management group, respectively, reaching the primary outcome. This trial is designed to yield a two-sided type I error of 0.05 and a power of 85% to detect the presumed significant difference. Considering a 5% drop-out rate, a total number of 224 patients (112 patients each group) is estimated to be enrolled in the CHABLIS-T II trial.

Statistical analysis
The primary analysis will be based on an intention-to-treat principle. Missing values will remain missing. All of the statistical analysis will be performed on SAS V.9.4 (or above) or Stata V.13.0 (or above). A two-sided statistical significance threshold of 0.05 is adopted. Continuous variables will be summarised by use of mean and SD if normally distributed or median and IQR if skewedly distributed. Categorical variables will be summarised as frequencies and percentages. The null hypothesis for the primary objective is that both treatment arms (ie, the 0.25 mg/kg TNK group and the best management group) have equal proportion of patients achieving the primary outcome. Differences between the two groups in the proportion of primary outcome will be compared using modified Poisson regression with log-link and robust error estimation adjusted for time from last known well to randomisation (4.5–9 hours vs 9–24 hours), site of vessel occlusion (ICA vs Other vessels), and endovascular treatment (EVT, Yes vs No). Adjusted relative risks (aRR) with 95% CIs between treatment groups will be estimated and presented.

Analysis of the secondary outcomes will be performed per standard statistical principles of modified Poisson regression model, median regression model and ordinal logistic regression model as appropriate.

Subgroup analysis will be carried out among four previously planned subgroups each at a time: occlusion site (ICA vs others), time from last known well to randomisation (4.5–9 hours vs 9–24 hours), witnessed versus unwitnessed stroke, and EVT vs non-EVT. The statistical method will follow the main analysis with an interaction term between treatment and each of the four subgroup factors additionally adding into the model (eg, treatment+subgroup + treatment×subgroup).

The primary outcome will be reanalysed under a range of assumptions about the missing data in the sensitivity analysis.

Data safety monitoring
A DSMB has been commissioned to monitor the study progress and ensure the safety of subjects by tracking the performance of the trial and data, reviewing serious adverse events and identifying any clinically relevant trends. It is consisted of international academicians with expertise in stroke neurology or biostatistics. Though no formal interim analyses for efficacy or futility is scheduled ahead, the board will consider advising an early termination of the trial out of safety concerns in case of high rate of AEs.

Study organisation and funding
This investigator-initiated trial is managed by the steering committee and is supported by the Clinical Research Plan of Shanghai Hospital Development Center (SHDC2020CR1041B). TNK is donated by Guangzhou Recomgen Biotech Co.

DISCUSSION
TNK has currently been considered as an alternative thrombolytic agent of alteplase, with its bolus nature and higher specificity of fibrin. Evidence has proven the effectiveness of alteplase in an extended time window using perfusion imaging or MRI.4,14 It has been acknowledged that TNK has a higher recanalisation efficacy compared with alteplase in AIS patients with large vessel occlusion(s).6,9 Therefore, intravenous thrombolysis in an extended time window using TNK for patients with large vessel occlusion(s) can bring potential benefit concerning rapid recanalisation and clinical improvements.

CHABLIS-T II is a multicentre, randomised trial, exploring the efficacy and safety of TNK at the dose of 0.25 mg/kg in Chinese patients who had an AIS with large/medium vessel occlusion in an extended time window. The primary endpoint is major reperfusion and absence of sICH. Since EVT has become a routine practice for patients who had an AIS in the extended time window, we allow patients with intention for EVT to be included in the CHABLIS-T II trial for the better clinical prognosis of patients. Chinese patients who had an AIS with large vessel occlusion(s) have been faced with the challenge of a longer reperfusion time of EVT compared with the Western population.15–19 One of the main reasons may be related to the high prevalence of in-situ thrombosis due to intracranial atherosclerotic disease (ICAD) in Asian patients with acute large vessel occlusion,20 which is refractory to the current stent retriever or aspiration systems.21 Additionally, the number of comprehensive stroke centres with capability of endovascular treatment is still insufficient considering the broad landscape of China, and the yet underdeveloped drip-and-ship transfer system. Therefore, given the its performance in large vessel occlusion and its potential to extend the therapeutic time window for intravenous thrombolysis, TNK can be a great candidate for reperfusion therapy to improve the clinical outcome of Chinese patients who had an AIS with large vessel occlusion. The allowance of EVT will encourage participants recruitment not only in the primary but also in the comprehensive stroke centres, ensuring the generalisability and representativeness of this trial. However, the clinical effect of EVT will be too robust to be balanced and a very large sample size will be needed if 3-month functional outcome is set as the primary outcome. Therefore, a composite primary endpoint taking both reperfusion efficacy and safety
into account is decided on in this trial. Additionally, the effect of EVT procedures may have a great impact on our primary and secondary outcome, especially for bleeding events. Therefore, a subgroup analysis concerning participants with and without EVT is planned.

Reperfusion status will be assessed through catheter angiography for patients with further endovascular treatment and through repeated perfusion imaging for patients with TNK therapy only. The reason that 4–6 hours post thrombolysis is chosen as the time-point to assess reperfusion is that one previous study has demonstrated that the rate of 3-month good clinical outcome for patients recanalised between 6 and 24 hours might be close to that of non-recanalised patients. Another study has reported a potentially higher haemorrhagic risk with patients recanalised within the 6–24 hours time window. Therefore, 4–6 hours is considered as an appropriate time-point to assess both the velocity and the clinical benefit of reperfusion. Additionally, reperfusion assessed by repeated perfusion imaging has demonstrated a predictive ability of clinical outcome not worse than that of reperfusion assessed by catheter angiography. Hence, reperfusion evaluation through either catheter angiography or repeated perfusion imaging is feasible and reasonable.

CHABLIS-T II is an open-label, blinded-endpoint randomised trial. Though a double-blinded design would be optimal for a clinical trial, the budget of manufacturing, quality control and administration monitoring of placebo is beyond the financial support of this trial. In order to eliminate the bias of treatment allocation awareness, blinded evaluation of all the clinical and radiological assessments concerning primary and secondary endpoints will be warranted by trained neurologists and radiologists. There are other ongoing and nearly completed trials exploring the treatment effect of TNK in the extended time window (TRACE III, TIMELESS and ETERNAL-LVO (NCT04454788)). Though with similar study objectives, these trials differ in specific imaging inclusion criteria, exclusion criteria regarding to planned EVT and primary outcomes. The ongoing trials, together with CHABLIS-T II, shall provide important evidence of thrombolytic therapy with TNK for patients who had an AIS in the extended time window.

CONCLUSIONS
The results of CHABLIS-T II will have a major impact on the reperfusion therapy in an extended time window, which will provide important evidence of intravenous thrombolysis with TNK for patients within 4.5–24 hours from time last known well.

Contributors All the authors have contributed significantly.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by 2021(643) The Ethics Committee of Huashan Hospital. Participants gave informed consent to participate in the study before taking part.

REFERENCES

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Data availability statement Data sharing not applicable as no datasets generated and/or analysed for this study.

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