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Tenecteplase thrombolysis for stroke up to 24 hours after onset with perfusion imaging selection: the umbrella phase IIa CHABLIS-T randomised clinical trial

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ABSTRACT

Background The performance of intravenous tenecteplase in patients who had an acute ischaemic stroke with large/medium vessel occlusion or severe stenosis in an extended time window remains unknown. We investigated the promise of efficacy and safety of different doses of tenecteplase manufactured in China, in patients who had an acute ischaemic stroke with large/medium vessel occlusion beyond 4.5-hour time window.

Methods The CHinese Acute tissue-Based imaging selection for Lysis In Stroke-Tenecteplase was an investigator-initiated, umbrella phase IIa, open-label, blinded-endpoint, Simon's two-stage randomised clinical trial in 13 centres across mainland China. Participants who had salvageable brain tissue on automated perfusion imaging and presented within 4.5–24 hours from time of last seen well were randomised to receive 0.25 mg/kg tenecteplase or 0.32 mg/kg tenecteplase, both with a bolus infusion over 5–10 s. The primary outcome was proportion of patients with promise of efficacy and safety defined as reaching major reperfusion without symptomatic intracranial haemorrhage at 24–48 hours after thrombolysis. Assessors were blinded to treatment allocation. All participants who received tenecteplase were included in the analysis.

Results A total of 86 patients who had an acute ischaemic stroke identified with anterior large/medium vessel occlusion or severe stenosis were included in this study from November 2019 to December 2021. All of the 86 patients enrolled either received 0.25 mg/kg (n=43) or 0.32 mg/kg (n=43) tenecteplase, and were available for primary outcome analysis. Fourteen out of 43 patients in the 0.25 mg/kg tenecteplase group and 10 out of 43 patients in the 0.32 mg/kg tenecteplase group reached the primary outcome, providing promise of efficacy and safety for both doses based on Simon's two-stage design.

Discussion Among patients with anterior large/medium vessel occlusion and significant penumbral mismatch presented within 4.5–24 hours from time of last seen well, tenecteplase 0.25 mg/kg and 0.32 mg/kg both provided sufficient promise of efficacy and safety.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Tenecteplase has now been proven to be non-inferior to alteplase, in patients who had an acute ischaemic stroke within the 4.5-hour time window. However, its performance in patients who had an acute stroke with large/medium vessel occlusion or severe stenosis in an extended time window remains unknown.

WHAT THIS STUDY ADDS

⇒ Among patients with anterior large/medium vessel occlusion and significant penumbral mismatch presented within 4.5–24 hours from time of last seen well, tenecteplase 0.25 mg/kg and 0.32 mg/kg both provided sufficient promise of efficacy and safety.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ It seems feasible to extend the time window of intravenous tenecteplase thrombolysis to 24 hours after last seen well through perfusion imaging selection.

Trial registration number ClinicalTrials.gov Registry (NCT04086147, https://clinicaltrials.gov/ct2/show/NCT04086147).

INTRODUCTION

Thrombolysis with alteplase is limited by its short half-life and low recanalisation rate of large vessel occlusion.[1] Tenecteplase—a genetically modified variant of alteplase—has gained increasing interest as an alternative for alteplase over the past decade. This is mainly due to its practical advantages (single bolus, rather than 1-hour infusion) and a number of hypothetical advantages over alteplase, including greater fibrin specificity.[2] It has been demonstrated that tenecteplase is non-inferior to alteplase in unselected patients who had an ischaemic stroke,[3–5]

and may be superior to alteplase in acute large vessel occlusion.[6] Prior tenecteplase trials have recruited acute ischaemic stroke presenting no longer than 6 hours from symptom onset.[3 5 7–15] Thus, the evidence of performance of tenecteplase beyond 4.5 hours still remains scarce. Perfusion imaging selection has been shown to extend the time for thrombolysis,[16] though increasing evidence suggests the optimal dose of tenecteplase in acute stroke of the Western population is 0.25 mg/kg[7 8 10 13] and the Tenecteplase in Stroke Patients Between 4.5 and 24 Hours (TIMELESS, NCT03785678) trial has reported that 0.25 mg/kg tenecteplase could improve recanalisation compared with placebo.[17] The appropriate dose in the Chinese patients with acute large/medium vessel occlusion or severe stenosis was unknown when the trial started.

Therefore, our hypothesis is that tenecteplase administered to patients who had an ischaemic stroke with a favourable penumbral profile on perfusion CT (CTP) between 4.5 and 24 hours from time of last seen well would be safe and beneficial.

We conducted the CHinese Acute tissue-Based imaging selection for Lysis In Stroke-Tenecteplase (CHABLIS-T) umbrella phase IIa randomised clinical trial. The goal of CHABLIS-T was to investigate the promise of efficacy and safety of various doses of tenecteplase in Chinese patients who had an acute ischaemic stroke with large/medium vessel occlusion or severe stenosis in the anterior circulation and a favourable penumbral profile between 4.5 and 24 hours from time of last seen well. The tenecteplase used in the trial was produced and approved for treating acute myocardial infarction in China and has been used in Tenecteplase Reperfusion therapy in Acute ischemic Cerebrovascular Events (TRACE) trials,[5 11] which had the same terminal amino acid sequence and different production process to the tenecteplase made by Boehringer (Metalyse) and Genentech (TNKase).

METHODS

Study design

When the trial started, the appropriate dose of tenecteplase in acute stroke, especially in Asian population, was unknown. Moreover, the bioequivalence of tenecteplase produced in China was also unclear. The CHABLIS-T trial adopted a Simon's two-stage design for individual dose stratum. The purpose of Simon's two-stage design is to determine whether tenecteplase has sufficient promise against acute stroke with large/medium vessel occlusion or severe stenosis in an extended time window in Chinese patients to warrant further investigation. Under this design, a small group of patients were enrolled in the first stage, and the activation of the second stage depended on a prespecified number of positive responses observed from the first stage. The trial was conducted in 13 centres across mainland China from November 2019 to December 2021.

The protocol and statistical analysis plan are provided in online supplemental file.

Participants

Patients were considered eligible if they (1) presented with acute ischaemic stroke within 4.5–24 hours from time of last seen well and were aged 18 years or older; (2) had a clinically significant acute neurological deficit measured by the baseline National Institutes of Health Stroke Scale (NIHSS) score; (3) had a prestroke modified Rankin Scale (mRS) 0–2; (4) fulfilled the 'dual target' imaging criteria, on baseline multimodal CT imaging.

Participants were identified with anterior circulation large/medium vessel occlusion or severe stenosis (defined as more than 70% narrowing compared with the adjacent vessel calibre), including the extracranial or intracranial internal carotid artery (ICA-IC/EC), first or second segment of the middle cerebral artery (MCA-M1/M2), and first or second segment of the anterior cerebral artery (ACA-A1/A2) on CT angiography. Additionally, a favourable penumbral profile was required on CTP imaging using automated real-time perfusion volumetric software, AutoMIStar (Apollo Medical Imaging Technology, Melbourne, Victoria, Australia). A favourable penumbral profile was a hypoperfusion lesion volume (delay time >3 s)[18] to infarct core volume (relative cerebral blood flow <30%) ratio that was greater than 1.2 with an absolute difference of volume greater than 10 mL, and an ischaemic core volume less than 70 mL.

Detailed inclusion and exclusion criteria are listed in online supplemental file.

Randomisation, intervention and blinding

Eligible patients were enrolled and randomly assigned in a 1:1 ratio to either dose stratum (0.25 mg/kg, maximum 25 mg, and 0.32 mg/kg, maximum 40 mg) of tenecteplase (Guangzhou Recomgen Biotech Co), as a bolus over 5–10 s and a following 2 mL bolus of saline for injection. Randomisation was performed using permuted blocks through a centralised website by local stroke neurologists. Patients were stratified according to time from last seen well (4.5–12 hours, 12–24 hours) and site of occlusion or severe stenosis (ICA-IC and MCA-M1; ICA-EC, MCA-M2 and ACA). The dosage of tenecteplase was open label to the patients and the clinicians involved in the treatment of participants. Bridging endovascular treatment was performed according to local guidelines. Guideline-based intensive care for patients who had an acute ischaemic stroke with intravenous thrombolysis was recommended for every patient. The investigators involved in the subsequent radiological and clinical evaluation were blinded to the allocation.

Outcomes

The primary outcome was a binary composite of efficacy and safety, that is, presence of major reperfusion at the initial catheter angiography or repeated CTP 4–6 hours in the absence of symptomatic intracerebral

haemorrhage (sICH) at 24–48 hours after intravenous tenecteplase. Major reperfusion was defined as the restoration of blood flow of greater than 50% of the involved territory. For patients not transferred to the catheter laboratory after thrombolysis, major reperfusion was considered as the hypoperfusion lesion volume (delay time >3 s) of the repeated CTP 4–6 hours decreased to less than 50% of the hypoperfusion lesion volume of the baseline CTP. For patients transferred to the catheter laboratory, major reperfusion was evaluated as a modified Treatment In Cerebral Ischemia (mTICI) score 2b/3 at the initial catheter angiography. SICH was defined according to the European Co-operative Acute Stroke Study-II criteria.[19]

The secondary radiological efficacy outcomes included recanalisation and infarct growth. The secondary clinical efficacy outcomes included mRS 0–1, mRS 0–2 and mRS distribution at 90 days, major neurological improvement at 24–48 hours and change in the NIHSS score within 24–48 hours. The secondary radiological safety outcomes included parenchymal haematoma type 2 (PH2), sICH[19] and any ICH at 24–48 hours post-treatment. The secondary clinical safety outcomes included mRS 5–6 at 90 days and systemic bleeding. Additionally, Barthel Index at 90 days was also collected. The details of the secondary outcomes can be found in online supplemental file.

The imaging protocol of multimodal CT at each centre was centrally standardised through careful quality control. All of the imaging results were centrally analysed in a core laboratory. Baseline multimodal CT imaging was reanalysed to make sure that the entry criteria were met. The radiological outcome measurements were evaluated by two independent neuroradiologists, and a third independent rater was consulted in cases of disagreement.

Sample size calculations

The sample size was calculated based on the results derived from the Tenecteplase vs Alteplase before Endovascular Therapy for Ischemic Stroke (EXTEND-IA TNK) trial,[7] where 22% of patients in the intravenous tenecteplase group and 10% of patients in the intravenous alteplase group reached major reperfusion, and 1% of patients, respectively, in both groups 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 two or fewer positive responses in these 18 patients, 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 eight 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 enrolment of 86 patients equally distributed between the two dose strata (figure 1).

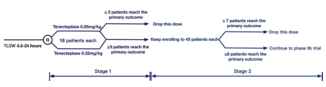

Figure 1 Study design: umbrella Simon's two-stage design. TLSW, time from last seen well.

Statistical analysis

All participants receiving tenecteplase were included in the analysis. For the analysis of the primary endpoint, as described above, if 8 or more out of the 43 patients reached the primary endpoint within a given stratum, the respective tenecteplase dose could be considered of being sufficient promise in terms of efficacy and safety and as a qualified candidate dose for the subsequent phase IIb trial.

Since the design of this study did not aim to compare the safety and efficacy of the two tenecteplase doses within the umbrella design, the analyses of secondary outcomes were descriptive. Secondary outcomes were described using percentages, mean with standard deviation (SD) and median with interquatile range (IQR as appropriate. Normality was tested using Shapiro-Wilk test.

In order to inform the design and planning for the subsequent phase IIb trial, we also estimated the proportions of participants achieving primary and secondary outcomes in patients without severe stenosis in the two tenecteplase dose strata.

No missing data for baseline information and primary outcome analysis were observed. Although 13.95% of participants had missing data on Barthel Index, its influence was considered to be minimal since it is a secondary outcome and the analyses did not involve a comparison between tenecteplase dose strata.[20]

The statistical analysis was performed on STATA V.15.1 (StataCorp, College Station, Texas, USA).

A data safety monitoring board oversaw the enrolment of the total 86 patients in this trial.

RESULTS

From 27 November 2019 to 30 September 2021, a total of 2193 patients who had an acute ischaemic stroke presented within 4.5–24 hours from time of last seen well at 13 sites, where 86 patients were randomised and received tenecteplase treatment in this trial. The flow diagram of patient selection is shown in figure 2. The preplanned interim analysis according to Simon's two-stage design was conducted in December 2020, demonstrating that each tenecteplase dose stratum had three or more patients reaching the primary endpoint, enabling

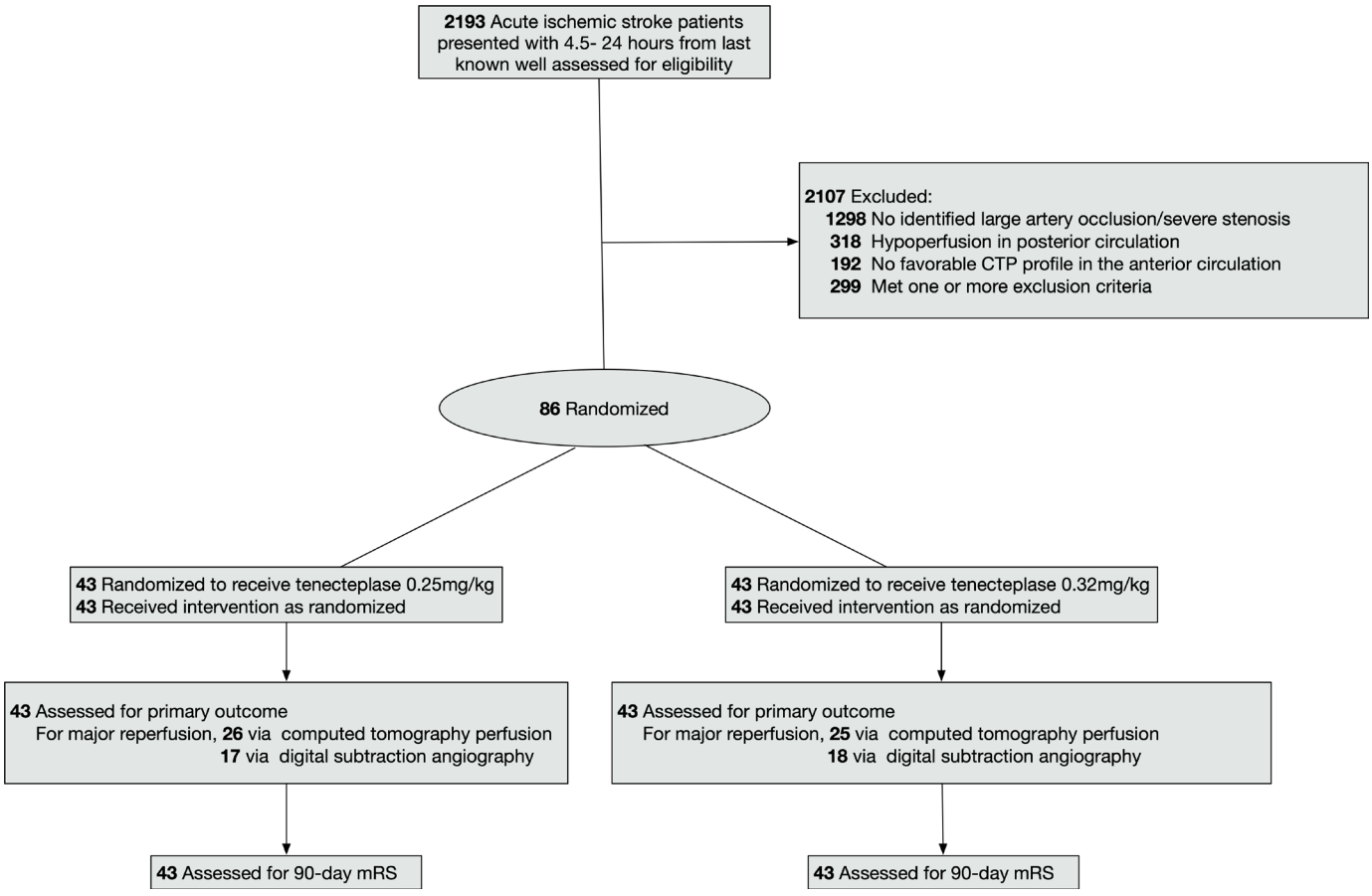

Figure 2 Trial profile. CTP, perfusion CT; mRS, modified Rankin Scale

progression to stage 2 in both dose strata. Forty-three patients were assigned to each 0.25 mg/kg tenecteplase and 0.32 mg/kg tenecteplase strata, and received corresponding tenecteplase treatment as allocated. The baseline demographic, clinical and imaging characteristics of the two tenecteplase dose strata are listed in table 1.

All of the 86 patients were available for the primary outcome analysis. The primary outcome analysis showed that 14 out of 43 (32.6%, 95% CI 20.2% to 48.0%) patients in the 0.25 mg/kg tenecteplase stratum and 10 out of 43 (23.3%, 95% CI 12.9% to 38.3%) patients in the 0.32 mg/kg tenecteplase stratum reached major reperfusion without sICH after tenecteplase thrombolysis. Additionally, regardless of the occurrence of haemorrhagic events, the numbers of patients achieving major reperfusion were 15 (34.9%, 95% CI 22.1% to 50.3%) and 12 (27.9%, 95% CI 16.5% to 43.2%) in the 0.25 mg/kg and 0.32 mg/kg dose strata, respectively.

Prespecified secondary efficacy and safety outcomes are presented in table 2. Recanalisation was attained in 18 (43.9%, 95% CI 29.5% to 59.4%) patients in both tenecteplase dose strata (with 41 patients available for analysis each arm) post-thrombolysis. Excellent functional outcome (mRS 0–1) was achieved in 12 (27.9%, 95% CI 16.5% to 43.2%) patients in the 0.25 mg/kg tenecteplase stratum and in 21 (48.8%, 95% CI 34.2% to 63.6%) patients in the 0.32 mg/kg tenecteplase stratum at 90 days. sICH occurred in 4 (9.3%, 95% CI 3.5% to 22.6%) patients in the 0.25 mg/kg tenecteplase stratum and in 4 (9.3%, 95% CI 3.5% to 22.6%) in the 0.32 mg/kg tenecteplase stratum. Details of severe adverse events are displayed in online supplemental table 1.

When excluding 13 patients with severe arterial stenosis, the baseline characteristics of the 73 patients with complete artery occlusion are listed in online supplemental table 2. The primary outcome was still achieved in 9 out of 35 (25.7%, 95% CI 13.8% to 42.8%) patients in the 0.25 mg/kg tenecteplase stratum and in 9 out of 38 (23.7%, 95% CI 12.7% to 39.9%) patients in the 0.32 mg/kg tenecteplase stratum. Secondary outcomes in patients with complete artery occlusion of two tenecteplase dose strata are shown in online supplemental table 3. Additionally, the baseline characteristics of the 52 patients without bridging endovascular treatment were listed in online supplemental table 4.

DISCUSSION

In this randomised trial, among 86 patients recruited, 14 out of 43 patients in the 0.25 mg/kg dose stratum and 10 out of 43 patients in the 0.32 mg/kg dose stratum achieved major reperfusion without occurrence of sICH, both surpassing the predefined eight-patient threshold of reaching the primary outcome. Therefore, both

Table 1 Baseline characteristics of the patients randomised

	Tenecteplase 0.25 mg/kg (n=43)	Tenecteplase 0.32 mg/kg (n=43)
Age, years	68.3 (13.1)	67.1 (11.5)
Male sex	25 (58.1%)	31 (72.1%)
NIHSS score at randomisation	11 (8–15)	9 (6–13)
Stroke aetiology		
Cardioembolism	15 (34.9%)	7 (16.3%)
Large artery atherosclerosis	20 (46.5%)	28 (65.2%)
Undetermined aetiology	8 (18.6%)	8 (18.6%)
Medical history		
Atrial fibrillation	14 (32.6%)	4 (9.3%)
Hypertension	27 (62.8%)	29 (67.4%)
Diabetes	12 (27.9%)	16 (37.2%)
Smoking	15 (34.9%)	25 (58.1%)
Ischaemic stroke or transient ischaemic attack	6 (14.0%)	5 (11.6%)
TLSW to randomisation		
4.5–12 hours	25 (58.1%)	26 (60.5%)
12- 24 hours	18 (41.9%)	17 (39.5%)
Witnessed stroke	21 (48.8%)	28 (65.1%)
Transferred to catheter lab	17 (39.5%)	18 (41.9%)
Underwent endovascular treatment	17 (39.5%)	17 (39.5%)
TLSW to hospital arrival, min	497 (310–815)	513 (394–632)
TLSW to initiation of intravenous therapy, min	645 (481–973)	674 (516–808)
Time from hospital arrival to initiation of intravenous therapy, min	130 (99–159)	140 (111–217)
Time from initiation of intravenous thrombolysis to initial angiographic assessment, min*	69.0 (45.0–94.5)	62.5 (41.8–93.3)
Site of artery occlusion or severe stenosis		
Extracranial segment of intracranial carotid artery	3 (7.0%)	6 (14.0%)
Intracranial segment of intracranial carotid artery	3 (7.0%)	4 (9.3%)
First segment of middle cerebral artery	25 (58.1%)	15 (34.9%)
Second segment of middle cerebral artery	9 (20.9%)	11 (25.6%)
Anterior cerebral artery	3 (7.0%)	6 (14.0%)
Tandem occlusion	0 (0.0%)	1 (2.3%)
Vessel severe stenosis at baseline	8 (18.6%)	5 (11.6%)
Hypoperfusion lesion volume at baseline, mL	77 (50–114)	76 (46–120)
Ischaemic core volume at baseline, mL	8 (4–15)	8 (4–19)

Data are mean (SD), n (%), median (IQR).
*Tenecteplase 0.25 mg/kg: n=17; tenecteplase 0.32 mg/kg: n=18.
IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; SD, standard deviation; TLSW, time from last seen well.

tenecteplase 0.25 mg/kg and tenecteplase 0.32 mg/kg demonstrated sufficient promise of efficacy and safety. CHABLIS-T is also one of the few trials that reported the performance of tenecteplase thrombolysis in the extended time window.

In TRACE Study, three doses of 0.1, 0.25, 0.32 mg/kg tenecteplase were compared with 0.9 mg/kg alteplase in Chinese patients with acute ischaemic stroke within 3 hours from symptom onset, which showed similar safety profiles.[11] However, in another phase IIb trial, two doses of 0.1 and 0.25 mg /kg tenecteplase were compared with alteplase, which showed the higher dose of tenecteplase was superior to the lower dose and to alteplase for all efficacy outcomes.[10] Therefore, we abandoned the lower dose of 0.1 mg/kg tenecteplase, and examined the promise of efficacy and safety in the other higher doses of tenecteplase, that is, 0.25 mg/kg and 0.32 mg/kg.

Table 2 Primary and secondary efficacy and safety outcomes

	Tenecteplase 0.25 mg/kg (n=43)	Tenecteplase 0.32 mg/kg (n=43)
Primary outcome* (major reperfusion without the occurrence of symptomatic ICH)	14 (32.6%)	10 (23.3%)
Secondary outcome		
Efficacy		
Recanalisation†	18 (43.9%)	18 (43.9%)
Infarct growth at 3–5 days, mL‡	23.9 (3.5–55.3)	16.9 (6.7–81.0)
Major neurological improvement at 24–48 hours§	7 (17.1%)	8 (18.6%)
Change in NIHSS score at 24–48 hours compared with baseline§	–1.0 (–6.5, 2.0)	0.0 (–3.0, 2.0)
mRS score 0–1 at 90 days	12 (27.9%)	21 (48.8%)
mRS score 0–2 at 90 days	20 (46.5%)	26 (60.5%)
mRS score at 90 days		
0	7 (16.3%)	8 (18.6%)
1	5 (11.6%)	13 (30.2%)
2	8 (18.6%)	5 (11.6%)
3	5 (11.6%)	3 (7.0%)
4	7 (16.3%)	7 (16.3%)
5	5 (11.6%)	5 (11.6%)
6	6 (14.0%)	2 (4.7%)
Safety		
Symptomatic ICH	4 (9.3%)	4 (9.3%)
Any ICH	21 (48.8%)	13 (30.2%)
Parenchymal haematoma type 2	5 (11.6%)	1 (2.3%)
mRS score 5–6 at 90 days	11 (25.6%)	7 (16.3%)
Systematic haemorrhage	3 (7.0%)	1 (2.3%)
Barthel Index at 90 days¶	95.0 (50.0–100.0)	95.0 (47.5–100.0)

Data are n (%), median (IQR).
*Major reperfusion was assessed at the initial catheter angiography or repeated CTP 4–6 hours and symptomatic ICH was assessed at 24–48 hours after intravenous tenecteplase treatment.
†Tenecteplase 0.25 mg/kg: n=41; tenecteplase 0.32 mg/kg: n=41.
‡Tenecteplase 0.25 mg/kg: n=36; tenecteplase 0.32 mg/kg: n=41.
§Tenecteplase 0.25 mg/kg: n=41.
¶Tenecteplase 0.25 mg/kg: n=33; tenecteplase 0.32 mg/kg: n=41.
CTP, perfusion CT; ICH, intracranial haemorrhage; IQR, interquartile range; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale.

Prompt reperfusion is of great importance for patients who had an acute ischaemic stroke with large vessel occlusion. Previous studies have shown that tenecteplase, as a single bolus administration, could achieve more rapid and substantial reperfusion compared with alteplase.[21–23] The recently reported TIMELESS trial (NCT03785678) has also reported a higher rate of recanalisation at 24 hours in the tenecteplase group than the placebo group.[17] The current trial showed that both 0.25 mg/kg and 0.32 mg/kg tenecteplase dose groups could reach a substantial reperfusion rate of around 30%, compared with the 20% in the EXTEND-IA TNK trials. One possible explanation is that we included medium vessel occlusion or severe stenosis, which could have higher reperfusion rate treated by tenecteplase. Another possible explanation is that the time window of reperfusion assessment in CHABLIS-T was around 60 min for patients transferred for catheter angiography (similar to EXTEND-IA TNK), and 4–6 hours for patients not transferred (60% of CHABLIS-T participants).[7 8] Apart from the time window, the imaging modality of reperfusion assessments may also play a part, since reperfusion status was mainly assessed through repeated perfusion imaging rather than catheter angiography in the CHABLIS-T trial, which was the opposite to the EXTEND-IA TNK trials.[7 8] However, reperfusion status assessed through perfusion imaging has been proven to have at least equivalent predictive ability for functional outcome compared with mTICI scores in catheter angiography.[24 25] As for the safety concerns, we did not observe a higher rate of

sICH or PH2 in the higher dose stratum. However, the percentages of haemorrhagic transformation (including symptomatic and asymptomatic) in this trial were notably higher than those of randomised controlled trials with patients with large vessel occlusion treated with intravenous thrombolysis.[7–10 16 26] Since the thrombolytic time windows of prior trials range from ≤4.5 hours to ≤9 hours after last seen well, the higher risk of haemorrhagic transformation in this trial can be partly explained by the longer onset-to-reperfusion time.[27] Additionally, though patients in CHABLIS-T have been selected with benign perfusion profiles, they may exhibit risk factors of sICH that could not be detected simply by automatically post-processed perfusion imaging (ie, larger volumes of very low cerebral blood flow),[28] which should be further explored by post-hoc analysis. Despite the higher sICH rates, both doses of 0.25 mg/kg and 0.32 mg/kg tenecteplase appear to be of sufficient promise in patients who had an acute ischaemic stroke with large/medium vessel occlusion or severe stenosis in the extended time window. Though the design of the study was not driven by the direct comparison between 0.25 mg/kg and 0.32 mg/kg tenecteplase, more patients in the 0.25 mg/kg tenecteplase dose stratum reached the primary outcome but failed to achieve 3-month mRS 0–1 or 0–2, compared with patients in the 0.32 mg/kg dose stratum. This discrepancy may be explained by the higher baseline NIHSS, higher prevalence of cardioembolic stroke and higher rate of PH2 in the 0.25 mg/kg dose stratum.

In the CHABLIS-T trial, we included both patients with complete large/medium vessel occlusion as well as those with severe stenosis. The reason why patients with severe stenosis were also eligible for this trial is that a considerable number of acute ischaemic stroke events in East Asian population are due to acute in-situ thrombosis with underlying chronic stenosis resulting from intracranial large artery atherosclerosis. Such patients were also candidates for acute reperfusion therapy and as such excluding patients with severe stenosis would undermine the generalisability of this trial. Notably, when excluding patients with severe stenosis, the primary outcome was still achieved in more than seven patients of each dose stratum in patients with complete artery occlusion.

A novel feature of CHABLIS-T trial was using the umbrella Simon's two-stage trial design in order to investigate the clinical promise of two doses of tenecteplase. Though not ever applied in stroke previously, Simon's two-stage design is acknowledged as a simple and effective dose selection method allowing modest sample size,[29] especially in oncology trials.

The study has the following limitations. First of all, the study design did not have an alteplase control group. However, the novel design (for a stroke trial) was aimed for dose finding rather than treatment comparison. Second, the design of this trial does not enable a direct comparison between 0.25 mg/kg and 0.32 mg/kg tenecteplase. With both dose strata reaching the predefined threshold for the primary outcome, both doses can be considered to be of sufficient promise. These results are in accordance with those of EXTEND-IA TNK Part2 trial, where 0.4 mg/kg tenecteplase had similar effect on reperfusion and other outcomes in patients with large vessel occlusions, compared with 0.25 mg/kg tenecteplase.[8] Third, the sample size of this study was underpowered to make reliable conclusions regarding the effect of tenecteplase on long-term functional outcomes (mRS at 90 days). Further, the primary outcome in this dose-finding study was different from that of phase III trials, making direct comparisons unreliable. However, the imaging composite primary outcome of both efficacy and safety is objective, immediate and straightforward, and more suitable for an adaptive sample size re-estimation early phase II design. Moreover, reperfusion without sICH usually translates to a good functional outcome at 90 days. Fourth, the tenecteplase used in the trial is manufactured locally, which limits its generalisability to other countries. Last but not least, the sample size calculation was based on a trial with shorter thrombolysis-to-reperfusion assessment time, while the reperfusion rate might be higher in CHABLIS-T than the assumed rate. However, the number of patients needed would be smaller than the 86 patients if higher reperfusion rate had been considered. Therefore, CHABLIS-T, with the current sample size, had sufficient power to detect the promise of efficacy and safety of tenecteplase.

In conclusion, among patients with large/medium vessel occlusion or severe stenosis in the anterior circulation and a favourable penumbral profile presenting within 4.5–24 hours from time of last seen well, both tenecteplase 0.25 mg/kg and 0.32 mg/kg doses demonstrated sufficient promise to achieve substantial reperfusion without sICH. Currently, other tenecteplase-related thrombolytic randomised controlled trials in patients with large vessel occlusion, including TIMELESS (NCT03785678), Extending the Time Window for Tenecteplase by Effective Reperfusion in Patients With Large Vessel Occlusion (ETERNAL-LVO, NCT04454788) and TRACE III (NCT05141305), choose 0.25 mg/kg as the experimental tenecteplase dosage. Additionally, Tenecteplase vs alteplase for the management of acute ischaemic stroke in Norway (NOR-TEST 2) trial failed to demonstrate superiority of 0.40 mg/kg tenecteplase compared with alteplase with a higher risk of bleeding events and worse functional outcomes in moderate and severe stroke.[13] Based on the results of CHABLIS-T, TRACE and other completed tenecteplase-related randomised controlled trials, a subsequent phase IIb trial, CHinese Acute tissue-Based imaging selection for Lysis In Stroke-Tenecteplase II (CHABLIS-T II) trial, is ongoing to investigate the performance of tenecteplase 0.25 mg/kg in comparison with best medical treatment (NCT04516993). The recent reported TIMELESS trial did not show superiority of tenecteplase in improvement of 3-month outcome though recanalisation rate was improved, which is probably due to the strong clinical benefit of endovascular treatment.[17] Therefore, CHABLIS-T II, together with other ongoing

trials, may help to further explore the optimal clinical setting where the recanalisation benefit of tenecteplase can be maximally translated into clinical benefit.

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Contributors XC and DQ take full responsibility for the work and the conduct of the study, have access to the data, and controll the decision to publish.XC, QD, YW and MP conceptualised the study. XC, LH, LL, YL, LY, YW, QD and MP curated and verified the data. LH, LC and H-QG did the formal analyses. XC and QD acquired funding. XC, LH, LL, LC and MP wrote the methods. XC, LH, LC, LL, YL, LY, YW, MP and QD conducted project administration. XC, LH, YL, JZ, JY, YG, DW, XL, XZ, YZ, QZ, LZ, YC, YG, XY, FG, YS, GL and QD recruited patients. XC, LH, LL, MP and QD wrote the article. XC, LH, LC, H-QG, MP and QD had full access to study data. XC and QD take final responsibility for the submitted publication.

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REFERENCES

1 Bhatia R, Hill MD, Shobha N, *et al*. Low rates of acute recanalization with intravenous recombinant tissue plasminogen activator in ischemic stroke: real-world experience and a call for action. *Stroke* 2010;41:2254–8.
2 Tanswell P, Modi N, Combs D, *et al*. Pharmacokinetics and pharmacodynamics of tenecteplase in fibrinolytic therapy of acute myocardial infarction. *Clin Pharmacokinet* 2002;41:1229–45.
3 Menon BK, Buck BH, Singh N, *et al*. Intravenous tenecteplase compared with alteplase for acute ischaemic stroke in Canada (AcT): a pragmatic, multicentre, open-label, Registry-linked, randomised, controlled, non-inferiority trial. *Lancet* 2022;400:161–9.
4 Burgos AM, Saver JL. Evidence that tenecteplase is noninferior to alteplase for acute ischemic stroke: meta-analysis of 5 randomized trials. *Stroke* 2019;50:2156–62.
5 Wang Y, Li S, Pan Y, *et al*. Tenecteplase versus alteplase in acute ischaemic cerebrovascular events (TRACE-2): a phase 3, multicentre, open-label, randomised controlled, non-inferiority trial. *Lancet* 2023;401:645–54.
6 Katsanos AH, Safouris A, Sarraj A, *et al*. Intravenous thrombolysis with tenecteplase in patients with large vessel occlusions: systematic review and meta-analysis. *Stroke* 2021;52:308–12.
7 Campbell BCV, Mitchell PJ, Churilov L, *et al*. Tenecteplase versus alteplase before thrombectomy for ischemic stroke. *N Engl J Med* 2018;378:1573–82.
8 Campbell BCV, Mitchell PJ, Churilov L, *et al*. Effect of intravenous tenecteplase dose on cerebral reperfusion before thrombectomy in patients with large vessel occlusion ischemic stroke: the EXTEND-IA TNK part 2 randomized clinical trial. *JAMA* 2020;323:1257–65.
9 Huang X, Cheripelli BK, Lloyd SM, *et al*. Alteplase versus tenecteplase for thrombolysis after ischaemic stroke (ATTEST): a phase 2, randomised, open-label, blinded endpoint study. *Lancet Neurol* 2015;14:368–76.
10 Parsons M, Spratt N, Bivard A, *et al*. A randomized trial of tenecteplase versus alteplase for acute ischemic stroke. *N Engl J Med* 2012;366:1099–107.
11 Li S, Pan Y, Wang Z, *et al*. Safety and efficacy of tenecteplase versus alteplase in patients with acute ischaemic stroke (TRACE): a multicentre, randomised, open label, blinded-endpoint (PROBE) controlled phase II study. *Stroke Vasc Neurol* 2022;7:47–53.
12 Haley EC Jr, Thompson JLP, Grotta JC, *et al*. Phase IIB/III trial of tenecteplase in acute ischemic stroke: results of a prematurely terminated randomized clinical trial. *Stroke* 2010;41:707–11.

13 Kvistad CE, Næss H, Helleberg BH, *et al*. Tenecteplase versus alteplase for the management of acute ischaemic stroke in Norway (NOR-TEST 2, part A): a phase 3, randomised, open-label, blinded endpoint, non-inferiority trial. *Lancet Neurol* 2022;21:511–9.
14 Bivard A, Zhao H, Churilov L, *et al*. Comparison of tenecteplase with alteplase for the early treatment of ischaemic stroke in the melbourne mobile stroke unit (TASTE-A): a phase 2, randomised, open-label trial. *Lancet Neurol* 2022;21:520–7.
15 Logallo N, Novotny V, Assmus J, *et al*. Tenecteplase versus alteplase for management of acute ischaemic stroke (NOR-TEST): a phase 3, randomised, open-label, blinded endpoint trial. *Lancet Neurol* 2017;16:781–8.
16 Ma H, Campbell BCV, Parsons MW, *et al*. Thrombolysis guided by perfusion imaging up to 9 hours after onset of stroke. *N Engl J Med* 2019;380:1795–803.
17 ESOC 2023 – late breaking abstracts. *Eur Stroke J* 2023;8:670–711.
18 Lin L, Bivard A, Kleinig T, *et al*. Correction for delay and dispersion results in more accurate cerebral blood flow ischemic core measurement in acute stroke. *Stroke* 2018;49:924–30.
19 Hacke W, Kaste M, Fieschi C, *et al*. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). *Lancet* 1998;352:1245–51.
20 Jakobsen JC, Gluud C, Wetterslev J, *et al*. When and how should multiple imputation be used for handling missing data in randomised clinical trials - a practical guide with flowcharts. *BMC Med Res Methodol* 2017;17:162.
21 Bivard A, Huang X, Levi CR, *et al*. Tenecteplase in ischemic stroke offers improved recanalization: analysis of 2 trials. *Neurology* 2017;89:62–7.
22 Gerschenfeld G, Smadja D, Turc G, *et al*. Functional outcome, recanalization, and hemorrhage rates after large vessel occlusion stroke treated with tenecteplase before thrombectomy. *Neurology* 2021;97:e2173–84.
23 Seners P, Caroff J, Chausson N, *et al*. Recanalization before thrombectomy in tenecteplase vs. Alteplase-treated drip-and-ship patients. *J Stroke* 2019;21:105–7.
24 Tan Z, Parsons M, Bivard A, *et al*. Optimal tissue reperfusion estimation by computed tomography perfusion post-thrombectomy in acute ischemic stroke. *Stroke* 2021;52:e760–3.
25 Hong L, Ling Y, Zhang Y, *et al*. Reperfusion measurements, treatment time, and outcomes in patients receiving endovascular treatment within 24 hours of last known well. *CNS Neurosci Ther* 2023;29:1067–74.
26 Ringleb P, Bendszus M, Bluhmki E, *et al*. Extending the time window for intravenous thrombolysis in acute ischemic stroke using magnetic resonance imaging-based patient selection. *Int J Stroke* 2019;14:483–90.
27 Jahan R, Saver JL, Schwamm LH, *et al*. Association between time to treatment with endovascular reperfusion therapy and outcomes in patients with acute ischemic stroke treated in clinical practice. *JAMA* 2019;322:252–63.
28 Campbell BCV, Christensen S, Parsons MW, *et al*. Advanced imaging improves prediction of hemorrhage after stroke thrombolysis. *Ann Neurol* 2013;73:510–9.
29 Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989;10:1–10.