

Appendix 2

ROSE-TNK Protocol

The MRI-guided thrombolysis for Stroke beyond Time Window by TNK (ROSE-TNK): a phase 2, randomized, multicenter trial

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Abstract

Rationale: Recent studies have demonstrated the benefit of intravenous alteplase for acute ischemic stroke (AIS) beyond the time window of 4.5 hours, but the effect of intravenous tenecteplase (TNK) in AIS patients with the extended time window is not well demonstrated.

Aim: To explore the efficacy and safety of intravenous TNK in AIS patients within 4.5 to 24 hours of onset by neuroimaging guiding.

Methods and Design: In this phase 2, multicenter, open-label, blinded-endpoint, randomized, control trial, eligible AIS patients within 4.5 to 24 hours of symptom onset and a mismatch between diffusion-weighted imaging (DWI) and fluid attenuated inversion recovery (FLAIR) were randomly assigned (1:1) into TNK group, and control group only receiving stand stroke care.

Study Outcomes: The primary efficacy outcome is excellent functional outcome, defined as modified Rankin Scale score 0 to 1 at 90 days. The primary safety outcome is symptomatic intracranial hemorrhage.

Keywords: tenecteplase, acute ischemic stroke, protocol

Introduction

Currently, the guideline recommended intravenous alteplase as one of the most effective treatment for acute ischemic stroke (AIS) within 4.5 hours of onset.¹ Tenecteplase (TNK) is a genetic variant of alteplase with higher fibrin specificity, greater conservation of fibrinogen, greater resistance to inhibitors of tissue plasminogen activator, more rapid thrombolysis, and longer half-life.² Accumulating studies demonstrated the noninferiority of TNK to alteplase in intravenous thrombolysis to treat AIS within 4.5 hours after symptom onset.^{3,4} Beyond the time window of 4.5 hours, two neuroimage-based screening randomized studies (WAKE-UP and EXTEND) also demonstrated the benefit of intravenous alteplase for AIS within the extended time window.^{5,6} Two prospective, non-randomized studies with small sample suggested the feasibility of intravenous TNK for AIS within 4 to 24 hours after onset based on the neuroimaging selection.^{7,8} To date, no randomized studies are found to report the effect of intravenous TNK in stroke patients beyond 4.5 hours of symptom onset.

In this context, this prospective, randomized, blinded-endpoint assessment, multi-center trial was conducted to explore the efficacy and safety of intravenous TNK for MRI-guided AIS within 4.5 to 24 hours of symptom onset.

Methods

Design

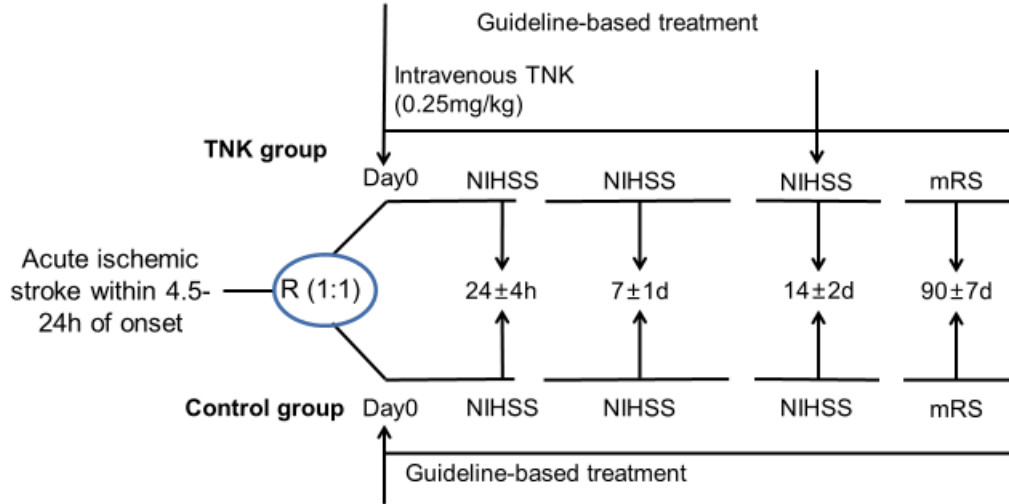
The MRI-guided thrombolysis for Stroke beyond Time Window by TNK (ROSE-TNK) is a phase 2, prospective, multicenter, open-label, blinded-endpoint, randomized control study to assess the efficacy and safety of intravenous TNK for MRI-guided AIS within 4.5 to 24 hours of symptom onset.

Intervention

In this trial, eligible patients will be randomly assigned (1:1) using a computer-generated randomization sequence with block size of four and sealed envelopes, prepared by an independent statistician, into either TNK group receiving intravenous TNK (0.25mg/kg, a maximum of 25 mg) as a single bolus over 5-10 seconds immediately after randomization., or control group only receiving standard stroke care

(Figure 1). Both patients will be treated according to AHA/ASA guidelines for early management of ischemic stroke.¹

Figure 1. Study schema



TNK: tenecteplase. mRS: modified Rankin Scale. NIHSS: National Institutes of Health Stroke Scale.

Patient Population

A total of 80 patients with acute moderate ischemic stroke in ten centers are expected in China between January 2021 and March 2022. There are 40 subjects in the experimental group and control group, respectively. The detailed inclusion/exclusion criteria are listed in Table 1.

Table 1. The Inclusion/Exclusion Criteria

Inclusion criteria:
1) Patient age: 18 - 80 years;
2) Acute ischemic stroke confirmed by CT or MRI;
3) The time from onset to treatment: 4.5 - 24 h;
4) NIHSS: 6-25, or NIHSS score ≤ 5 but responsible vessel occlusion or severe stenosis on CTA/MRA;
5) Imaging requirements: (a) DWI infarct region: no more than 1/3 of middle cerebral artery territory or 1/2 of the anterior cerebral artery territory or 1/2 of the

posterior cerebral artery territory; (b) DWI infarct volume <70 ml; (c) presence of DWI/FLAIR mismatch: DWI high signal and FLAIR visually normal;
6) First stroke onset or past stroke without obvious neurological deficit (mRS≤1);
7) Signed informed consent from the patients, or their legally authorized representative.
Exclusion criteria:
1) Planned endovascular treatment;
2) Serious neurological deficits before onset (mRS≥2);
3) Obvious head injuries or strokes within 3 months;
4) Subarachnoid or intracranial hemorrhage;
5) History of intracranial hemorrhage;
6) Intracranial tumor, arteriovenous malformation or aneurysm;
7) Intracranial or spinal cord surgery within 3 months;
8) Arterial puncture at a noncompressible site within the previous seven days;
9) Active internal hemorrhage;
10) Coagulation abnormalities: platelet count of <100000/mm ³ ;
11) Aortic arch dissection;
12) Heparin therapy within 24 hours;
13) Infective endocarditis;
14) Oral warfarin is being taken and INR>1.6 or APTT abnormal; oral anticoagulation therapy;
15) Systolic pressure ≥185 mmHg or diastolic pressure ≥110 mmHg;
16) Blood glucose < 50 mg/dl (2.7mmol/L);
17) Pregnancy;
18) Neurological deficit after epileptic seizures;
19) Major surgery within 1 month;
20) Gastrointestinal or urinary tract hemorrhage within the previous 30 days;
21) Myocardial infarction within 3 months; allergy to study drugs;
22) Contradictory to MRI examination;

23) MRI image not qualified for evaluation;
24) Other serious illness;
25) Participating in other clinical trials within 3 months;
26) Patients not suitable for this clinical study considered by researcher.

CT: Computed tomography; MRI: Magnetic resonance imaging; CTA: Computed tomographic angiography; MRA: Magnetic resonance arteriography; DWI: Diffusion Weighted Imaging; FLAIR: Fluid attenuated inversion recovery; INR: International normalized ratio; APTT: Activated partial thromboplastin time; mRS: modified Rankin Scale; NIHSS: National Institutes of Health Stroke Scale.

Standard Protocol Approvals, Registrations, and Patient Consents

The trial was registered on clinicaltrial.gov (NCT04752631). The protocol and data collection of the trial have been approved by the ethics committee of General Hospital of Northern Theater Command and all participating sites. All subjects or their representatives will provide written informed consents before inclusion into the trial.

Data Collection and Follow-up

Demography and clinically relevant data are recorded at the time of entry into the study. At baseline, demographic characteristics, routine laboratory tests, and neuroimaging will be collected and NIHSS and mRS will be evaluated. NIHSS scoring will be assessed at 24 ± 4 hours, 48 ± 8 hours, 7 ± 1 days, and 14 ± 2 days, and mRS will be assessed at 90 ± 7 days. All clinical assessments including NIHSS and mRS were evaluated by certified assessors according to a standardized procedure manual in each study center. 90-day mRS will be evaluated by face-to-face or by telephone interview (if a face-to-face interview was not possible). Concomitant medications and adverse events within 90 days after randomization will be recorded in detail by investigators and adjudicated by certified assessors.

Outcomes

The primary endpoint was an excellent function outcome, which was defined as modified Rankin Scale score 0 to 1 at 90 days. The secondary endpoints included favorable function outcome, defined as modified Rankin Scale score 0 to 2 at 90 days, distribution of mRS at 90 days, changes in NIHSS at 24 hours and 7 days, and early neurological improvement (ENI), defined as more than 4 point decrease in NIHSS within 24 hours.⁹

Prespecified safety outcomes included symptomatic intracranial hemorrhage (sICH) within 48 hours, defined as an increase in the NIHSS score of ≥ 4 points as a result of the intracranial hemorrhage,¹⁰ proportion of parenchymal hemorrhage (PH1, PH2) within 48 hours,¹⁰ proportion of hemorrhagic transformation within 7 days, any bleeding events within 7 days, and mortality within 14 days.

Quality Control

Before the beginning of the study, all the investigators at each center attended training sessions to review the protocol and procedures. An independent Data Monitoring Committee (DMC) will perform to assure fidelity of conduct of the study according to the protocol and Good Clinical Practice (GCP). The primary purpose of the termination is to protect the rights and interests of the subjects and to avoid unnecessary economic losses. If the therapy shows a statistically significant difference of efficacy and/or safety over the other, the DMC has the right to terminate the study unconditionally.

Data Management and Monitoring

Data will be stored in the case report form (CRF). DMC is established to ensure ongoing monitoring of data security, such as hemorrhagic events and other adverse events, etc. Clinical outcome events (stroke, death, intracerebral hemorrhage, etc.) will also be adjudicated by the independent DMC. All neuroimaging will be sent to the central reader who are blinded to treatment assignment (Y.J.D.).

All AEs Monitoring

All information about AEs should be recorded on the AEs page of the case report.

All relevant SAEs are reviewed and adjudicated centrally in order to ensure that they meet the same diagnostic criteria.

Sample Size Determination

No formal sample size calculation was performed due to no relevant data from previous trial. For this exploratory trial, the sample size (40 patients per group) was determined after discussion with the Steer Committee.

Statistical Analysis

All efficacy analyses will be performed according to the intention-to-treat principle, which comprises patients who received the allocated treatment and completed the assessment period. Baseline characteristics and procedural details will be compared with Student's t test if normally distributed or rank sum test if non-normally distributed. Treatment effect will be presented as odds ratio (95% CI) of TNK group versus control group, analyzed by binary logistic regression. The primary and secondary outcomes such as mRS (0-2) at 90 days, occurrence of ENI, proportion of sICH within 48 hours, proportion of parenchymal hemorrhage within 48 hours, proportion of hemorrhagic transformation within 7 days, any bleeding events within 7 days, and mortality within 14 days will be estimated using a binary logistic regression adjusted for age, sex, systolic blood pressure, stroke history, NIHSS score at admission and time from stroke onset to randomization. Change in NIHSS score between two groups will be compared using a generalized linear model. Shift analysis of the mRS scores at 90 days will be performed using ordinal logistic regression. Descriptive statistics of proportions will be used for the safety data. Continuous data are presented as mean (standard deviation) or median (interquartile range) as appropriate. For categorical variables, absolute and relative frequencies are presented. there is statistical significance if P value < 0.05 . Analyses will be performed with statistical software of IBM SPSS version 20.

Study Organization and Funding

The protocol was designed by Hui-Sheng Chen and discussed by the academic team.

Steering Committee is made up of external scientific advisors, and will monitor the research and data regularly. The trial is initiated by Cerebrovascular Disease Collaboration & Innovation Alliance (CDCIA) of Liaoning, and supported by grants from the Science and Technology Project Plan of Liaoning Province (2019JH2/10300027).

Discussion

Several studies have demonstrated the benefit of intravenous alteplase to treat AIS beyond 4.5 hours time window.^{5,6} However, the efficacy and safety of intravenous TNK in the patients with the extended time windows are not fully investigated. Two prospective, non-randomized studies with small sample supported the feasibility of intravenous TNK for AIS within 12 to 24 hours after onset.^{7,8}

The current study is a phase 2, randomized, clinical trial to investigate the efficacy and safety of intravenous TNK in AIS patients within 4.5-24 hours of onset, which is quite different from two previous studies.^{7,8} First, the randomized control design was adopted in this study, while non-randomized design was in two previous studies. Second, the mismatch between DWI and FLAIR was set as neuroimaging criteria, while non-contrast CT⁷ or perfusion computed tomography/magnetic resonance imaging⁸ were used in previous studies. Finally, the multi-center design was used in this trial, while single center design was in a previous study.⁸

In conclusion, the results of ROSE-TNK will provide us important information about the effect of intravenous TNK in MRI-guided AIS patients within 4.5-24 hours.

References

- 1 Powers WJ, Rabinstein AA, Ackerson T, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American heart Association/American stroke association. *Stroke* 2019;50:e344–418.
- 2 Benedict, C.R., et al., New variant of human tissue plasminogen activator (TPA) with enhanced efficacy and lower incidence of bleeding compared with recombinant human TPA. *Circulation*, 1995. 92(10): p. 3032-40.
- 3 3. 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(10): 781-788.
- 4 Coutts SB, Berge E, Campbell BC, Muir KW, Parsons MW. Tenecteplase for the treatment of acute ischemic stroke: A review of completed and ongoing randomized controlled trials. *Int J Stroke*. 2018;13(9):885-892.
- 5 Ma H, Campbell BCV, Parsons MW, Churilov L, Levi CR, Hsu C, et al; EXTEND Investigators. Thrombolysis guided by perfusion imaging up to 9 hours after onset of stroke. *N Engl J Med*. 2019;380:1795–1803. doi: 10.1056/NEJMoa1813046
- 6 Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B, et al; WAKE-UP Investigators. MRI-guided thrombolysis for stroke with unknown time of onset. *N Engl J Med*. 2018;379:611–622.
- 7 Coutts SB, Dubuc V, Mandzia J, et al. Tenecteplase-tissue-type plasminogen activator evaluation for minor ischemic stroke with proven occlusion. *Stroke*. 2015;46(3):769-774.
- 8 Kate M, Wannamaker R, Kamble H, et al. Penumbra Imaging-Based Thrombolysis with Tenecteplase Is Feasible up to 24 Hours after Symptom Onset. *J Stroke*. 2018;20(1):122-130.
9. Kharitonova T, Mikulik R, Roine RO, et al. Association of early National Institutes of Health Stroke Scale improvement with vessel recanalization and

functional outcome after intravenous thrombolysis in ischemic stroke. *Stroke* 2011;42(6):1638-43.

10. Hacke W, Kaste M, Bluhmki E, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008;359(13):1317-29.