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Efficacy and Safety of Tirofiban in Patients with Acute Branch Atherosclerotic Disease(BAD)-Related Stroke (BRANT): Protocol for a randomized controlled trial

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Efficacy and Safety of Tirofiban in Patients with Acute Branch Atherosclerotic Disease(BAD)-Related Stroke (BRANT): Protocol for a randomized controlled trial

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Strengths and limitations of this study

1. Most published clinical studies classed BAD into small-vessel occlusion or undetermined etiology based on TOAST system. This study focuses on patients with acute BAD-related stroke, with the aid of magnetic resonance imaging.

2. Currently, there is no effective regimen to treat BAD-related stroke, and reduce disability. This study aims to addressing the current treatment dilemma in acute phase. Intervention will be prescribed within 48 hours after onset. 90-day modified Rankin Scale is set as primary outcome.

3. Lack of double blinded design is a limitation, but the endpoints are measured in a blind manner. An independent Clinical Event Committee is established to assess clinical events.

Abstract

Introduction: Branch atheromatous disease (BAD)-related stroke is increasingly becoming a clinical entity and prone to early neurological deterioration (END) and poor prognosis. There are no effective regimen in acute phase to reduce the disability caused by BAD-related stroke. Recent studies have indicated the efficacy of tirofiban in acute ischemic stroke, but have not been validated in patients with BAD-related stroke. Thus, we aim to test whether intravenous tirofiban initiated within 48 hours after onset would safely improve the functional outcome in patients with acute BAD-related stroke, in comparison with standard antiplatelet therapy based on guideline.

Methods and analysis: BRANT is a multicenter, randomized, open-label, blinded endpoint, parallel controlled phase III trial in China. Participants with acute BAD-related stroke are randomized 1:1 to tirofiban or control group. The primary outcome is the excellent functional outcome (modified Rankin Scale score: 0-1) at 90 days. Secondary outcomes include END, major bleeding, stroke, death, functional status, serious adverse events, and change in bleeding-related markers. A total of 516 participants assuming the rates of primary outcome to be 74% in the tirofiban group and 62% in the control group are needed for 0.8 power (two-sided 0.05 alpha).

Discussion: BRANT aims to provide direct evidence on the efficacy and safety of early intravenous tirofiban in acute BAD-related stroke, thus addressing the current treatment dilemma for improving functional outcome.

Ethics and dissemination: BRANT study has been approved by the Ethics Committee of Peking Union Medical College Hospital. Written informed consent is required for

all patients before enrollment. Results of the study will be published in a peer-reviewed journal.

Keywords: Branch Atheromatous Disease, Acute ischemic stroke, Early neurological deterioration, Functional outcome, Tirofiban, Treatment

Introduction

Branch atheromatous disease (BAD), first described by Caplan in 1989 as a concept, is becoming a clinical entity with the aid of advanced neuroimaging.[1-3] BAD-related stroke, characterized by subcortical single infarcts without severe stenosis of large artery, accounts for 20.4% of all ischemic stroke cases in Asian population.[2] Different from lacunar infarct due to lipohyalinosis, BAD-related stroke is caused by parent arterial plaque occlusion of the perforating orifice or proximal atherosclerotic occlusion of the perforating artery.[1-3]

High incidence of early neurological deterioration (END) was observed in BAD-related stroke, and was strongly associated with poor prognosis.[4 5] The rate of END is higher in BAD-related stroke than that in lacunar infarct (26.8-37.5% vs. 6.3-18.6%), and thrombolysis itself cannot prevent the occurrence of END.[4-6] In addition, it remains unclear whether the intravenous thrombolysis could improve the clinical outcome in BAD-related stroke.[7] Its rate of disability could reach 61%.[2] However, there are no high-level recommendations for acute-phase treatment of BAD-related stroke, and no RCT has examined BAD as a separate disease. Current practice-based on limited observational data and expert opinion-is heterogeneous, including anticoagulants and dual antiplatelet therapy, whose efficacy is uncertain for BAD-related stroke.[8 9]

Historical evidence suggests that intravenous tirofiban, a selective and reversible antagonist of GP IIb/IIIa inhibitors on platelet, increases recanalization rate and improves functional prognosis in stroke patients with endovascular therapy, without increased bleeding risk [10-12]. A large randomized trial of stroke without large or

medium-sized vessel occlusion also reported the efficacy of tirofiban [13]. Though atherosclerotic mechanism is presumed between BAD and large artery atherosclerosis, this evidence may not generalize to BAD-related stroke, for selection bias, retrospective data or small sample.[10 14] Another limitation is that tirofiban was often prescribed after END, which might cause irreversible ischemic lesion and neurologic deficit.[10 13] Randomized controlled trials of acute BAD-related stroke are therefore needed, and have been called for by researchers.[3 15]

The BTANT trial aims to establish the efficacy and safety of intravenous tirofiban in improving functional outcome in patients with acute BAD-related stroke.

Methods

Design

This is a multicenter, randomized, open label, blinded endpoint, parallel controlled phase III trial. BRANT study has started enrollment on November 9, 2023, and the anticipated date of study completion is October 31, 2025. This protocol has been registered at ClinicalTrials.gov (NCT06037889).

Patient population

BRANT will enroll 516 participants with BAD-related stroke within 48 hours of onset, from 21 centers in China.

Inclusion criteria

- Age: 18-75 years old
- Acute ischemic stroke

- Time from onset to randomization ≤ 48h; if onset time is unknown, time from last known well to randomization ≤48h
- Meet the following BAD Diagnostic Imaging Criteria

1. DWI infarcts: single (isolated) deep (subcortical) infarcts;

2. The culprit arteries are either Lenticulostriate artery (LSA) or Paramedian pontine artery (PPA), and the infarct lesion on DWI conforms to one of the following characteristics (A/B):

A. LSA: "Comma-like" infarct lesions with "Fan-shaped" extension from bottom to top in the coronary position; or ≥ 3 layers (layer thickness 5– 7 mm) on axial DWI brain images;

B. PPA: The infarct lesion extends from the deep pons to the ventral pons on the axial DWI brain images;

- No more than 50% stenosis on the parent artery of the criminal artery (i.e. corresponding basilar or middle cerebral artery) (Confirmed by MRA/CTA/DSA)
- Signed informed consent by the patient or legally authorized representatives.

Exclusion criteria

- Transient ischemic attack (TIA)
- Intracranial hemorrhagic diseases, vascular malformations, aneurysms, brain abscesses, malignant space-occupying lesions, or other non- ischemic intracranial lesions detected by baseline CT/MRI, or MRA/CTA/ DSA

- Presence of ≥ 50% stenosis in extracranial artery in tandem relationship ipsilateral to the lesion
- Cardiogenic embolism: atrial fibrillation, myocardial infarction, heart valve disease, dilated cardiomyopathy, infective endocarditis, atrioventricular block disease, heart rate less than 50 beats per minute
- Have received or plan to receive endovascular therapy or thrombolysis after onset
- Stroke of other clear causes, e.g., moyamoya disease, arterial entrapment, vasculitis, etc.
- modified Rankin Scale ≥2 before onset
- Use of tirofiban within 1 week before or after onset
- Low platelets (<100×10^9/L), or prothrombin time >1.3 times of the upper normal limit, or INR >1.5, or other systemic hemorrhagic tendencies such as hematologic disorders
- Elevation of ALT or AST more than 1.5 times the upper normal limit
- Glomerular filtration rate <60 ml/min/1.73m^2
- Known malignant tumors
- History of trauma or major surgical intervention within 6 weeks prior to onset
- History of intracranial hemorrhage
- Active or recent history (within 30 days prior to onset) of clinical bleeding (e.g., gastrointestinal bleeding)

- Malignant hypertension (systolic blood pressure >200 mmHg, or diastolic blood pressure >120 mmHg)
- Life expectancy ≤ 6 months
- Contraindications of 3 T MRI examination
- Pregnant or lactating women
- Have participated in another clinical trial within 3 months prior to the date of informed consent, or are participating in another clinical trial

Randomization

Participants will be randomized at a ratio of 1:1 using a dynamic block randomization method via an independent central website. Block sizes were set to 6, 8, and 12. The allocation sequence was stored in the central website and the participant was issued to intervention or control group by 1:1 ratio according to the order of enrollment time.

Intervention

Tirofiban group: Intravenous tirofiban will be administered immediately after randomization for a total duration of 48 hours with a loading dose of 0.4ug/kg/min*30min, followed by a maintenance dose of 0.1ug/kg/min*47.5h (Figrue1).

Control group: Standard antiplatelet therapy based on Chinese stroke guideline will be initiated after randomization for a total duration of 48 hours, as the two following types: 1) aspirin 150-300 mg qd, or 2) aspirin 100 mg qd plus clopidogrel 75 mg qd [16]. Its

initiation will be determined based on the last administration time of antithrombotic drugs, but the drug should be given as soon as possible.

Primary outcomes

The primary outcome is excellent functional outcome at 90 days, defined as modified Rankin Scale score: 0-1. Primary outcome will be measured by the qualified evaluators, who are blinded to all procedures.

Secondary outcomes

Secondary efficacy outcomes include END, National Institutes of Health Stroke Scale (NIHSS) score, Barthel index score, ischemic stroke, stroke, TIA, and a composite event of new-onset stroke, myocardial infarction, and all-cause death. The safety outcomes include the proportion of major bleeding defined by the PLATO criteria, adverse events, all-cause death, and changes in bleeding-related markers[17]. Evaluators after randomization are not aware of treatment assignment. All the clinical and safety events will be re-examined by the independent Clinical Event Committee (CEC), who are blinded during all procedures.

The presence of END is determined by an increase of ≥ 4 points in the NIHSS or an increase of ≥ 2 points in the NIHSS motor score. In addition, NIHSS motor score refers to bilateral upper and lower extremity mobility scores. The baseline NIHSS score for the calculation of END is the first clinician-evaluated and recorded NIHSS score after onset. The time frame for post-randomization END is within 7 days of randomization.

Study protocol and data management

A study flow is shown in Figrue1 and Table S1. At visit 1, trained investigators will recruit the patient by screening age, onset time, magnetic resonance imaging, and other enrollment criteria (ie, intracranial artery, and electrocardiogram). Then, the investigator will explain the BRANT study in details, including contents of each visits and interventions to patients. After obtaining written informed consent, participants will be assigned to tirofiban or control group via a central website-based randomization system. Patients are encouraged to undergo on-site follow-up at 90 days. Demographic, clinical, radiological, laboratory and clinical event data at each visit (Table S1) will be collected and stored in electronic case report form via a website (http://117.78.2.36:5010/). All CRFs will be checked by local investigators for completeness and correction before data entry. Data will be checked dynamically by investigator (Jun Ni), with the aid of research assistants.

Data Monitoring Board

An independent Data Security Monitoring Board (DSMB), including academic experts and statisticians, has been established to protect the interests of the participants during the study. The DSMB needs to review the overall implementation of the clinical study, and assess the risks and benefits regularly and dynamically, especially unexpected adverse event. DSMB reports to the Executive Committee and provides professional advice.

Sample size estimates

Based on previous studies and clinical practice, we assumed the rates of primary outcome to be 62% and 74% in the control group and tirofiban group, respectively.[2 10-12 14 18-20] Thus, 234 per arm is needed for a two-sided test at alpha 0.05 and power 0.8. Considering a 10% dropout rate, 516 patients will be required.

Statistical analyses

According to the principle of intention-to-treat analysis, all subjects randomized into the groups with more than one efficacy evaluation will be included in the full analysis set. The estimation of missing values will be conducted by the carry-over based on last observation carried forward (LOCF) estimation method. The proportion of excellent outcome at 90 days will be compared using the chi-square tests, and shown as frequency (percentage). Most secondary outcome analyses will also use the primary outcome analysis strategy. Survival data will be calculated by Kaplan-Meier method to estimate its survival rate in each group and the efficacy are assessed by Log-rank test. Hazard ratio and 95% CI will be calculated by cox proportional hazards model. Non-survival data will be calculated by chi-square test and odds ratios and 95%CI also will be calculated. Continuous variables will be compared by Student t -test or Wilcoxon rank sum test between the two groups. The influence of covariables will be evaluated via the subgroup analyses. All analysis will be performed using SAS 9.4 and a two-sided $P < 0.05$ is considered significant.

Patient and public involvement statement

None.

Ethics and dissemination: BRANT study has been approved by the Ethics Committee of Peking Union Medical College Hospital on July 20, 2023. Written informed consent is required for all patients before enrollment. BRANT is carried out according to Good Clinical Practice and the Declaration of Helsinki. Protocol amendments will be reported to the institutional ethics committee. The trial sponsor is Peking Union Medical College Hospital. Trial results will be published in a peer-reviewed journal.

Discussion

The BRANT trial is a multicenter RCT, addressing the important treatment dilemma that how to improve the functional outcome of BAD-related stroke.

BAD was first described by Caplan in 1989, when BAD was just a concept, distinct from lacuna infarct.[1] However, in past three decades, most clinical studies classed BAD into small-vessel occlusion or undetermined etiology based on TOAST system.[15 21] Few studies focused on acute BAD-related stroke, probably due to discrepant definitions.[3] Recently, increasing observational studies found distinct clinical, radiologic, and prognostic features that BAD-related stroke were prone to END and poor prognosis.[2 22]

Due to the limitation of neuroimaging technique, there were no direct visualization of perforating artery, such as LSA and PPA. Radiological diagnosis was based on vascular

territory, dimension, or shape of the lesion,[3 23] which resulted in a huge variation among BAD definitions. With the aid of neuroimaging and clinical practice, Asian neurologists proposed radiological diagnosis criteria for BAD.[24 25] Our previous study also found that ≥4 consecutive slices on axial view was more effective than transversal diameter to differentiate atherosclerotic mechanisms of single subcortical infarction in LSA territory.[26] Considering generalization and diagnostic accuracy of our study, we used ≥ 3 consecutive layers on axial DWI series instead of lesion diameter to define BAD-related stroke in LSA territory.[27] As direct evidence of LSA and PPA are not technically feasible at present, our inclusion criteria based on MRI shows considerable accuracy and representativeness.

About 78.6%-90.9% of END occurred within 48 hours after onset.[28 29] Our preliminary results of BAD-related stroke cohort found that the median hours from onset to END was 38 [30]. We hypothesize that early tirofiban could improve functional prognosis via preventing the occurrence of END. Thus, tirofiban should be initiated within 48 hours after onset. Considering the predictive value of END, we adopted the widely used and conservative definition of END in BRANT study.[31] The effect of tirofiban on reducing END risk is a key secondary outcome.

We set 90-day excellent outcome instead of END or new-onset stroke as the primary outcome for: 1) historical evidence indicated that tirofiban improved the functional outcome of ischemic stroke;[10 13] 2) END is an intermediate indicators;[4] 3) 90-day rate of recurrent stroke is 1.8% in our preliminary analysis of BAD-related stroke cohort, and probably less than 3.8% in other cohorts,[22] which is relatively low. Thus, BRANT

study would provide direct evidence on how to reduce disability caused by BAD, which is the major challenge in current clinical practice.

As oral mono antiplatelet therapy is unethical and against Chinese stroke guidelines for patients with NIHSS ≤ 3, there are two types of antiplatelet therapy in control group.[16] Double-blind design would markedly increase the complexity of the trial procedure. Therefore, we selected PROBE design for BRANT. We trained independent senior neurologists for the evaluation of primary outcome blinded to the procedure information. Independent CEC was established to centrally re-examine all clinical events after randomization. Some local investigators may know the treatment allocation, but all evaluators of subjective indicators are blinded to treatment allocation.

Conclusions

BRANT is designed to test the efficacy and safety of early intravenous tirofiban in patients with acute BAD-related stroke, aiming at effectively improving the functional outcome.

Contributors: Shengde Li, and Jun Ni designed the study. Shengde Li drafted the manuscript. Dingding Zhang designed the statistical method. Yuhui Sha, Lixin Zhou, Yicheng Zhu, and Bin Peng critically revised the study protocol and the manuscript. The entire project will be supervised by Jun Ni.

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Disclaimer: The funder has no role in this study.

Competing interests: None declared.

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Figure I. Study Flow

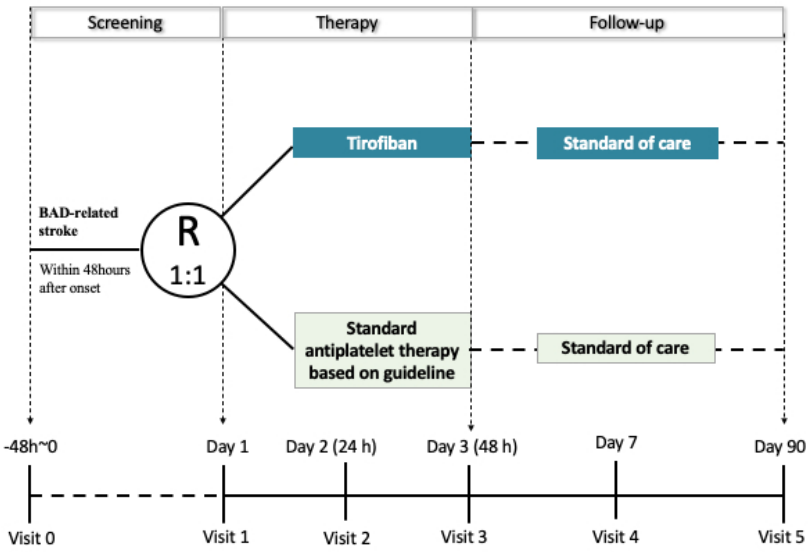

Figure 1. Study Flow

338x190mm (54 x 54 DPI)

Supplementary materials

Table S1. Study Procedure of STRATEGY trial

Measurement	Day 1	Day 2	Day 3	Day 7	Day 90
Demographic characteristics	X				
Current medical history taking	X				
Body temperature measurement	X				
Physical examination	X			X	X
Past medical history	X				
Pre-randomization medication after onset	X				
Regular blood pressure monitoring	X	X			
NIHSS score	X			X	X
mRS score	X			X	X
Barthel index score	X				X
Magnetic resonance image	X				
Evaluation of Intracranial vessels	X				
Evaluation of extracranial vessels	X				
Laboratory tests*	X				
ECG*	X			X	
Verification of inclusion/exclusion criteria	X				
Signed informed consent	X				
Randomization	X				
Blood tests after enrollment		X	X		

Urine and fecal examination			**X**		
Compliance			**X**		
Concomitant medication				**X**	**X**
Early neurological deterioration				**X**	
Major bleeding				**X**	**X**
Adverse Events/ Serious Adverse Events				**X**	**X**
*Remarks: ECG and laboratory data performed within 48 hours of onset before signing the informed consent form can be used as trial data.					

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	Clinical Trials.gov
Protocol version	3	Date and version identifier	12
Funding	4	Sources and types of financial, material, and other support	12
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	15
	5b	Name and contact information for the trial sponsor	Clinical Trials.gov
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	15
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	10-11
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-6
	6b	Explanation for choice of comparators	5

Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	In Protocol, not shown in article
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	In Protocol, not shown in article
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	In Protocol, not shown in article
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	In Protocol, not shown in article
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	112
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	11
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	13
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13

	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	In protocoal, not shown in article
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	In protocoal, not shown in article
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
	31b	Authorship eligibility guidelines and any intended use of professional writers	In protocoal, not shown in article
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Consent form has been approved.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the

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Efficacy and Safety of Tirofiban in Patients with Acute Branch Atherosclerotic Disease(BAD)-Related Stroke (BRANT): Protocol for a Randomized Controlled Trial

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Efficacy and Safety of Tirofiban in Patients with Acute Branch Atherosclerotic Disease(BAD)-Related Stroke (BRANT): Protocol for a Randomized Controlled Trial

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Abstract

Introduction: Branch atheromatous disease (BAD)-related stroke is increasingly becoming a clinical entity and prone to early neurological deterioration (END) and poor prognosis. There are no effective regimens to reduce the disability caused by BAD-related stroke in acute phase. Recent studies have indicated the efficacy of tirofiban in acute ischemic stroke, however, its efficacy has not been validated in patients with BAD-related stroke. Thus, we aim to test whether intravenous tirofiban initiated within 48 hours after onset would safely improve the functional outcome in patients with acute BAD-related stroke, in comparison with standard antiplatelet therapy based on the current guideline.

Methods and analysis: BRANT is a multicenter, randomized, open-label, blinded endpoint, parallel-controlled, phase III trial conducted in 21 hospitals in China. Participants aged 18-75 years with acute BAD-related stroke within 48 h after stroke onset are randomized in a 1:1 ratio to the tirofiban or control group. The treatment period is 48 hours in both groups. The primary outcome is the excellent functional outcome (modified Rankin Scale score: 0-1) at 90 days. The secondary outcomes include END, major bleeding, stroke, death, functional status, serious adverse events, and change in bleeding-related markers. Assuming the rates of the primary outcome to be 74% in the tirofiban group and 62% in the control group, a total of 516 participants are needed for 0.8 power (two-sided 0.05 alpha).

Ethics and dissemination: BRANT study has been approved by the Ethics Committee of the Peking Union Medical College Hospital. Written informed consent is required

for all patients before enrollment. The results of the study will be published in a peer-reviewed journal.

Trial registration number ClinicalTrials.gov (NCT06037889)

Keywords: Branch Atheromatous Disease, Acute ischemic stroke, Early neurological deterioration, Functional outcome, Tirofiban, Treatment

Strengths and limitations of this study

1. With the aid of magnetic resonance imaging, this study focuses on patients with acute BAD-related stroke, which had been inappropriately classified as small-vessel occlusion or an undetermined aetiology by the TOAST system in previous studies.

2. This study is designed to test the efficacy of tirofiban initiated within 48 h of onset, with the aim of addressing the current treatment dilemma in the acute phase.

3. Lack of double-blinded design is a limitation, but the endpoints are measured in a blinded manner.

Introduction

Branch atheromatous disease (BAD), first described conceptually by Caplan in 1989, is being confirmed as a clinical entity with the aid of advanced neuroimaging.[1-3] BAD-related stroke, characterized by subcortical single infarcts in penetrating artery territories without severe stenosis of the large parent artery, accounts for 20.4% of all ischaemic stroke cases in Asian populations[2 4]. Differing from lacunar infarct due to lipohyalinosis, BAD-related stroke is caused by parent arterial plaque occlusion of the perforating orifice or proximal atherosclerotic occlusion of the perforating artery[1-3], which could be identified from small-vessel occlusion or stroke of undetermined source in the TOAST system[5 6].

High incidence of early neurological deterioration (END) has been observed in BAD-related stroke and is strongly associated with poor prognosis[7 8]. The rate of END is higher in BAD-related stroke than lacunar infarct (26.8-37.5% vs. 6.3-18.6%), and thrombolysis itself cannot prevent the occurrence of END[7-9]. In addition, it remains unclear whether intravenous thrombolysis could improve the clinical outcome in BAD-related stroke[10]. The rate of disability can reach 61%[2]. However, there are no high-level recommendations for acute-phase treatment of BAD-related stroke, and no RCT has examined BAD as a separate disease. Current practice—based on limited observational data and expert opinion—is heterogeneous, including anticoagulant and mono/dual antiplatelet therapy, the efficacy of which is uncertain for BAD-related stroke[11 12].

Tirofiban, a selective and reversible antagonist of glycoprotein IIb/IIIa inhibitors on

platelets, might be more effective than conventional agents (such as aspirin or clopidogrel) by blocking the final common pathway of platelet aggregation at the pathophysiological level[13]. In clinical studies, historical evidence has also reported that tirofiban increases the recanalization rate and improves functional prognosis in stroke patients with endovascular therapy without increasing bleeding risk[14-16]. A large randomized trial of patients with stroke without large or medium-sized vessel occlusion also reported the efficacy of tirofiban[17]. However, though an atherosclerotic mechanism is presumed to exist between BAD and large artery atherosclerosis, this evidence may not be generalized to BAD-related stroke, as retrospective data or small samples may introduce selection bias[14 18]. Moreover, about 78.6%-90.9% of END occurs within 48 h after onset[19 20]. Our preliminary results of a cohort with BAD-related stroke found that the median time from onset to END was 38 h. We hypothesised that early tirofiban administration could improve functional prognosis by preventing the occurrence of END. However, tirofiban was often prescribed after END in previous studies, which might cause irreversible ischemic lesions and neurologic deficits[14 17]. Thus, randomised controlled trials of acute BAD-related stroke are needed and have been requested by researchers[3 4]. In addition, we speculated that tirofiban should be initiated within 48 h after onset to prevent the occurrence of END.

The BRANT trial aims to establish the efficacy and safety of intravenous tirofiban for improving functional outcome in patients with acute BAD-related stroke.

Methods

Design

This is a multicenter, randomized, open-label, blinded-endpoint, parallel-controlled phase III trial. The BRANT study began enrolment on November 9, 2023, and the anticipated date of study completion is October 31, 2025.

Patient population

BRANT will enroll 516 participants with BAD-related stroke within 48 hours of onset, from 21 centers in China.

Inclusion criteria

- Age: 18-75 years old [21 22]
- Acute ischemic stroke
- Time from onset to randomization ≤ 48h; if onset time is unknown, time from last known well to randomization ≤48h
- Meet the following BAD Diagnostic Imaging Criteria

1. Diffusion Weighted Imaging (DWI) infarcts: single (isolated) deep (subcortical) infarcts;

2. The culprit arteries are either lenticulostriate artery (LSA) or paramedian pontine artery (PPA), and the infarct lesion on DWI conforms to one of the following characteristics (A/B):

A. LSA: “Comma-like” infarct lesions with “fan-shaped” extension from bottom to top in the coronary position; or ≥ 3 layers (layer thickness 5– 7 mm) on axial DWI brain images;

B. PPA: The infarct lesion extends from the deep pons to the ventral pons on the axial DWI brain images;

- No more than 50% stenosis on the parent artery of the criminal artery (i.e. corresponding basilar or middle cerebral artery) (Confirmed by magnetic resonance angiography[MRA]/ computed tomography angiography [CTA]/ digital subtraction angiography [DSA])
- Signed informed consent by the patient or legally authorized representatives.

Exclusion criteria

- Transient ischemic attack (TIA)
- Intracranial hemorrhagic diseases, vascular malformations, aneurysms, brain abscesses, malignant space-occupying lesions, or other non- ischemic intracranial lesions detected by baseline computed tomography(CT)/ magnetic resonance imaging (MRI), or MRA/CTA/ DSA
- Presence of ≥ 50% stenosis in extracranial artery in tandem relationship ipsilateral to the lesion
- Cardiogenic embolism: atrial fibrillation, myocardial infarction, heart valve disease, dilated cardiomyopathy, infective endocarditis, atrioventricular block disease, heart rate less than 50 beats per minute
- Have received or plan to receive endovascular therapy or thrombolysis after onset
- Stroke of other clear causes, e.g., moyamoya disease, arterial entrapment, vasculitis, etc.

- modified Rankin Scale ≥2 before onset
- Use of tirofiban within 1 week before or after onset
- Low platelets (<100×109/L), or prothrombin time >1.3 times of the upper normal limit, or international normalised ratio (INR) >1.5, or other systemic hemorrhagic tendencies such as hematologic disorders
- Elevation of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) more than 1.5 times the upper normal limit
- Glomerular filtration rate <60 mL/min/1.73m2
- Known malignant tumors
- History of trauma or major surgical intervention within 6 weeks prior to onset
- History of intracranial hemorrhage
- Active or recent history (within 30 days prior to onset) of clinical bleeding (e.g., gastrointestinal bleeding)
- Malignant hypertension (systolic blood pressure >200 mmHg, or diastolic blood pressure >120 mmHg)
- Life expectancy ≤ 6 months
- Contraindications of 3 T MRI examination
- Pregnant or lactating women
- Have participated in another clinical trial within 3 months prior to the date of informed consent or are participating in another clinical trial

Randomization

Participants will be randomized in a 1:1 ratio using a dynamic block randomization method via an independent central website. The block sizes were set to 6, 8, and 12. The allocation sequence is stored on the central website and the participant will be assigned to the intervention or control group in a 1:1 ratio according to the order of enrolment.

Intervention

Tirofiban group: Intravenous tirofiban will be administered immediately after randomization for a total duration of 48 hours with a loading dose of 0.4μg /kg/min×30min, followed by a maintenance dose of 0.1μg /kg/min×47.5h (Figure1).

Control group: Standard antiplatelet therapy based on Chinese stroke guideline will be initiated after randomization for a total duration of 48 hours, as the two following types: (1) aspirin 150-300 mg qd, or (2) aspirin 100 mg qd plus clopidogrel 75 mg qd.[23] Its initiation will be determined based on the last administration time of antithrombotic drugs; however, the drug should be administered as soon as possible.

After a 48-hour treatment period in both groups, the standard of care, including an antithrombotic regimen, will be performed based on current guidelines and recorded in detail (Figure 1).

Primary outcomes

The primary outcome is excellent functional outcome at 90 days, defined as modified Rankin Scale score of 0-1.[24] Primary outcome will be measured by the qualified evaluators who are blinded to all procedures.

Secondary outcomes

Secondary efficacy outcomes include END, National Institutes of Health Stroke Scale (NIHSS) score, Barthel index score, ischemic stroke, stroke, TIA, and a composite event of new-onset stroke, myocardial infarction, and all-cause death. Safety outcomes include the proportion of major bleeding as defined by the PLATO criteria, adverse events, all-cause death, and changes in bleeding-related markers[25]. The evaluators will not be aware of the treatment assignment after randomization. All the clinical and safety events will be re-examined by the independent Clinical Event Committee (CEC), who will be blinded during all procedures.

Considering the predictive value of END, we adopted the widely used and conservative definition of END for the BRANT study[26]. The presence of END is determined by an increase of ≥ 4 points in the NIHSS or an increase of ≥ 2 points in the NIHSS motor score. The NIHSS motor score refers to bilateral upper and lower extremity mobility scores. The baseline NIHSS score for the calculation of END is the first clinician evaluated and recorded NIHSS score after onset. The time frame for post-randomisation END is within 7 days of randomization.

Study protocol and data management

A study flowchart is shown in Figure 1 and Table S1. At visit 1, trained investigators will recruit patients based on screening age, onset time, MRI, and other enrolment criteria (i.e. intracranial artery and electrocardiogram). The investigator will then explain the BRANT study to the patient in detail, including the contents of each visit

and the interventions. After obtaining written informed consent, the participants will be assigned to the tirofiban or control groups via a central website-based randomization system. Patients are encouraged to undergo on-site follow-up at 90 days. Demographic, clinical, radiological, laboratory, and clinical event data at each visit (Table S1) will be collected and stored in an electronic case report form (CRF) via a secure website. All CRFs will be checked by local investigators for completeness and correction prior to data entry. The data will be checked dynamically by the principal investigator (Jun Ni) with the aid of research assistants.

Data Monitoring Board

An independent Data Security Monitoring Board (DSMB), including academic experts and statisticians, has been established to protect the interests of the participants during the study. The DSMB will review the overall implementation of the clinical study and regularly and dynamically assess the risks and benefits, particularly unexpected adverse events. The DSMB reports to the Executive Committee and provides professional advice.

Sample size estimates

Based on previous studies and clinical practice, we assumed the rates of the primary outcome to be 62% and 74% in the control and tirofiban groups, respectively[2 14-16 18 27-29]. Thus, 234 participants per arm are needed for a two-sided test at alpha 0.05 and power 0.8. Considering a dropout rate of 10%, 516 patients will be required.

Statistical analyses

According to the principle of intention-to-treat analysis, all participants who are randomized into groups with more than one efficacy evaluation will be included in the full analysis set. The estimation of missing values will be conducted by the carry-over based on last observation carried forward (LOCF) estimation method. The proportion of excellent outcomes at 90 days will be compared using the chi-square tests, and shown as frequency (percentage). Most secondary outcome analyses will also use the primary outcome analysis strategy. Survival data will be calculated using the Kaplan-Meier method to estimate the survival rate in each group, and efficacy will be assessed using the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CI) will be calculated using the Cox proportional hazards model. Non-survival data will be analysed using the chi-square test, and odds ratios and 95% CIs will be calculated. Continuous variables will be compared between the two groups using the Student's t-test or Wilcoxon rank-sum test. The influence of covariables will be evaluated using subgroup analysis. All analyses will be performed using SAS 9.4, and a two-sided $P < 0.05$ is considered significant.

Patient and public involvement statement

None.

Ethics and dissemination: The BRANT study was approved by the Ethics Committee of Peking Union Medical College Hospital on July 20, 2023. Written informed consent is required from all patients before enrolment. BRANT will be carried out according to Good Clinical Practice and the Declaration of Helsinki. Protocol amendments will be reported to the institutional ethics committee. The trial sponsor is Peking Union Medical College Hospital. The trial results will be published in a peer-reviewed journal.

Discussion

The BRANT trial is a multicentre RCT that addresses the important treatment dilemma of improving the functional outcomes of BAD-related stroke.

BAD was first proposed by Caplan in 1989 to be distinct from lacunar infarct[1]. However, in the past three decades, most clinical studies have classified BAD as small-vessel occlusion or undetermined etiology based on the TOAST system[4 30]. Few studies focused on acute BAD-related stroke, probably due to discrepant definitions[3]. Recently, an increasing number of observational studies found distinct clinical, radiologic, and prognostic features that patients with BAD-related stroke are prone to END and poor prognosis[2 31].

Owing to the limitations of neuroimaging techniques, the perforating artery, such as the LSA or PPA, cannot be directly visualized. Radiological diagnosis is based on the vascular territory, dimension, or shape of the lesion[3 32], which results in a huge variations among BAD definitions. With the aid of neuroimaging and clinical practice, Asian neurologists proposed radiological diagnosis criteria for BAD[33 34]. Our

previous study also found that ≥4 consecutive slices on axial view are more effective than transversal diameter to differentiate atherosclerotic mechanisms of single subcortical infarction in the LSA territory[35]. Considering the generalisation and diagnostic accuracy of our study, we used ≥ 3 consecutive layers on axial DWI series instead of lesion diameter to define BAD-related stroke in the LSA territory[36]. Because obtaining direct evidence of the LSA and PPA is currently not technically feasible, our inclusion criteria based on MRI show considerable accuracy and representativeness.

In addition, our study uses simplified operationalised criteria to exclude cardiogenic embolism, and patients with these comorbidities will not be included. Some conditions seem general, which is a limitation of our study; however, this facilitates the researcher's ability to complete screening within a limited timeframe with low inconsistency.

We set a 90-day excellent outcome instead of END or new-onset stroke as the primary outcome for the following reasons: (1) historical evidence indicated that tirofiban improved the functional outcome of ischemic stroke[14 17]; (2) END is an intermediate indicators[7]; (3) the 90-day rate of recurrent stroke is 1.8% in our preliminary analysis of a BAD-related stroke cohort and probably less than 3.8% in other cohorts[31], which is relatively low. Thus, the BRANT study will provide direct evidence on how to reduce disability caused by BAD, which is the major challenge in current clinical practice.

As oral mono antiplatelet therapy is unethical and against Chinese stroke guidelines for patients with NIHSS ≤ 3, there are two types of antiplatelet therapy in the control

group[23]. A double-blind design would markedly increase the complexity of the trial procedure. Therefore, we selected a prospective randomized open blinded end-point design (PROBE) for BRANT. Independent senior neurologists who will be blinded to the procedure information have been trained to evaluate the primary outcome. An independent CEC has been established to centrally re-examine all clinical events after randomization. Some local investigators may know the treatment allocation; however, all evaluators of the subjective indicators will be blinded to the treatment allocation.

Contributors: Shengde Li, and Jun Ni designed the study. Shengde Li drafted the manuscript. Dingding Zhang designed the statistical method. Yuhui Sha, Lixin Zhou, Yicheng Zhu, and Bin Peng critically revised the study protocol and the manuscript. The entire project will be supervised by Jun Ni.

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Disclaimer: The funder has no role in this study.

Competing interests: None declared.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author on reasonable request.

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Figure I. Study Flow

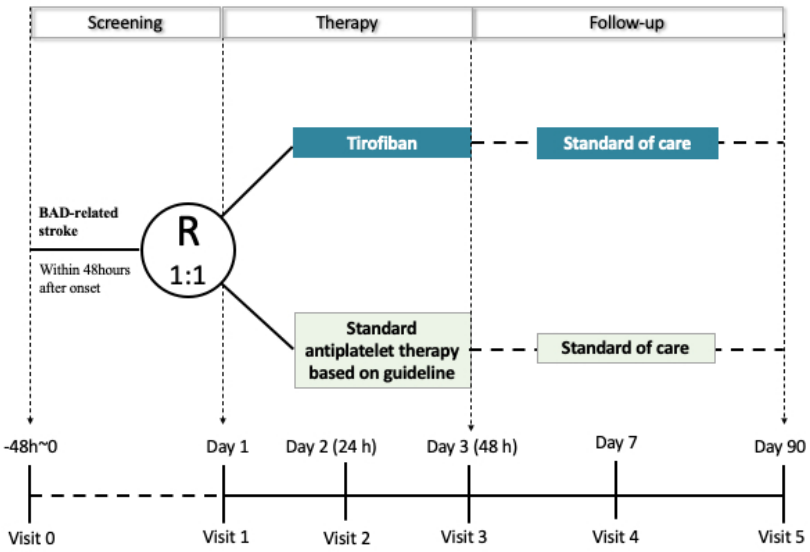

Figure 1. Study Flow

338x190mm (54 x 54 DPI)

Supplementary materials

Table S1. Study Procedure of BRANT trial

Measurement	Day 1	Day 2	Day 3	Day 7	Day 90
Demographic characteristics	X				
Current medical history taking	X				
Body temperature measurement	X				
Physical examination	X			X	X
Past medical history	X				
Pre-randomization medication after onset	X				
Regular blood pressure monitoring	X	X			
NIHSS score	X			X	X
mRS score	X			X	X
Barthel index score	X				X
Magnetic resonance image	X				
Evaluation of Intracranial vessels	X				
Evaluation of extracranial vessels	X				
Laboratory tests*	X				
ECG*	X			X	
Verification of inclusion/exclusion criteria	X				
Signed informed consent	X				
Randomization	X				
Blood tests after enrollment		X	X		

Urine and fecal examination			X		
Compliance			X		
Concomitant medication				X	X
Early neurological deterioration				X	
Major bleeding				X	X
Adverse Events/ Serious Adverse Events				X	X
*Remarks: ECG and laboratory data performed within 48 hours of onset before signing the informed consent form can be used as trial data.					

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2-3
	2b	All items from the World Health Organization Trial Registration Data Set	Clinical Trials.gov
Protocol version	3	Date and version identifier	In Protocol, not shown in article
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	16
	5b	Name and contact information for the trial sponsor	Clinical Trials.gov
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	10-11
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-6
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	In Protocol, not shown in article
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	In Protocol, not shown in article
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	In Protocol, not shown in article
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-11

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	In Protocol, not shown in article
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9-10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9-10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9-10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10-11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	11-12

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11-12
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11-12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	3
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	3

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	3
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	In protocoal, not shown in article
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	In protocoal, not shown in article
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	See consent from 3
	31b	Authorship eligibility guidelines and any intended use of professional writers	In protocoal, not shown in article
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	We provide a model consent from
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy and Safety of Tirofiban in Patients with Acute Branch Atherosclerotic Disease(BAD)-Related Stroke (BRANT): Protocol for a Randomized Controlled Trial

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Efficacy and Safety of Tirofiban in Patients with Acute Branch Atherosclerotic Disease(BAD)-Related Stroke (BRANT): Protocol for a Randomized Controlled Trial

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Abstract

Introduction: Branch atheromatous disease (BAD)-related stroke is increasingly becoming a clinical entity and prone to early neurological deterioration (END) and poor prognosis. There are no effective regimens to reduce the disability caused by BAD-related stroke in acute phase. Recent studies have indicated the efficacy of tirofiban in acute ischemic stroke, however, its efficacy has not been validated in patients with BAD-related stroke. Thus, we aim to test whether intravenous tirofiban initiated within 48 hours after onset would safely improve the functional outcome in patients with acute BAD-related stroke, in comparison with standard antiplatelet therapy based on the current guideline.

Methods and analysis: BRANT is a multicenter, randomized, open-label, blinded endpoint, parallel-controlled, phase III trial conducted in 21 hospitals in China. Participants aged 18-75 years with acute BAD-related stroke within 48 h after stroke onset are randomized in a 1:1 ratio to the tirofiban or control group. The treatment period is 48 hours in both groups. The primary outcome is the excellent functional outcome (modified Rankin Scale score: 0-1) at 90 days. The secondary outcomes include END, major bleeding, stroke, death, functional status, serious adverse events, and change in bleeding-related markers. Assuming the rates of the primary outcome to be 74% in the tirofiban group and 62% in the control group, a total of 516 participants are needed for 0.8 power (two-sided 0.05 alpha).

Ethics and dissemination: BRANT study has been approved by the Ethics Committee of the Peking Union Medical College Hospital (I-23PJ1242). Written informed consent

is required for all patients before enrollment. The results of the study will be published in a peer-reviewed journal.

Trial registration number ClinicalTrials.gov (NCT06037889)

Keywords: Branch Atheromatous Disease, Acute ischemic stroke, Early neurological deterioration, Functional outcome, Tirofiban, Treatment

Strengths and limitations of this study

1. With the aid of magnetic resonance imaging, this study focuses on patients with acute BAD-related stroke, which had been inappropriately classified as small-vessel occlusion or an undetermined aetiology by the TOAST system in previous studies.

2. Intervention will be initiated within 48 hours of onset， which is more in line with the timeliness of BAD treatment.

3. Lack of double-blinded design is a limitation, but the endpoints are measured in a blinded manner.

Introduction

Branch atheromatous disease (BAD), first described conceptually by Caplan in 1989, is being confirmed as a clinical entity with the aid of advanced neuroimaging.[1-3] BAD-related stroke, characterized by subcortical single infarcts in penetrating artery territories without severe stenosis of the large parent artery, accounts for 20.4% of all ischaemic stroke cases in Asian populations[2 4]. Differing from lacunar infarct due to lipohyalinosis, BAD-related stroke is caused by parent arterial plaque occlusion of the perforating orifice or proximal atherosclerotic occlusion of the perforating artery[1-3], which could be identified from small-vessel occlusion or stroke of undetermined source in the Trial Org 10172 in Acute Stroke Treatment (TOAST) system[5 6].

High incidence of early neurological deterioration (END) has been observed in BAD-related stroke and is strongly associated with poor prognosis[7 8]. The rate of END is higher in BAD-related stroke than lacunar infarct (26.8-37.5% vs. 6.3-18.6%), and thrombolysis itself cannot prevent the occurrence of END[7-9]. In addition, it remains unclear whether intravenous thrombolysis could improve the clinical outcome in BAD-related stroke[10]. The rate of disability can reach 61%[2]. However, there are no high-level recommendations for acute-phase treatment of BAD-related stroke, and no Randomized Controlled Trial (RCT) has examined BAD as a separate disease. Current practice—based on limited observational data and expert opinion—is heterogeneous, including anticoagulant and mono/dual antiplatelet therapy, the efficacy of which is uncertain for BAD-related stroke[11 12].

Tirofiban, a selective and reversible antagonist of glycoprotein IIb/IIIa inhibitors on

platelets, might be more effective than conventional agents (such as aspirin or clopidogrel) by blocking the final common pathway of platelet aggregation at the pathophysiological level[13]. In clinical studies, historical evidence has also reported that tirofiban increases the recanalization rate and improves functional prognosis in stroke patients with endovascular therapy without increasing bleeding risk[14-16]. A large randomized trial of patients with stroke without large or medium-sized vessel occlusion also reported the efficacy of tirofiban[17]. However, though an atherosclerotic mechanism is presumed to exist between BAD and large artery atherosclerosis, this evidence may not be generalized to BAD-related stroke, as retrospective data or small samples may introduce selection bias[14 18]. Moreover, about 78.6%-90.9% of END occurs within 48 h after onset[19 20]. Our preliminary results of a cohort with BAD-related stroke found that the median time from onset to END was 38 h. We hypothesised that early tirofiban administration could improve functional prognosis by preventing the occurrence of END. However, tirofiban was often prescribed after END in previous studies, which might cause irreversible ischemic lesions and neurologic deficits[14 17]. Thus, randomised controlled trials of acute BAD-related stroke are needed and have been requested by researchers[3 4]. In addition, we speculated that tirofiban should be initiated within 48 h after onset to prevent the occurrence of END.

The BRANT trial aims to establish the efficacy and safety of intravenous tirofiban for improving functional outcome in patients with acute BAD-related stroke.

Methods

Design

This is a multicenter, randomized, open-label, blinded-endpoint, parallel-controlled phase III trial. The BRANT study began enrolment on November 9, 2023, and the anticipated date of study completion is October 31, 2025.

Patient population

BRANT will enroll 516 participants with BAD-related stroke within 48 hours of onset, from 21 centers in China.

Inclusion criteria

- Age: 18-75 years old [21 22]
- Acute ischemic stroke
- Time from onset to randomization ≤ 48h; if onset time is unknown, time from last known well to randomization ≤48h
- Meet the following BAD Diagnostic Imaging Criteria

1. Diffusion Weighted Imaging (DWI) infarcts: single (isolated) deep (subcortical) infarcts;

2. The culprit arteries are either lenticulostriate artery (LSA) or paramedian pontine artery (PPA), and the infarct lesion on DWI conforms to one of the following characteristics (A/B):

A. LSA: “Comma-like” infarct lesions with “fan-shaped” extension from bottom to top in the coronary position; or ≥ 3 layers (layer thickness 5– 7 mm) on axial DWI brain images;

B. PPA: The infarct lesion extends from the deep pons to the ventral pons on the axial DWI brain images;

- No more than 50% stenosis on the parent artery of the criminal artery (i.e. corresponding basilar or middle cerebral artery) (Confirmed by magnetic resonance angiography[MRA]/ computed tomography angiography [CTA]/ digital subtraction angiography [DSA])
- Signed informed consent by the patient or legally authorized representatives.

Exclusion criteria

- Transient ischemic attack (TIA)
- Intracranial hemorrhagic diseases, vascular malformations, aneurysms, brain abscesses, malignant space-occupying lesions, or other non- ischemic intracranial lesions detected by baseline computed tomography(CT)/ magnetic resonance imaging (MRI), or MRA/CTA/ DSA
- Presence of ≥ 50% stenosis in extracranial artery in tandem relationship ipsilateral to the lesion
- Cardiogenic embolism: atrial fibrillation, myocardial infarction, heart valve disease, dilated cardiomyopathy, infective endocarditis, atrioventricular block disease, heart rate less than 50 beats per minute
- Have received or plan to receive endovascular therapy or thrombolysis after onset
- Stroke of other clear causes, e.g., moyamoya disease, arterial entrapment, vasculitis, etc.

- modified Rankin Scale score ≥2 before onset
- Use of tirofiban within 1 week before or after onset
- Low platelets (<100×109/L), or prothrombin time >1.3 times of the upper normal limit, or international normalised ratio (INR) >1.5, or other systemic hemorrhagic tendencies such as hematologic disorders
- Elevation of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) more than 1.5 times the upper normal limit
- Glomerular filtration rate <60 mL/min/1.73m2
- Known malignant tumors
- History of trauma or major surgical intervention within 6 weeks prior to onset
- History of intracranial hemorrhage
- Active or recent history (within 30 days prior to onset) of clinical bleeding (e.g., gastrointestinal bleeding)
- Malignant hypertension (systolic blood pressure >200 mmHg, or diastolic blood pressure >120 mmHg)
- Life expectancy ≤ 6 months
- Contraindications of 3 T MRI examination
- Pregnant or lactating women
- Have participated in another clinical trial within 3 months prior to the date of informed consent or are participating in another clinical trial

Randomization

Participants will be randomized in a 1:1 ratio using a dynamic block randomization method via an independent central website. The block sizes were set to 6, 8, and 12. The allocation sequence is stored on the central website and the participant will be assigned to the intervention or control group in a 1:1 ratio according to the order of enrolment.

Intervention

Tirofiban group: Intravenous tirofiban will be administered immediately after randomization for a total duration of 48 hours with a loading dose of 0.4μg /kg/min×30min, followed by a maintenance dose of 0.1μg /kg/min×47.5h (Figure1).

Control group: Standard antiplatelet therapy based on Chinese stroke guideline will be initiated after randomization for a total duration of 48 hours, as the two following types: (1) aspirin 150-300 mg qd, or (2) aspirin 100 mg qd plus clopidogrel 75 mg qd.[23] Its initiation will be determined based on the last administration time of antithrombotic drugs; however, the drug should be administered as soon as possible.

After a 48-hour treatment period in both groups, the standard of care, including an antithrombotic regimen, will be performed based on current guidelines and recorded in detail (Figure 1).

Primary outcomes

The primary outcome is excellent functional outcome at 90 days, defined as modified Rankin Scale score of 0-1[24]. Primary outcome will be measured by the qualified evaluators who are blinded to all procedures.

Secondary outcomes

Secondary efficacy outcomes include END, National Institutes of Health Stroke Scale (NIHSS) score, Barthel index score, ischemic stroke, stroke, TIA, and a composite event of new-onset stroke, myocardial infarction, and all-cause death. Excellent functional outcome at 7 days is also listed as a secondary efficacy outcome. Safety outcomes include the proportion of major bleeding as defined by the PLATO criteria, adverse events, all-cause death, and changes in bleeding-related markers[25]. The evaluators will not be aware of the treatment assignment after randomization. All the clinical and safety events will be re-examined by the independent Clinical Event Committee (CEC), who will be blinded during all procedures.

Considering the predictive value of END, we adopted the widely used and conservative definition of END for the BRANT study[26]. The presence of END is determined by an increase of ≥ 4 points in the NIHSS or an increase of ≥ 2 points in the NIHSS motor score. The NIHSS motor score refers to bilateral upper and lower extremity mobility scores. The baseline NIHSS score for the calculation of END is the first clinician evaluated and recorded NIHSS score after onset. The time frame for post-randomisation END is within 7 days of randomization.

Study protocol and data management

A study flowchart is shown in Figure 1 and Table S1. At visit 1, trained investigators will recruit patients based on screening age, onset time, MRI, and other enrolment criteria (i.e. intracranial artery and electrocardiogram). The investigator will then

explain the BRANT study to the patient in detail, including the contents of each visit and the interventions. After obtaining written informed consent, the participants will be assigned to the tirofiban or control groups via a central website-based randomization system. Patients are encouraged to undergo on-site follow-up at 90 days. Demographic, clinical, radiological, laboratory, and clinical event data at each visit (Table S1) will be collected and stored in an electronic case report form (CRF) via a secure website. All CRFs will be checked by local investigators for completeness and correction prior to data entry. The data will be checked dynamically by the principal investigator (Jun Ni) with the aid of research assistants.

Data Monitoring Board

An independent Data Security Monitoring Board (DSMB), including academic experts and statisticians, has been established to protect the interests of the participants during the study. The DSMB will review the overall implementation of the clinical study and regularly and dynamically assess the risks and benefits, particularly unexpected adverse events. The DSMB reports to the Executive Committee and provides professional advice.

Sample size estimates

Based on previous studies and clinical practice, we assumed the rates of the primary outcome to be 62% and 74% in the control and tirofiban groups, respectively[2 14-16

18 27-29]. Thus, 234 participants per arm are needed for a two-sided test at alpha 0.05 and power 0.8. Considering a dropout rate of 10%, 516 patients will be required.

Statistical analyses

According to the principle of intention-to-treat analysis, all participants who are randomized into groups with more than one efficacy evaluation will be included in the full analysis set. The estimation of missing values will be conducted by the carry-over based on last observation carried forward (LOCF) estimation method. The proportion of excellent outcomes at 90 days will be compared using the chi-square tests, and shown as frequency (percentage). Most secondary outcome analyses will also use the primary outcome analysis strategy. Survival data will be calculated using the Kaplan-Meier method to estimate the survival rate in each group, and efficacy will be assessed using the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CI) will be calculated using the Cox proportional hazards model. Non-survival data will be analysed using the chi-square test, and odds ratios and 95% CIs will be calculated. Continuous variables will be compared between the two groups using the Student's t-test or Wilcoxon rank-sum test. The influence of covariables will be evaluated using subgroup analysis. No interim analysis is planned in this trial. All analyses will be performed using SAS 9.4, and a two-sided $P < 0.05$ is considered significant.

Patient and public involvement statement

None.

Ethics and dissemination: The BRANT study was approved by the Ethics Committee of Peking Union Medical College Hospital (I-23PJ1242) on July 20, 2023. Written informed consent is required from all patients before enrolment. BRANT will be carried out according to Good Clinical Practice and the Declaration of Helsinki. Protocol amendments will be reported to the institutional ethics committee. The trial sponsor is Peking Union Medical College Hospital. The trial results will be published in a peer-reviewed journal.

Discussion

The BRANT trial is a multicentre RCT that addresses the important treatment dilemma of improving the functional outcomes of BAD-related stroke.

BAD was first proposed by Caplan in 1989 to be distinct from lacunar infarct[1]. However, in the past three decades, most clinical studies have classified BAD as small-vessel occlusion or undetermined etiology based on the TOAST system[4 30]. Few studies focused on acute BAD-related stroke, probably due to discrepant definitions[3]. Recently, an increasing number of observational studies found distinct clinical, radiologic, and prognostic features that patients with BAD-related stroke are prone to END and poor prognosis[2 31].

Owing to the limitations of neuroimaging techniques, the perforating artery, such as the LSA or PPA, cannot be directly visualized. Radiological diagnosis is based on the vascular territory, dimension, or shape of the lesion[3 32], which results in a huge

variations among BAD definitions. With the aid of neuroimaging and clinical practice, Asian neurologists proposed radiological diagnosis criteria for BAD[33 34]. Our previous study also found that ≥4 consecutive slices on axial view are more effective than transversal diameter to differentiate atherosclerotic mechanisms of single subcortical infarction in the LSA territory[35]. Considering the generalisation and diagnostic accuracy of our study, we used ≥ 3 consecutive layers on axial DWI series instead of lesion diameter to define BAD-related stroke in the LSA territory[36]. Because obtaining direct evidence of the LSA and PPA is currently not technically feasible, our inclusion criteria based on MRI show considerable accuracy and representativeness.

In addition, our study uses simplified operationalised criteria to exclude cardiogenic embolism, and patients with these comorbidities will not be included. Some conditions seem general, which is a limitation of our study; however, this facilitates the researcher's ability to complete screening within a limited timeframe with low inconsistency.

We set a 90-day excellent outcome instead of END or new-onset stroke as the primary outcome for the following reasons: (1) historical evidence indicated that tirofiban improved the functional outcome of ischemic stroke[14 17]; (2) END is an intermediate indicators[7]; (3) the 90-day rate of recurrent stroke is 1.8% in our preliminary analysis of a BAD-related stroke cohort and probably less than 3.8% in other cohorts[31], which is relatively low. Thus, the BRANT study will provide direct evidence on how to reduce disability caused by BAD, which is the major challenge in current clinical practice.

As oral mono antiplatelet therapy is unethical and against Chinese stroke guidelines for patients with NIHSS ≤ 3, there are two types of antiplatelet therapy in the control group[23]. A double-blind design would markedly increase the complexity of the trial procedure. Therefore, we selected a prospective randomized open blinded end-point design (PROBE) for BRANT. Independent senior neurologists who will be blinded to the procedure information have been trained to evaluate the primary outcome. An independent CEC has been established to centrally re-examine all clinical events after randomization. Some local investigators may know the treatment allocation; however, all evaluators of the subjective indicators will be blinded to the treatment allocation.

Contributors: Shengde Li, and Jun Ni designed the study. Shengde Li drafted the manuscript. Dingding Zhang designed the statistical method. Yuhui Sha, Lixin Zhou, Yicheng Zhu, and Bin Peng critically revised the study protocol and the manuscript. The entire project will be supervised by Jun Ni.

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Disclaimer: The funder has no role in this study.

Competing interests: None declared.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author on reasonable request.

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Figure I. Study Flow

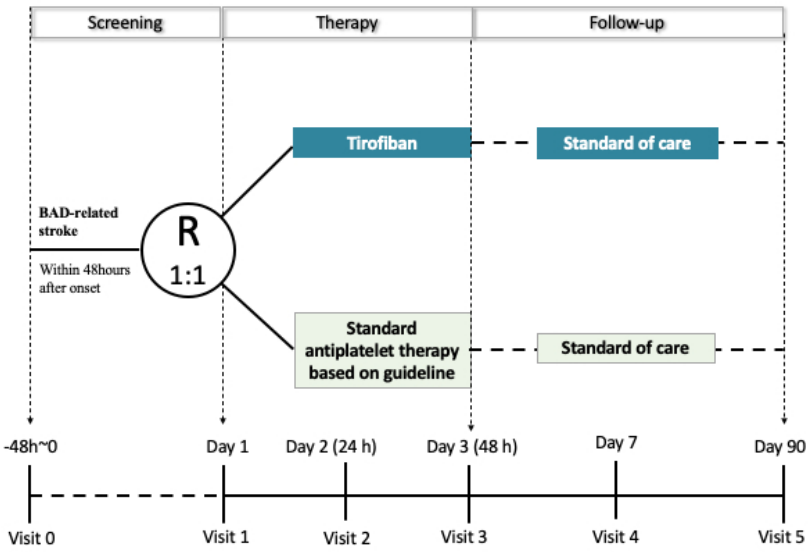

Figure 1. Study Flow

338x190mm (54 x 54 DPI)

Supplementary materials

Table S1. Study Procedure of BRANT trial

Measurement	Day 1	Day 2	Day 3	Day 7	Day 90
Demographic characteristics	X				
Current medical history taking	X				
Body temperature measurement	X				
Physical examination	X			X	X
Past medical history	X				
Pre-randomization medication after onset	X				
Regular blood pressure monitoring	X	X			
NIHSS score	X			X	X
mRS score	X			X	X
Barthel index score	X				X
Magnetic resonance image	X				
Evaluation of Intracranial vessels	X				
Evaluation of extracranial vessels	X				
Laboratory tests*	X				
ECG*	X			X	
Verification of inclusion/exclusion criteria	X				
Signed informed consent	X				
Randomization	X				
Blood tests after enrollment		X	X		

Urine and fecal examination			X		
Compliance			X		
Concomitant medication				X	X
Early neurological deterioration				X	
Major bleeding				X	X
Adverse Events/ Serious Adverse Events				X	X
*Remarks: ECG and laboratory data performed within 48 hours of onset before signing the informed consent form can be used as trial data.					

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2-3
	2b	All items from the World Health Organization Trial Registration Data Set	Clinical Trials.gov
Protocol version	3	Date and version identifier	In Protocol, not shown in article
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	16
	5b	Name and contact information for the trial sponsor	Clinical Trials.gov
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	10-11
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-6
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	In Protocol, not shown in article
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	In Protocol, not shown in article
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	In Protocol, not shown in article
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-11

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	In Protocol, not shown in article
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9-10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9-10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9-10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10-11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	11-12

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11-12
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11-12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	3
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	3

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	3
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	In protocoal, not shown in article
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	In protocoal, not shown in article
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	See consent from 3
	31b	Authorship eligibility guidelines and any intended use of professional writers	In protocoal, not shown in article
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	We provide a model consent from
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy and Safety of Tirofiban in Patients with Acute Branch Atherosclerotic Disease(BAD)-Related Stroke (BRANT): Protocol for a Randomized Controlled Trial

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Efficacy and Safety of Tirofiban in Patients with Acute Branch Atherosclerotic Disease(BAD)-Related Stroke (BRANT): Protocol for a Randomized Controlled Trial

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Abstract

Introduction: Branch atheromatous disease (BAD)-related stroke is increasingly becoming a clinical entity and prone to early neurological deterioration (END) and poor prognosis. There are no effective regimens to reduce the disability caused by BAD-related stroke in acute phase. Recent studies have indicated the efficacy of tirofiban in acute ischemic stroke, however, its efficacy has not been validated in patients with BAD-related stroke. Thus, we aim to test whether intravenous tirofiban initiated within 48 hours after onset would improve the functional outcome in patients with acute BAD-related stroke, in comparison with standard antiplatelet therapy based on the current guideline.

Methods and analysis: BRANT is a multicenter, randomized, open-label, blinded endpoint, parallel-controlled, phase III trial conducted in 21 hospitals in China. Participants aged 18-75 years with acute BAD-related stroke within 48 h after stroke onset are randomized in a 1:1 ratio to the tirofiban or control group. The treatment period is 48 hours in both groups. The primary outcome is the excellent functional outcome (modified Rankin Scale score: 0-1) at 90 days. The secondary outcomes include END, major bleeding, stroke, death, functional status, serious adverse events, and change in bleeding-related markers. Assuming the rates of the primary outcome to be 74% in the tirofiban group and 62% in the control group, a total of 516 participants are needed for 0.8 power (two-sided 0.05 alpha).

Ethics and dissemination: BRANT study has been approved by the Ethics Committee of the Peking Union Medical College Hospital (I-23PJ1242). Written informed consent

is required for all patients before enrollment. The results of the study will be published in a peer-reviewed journal.

Trial registration number ClinicalTrials.gov (NCT06037889)

Keywords: Branch Atheromatous Disease, Acute ischemic stroke, Early neurological deterioration, Functional outcome, Tirofiban, Treatment

Strengths and limitations of this study

1. With the aid of magnetic resonance imaging, this study focuses on patients with acute BAD-related stroke, which had been inappropriately classified as small-vessel occlusion or an undetermined aetiology by the TOAST system in previous studies.

2. Intervention will be initiated within 48 hours of onset， which is more in line with the timeliness of BAD treatment.

3. Lack of double-blinded design is a limitation, but the endpoints are measured in a blinded manner.

Introduction

Branch atheromatous disease (BAD), first described conceptually by Caplan in 1989, is being confirmed as a clinical entity with the aid of advanced neuroimaging.[1-3] BAD-related stroke, characterized by subcortical single infarcts in penetrating artery territories without severe stenosis of the large parent artery, accounts for 20.4% of all ischaemic stroke cases in Asian populations[2 4]. Differing from lacunar infarct due to lipohyalinosis, BAD-related stroke is caused by parent arterial plaque occlusion of the perforating orifice or proximal atherosclerotic occlusion of the perforating artery[1-3], which could be identified from small-vessel occlusion or stroke of undetermined source in the Trial Org 10172 in Acute Stroke Treatment (TOAST) system[5 6].

High incidence of early neurological deterioration (END) has been observed in BAD-related stroke and is strongly associated with poor prognosis[7 8]. The rate of END is higher in BAD-related stroke than lacunar infarct (26.8-37.5% vs. 6.3-18.6%), and thrombolysis itself cannot prevent the occurrence of END[7-9]. In addition, it remains unclear whether intravenous thrombolysis could improve the clinical outcome in BAD-related stroke[10]. The rate of disability can reach 61%[2]. However, there are no high-level recommendations for acute-phase treatment of BAD-related stroke, and no Randomized Controlled Trial (RCT) has examined BAD as a separate disease. Current practice—based on limited observational data and expert opinion—is heterogeneous, including anticoagulant and mono/dual antiplatelet therapy, the efficacy of which is uncertain for BAD-related stroke[11 12].

Tirofiban, a selective and reversible antagonist of glycoprotein IIb/IIIa inhibitors on

platelets, might be more effective than conventional agents (such as aspirin or clopidogrel) by blocking the final common pathway of platelet aggregation at the pathophysiological level[13]. In clinical studies, historical evidence has also reported that tirofiban increases the recanalization rate and improves functional prognosis in stroke patients with endovascular therapy without increasing bleeding risk[14-16]. A large randomized trial of patients with stroke without large or medium-sized vessel occlusion also reported the efficacy of tirofiban[17]. However, though an atherosclerotic mechanism is presumed to exist between BAD and large artery atherosclerosis, this evidence may not be generalized to BAD-related stroke, as retrospective data or small samples may introduce selection bias[14 18]. Moreover, about 78.6%-90.9% of END occurs within 48 h after onset[19 20]. Our preliminary results of a cohort with BAD-related stroke found that the median time from onset to END was 38 h. We hypothesised that early tirofiban administration could improve functional prognosis by preventing the occurrence of END. However, tirofiban was often prescribed after END in previous studies, which might cause irreversible ischemic lesions and neurologic deficits[14 17]. Thus, randomised controlled trials of acute BAD-related stroke are needed and have been requested by researchers[3 4]. In addition, we speculated that tirofiban should be initiated within 48 h after onset to prevent the occurrence of END.

The BRANT trial aims to establish the efficacy and safety of intravenous tirofiban for improving functional outcome in patients with acute BAD-related stroke.

Methods

Design

This is a multicenter, randomized, open-label, blinded-endpoint, parallel-controlled phase III trial. The BRANT study began enrolment on November 9, 2023, and the anticipated date of study completion is October 31, 2025.

Patient population

BRANT will enroll 516 participants with BAD-related stroke within 48 hours of onset, from 21 centers in China.

Inclusion criteria

- Age: 18-75 years old [21 22]
- Acute ischemic stroke
- Time from onset to randomization ≤ 48h; if onset time is unknown, time from last known well to randomization ≤48h
- Meet the following BAD Diagnostic Imaging Criteria

1. Diffusion Weighted Imaging (DWI) infarcts: single (isolated) deep (subcortical) infarcts;

2. The culprit arteries are either lenticulostriate artery (LSA) or paramedian pontine artery (PPA), and the infarct lesion on DWI conforms to one of the following characteristics (A/B):

A. LSA: “Comma-like” infarct lesions with “fan-shaped” extension from bottom to top in the coronary position; or ≥ 3 layers (layer thickness 5– 7 mm) on axial DWI brain images;

B. PPA: The infarct lesion extends from the deep pons to the ventral pons on the axial DWI brain images;

- No more than 50% stenosis on the parent artery of the criminal artery (i.e. corresponding basilar or middle cerebral artery) (Confirmed by magnetic resonance angiography[MRA]/ computed tomography angiography [CTA]/ digital subtraction angiography [DSA])
- Signed informed consent by the patient or legally authorized representatives.

Exclusion criteria

- Transient ischemic attack (TIA)
- Intracranial hemorrhagic diseases, vascular malformations, aneurysms, brain abscesses, malignant space-occupying lesions, or other non- ischemic intracranial lesions detected by baseline computed tomography(CT)/ magnetic resonance imaging (MRI), or MRA/CTA/ DSA
- Presence of ≥ 50% stenosis in extracranial artery in tandem relationship ipsilateral to the lesion
- Cardiogenic embolism: atrial fibrillation, myocardial infarction, heart valve disease, dilated cardiomyopathy, infective endocarditis, atrioventricular block disease, heart rate less than 50 beats per minute
- Have received or plan to receive endovascular therapy or thrombolysis after onset
- Stroke of other clear causes, e.g., moyamoya disease, arterial entrapment, vasculitis, etc.

- modified Rankin Scale score ≥2 before onset
- Use of tirofiban within 1 week before or after onset
- Low platelets (<100×109/L), or prothrombin time >1.3 times of the upper normal limit, or international normalised ratio (INR) >1.5, or other systemic hemorrhagic tendencies such as hematologic disorders
- Elevation of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) more than 1.5 times the upper normal limit
- Glomerular filtration rate <60 mL/min/1.73m2
- Known malignant tumors
- History of trauma or major surgical intervention within 6 weeks prior to onset
- History of intracranial hemorrhage
- Active or recent history (within 30 days prior to onset) of clinical bleeding (e.g., gastrointestinal bleeding)
- Malignant hypertension (systolic blood pressure >200 mmHg, or diastolic blood pressure >120 mmHg)
- Life expectancy ≤ 6 months
- Contraindications of 3 T MRI examination
- Pregnant or lactating women
- Have participated in another clinical trial within 3 months prior to the date of informed consent or are participating in another clinical trial

Randomization

Participants will be randomized in a 1:1 ratio using a dynamic block randomization method via an independent central website. The block sizes were set to 6, 8, and 12. The allocation sequence is stored on the central website and the participant will be assigned to the intervention or control group in a 1:1 ratio according to the order of enrolment.

Intervention

Tirofiban group: Intravenous tirofiban will be administered immediately after randomization for a total duration of 48 hours with a loading dose of 0.4μg /kg/min×30min, followed by a maintenance dose of 0.1μg /kg/min×47.5h (Figure1).

Control group: Standard antiplatelet therapy based on Chinese stroke guideline will be initiated after randomization for a total duration of 48 hours, as the two following types: (1) aspirin 150-300 mg qd, or (2) aspirin 100 mg qd plus clopidogrel 75 mg qd.[23] Its initiation will be determined based on the last administration time of antithrombotic drugs; however, the drug should be administered as soon as possible.

After a 48-hour treatment period in both groups, the standard of care, including an antithrombotic regimen, will be performed based on current guidelines and recorded in detail (Figure 1).

Primary outcomes

The primary outcome is excellent functional outcome at 90 days, defined as modified Rankin Scale score of 0-1[24]. Primary outcome will be measured by the qualified evaluators who are blinded to all procedures.

Secondary outcomes

Secondary efficacy outcomes include END, National Institutes of Health Stroke Scale (NIHSS) score, Barthel index score, ischemic stroke, stroke, TIA, and a composite event of new-onset stroke, myocardial infarction, and all-cause death. Excellent functional outcome at 7 days is also listed as a secondary efficacy outcome. Safety outcomes include major bleeding as defined by the PLATO criteria, adverse events, all-cause death, and changes in bleeding-related markers[25]. The evaluators will not be aware of the treatment assignment after randomization. All the clinical and safety events will be re-examined by the independent Clinical Event Committee (CEC), who will be blinded during all procedures.

Considering the predictive value of END, we adopted the widely used and conservative definition of END for the BRANT study[26]. The presence of END is determined by an increase of ≥ 4 points in the NIHSS or an increase of ≥ 2 points in the NIHSS motor score. The NIHSS motor score refers to bilateral upper and lower extremity mobility scores. The baseline NIHSS score for the calculation of END is the first clinician evaluated and recorded NIHSS score after onset. The time frame for post-randomisation END is within 7 days of randomization.

Study protocol and data management

A study flowchart is shown in Figure 1 and Table S1. At visit 1, trained investigators will recruit patients based on screening age, onset time, MRI, and other enrolment criteria (i.e. intracranial artery and electrocardiogram). The investigator will then

explain the BRANT study to the patient in detail, including the contents of each visit and the interventions. After obtaining written informed consent, the participants will be assigned to the tirofiban or control groups via a central website-based randomization system. Patients are encouraged to undergo on-site follow-up at 90 days. Demographic, clinical, radiological, laboratory, and clinical event data at each visit (Table S1) will be collected and stored in an electronic case report form (CRF) via a secure website. All CRFs will be checked by local investigators for completeness and correction prior to data entry. The data will be checked dynamically by the principal investigator (Jun Ni) with the aid of research assistants.

Data Monitoring Board

An independent Data Security Monitoring Board (DSMB), including academic experts and statisticians, has been established to protect the interests of the participants during the study. The DSMB will review the overall implementation of the clinical study and regularly and dynamically assess the risks and benefits, particularly unexpected adverse events. The DSMB reports to the Executive Committee and provides professional advice.

Sample size estimates

Based on previous studies and clinical practice, we assumed the rates of the primary outcome to be 62% and 74% in the control and tirofiban groups, respectively[2 14-16 18

[27-29]. Thus, 234 participants per arm are needed for a two-sided test at alpha 0.05 and power 0.8. Considering a dropout rate of 10%, 516 patients will be required.

Statistical analyses

According to the principle of intention-to-treat analysis, all participants who are randomized into groups with more than one efficacy evaluation will be included in the full analysis set. The estimation of missing values will be conducted by the carry-over based on last observation carried forward (LOCF) estimation method. The proportion of excellent outcomes at 90 days will be compared using the chi-square tests, and shown as frequency (percentage). Most secondary outcome analyses will also use the primary outcome analysis strategy. Survival data will be calculated using the Kaplan-Meier method to estimate the survival rate in each group, and efficacy will be assessed using the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CI) will be calculated using the Cox proportional hazards model. Non-survival data will be analysed using the chi-square test, and odds ratios and 95% CIs will be calculated. Continuous variables will be compared between the two groups using the Student's t-test or Wilcoxon rank-sum test. The influence of covariables will be evaluated using subgroup analysis. No interim analysis is planned in this trial. All analyses will be performed using SAS 9.4, and a two-sided $P < 0.05$ is considered significant.

Patient and public involvement statement

None.

Ethics and dissemination: The BRANT study was approved by the Ethics Committee of Peking Union Medical College Hospital (I-23PJ1242) on July 20, 2023. Written informed consent is required from all patients before enrolment. BRANT will be carried out according to Good Clinical Practice and the Declaration of Helsinki. Protocol amendments will be reported to the institutional ethics committee. The trial sponsor is Peking Union Medical College Hospital. The trial results will be published in a peer-reviewed journal.

Discussion

The BRANT trial is a multicentre RCT that addresses the important treatment dilemma of improving the functional outcomes of BAD-related stroke.

BAD was first proposed by Caplan in 1989 to be distinct from lacunar infarct[1]. However, in the past three decades, most clinical studies have classified BAD as small-vessel occlusion or undetermined etiology based on the TOAST system[4 30]. Few studies focused on acute BAD-related stroke, probably due to discrepant definitions[3]. Recently, an increasing number of observational studies found distinct clinical, radiologic, and prognostic features that patients with BAD-related stroke are prone to END and poor prognosis[2 31].

Owing to the limitations of neuroimaging techniques, the perforating artery, such as the LSA or PPA, cannot be directly visualized. Radiological diagnosis is based on the vascular territory, dimension, or shape of the lesion[3 32], which results in a huge

variations among BAD definitions. With the aid of neuroimaging and clinical practice, Asian neurologists proposed radiological diagnosis criteria for BAD[33 34]. Our previous study also found that ≥4 consecutive slices on axial view are more effective than transversal diameter to differentiate atherosclerotic mechanisms of single subcortical infarction in the LSA territory[35]. Considering the generalisation and diagnostic accuracy of our study, we used ≥ 3 consecutive layers on axial DWI series instead of lesion diameter to define BAD-related stroke in the LSA territory[36]. Because obtaining direct evidence of the LSA and PPA is currently not technically feasible, our inclusion criteria based on MRI show considerable accuracy and representativeness.

In addition, our study uses simplified operationalised criteria to exclude cardiogenic embolism, and patients with these comorbidities will not be included. Some conditions seem general, which is a limitation of our study; however, this facilitates the researcher's ability to complete screening within a limited timeframe with low inconsistency.

We set a 90-day excellent outcome instead of END or new-onset stroke as the primary outcome for the following reasons: (1) historical evidence indicated that tirofiban improved the functional outcome of ischemic stroke[14 17]; (2) END is an intermediate indicators[7]; (3) the 90-day rate of recurrent stroke is 1.8% in our preliminary analysis of a BAD-related stroke cohort and probably less than 3.8% in other cohorts[31], which is relatively low. Thus, the BRANT study will provide direct evidence on how to reduce disability caused by BAD, which is the major challenge in current clinical practice.

As oral mono antiplatelet therapy is unethical and against Chinese stroke guidelines for patients with NIHSS ≤ 3, there are two types of antiplatelet therapy in the control group[23]. A double-blind design would markedly increase the complexity of the trial procedure. Therefore, we selected a prospective randomized open blinded end-point design (PROBE) for BRANT. Independent senior neurologists who will be blinded to the procedure information have been trained to evaluate the primary outcome. An independent CEC has been established to centrally re-examine all clinical events after randomization. Some local investigators may know the treatment allocation; however, all evaluators of the subjective indicators will be blinded to the treatment allocation.

Contributors: Shengde Li, and Jun Ni designed the study. Shengde Li drafted the manuscript. Dingding Zhang designed the statistical method. Yuhui Sha, Lixin Zhou, Yicheng Zhu, and Bin Peng critically revised the study protocol and the manuscript. The entire project will be supervised by Jun Ni.

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Disclaimer: The funder has no role in this study.

Competing interests: None declared.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author on reasonable request.

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Figure I. Study Flow

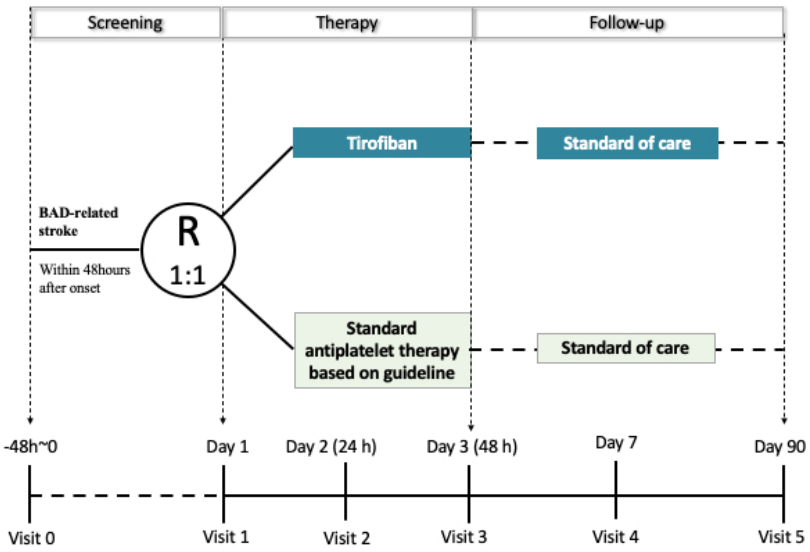

Figure 1. Study Flow

338x190mm (54 x 54 DPI)

Supplementary materials

Table S1. Study Procedure of BRANT trial

Measurement	Day 1	Day 2	Day 3	Day 7	Day 90
Demographic characteristics	X				
Current medical history taking	X				
Body temperature measurement	X				
Physical examination	X			X	X
Past medical history	X				
Pre-randomization medication after onset	X				
Regular blood pressure monitoring	X	X			
NIHSS score	X			X	X
mRS score	X			X	X
Barthel index score	X				X
Magnetic resonance image	X				
Evaluation of Intracranial vessels	X				
Evaluation of extracranial vessels	X				
Laboratory tests*	X				
ECG*	X			X	
Verification of inclusion/exclusion criteria	X				
Signed informed consent	X				
Randomization	X				
Blood tests after enrollment		X	X		

Urine and fecal examination			X		
Compliance			X		
Concomitant medication				X	X
Early neurological deterioration				X	
Major bleeding				X	X
Adverse Events/ Serious Adverse Events				X	X
*Remarks: ECG and laboratory data performed within 48 hours of onset before signing the informed consent form can be used as trial data.					

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2-3
	2b	All items from the World Health Organization Trial Registration Data Set	Clinical Trials.gov
Protocol version	3	Date and version identifier	In Protocol, not shown in article
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	16
	5b	Name and contact information for the trial sponsor	Clinical Trials.gov
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	10-11
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-6
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	In Protocol, not shown in article
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	In Protocol, not shown in article
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	In Protocol, not shown in article
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-11

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	In Protocol, not shown in article
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9-10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9-10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9-10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10-11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	11-12

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11-12
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11-12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	3
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	3

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	3
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	In protocoal, not shown in article
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	In protocoal, not shown in article
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	See consent from 3
	31b	Authorship eligibility guidelines and any intended use of professional writers	In protocoal, not shown in article
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	We provide a model consent from
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.