PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Efficacy and Safety of Tirofiban in Patients with Acute Branch Atherosclerotic Disease(BAD)-Related Stroke (BRANT): Protocol for a Randomized Controlled Trial
AUTHORS	Li, Shengde; dingding, zhang; Sha, Yuhui; yicheng, zhu; Zhou, Lixin; Peng, Bin; Ni, Jun

VERSION 1 – REVIEW

REVIEWER	Sadeghipour, Parham Rajaie Cardiovascular Medical and Research Center, Vascular Disease and Thrombosis Research Center
REVIEW RETURNED	14-Dec-2023

GENERAL COMMENTS	The authors present their study protocol on the use of Tirofiban on patients with Branch Atherosclerotic Disease(BAD)-Related Stroke. Please find my concerns: 1. I suggest to provide potential pathophysiological explanation on why GPI might have a better efficacy compared to other antithrombotic regimens. 2. Please explain why a limitation of 75 years of age was defined. 3. I think the exclusion criteria “cardiac embolism” need to be clarified. Will patients with these comorbidities be excluded? Or just when they were stated as the source of emboli? Some of the conditions are too general (e.g., heart valve diseases and myocardial infarction) and for some, a plausibility should be stated (e.g., HR below 50 bpm). 4. I suggest to add recent history of “major” bleeding as an exclusion criteria 5. Is previous use of anticoagulation set as an exclusion criteria? 6. After 48-hour of Tirofiban, what will be the antithrombotic regimen of the intervention group? 7. Did the authors define stopping rules for the DSMB?

REVIEWER	Uchiyama, , Shinichiro International University of Health and Welfare, Clinical Research Center for Medicine
REVIEW RETURNED	11-Jan-2024

GENERAL COMMENTS	This trial is well designed and organized, and may provide useful information in clinical practice. I raised the following comments to be addressed. 1. The authors should explain more clearly how BAD is classified in the TOAST classification (unclassified? other causes?). Otherwise, Western readers do not well understand nor recognize in the nosological position of BAD.

	2. In exclusion criteria, cardiogenic embolism should be more precisely defined according to the established criteria widely accepted. 3. The efficacy and safety would greatly different between aspirin alone and aspirin plus clopidogrel versus tirofiban. How is this issue overcome with the present study design? 4. Are CYP2C19 genetic polymorphisms included in this trial as a subgroup analysis? 5. It should be stated who will adjudicate the outcomes in a blinded manner. 6. References should be described according to the guidelines to authors of this journal.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Parham Sadeghipour, Heshmat Cardiovascular Medical Center,
Comments to the Author:
The authors present their study protocol on the use of Tirofiban on patients with Branch Atherosclerotic Disease(BAD)-Related Stroke. Please find my concerns:
Q1. I suggest to provide potential pathophysiological explanation on why GPI might have a better efficacy compared to other antithrombotic regimens.
A1. We add the sentence in the introduction part of the revised manuscript as follows: “Tirofiban, a selective and reversible antagonist of GP IIb/IIIa inhibitors on platelet, which block the final common pathway to platelet aggregation, might be more effective than the conventional agents (such as aspirin, or clopidogrel) with blockade of single pathway of platelet aggregation at pathophysiological level.”

Q2. Please explain why a limitation of 75 years of age was defined.
A2. Thank you for your critical and valuable comment. Before the study design, we searched Pubmed for this question “how to set the upper limit of age in BRANT study”. First, Lin Y et al found that standard-dose tirofiban increase the major bleeding risk in patient with more than 80 years (Acta Pharmacol Sin. 2009 May;30(5):553-8). Second, J. L. Januzzi reported the increasing bleeding risk between age and tirofiban (Figure below). Third, Linxin Li, et al found the major bleeding risk steeply elevated in age after 75 years (Lancet. 2017 Jul 29;390(10093):490-499). After thorough discussion with academic committee, we set the upper age limit at 75 years, which could reduce the bleeding risk of tirofiban in elderly. We cited two article in the including criterial, please check.

Q3. I think the exclusion criteria “cardiac embolism” need to be clarified. Will patients with these comorbidities be excluded? Or just when they were stated as the source of emboli? Some of the conditions are too general (e.g., heart valve diseases and myocardial infarction) and for some, a plausibility should be stated (e.g., HR below 50 bpm).
A3. Thank you for your scientific and meaningful suggestions. We had the same concerns as you do during the process of study design. Considering that the evaluation of cardiogenic embolism and brain MRI are required to be completed in less than 48 hours after stroke onset. Some hospitals were unable to complete the entire standard cardiac embolism assessment process, such as completing a Holter and echocardiogram. And specific TOAST classification was not the focus of the study. Thus, we used the suggested simple criteria to exclude the possible cardiogenic embolism. We also noticed the limitation of this exclusion criteria “Cardiogenic embolism: atrial fibrillation, myocardial infarction, heart valve disease, dilated cardiomyopathy, infective endocarditis, atrioventricular block disease, heart rate less than 50 beats per minute”. We add sentences in the discussion part “In addition, our

study used a simple, operationalized criteria to exclude cardiogenic embolism, that patients with these comorbidities will not be included. Some conditions seem general, which is a limitation in our study, but this facilitates the researcher's ability to complete screening in a limited time frame with low inconsistency." to remind the readers.

Q4. I suggest to add recent history of "major" bleeding as an exclusion criteria
A4. Many thanks for your suggestion. Based on the drug instructions of tirofiban, we used the item in exclusion part "Active or recent history (within 30 days prior to onset) of clinical bleeding (e.g., gastrointestinal bleeding)" to exclude the major bleeding. Please check it.

Q5. Is previous use of anticoagulation set as an exclusion criteria?
A5. Previous use of anticoagulation drugs is not listed as an exclusion criteria.

Q6. After 48-hour of Tirofiban, what will be the antithrombotic regimen of the intervention group?
A6. We added this sentence "After 48-hour treatment period in both groups, standard of care, including antithrombotic regimen, will be performed based on guideline and recorded in detail." In the "Intervention part".

Q7. Did the authors define stopping rules for the DSMB?
A7. The study does not set a pre-determined interim analysis and will not be terminated early for English validity or invalidity. However, it may be possible to terminate the study early because of safety issue, e.g., if serious adverse events occur in the tirofiban group, and statistically significant higher than that in the control group.

Reviewer: 2
Dr. Shinichiro Uchiyama, , International University of Health and Welfare
Comments to the Author:
This trial is well designed and organized, and may provide useful information in clinical practice. I raised the following comments to be addressed.
Q1. The authors should explain more clearly how BAD is classified in the TOAST classification (unclassified? other causes?). Otherwise, Western readers do not well understand nor recognize in the nosological position of BAD.
A1. Your suggestion is very important for western readers and help readers to understand the relationship between BAD and TOAST system. We add sentences in introduction part to clarify this point:1) BAD-related stroke, characterized by subcortical single infarcts in penetrating artery territory without severe stenosis of parent 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, which could be identified from small-vessel occlusion or embolic stroke of undetermined source in TOAST system.

Q2. In exclusion criteria, cardiogenic embolism should be more precisely defined according to the established criteria widely accepted.
A2. Thank you for this important comment. And this is a limitation of our study. We had the same concerns as you do during the process of study design. Considering that the evaluation of cardiogenic embolism and brain MRI are required to be completed in less than 48 hours after onset and some hospitals were unable to complete the standard cardiac embolism assessment process, such as completing a Holter and echocardiogram. And specific TOAST classification was not the focus of the study. Thus, we used the suggested simplified criteria [Cardiogenic embolism: atrial fibrillation, myocardial infarction, heart valve disease, dilated cardiomyopathy, infective endocarditis, atrioventricular block disease, heart rate less than 50 beats per minute] to exclude cardiogenic

embolism. We also noticed the limitation of this exclusion criteria “Cardiogenic embolism: atrial fibrillation, myocardial infarction, heart valve disease, dilated cardiomyopathy, infective endocarditis, atrioventricular block disease, heart rate less than 50 beats per minute”. We add sentences in the discussion part “In addition, our study used a simple, operationalized criteria to exclude cardiogenic embolism, that patients with these comorbidities will not be included. Some conditions seem general, which is a limitation in our study, but this facilitates the researcher's ability to complete screening in a limited time frame with low inconsistency.” to remind readers.

Q3. The efficacy and safety would greatly different between aspirin alone and aspirin plus clopidogrel versus tirofiban. How is this issue overcome with the present study design?
A3. This is our concern as well when the study was designed. and repeatedly discussed which antiplate would be a more reasonable and ethical choice for the control group. Indeed, the results of choosing mono- and dual- antiplatelet therapy as the control group might be different, but given the ethical rigor of clinical research, we need to follow the current guidelines for the diagnosis and treatment of acute ischemic stroke in the control group to provide antiplatelet drugs. On the other hand, there is a lack of evidence-based medical evidence whether mono- or dual-antiplatelet therapy is more reasonable in BAD stroke. The aim of our study is to compare tirofiban with the standard guideline-based antiplatelet therapy. Thus, we decided to use 1) aspirin alone OR 2) aspirin plus clopidogrel in our study. We plan to conduct post hoc analysis to assess the efficacy of mono and dual antiplatelet therapy in improving functional outcome in BAD-related stroke.

Q4. Are CYP2C19 genetic polymorphisms included in this trial as a subgroup analysis?
A4. Thanks for your nice suggestion. However, CYP2C19 genetic polymorphisms was not included in this trial as a subgroup analysis.

Q5. It should be stated who will adjudicate the outcomes in a blinded manner.
A5. Thank you for this important comment. These information was stated in the part of “primary outcomes” and “secondary outcomes.” Please check in the revised manuscript.

Q6. References should be described according to the guidelines to authors of this journal.
A6. References have been re-checked according to the guidelines of BMJ OPEN.

VERSION 2 – REVIEW

REVIEWER	Sadeghipour, Parham Rajaie Cardiovascular Medical and Research Center, Vascular Disease and Thrombosis Research Center
REVIEW RETURNED	02-Mar-2024

GENERAL COMMENTS	The authors has completely answer my queries expect for the DSMB. In think in a trial with potential risk of added bleeding, DSMB, stopping rules and interim analysis is a must and cannot be ignored.

REVIEWER	Uchiyama, Shinichiro International University of Health and Welfare, Clinical Research Center for Medicine
REVIEW RETURNED	26-Feb-2024

GENERAL COMMENTS	The authors well responded to my comments.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Comments to the Author:

The authors has completely answer my queries expect for the DSMB. In think in a trial with potential risk of added bleeding, DSMB, stopping rules and interim analysis is a must and cannot be ignored.

Reply:

A7. Dear reviewer, many thanks for your suggestion. Your issue is very important at both clinical and research levels. The safety of subjects is also a major concern for us. Before the study design, we had a detailed discussion with the DSMB and stroke experts for the potential risk of bleeding.

First, the drugs used in this project are post-marketing drugs with relatively predictable side effects (N Engl J Med. 2013 Jul 4;369(1):11-9; Neuroradiology. 2021 Jan;63(1):17-25.). We also searched published studies on thrombolytic drugs and found some open-label studies did not set up an interim analysis (TRACE II, Stroke & Vascular Neurology 2022;7: 001074. doi:10.1136/ svn-2021-001074; Lancet. 2023 Feb 25;401(10377):645-654.). The second and most important point is that our study is an open-label study and there is no hidden risk of bleeding due to blinding design. To further decrease the risk of bleeding, We set the upper age limit at 75 years. Evaluators and investigators could report bleeding or adverse events in the system and submit them to the Clinical Event Committee (CEC) and DSMB. The DSMB will evaluate the risk and benefit of study drugs and decide whether to terminate the study or not. Third, this study was conducted under the supervision of the DSMB, which is described in further detail in the article “The DSMB will review the overall implementation of the clinical study and regularly and dynamically assess the risks and benefits, particularly unexpected adverse events.” It may be possible to terminate the study early because of safety issue, e.g., if serious adverse events occur in the tirofiban group, and statistically significant higher than that in the control group.

Also, to remove ambiguity, we have added a sentence “No interim analysis is planned in this trial” in the statistical analysis section.