BMJ Open

Personalized antiplatelet therapy based on pharmacogenomics in acute ischemic minor stroke and transient ischemic attack: study protocol for a randomised controlled trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-028595
Article Type:	Protocol
Date Submitted by the Author:	27-Dec-2018
Complete List of Authors:	Yue, Yun-hua; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Zhang, Xiao-guang ; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Zhu, Xiao-qiong; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Xue, Jie; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Li, Zhi-zhang; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Jiang, Hua-yu; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Hu, Liang; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine
Keywords:	personlized antiplatelet therapy, clopidogrel, pharmacogenomics, acute ischemic minor stroke, transient ischemic attack

Personalized antiplatelet therapy based on pharmacogenomics in acute ischemic minor stroke and transient ischemic attack: study protocol for a randomised controlled trial

Xiao-Guang Zhang, Xiao-Qiong Zhu, Jie Xue, Zhi-Zhang Li, Hua-Yu Jiang, Liang Hu, Yun-Hua Yue

Department of Neurology, Yangpu Hospital, Tongji University School of Medicine, No. 450 Tengyue Road, Shanghai 200090, China

X-GZ and X-QZ contributed equally to this work.

Correspondence to Dr. Liang Hu; 13585696649@163.com Dr. Yun-Hua Yue; 447879206@qq.com

ABSTRACT

Introduction: Antiplatelet therapy combining aspirin and clopidogrel is considered to be a key intervention for acute ischemic minor stroke (AIMS) and transient ischemic attack (TIA). However, the interindividual variability in response to clopidogrel resulting from the polymorphisms in clopidogrel metabolism-related genes has greatly limited its efficacy. To date, there are no reports on individualized antiplatelet therapy for AIMS and TIA based on the genetic testing and clinical features. Therefore, we conducted this randomized controlled trial (RCT) to validate the hypothesis that the individualized antiplatelet therapy selected on the basis of a combination of genetic information and clinical features would lead to better clinical outcomes compared to the standard care based only on clinical features in patients with AIMS or TIA.

Methods and analysis: This open blinded RCT will recruit 2268 patients with AIMS or TIA who meet eligibility criteria. Patients are randomly assigned in a 1:1 ratio to pharmacogenetic group and standard group. Both groups receive a loading dose of 100-300 mg of aspirin and 300 mg of clopidogrel on day 1, followed by 100 mg aspirin per day on days 2 to 365. The P2Y12 receptor antagonist is selected by the clinician according to the genetic information and clinical features for pharmacogenetic group and clinical features for the standard group on days 2 to 21. The primary endpoint is a new stroke event (ischemic or hemorrhagic) that happens within one year. The secondary endpoint is composite clinical vascular event (ischemic stroke, hemorrhagic

stroke, myocardial infarction, or vascular death). Baseline characteristics and outcomes after treatment will be evaluated.

Ethics and dissemination: This protocol has been approved by the ethics committee of Yangpu Hospital, Tongji University School of Medicine (No. LL-2018-KY-012). We will submit the results of this trial for publication in a peer-reviewed journal.

Trial registration number: ChiCTR1800019911

Strengths and limitations of this study

This is a well-designed study to assess the efficacy and safety of individualized antiplatelet therapy selected on the basis of a combination of genetic information and a patient's clinical features in patients with AIMS or TIA.

The results from this randomized open blinded end-point study will provide new evidence of the efficacy of individualized antiplatelet therapy on the basis of genetic information for AIMS or TIA.

This study will help clinicians predict and estimate the risk of clopidogrel resistance in patients with AIMS or TIA, and then take corresponding measures to reduce the risk of stroke recurrence.

One limitation is that we mainly focus on the CYP2C19 genotype (*2, *3 and *17 alleles), which might neglect the potential impact of other alleles.

Another limitation is that the trial implementation is not multi-centered, which might limit its generalisability.

INTRODUCTION

Acute ischemic minor stroke (AIMS) and transient ischemic attack (TIA) are common cerebrovascular events with a high tendency to cause disability. Patients with AIMS or TIA have a high risk of subsequent ischemic events, especially during the first 90 days after the cerebrovascular event.[1] During this acute phase, there is still a risk of previous ischemic tissue, with ruptured plaques in a state of high thrombus formation and high platelet activation, which may lead to more severe acute instability events.[2] Therefore, antiplatelet therapy is considered to be a key intervention for AIMS and TIA at present.

Clopidogrel is one of the commonly used anti-platelet drugs in clinic, which is recommended for the secondary prevention of ischemic stroke. The Clopidogrel in High-Risk Patients with Acute Nondisabling Cerebrovascular Events (CHANCE) trial reported that compared with aspirin alone, the combination of clopidogrel with aspirin decreased the risk of stroke among patients with AIMS or TIA who can be treated within 24 hours after the onset of symptoms.[3] However, there is interindividual variability in response to clopidogrel, which leads to the result that a large number of subsequent strokes still occur despite clopidogrel treatment and even among those treated with dual-antiplatelet agents.[4] Those patients who have poor response to clopidogrel against platelet aggregation and are therefore at greater risk of ischemic events are suffering from clopidogrel resistance.[5] Clopidogrel resistance is an important cause of failure in the prevention and treatment of patients with partial ischemic stroke, and will greatly increase the recurrence of ischemic stroke. To date, the mechanisms related to variability in clopidogrel responsiveness are not fully elucidated.[6]

Mounting evidence have showed that genetic factors may play a crucial role in mediating clopidogrel resistance.[7] As clopidogrel is a prodrug that requires hepatic cytochrome P450 (CYP) for its conversion into an active metabolite[8], polymorphisms of its encoded gene CYP2C19 have been identified as strong predictors of clopidogrel nonresponsiveness.[9] Among them, CYP2C19 loss-of-function genotype (*2 and/or *3 alleles) is found to be related to low responsiveness to clopidogrel, which is a risk factor for ischemic events, whereas the presence of gain-of function CYP2C19 allele (*17) is associated with a high platelet inhibition and increased risk of bleeding.[10] The genetic substudy of the CHANCE trial also showed that only in patients with AIMS or TIA who did not carry the CYP2C19 loss-of-function alleles, the combined treatment of

clopidogrel and aspirin could reduce the risk of a new stroke in comparison with aspirin alone.[11] This study provided evidence to support the genetic testing that may allow clinicians to personalize antiplatelet therapy, especially in East Asian patient populations for whom the prevalence of CYP2C19 loss-of-function allele is high.[11, 12]

To date, the clinical factors and genetic factors affecting clopidogrel responses have not reached a consistent conclusion. Besides, most of the study endpoints are cardiovascular events, and the research on cerebrovascular events is lacking. For example, Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines recommend that non-carriers of CYP2C19 loss-of-function alleles patients with acute coronary syndrome (ACS) continue to take clopidogrel 75 mg daily, and carriers are advised to increase the dose of clopidogrel or switch to other antiplatelet agents such as ticagrelor.[13] Wallentin et al. researched on the genetics of the CYP2C19 gene polymorphism in patients with ACS and found that compared with clopidogrel, treatment with ticagrelor significantly reduced the death rate from vascular causes in patients with CYP2C19 loss of function alleles.[10] However, for patients with AIMS or TIA with clopidogrel resistance, it is unclear whether there will be more clinical benefit when switching to ticagrelor. Based on the above, we conducted this randomized controlled trial to validate the hypothesis that the individualized antiplatelet therapy selected on the basis of a combination of genetic information and a patient's clinical features would lead to better clinical outcomes compared to the standard care based only on clinical features in patients with AIMS or TIA.

METHOD

Design

The design of study is shown in Fig. 1. The Pharmacogenetics of clopidogrel in patients with AIMS or TIA study is an open blinded end-point RCT, aiming to evaluate whether selecting antiplatelet therapy (label-recommended or doubled dosage of clopidogrel or ticagrelor) on the basis of a patient's both genetic and clinical features leads to more clinical benefits compared to the standard care which bases only on clinical features. Participation in this clinical trial is voluntary. Collection and genetic analysis of samples are subjected to informed consent from all patients and approval by ethics committee of Yangpu Hospital Tongji University School of medicine.

Patient population

Patients are included into this study if they meet all the following criteria: 1). age of 18 years or older; 2). diagnosis of an AIMS or TIA; AIMS is defined as a sudden focal neurological dysfunction caused by vascular causes, and score of 3 or less at the time of randomization on the National Institutes of Health Stroke Scale (NIHSS; scores range from 0 to 42, with higher scores indicating greater deficits).[3] TIA is defined as a transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia, without acute infarction;[14] 3). onset of the AIMS or TIA symptoms less than 72 h.

Patients are excluded from study participation if one of the following criteria is met: 1) hemorrhage; other conditions, such as vascular malformation, trauma, tumor, abscess, degenerative neurologic disease or other major nonischemic brain disease; 2) systemic infectious diseases, autoimmune diseases, severe heart, liver and kidney diseases; 3) any contraindication to the use of aspirin or P2Y12 receptor antagonists; 4) prior knowledge of the patients' CYP2C19*2, CYP2C19*3 or CYP2C19*17 genotype; 5) ongoing treatment in another observational or registry randomized trial; 6) an inability to provide informed consent or unavailability for follow-up.

Based on the PLATO trial and PHARMCLO trial exclusion criteria, ticagrelor is contraindicated in patients: 1) with active pathological bleeding; 2) with a history of intracranial bleeding; 3) requiring dialysis, 4) taking oral anticoagulant therapy that could not be stopped; 5) with known clinically important thrombocytopenia; 6) receiving fibrinolytic therapy within the previous 24 hours; and 7) taking concomitant therapy with strong CYP3A inhibitors or inducers.[15, 16]

Patients and public involvement

Patients in this trial will be not involved in the designment, recruitment and conduction of the study. All patients will be provided informed consent and clopidogrel genes of patients in pharmacogenetic group will be detected as soon as possible after the random assignment. The individual genetic information will inform the corresponding participant. We will not perform the structural evaluation on study patients' burden in RCTs.

Randomization and treatments

Immediately after the diagnosis of AIMS or TIA, the patients are randomly assigned in a 1:1 ratio to the strategy of selecting a P2Y12 receptor antagonist based on their clinical features plus the genotyping results, or on the basis of their clinical features alone. A blood sample is obtained from every participant right after randomisation. Three single-nucleotide polymorphisms (SNPs) for CYP2C19 (National Center for Biotechnology Information [NCBI] Genome build 37.1, GenBank NG_008384), including CYP2C19*2 (681G>A, dbSNP rs4244285), CYP2C19*3 (636G>A, dbSNP rs4986893), and CYP2C19*17 (−806C>T, dbSNP rs12248560), are genotyped in the participants assigned to the Pharmacogenetical strategy. Genotyping of the 3 SNPs is done at the Central laboratory of Yangpu Hospital Tongji University School of Medicine with microarray-based metho (CapitalBio Technology). Patients are categorized by CYP2C19 metabolizer status based on *2, *3, and *17 genotypes within 24 hours of admission. And they are divided into four metabolite types: ultra-metabolizers (UM, *1/*17, *17/*17), extensive metabolizers (EM, *1/*1), intermediate metabolizers (IM, *1/*2, *1/*3, *17/*2, *17/*3), poor metabolizers (PM, *2/*2, *2/*3, *3/ *3).

Both pharmacogenetic group and standard group receive a loading dose of 100-300 mg of aspirin and 300 mg of clopidogrel on day 1, followed by a dose of 100 mg of aspirin per day on days 2 to 365. Patients randomly assigned to the pharmacogenetic group receive a dose of 75 mg clopidogrel per day (UM and EM group), 150 mg clopidogrel per day (IM group) and ticagrelor 90 mg twice daily (PM group) on days 2 to 21, which can be further adjusted in combination with clinical features. While for standard group, the P2Y12 receptor antagonist is selected by the clinician according to the clinical features of the patients. The clinical features include age, weight, ischemic risk, prior history of stroke/TIA, bleeding risk, intracranial bleeding, active bleeding, history of bleeding, anemia, diabetes or chronic kidney disease.

Primary outcomes

The primary endpoint for this trial is a new stroke event (ischemic or hemorrhagic) that happens within one year. Ischemic stroke is defined as a sudden focal neurological dysfunction caused by vascular causes, duration ≥ 24 hours, or neurological dysfunction due to imaging and clinical symptoms caused by bloody infarction rather than cerebral hemorrhage found by imaging examination. Hemorrhagic stroke is

defined as acute extravasation of blood into the brain parenchyma or subarachnoid space with associated neurologic symptoms.

Secondary outcomes

The secondary endpoint was the composite of the primary endpoint plus new clinical vascular event (ischemic stroke, hemorrhagic stroke, myocardial infarction, or vascular death). The definition of vascular death is adapted from the CHANCE trial.[3] Briefly, vascular death is defined as death resulting from stroke (ischemic or hemorrhagic), systemic hemorrhage, myocardial infarction, congestive heart failure, pulmonary embolism, sudden death, or arrhythmia.

Safety assessments

Safety endpoint is a major bleeding event, according to the definitions in International Society on Thrombosis and Hemostasis[17] and Platelet-Oriented Inhibition in New TIA and Minor Ischemic Stroke (POINT) Trial[18]. Major hemorrhage is defined as symptomatic intracranial hemorrhage or intraocular bleeding causing loss of vision, requiring two or more units of red cells or equivalent amount of whole blood replacement, or requiring hospitalization or prolongation of an existing hospitalization, surgical intervention or death. An independent Clinical Event Adjudication Committee evaluates all components of the primary and secondary outcomes and safety endpoint.

Sample size

Based on data from the One-year outcomes of the CHANCE trial[19], a primary endpoint (ischemic or hemorrhagic stroke) rate of 10.6% is originally assumed at one year following randomization. The sample size is calculated based on the assumption that the cumulative event rate of the primary outcome in the standard group after one year would be 15%. Given a relative risk reduction in the pharmacogenomic group of 5%, 95% power and a two-sided type I error of 0.05, the calculated sample size is 1,134 patients in each group.

Statistical analyses

The distributions of baseline characteristics are compared between two study groups using T test. Proportions are used for categorical variables and continuous variables will be reported as median (inter-quartile range). Cox proportional-hazard model is used to estimate the hazard ratio and 95% confidence intervals relating to the primary and secondary outcomes. Schoenfeld residuals test is used to confirm the proportional hazards assumption for the Cox regression model. For estimating the cumulative incidence of endpoints during the 1-year follow-up, we perform the Kaplan–Meier analyses by means of Aalen-Johansen estimator. And Fine-Gray model is used to test the significance of the differences between the sub-distribution of the hazards. Values of $P < 0.05$ are considered statistically significant.

DISCUSSION

Currently, clopidogrel combined with aspirin has become the preferred treatment for patients with AIMS or TIA.[20] However, the pharmacokinetics of clopidogrel could be influenced by metabolic status. Poor metabolizers will cause insufficient anti-platelet effect and impaired clinical benefit.[21] At present, the mechanisms of clopidogrel resistence are not fully elucidated and evidences from the genetic substudy of CHANCE trail showed the correlation between CYP2C19 polymorphisms with clopidogrel nonresponsiveness. For patients with AIMS or TIA treated with clopidogrel and aspirin, CYP2C19 loss-of-function alleles carriers were prone to have increased risk for subsequent stroke and composite vascular events compared with noncarriers.[11] Although these genetic associations with clinical benefits have been widely replicated and the sample sizes are large enough to be predictive in the clinical setting, there are few examples using pharmacogenetic data concerning clopidogrel metabolism to guide clinical practice.[22-24] Thus, genotype-guided antiplatelet therapy may be regarded as a prospective alternative approach to personalized treatment in AIMS or TIA.

Given the fact that clopidogrel is currently the only antiplatelet agent approved for use in ischemic mild stroke or TIA with aspirin and there are indeed differences in reactivity between individuals, varying the dose of clopidogrel or shifting to new antiplatelet agents based on genetic datas may be alternatives, but it has not been adequately evaluated.[25] This RCT will provide evidence for the assumption that using pharmacogenetic data to select P2Y12 receptor antagonists can be successfully incorporated into the clinical care of patients with AIMS or TIA. The selection of a

P2Y12 receptor antagonist in our trial (label-recommended or doubled Dosage of clopidogrel or ticagrelor) is based on their pharmacogenetic data and individual clinical features in order to acquire the best trade-off between ischemic events and bleeding complications. Furthermore, for the sake of racial and geographical differences in genetic factors, we will collect patients of AIMS or TIA from both Han and Uygur population in different regions of Shanghai and Xinjiang of China. The North-South differences make the participants more representative, ensuring the effectiveness of clinical trial. In addition, due to the fact that the clinical course of most patients in clinical practice is long, we appropriately expand the time of patient enrollment, so that it can be applied to more people in the future.

Acknowledgements We thank Chang Shan (Department of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Rui-jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai, China) for her help in revising the manuscript.

Contributors LH and Y-hY conceived and designed this study. X-gZ, X-qZ, JX and Z-zL wrote the manuscript with contributions from all authors. H-yJ and Y-hY refined the protocol. All authors read and approved the final manuscript.

Funding This project is supported by Science and Technology Commission of Shanghai Municipality (18411970100).

Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethics committee, Yangpu Hospital, Tongji University School of Medicine, China.

REFERENCES

1 Coull AJ, Lovett JK, Rothwell PM. Population based study of early risk of stroke after transient ischaemic attack or minor stroke: implications for public education and organisation of services. *BMJ* 2004; 328:326.

2 Johnston SC. Clinical practice. Transient ischemic attack. *N Engl J Med* 2002; 347:1687-1692.

3 Wang Y, Wang Y, Zhao X, *et al*. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. *N Engl J Med* 2013; 369:11-19.

4 Mijajlovic MD, Shulga O, Bloch S, *et al*. Clinical consequences of aspirin and clopidogrel resistance: an overview. *Acta Neurol Scand* 2013; 128:213-219.

5 Guirgis M, Thompson P, Jansen S. Review of aspirin and clopidogrel resistance in peripheral arterial disease. *J Vasc Surg* 2017; 66:1576-1586.

6 Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, *et al*. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J Am Coll Cardiol* 2007; 49:1505-1516.

7 Juth V, Holman EA, Chan MK, *et al*. Genetics as a molecular window into recovery, its treatment, and stress responses after stroke. *J Investig Med* 2016; 64:983-988.

8 Kazui M, Nishiya Y, Ishizuka T, *et al*. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. *Drug Metab Dispos* 2010; 38:92-99.

9 Mega JL, Close SL, Wiviott SD, *et al*. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009; 360:354-362.

10 Wallentin L, James S, Storey RF, *et al*. Effect of CYP2C19 and ABCB1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial. *Lancet* 2010; 376:1320-1328.

11 Wang Y, Zhao X, Lin J, *et al*. Association Between CYP2C19 Loss-of-Function Allele Status and Efficacy of Clopidogrel for Risk Reduction Among Patients With Minor Stroke or Transient Ischemic Attack. *JAMA* 2016; 316:70-78.

12 Kitzmiller JP, Groen DK, Phelps MA, *et al*. Pharmacogenomic testing: relevance in medical practice: why drugs work in some patients but not in others. *Cleve Clin J Med* 2011; 78:243-257.

13 Scott SA, Sangkuhl K, Stein CM, *et al*. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. *Clin Pharmacol Ther* 2013; 94:317-323.

14 Easton JD, Saver JL, Albers GW, *et al*. Definition and evaluation of transient ischemic attack: a scientific statement for healthcare professionals from the

American Heart Association/American Stroke Association Stroke Council; Council on Cardiovascular Surgery and Anesthesia; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; and the Interdisciplinary Council on Peripheral Vascular Disease. The American Academy of Neurology affirms the value of this statement as an educational tool for neurologists. *Stroke* 2009; 40:2276-2293.

15 Shuldiner AR, Palmer K, Pakyz RE, *et al*. Implementation of pharmacogenetics: the University of Maryland Personalized Anti-platelet Pharmacogenetics Program. *Am J Med Genet C Semin Med Genet* 2014; 166c:76-84.

16 Notarangelo FM, Maglietta G, Bevilacqua P, *et al*. Pharmacogenomic Approach to Selecting Antiplatelet Therapy in Patients With Acute Coronary Syndromes: The PHARMCLO Trial. *J Am Coll Cardiol* 2018; 71:1869-1877.

17 Schulman S, Kearon C. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. *J Thromb Haemost* 2005; 3:692-694.

18 Johnston SC, Easton JD, Farrant M, *et al*. Platelet-oriented inhibition in new TIA and minor ischemic stroke (POINT) trial: rationale and design. *Int J Stroke* 2013; 8:479-483.

19 Wang Y, Pan Y, Zhao X, *et al*. Clopidogrel With Aspirin in Acute Minor Stroke or Transient Ischemic Attack (CHANCE) Trial: One-Year Outcomes. *Circulation* 2015; 132:40-46.

20 Kernan WN, Ovbiagele B, Black HR, *et al*. Guidelines for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2014; 45:2160-2236.

21 Holmes MV, Perel P, Shah T, *et al*. CYP2C19 genotype, clopidogrel metabolism, platelet function, and cardiovascular events: a systematic review and meta-analysis. *JAMA* 2011; 306:2704-2714.

22 Peterson JF, Field JR, Unertl KM, *et al*. Physician response to implementation of genotype-tailored antiplatelet therapy. *Clin Pharmacol Ther* 2016; 100:67-74.

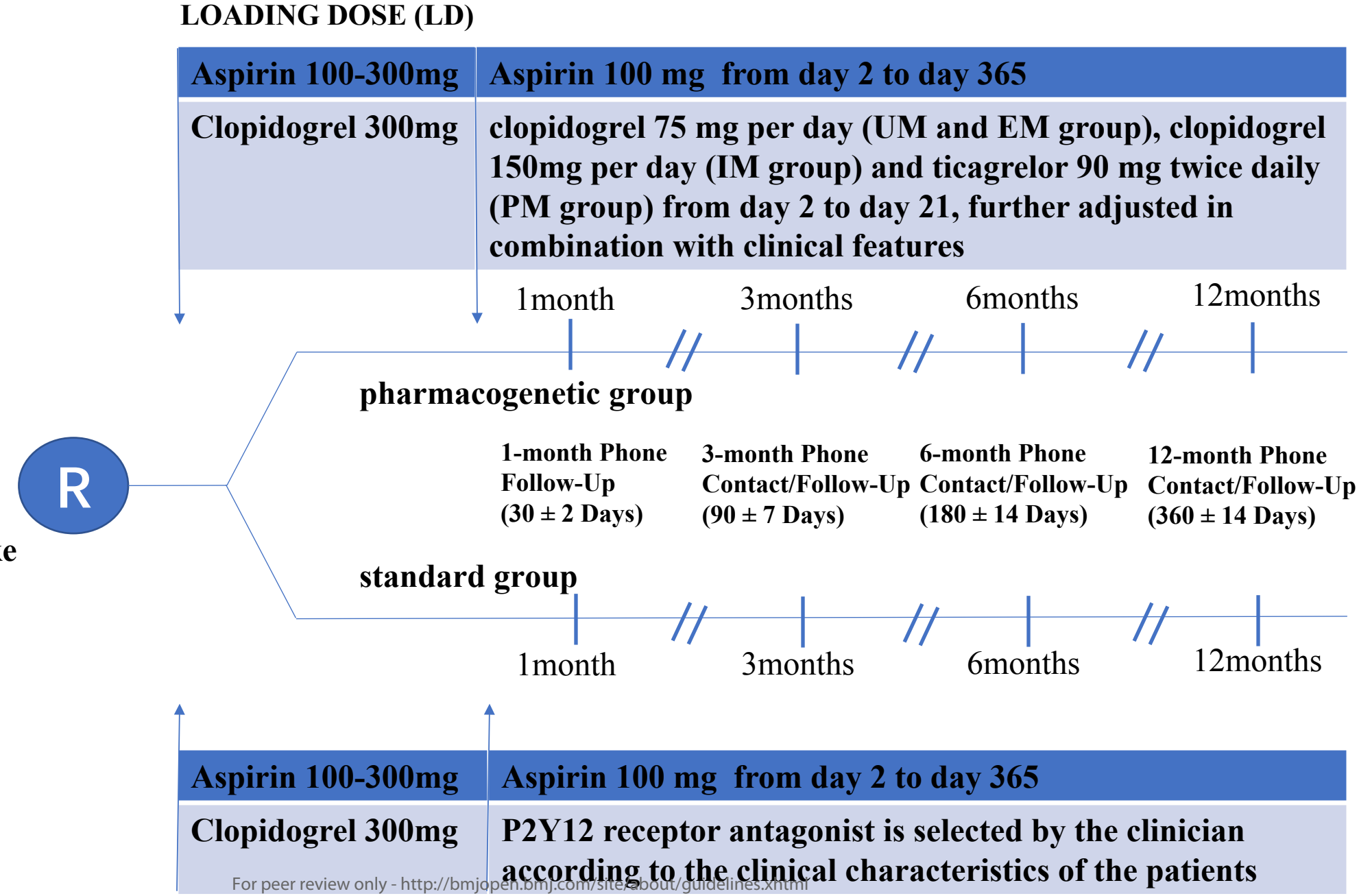
23 Price MJ, Berger PB, Teirstein PS, *et al*. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. *JAMA* 2011; 305:1097-1105.

24 Cavallari LH, Weitzel KW, Elsey AR, *et al*. Institutional profile: University of Florida Health Personalized Medicine Program. *Pharmacogenomics* 2017; 18:421-426.

25 Roberts JD, Wells GA, Le May MR, *et al*. Point-of-care genetic testing for personalisation of antiplatelet treatment (RAPID GENE): a prospective, randomised, proof-of-concept trial. *Lancet* 2012; 379:1705-1711.

Figure legend

Fig. 1. Study flowchart. TIA, transient ischemic attack; UM, ultra-metabolizers; EM, extensive metabolizers; IM, intermediate metabolizers; PM, poor metabolizers.



BMJ Open

Personalized antiplatelet therapy based on pharmacogenomics in acute ischemic minor stroke and transient ischemic attack: study protocol for a randomised controlled trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-028595.R1
Article Type:	Protocol
Date Submitted by the Author:	05-Mar-2019
Complete List of Authors:	Zhang, Xiao-guang ; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Zhu, Xiao-qiong; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Xue, Jie; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Li, Zhi-zhang; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Jiang, Hua-yu; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Hu, Liang; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Yue, Yun-hua; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Neurology
Keywords:	personlized antiplatelet therapy, clopidogrel, pharmacogenomics, acute ischemic minor stroke, transient ischemic attack

Personalized antiplatelet therapy based on pharmacogenomics in acute ischemic minor stroke and transient ischemic attack: study protocol for a randomised controlled trial

Xiao-Guang Zhang, Xiao-Qiong Zhu, Jie Xue, Zhi-Zhang Li, Hua-Yu Jiang, Liang Hu, Yun-Hua Yue

Department of Neurology, Yangpu Hospital, Tongji University School of Medicine, No. 450 Tengyue Road, Shanghai 200090, China

X-GZ and X-QZ contributed equally to this work.

Correspondence to Dr. Liang Hu; 13585696649@163.com Dr. Yun-Hua Yue; 447879206@qq.com

ABSTRACT

Introduction: Antiplatelet therapy combining aspirin and clopidogrel is considered to be a key intervention for acute ischemic minor stroke (AIMS) and transient ischemic attack (TIA). However, the interindividual variability in response to clopidogrel resulting from the polymorphisms in clopidogrel metabolism-related genes has greatly limited its efficacy. To date, there are no reports on individualized antiplatelet therapy for AIMS and TIA based on the genetic testing and clinical features. Therefore, we conduct this randomized controlled trial (RCT) to validate the hypothesis that the individualized antiplatelet therapy selected on the basis of a combination of genetic information and clinical features would lead to better clinical outcomes compared to the standard care based only on clinical features in patients with AIMS or TIA.

Methods and analysis: This trial will recruit 2382 patients with AIMS or TIA who meet eligibility criteria. Patients are randomly assigned in a 1:1 ratio to pharmacogenetic group and standard group. Both groups receive a loading dose of 300 mg aspirin and 300 mg clopidogrel on day 1, followed by 100 mg aspirin per day on days 2 to 365. The P2Y12 receptor antagonist is selected by the clinician according to the genetic information and clinical features for pharmacogenetic group and clinical features for the standard group on days 2 to 21. The primary efficacy endpoint is a new stroke event (ischemic or hemorrhagic) that happens within one year. The secondary efficacy endpoint is analyzed as the individual or composite outcomes of the new

clinical vascular event (ischemic stroke, hemorrhagic stroke, myocardial infarction, or vascular death). Baseline characteristics and outcomes after treatment will be evaluated.

Ethics and dissemination: This protocol has been approved by the ethics committee of Yangpu Hospital, Tongji University School of Medicine (No. LL-2018-KY-012). We will submit the results of this trial for publication in a peer-reviewed journal.

Trial registration number: ChiCTR1800019911

Strengths and limitations of this study

This is a study to assess the efficacy and safety of individualized antiplatelet therapy selected on the basis of a combination of genetic information and a patient's clinical features in patients with AIMS or TIA.

The results from this randomized study will provide new evidence of the efficacy of individualized antiplatelet therapy on the basis of genetic information for AIMS or TIA.

This study will help clinicians predict and estimate the risk of clopidogrel resistance in patients with AIMS or TIA, and then take corresponding measures to reduce the risk of stroke recurrence.

One limitation is that we mainly focus on the CYP2C19 genotype (*2, *3 and *17 alleles), which might neglect the potential impact of other alleles.

Another limitation is that the trial implementation is not multi-centered and only Chinese population are included, which might limit its generalisability.

INTRODUCTION

Acute ischemic minor stroke (AIMS) and transient ischemic attack (TIA) are common cerebrovascular events with a high tendency to cause disability. Patients with AIMS or TIA have a high risk of subsequent ischemic events, especially during the first 90 days after the cerebrovascular event.[1] During this acute phase, there is still a risk of previous ischemic tissue, with ruptured plaques in a state of high thrombus formation and high platelet activation, which may lead to more severe acute instability events.[2] Therefore, antiplatelet therapy is considered to be a key intervention for AIMS and TIA at present.

Clopidogrel is one of the commonly used anti-platelet drugs in clinic, which is recommended for the secondary prevention of ischemic stroke. The Clopidogrel in High-Risk Patients with Acute Nondisabling Cerebrovascular Events (CHANCE) trial reported that compared with aspirin alone, the combination of clopidogrel with aspirin decreased the risk of stroke among patients with AIMS or TIA who can be treated within 24 hours after the onset of symptoms.[3] However, there is interindividual variability in response to clopidogrel, which leads to the result that a large number of subsequent strokes still occur despite clopidogrel treatment and even among those treated with dual-antiplatelet agents.[4] Compared with the inhibition of platelet aggregation expected, poor inhibition using antiplatelet therapy is referred to high on-treatment platelet reactivity (HPR).[5] Those patients who show HPR to clopidogrel and are therefore at greater risk of ischemic events are suffering from clopidogrel resistance.[5, 6] Clopidogrel resistance is an important cause of failure in the prevention and treatment of patients with partial ischemic stroke, and will greatly increase the recurrence of ischemic stroke. To date, the mechanisms related to variability in clopidogrel responsiveness are not fully elucidated.[7]

Mounting evidence have shown that genetic factors may play a crucial role in mediating clopidogrel resistance.[8] As clopidogrel is a prodrug that requires hepatic cytochrome P450 (CYP) for its conversion into an active metabolite[9], polymorphisms of its encoded gene CYP2C19 have been identified as strong predictors of clopidogrel nonresponsiveness.[10] Among them, CYP2C19 loss-of-function genotype (*2 and/or *3 alleles) is found to be related to low responsiveness to clopidogrel, which is a risk factor for ischemic events, whereas the presence of gain-of function CYP2C19 allele (*17) is associated with a high platelet inhibition and increased risk of bleeding.[11] The genetic

substudy of the CHANCE trial also showed that only in patients with AIMS or TIA who did not carry the CYP2C19 loss-of-function alleles, the combined treatment of clopidogrel and aspirin could reduce the risk of a new stroke in comparison with aspirin alone.[12] This study provided evidence to support the genetic testing that may allow clinicians to personalize antiplatelet therapy, especially in East Asian patient populations for whom the prevalence of CYP2C19 loss-of-function allele is high.[12, 13]

To date, the clinical factors and genetic factors affecting clopidogrel responses have not reached a consistent conclusion. Besides, most of the study endpoints are cardiovascular events, and the research on cerebrovascular events is lacking. For example, Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines recommend that non-carriers of CYP2C19 loss-of-function alleles patients with acute coronary syndrome (ACS) continue to take clopidogrel 75 mg daily, and carriers are advised to increase the dose of clopidogrel or switch to other antiplatelet agents such as ticagrelor.[14] Wallentin et al. researched on the genetics of the CYP2C19 gene polymorphism in patients with ACS and found that compared with clopidogrel, treatment with ticagrelor significantly reduced the death rate from vascular causes in patients with CYP2C19 loss of function alleles.[11] However, for patients with AIMS or TIA with clopidogrel resistance, it is unclear whether there will be more clinical benefit when switching to ticagrelor. Based on the above, we conduct this randomized controlled trial to validate the hypothesis that the individualized antiplatelet therapy selected on the basis of a combination of genetic information and a patient's clinical features would lead to better clinical outcomes compared to the standard care based only on clinical features in patients with AIMS or TIA.

METHOD

Design

The design of study is shown in Fig. 1. The Pharmacogenetics of clopidogrel in patients with AIMS or TIA study is a prospective, open-label RCT, aiming to evaluate whether selecting antiplatelet therapy (label-recommended or doubled dosage of clopidogrel or ticagrelor) on the basis of a patient's both genetic and clinical features leads to more clinical benefits compared to the standard care which bases selection only on clinical features. Collection and genetic analysis of samples are subjected to

informed consent from all patients and approval by ethics committee of Yangpu Hospital Tongji University School of medicine.

Patient population

Patients are included into this study if they meet all the following criteria: 1). age of 18 years or older; 2). diagnosis of an AIMS or TIA; AIMS is defined as a sudden focal neurological dysfunction caused by vascular causes, and score of 3 or less at the time of randomization on the National Institutes of Health Stroke Scale (NIHSS; scores range from 0 to 42, with higher scores indicating greater deficits).[3] TIA is defined as a transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia, without acute infarction;[15] 3). onset of the AIMS or TIA symptoms less than 72 h.

Patients are excluded from study participation if one of the following criteria is met: 1) hemorrhage; other conditions, such as vascular malformation, trauma, tumor, abscess, degenerative neurologic disease or other major nonischemic brain disease; 2) systemic infectious diseases, autoimmune diseases, severe heart, liver and kidney diseases; 3) any contraindication to the use of aspirin or P2Y12 receptor antagonists; 4) prior knowledge of the patients' CYP2C19*2, CYP2C19*3 or CYP2C19*17 genotype; 5) ongoing treatment in another observational or registry randomized trial; 6) an inability to provide informed consent or unavailability for follow-up.

Based on the PLATO trial and PHARMCLO trial exclusion criteria, ticagrelor is contraindicated in patients: 1) with active pathological bleeding; 2) with a history of intracranial bleeding; 3) requiring dialysis, 4) taking oral anticoagulant therapy that could not be stopped; 5) with known clinically important thrombocytopenia; 6) receiving fibrinolytic therapy within the previous 24 hours; and 7) taking concomitant therapy with strong CYP3A inhibitors or inducers.[16, 17]

Patients and public involvement

Patients in this trial will not be involved in the design, recruitment and conduction of the study. Clopidogrel genes of patients in pharmacogenetic group will be detected as soon as possible after the random assignment. The individual genetic information and the corresponding anti-platelet aggregation drug adjustment regimen will be disseminated to study participants as soon as possible after the gene detection.

Satisfaction of the intervention and the burden of involvement in this RCT will be assessed as part of the evaluation.

Randomization and treatments

Patients are consented and randomized in a 1:1 ratio to pharmacogenetic or standard group as soon as feasible after the diagnosis of AIMS or TIA is made and no later 72 hours after initial symptom onset. A blood sample is obtained from every participant right after randomization. Three single-nucleotide polymorphisms (SNPs) for CYP2C19 (National Center for Biotechnology Information [NCBI] Genome build 37.1, GenBank NG_008384), including CYP2C19*2 (681G>A, dbSNP rs4244285), CYP2C19*3 (636G>A, dbSNP rs4986893), and CYP2C19*17 (−806C>T, dbSNP rs12248560), are genotyped in the participants assigned to the Pharmacogenetical strategy. Genotyping of the 3 SNPs is done at the Central laboratory of Yangpu Hospital Tongji University School of Medicine with microarray-based metho (CapitalBio Technology). Patients are categorized by CYP2C19 metabolizer status based on *2, *3, and *17 genotypes within 24 hours of admission. And they are divided into four metabolite types: ultra-metabolizers (UM, *1/*17, *17/*17), extensive metabolizers (EM, *1/*1), intermediate metabolizers (IM, *1/*2, *1/*3, *17/*2, *17/*3), poor metabolizers (PM, *2/*2, *2/*3, *3/ *3).

Both pharmacogenetic group and standard group receive a loading dose of 300 mg aspirin and 300mg clopidogrel on day 1, followed by a dose of 100 mg of aspirin per day on days 2 to 365. Patients randomly assigned to the pharmacogenetic group receive a dose of 75 mg clopidogrel per day (UM and EM group), 150 mg clopidogrel per day (IM group) or ticagrelor 90 mg twice daily (PM group) on days 2 to 21, which can be further adjusted in combination with clinical features. While for standard group, the P2Y12 receptor antagonist is selected by the clinician according to the clinical features of the patients. The clinical features include age, weight, ischemic risk, prior history of stroke/TIA, bleeding risk, intracranial bleeding, active bleeding, history of bleeding, anemia, diabetes or chronic kidney disease.

Primary efficacy endpoint

The primary efficacy endpoint for this trial is a new stroke event (ischemic or hemorrhagic) that happens within one year. Ischemic stroke is defined as a sudden focal

neurological dysfunction caused by vascular causes, duration ≥ 24 hours, or neurological dysfunction due to imaging and clinical symptoms caused by bloody infarction rather than cerebral hemorrhage found by imaging examination. Hemorrhagic stroke is defined as acute extravasation of blood into the brain parenchyma or subarachnoid space with associated neurologic symptoms.

Secondary efficacy endpoint

The secondary efficacy endpoint is analyzed as the individual or composite outcomes of the new clinical vascular event (ischemic stroke, hemorrhagic stroke, myocardial infarction, or vascular death). The definition of vascular death is adapted from the CHANCE trial.[3] Briefly, vascular death is defined as death resulting from stroke (ischemic or hemorrhagic), systemic hemorrhage, myocardial infarction, congestive heart failure, pulmonary embolism, sudden death, or arrhythmia.

Safety assessments

Safety endpoint is a major bleeding event, according to the definitions in International Society on Thrombosis and Hemostasis[18] and Platelet-Oriented Inhibition in New TIA and Minor Ischemic Stroke (POINT) Trial[19]. Major hemorrhage is defined as symptomatic intracranial hemorrhage or intraocular bleeding causing loss of vision, requiring two or more units of red cells or equivalent amount of whole blood replacement, or requiring hospitalization or prolongation of an existing hospitalization, surgical intervention or death. An independent Clinical Event Adjudication Committee evaluates all components of the primary and secondary outcomes and safety endpoint.

Sample size

Based on data from the One-year outcomes of the CHANCE trial with a primary endpoint (ischemic or hemorrhagic stroke) rate of 10.6%[20], and considering that the time point when patients start to receive treatments in our study is later than that in the CHANCE trial, we estimate a higher incidence of primary efficacy endpoint in the standard group and define it as 15%. As we expect an absolute risk reduction of 5% in the pharmacogenomic group, so we define it as 10%. Given a 5% missed follow-up rate, 95% power and a two-sided type I error of 0.05, the calculated sample size is 1,191 patients in each group.

Statistical analyses

The distributions of baseline characteristics are compared between two study groups using T test. Proportions and $\chi 2$ test are used for categorical variables and continuous variables will be reported as median (inter-quartile range). Cox proportional-hazard model is used to estimate the hazard ratio and 95% confidence intervals relating to the primary and secondary outcomes. Schoenfeld residuals test is used to confirm the proportional hazards assumption for the Cox regression model. For estimating the cumulative incidence of endpoints during the 1-year follow-up, we perform the Kaplan–Meier analyses by means of Aalen-Johansen estimator. And Fine-Gray model is used to test the significance of the differences between the sub-distribution of the hazards. Values of $P < 0.05$ are considered statistically significant.

DISCUSSION

Currently, clopidogrel combined with aspirin has become the preferred short-term treatment for patients with AIMS or TIA for many clinicians.[21] However, the pharmacokinetics of clopidogrel could be influenced by metabolic status. Poor metabolizers will cause insufficient anti-platelet effect and impaired clinical benefit.[22] At present, the mechanisms of clopidogrel resistence are not fully elucidated and evidences from the genetic substudy of CHANCE trail showed the correlation between CYP2C19 polymorphisms with clopidogrel nonresponsiveness. For patients with AIMS or TIA treated with clopidogrel and aspirin, CYP2C19 loss-of-function alleles carriers were prone to have increased risk for subsequent stroke and composite vascular events compared with noncarriers.[12] Although these genetic associations with clinical benefits have been widely replicated and the sample sizes are large enough to be predictive in the clinical setting, there are few examples using pharmacogenetic data concerning clopidogrel metabolism to guide clinical practice.[23-25] Regarding to cardiovascular diseases, mounting evidence have shown that for patients with CYP2C19 loss-of-function alleles, obtaining genotype data early after percutaneous coronary intervention (PCI) and thus making genotype-guided personalized antiplatelet therapeutic regimen could reduce risks for major adverse cardiovascular events (MACE).[26, 27] Thus, genotype-guided antiplatelet therapy may be regarded as a prospective alternative approach to personalized treatment in AIMS or TIA.

Given the fact that clopidogrel is currently the most widely used antiplatelet agent for AIMS or TIA with aspirin and there are indeed differences in reactivity among individuals, varying the dose of clopidogrel or shifting to new antiplatelet agents based on genetic datas may be alternatives, but it has not been adequately evaluated.[28] The ongoing Platelet Reactivity in Acute Non-disabling Cerebrovascular Events (PRINCE) study intends to investigate whether the combination of ticagrelor and aspirin is superior to the combination of clopidogrel and aspirin in reducing the 90-day HPR for AIMS or TIA, especially for carriers of CYP2C19 loss-of-function allele.[29] The interim results of PRINCE trial have shown that although ticagrelor could significantly reduce HPR better than clopidogrel, there were no significant differences between the two groups in reducing stroke and the composite endpoint events.[30] More future randomized studies of genotype-guided antiplatelet therapy may be of value. Thus, this RCT will provide evidence for the assumption that using pharmacogenetic data to select P2Y12 receptor antagonists can be successfully incorporated into the clinical care of patients with AIMS or TIA. The selection of a P2Y12 receptor antagonist in our trial (label-recommended or doubled Dosage of clopidogrel or ticagrelor) is based on their pharmacogenetic data and individual clinical features in order to acquire the best trade-off between ischemic events and bleeding complications. Furthermore, for the sake of racial and geographical differences in genetic factors, we will collect patients of AIMS or TIA from both Han and Uygur population in different regions of Shanghai and Xinjiang of China. The North-South differences make the participants more representative, ensuring the effectiveness of clinical trial. In addition, due to the fact that the clinical course of most patients in clinical practice is long, we appropriately expand the time of patient enrollment, so that it can be applied to more people in the future.

Acknowledgements We thank Chang Shan (Department of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Rui-jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai, China) for her help in revising the manuscript.

Contributors LH and Y-hY conceived and designed this study. X-gZ, X-qZ, JX and Z-zL wrote the manuscript with contributions from all authors. H-yJ and Y-hY refined the protocol. All authors read and approved the final manuscript.

Funding This project is supported by Science and Technology Commission of Shanghai Municipality (18411970100).

Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethics committee, Yangpu Hospital, Tongji University School of Medicine, China.

REFERENCES

1 Coull AJ, Lovett JK, Rothwell PM. Population based study of early risk of stroke after transient ischaemic attack or minor stroke: implications for public education and organisation of services. *BMJ* 2004; 328:326.

2 Johnston SC. Clinical practice. Transient ischemic attack. *N Engl J Med* 2002; 347:1687-1692.

3 Wang Y, Wang Y, Zhao X, *et al*. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. *N Engl J Med* 2013; 369:11-19.

4 Mijajlovic MD, Shulga O, Bloch S, *et al*. Clinical consequences of aspirin and clopidogrel resistance: an overview. *Acta Neurol Scand* 2013; 128:213-219.

5 Tantry US, Bonello L, Aradi D, *et al*. Consensus and update on the definition of on-treatment platelet reactivity to adenosine diphosphate associated with ischemia and bleeding. *J Am Coll Cardiol* 2013; 62:2261-2273.

6 Guirgis M, Thompson P,Jansen S. Review of aspirin and clopidogrel resistance in peripheral arterial disease. *J Vasc Surg* 2017; 66:1576-1586.

7 Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, *et al*. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J Am Coll Cardiol* 2007; 49:1505-1516.

8 Juth V, Holman EA, Chan MK, *et al*. Genetics as a molecular window into recovery, its treatment, and stress responses after stroke. *J Investig Med* 2016; 64:983-988.

9 Kazui M, Nishiya Y, Ishizuka T, *et al*. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of

clopidogrel to its pharmacologically active metabolite. *Drug Metab Dispos* 2010; 38:92-99.

10 Mega JL, Close SL, Wiviott SD, *et al*. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009; 360:354-362.

11 Wallentin L, James S, Storey RF, *et al*. Effect of CYP2C19 and ABCB1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial. *Lancet* 2010; 376:1320-1328.

12 Wang Y, Zhao X, Lin J, *et al*. Association Between CYP2C19 Loss-of-Function Allele Status and Efficacy of Clopidogrel for Risk Reduction Among Patients With Minor Stroke or Transient Ischemic Attack. *JAMA* 2016; 316:70-78.

13 Kitzmiller JP, Groen DK, Phelps MA, *et al*. Pharmacogenomic testing: relevance in medical practice: why drugs work in some patients but not in others. *Cleve Clin J Med* 2011; 78:243-257.

14 Scott SA, Sangkuhl K, Stein CM, *et al*. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. *Clin Pharmacol Ther* 2013; 94:317-323.

15 Easton JD, Saver JL, Albers GW, *et al*. Definition and evaluation of transient ischemic attack: a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association Stroke Council; Council on Cardiovascular Surgery and Anesthesia; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; and the Interdisciplinary Council on Peripheral Vascular Disease. The American Academy of Neurology affirms the value of this statement as an educational tool for neurologists. *Stroke* 2009; 40:2276-2293.

16 Shuldiner AR, Palmer K, Pakyz RE, *et al*. Implementation of pharmacogenetics: the University of Maryland Personalized Anti-platelet Pharmacogenetics Program. *Am J Med Genet C Semin Med Genet* 2014; 166c:76-84.

17 Notarangelo FM, Maglietta G, Bevilacqua P, *et al*. Pharmacogenomic Approach to Selecting Antiplatelet Therapy in Patients With Acute Coronary Syndromes: The PHARMCLO Trial. *J Am Coll Cardiol* 2018; 71:1869-1877.

18 Schulman S,Kearon C. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. *J Thromb Haemost* 2005; 3:692-694.

19 Johnston SC, Easton JD, Farrant M, *et al*. Platelet-oriented inhibition in new TIA and minor ischemic stroke (POINT) trial: rationale and design. *Int J Stroke* 2013; 8:479-483.

20 Wang Y, Pan Y, Zhao X, *et al*. Clopidogrel With Aspirin in Acute Minor Stroke or Transient Ischemic Attack (CHANCE) Trial: One-Year Outcomes. *Circulation* 2015; 132:40-46.

21 Kernan WN, Ovbiagele B, Black HR, *et al*. Guidelines for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2014; 45:2160-2236.

22 Holmes MV, Perel P, Shah T, *et al*. CYP2C19 genotype, clopidogrel metabolism, platelet function, and cardiovascular events: a systematic review and meta-analysis. *JAMA* 2011; 306:2704-2714.

23 Peterson JF, Field JR, Unertl KM, *et al*. Physician response to implementation of genotype-tailored antiplatelet therapy. *Clin Pharmacol Ther* 2016; 100:67-74.

24 Price MJ, Berger PB, Teirstein PS, *et al*. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. *JAMA* 2011; 305:1097-1105.

25 Cavallari LH, Weitzel KW, Elsey AR, *et al*. Institutional profile: University of Florida Health Personalized Medicine Program. *Pharmacogenomics* 2017; 18:421-426.

26 Cavallari LH, Lee CR, Beitelshees AL, *et al*. Multisite Investigation of Outcomes With Implementation of CYP2C19 Genotype-Guided Antiplatelet Therapy After Percutaneous Coronary Intervention. *JACC Cardiovasc Interv* 2018; 11:181-191.

27 Kheiri B, Abdalla A, Osman M, *et al*. Personalized antiplatelet therapy in patients with coronary artery disease undergoing percutaneous coronary intervention: A network meta-analysis of randomized clinical trials. *Catheter Cardiovasc Interv* 2019.

28 Roberts JD, Wells GA, Le May MR, *et al*. Point-of-care genetic testing for personalisation of antiplatelet treatment (RAPID GENE): a prospective, randomised, proof-of-concept trial. *Lancet* 2012; 379:1705-1711.

29 Wang Y, Lin Y, Meng X, *et al*. Effect of ticagrelor with clopidogrel on high on-treatment platelet reactivity in acute stroke or transient ischemic attack (PRINCE) trial: Rationale and design. *Int J Stroke* 2017; 12:321-325.

30 Wang Y, Meng X, Chen W, *et al*. Ticagrelor with Aspirin on Platelet Reactivity in Acute Nondisabling Cerebrovascular Events (PRINCE) Trial--Final Analysis. https://www.wesrch.com/medical/pdfME1XXF000PAZG. *International Stroke Conference* 2018.

Figure legend

Fig. 1. Study flowchart. TIA, transient ischemic attack; UM, ultra-metabolizers; EM, extensive metabolizers; IM, intermediate metabolizers; PM, poor metabolizers.

LOADING DOSE (LD)

Aspirin 300mg	Aspirin 100 mg from day 2 to day 365
Clopidogrel 300mg	clopidogrel 75 mg per day (UM and EM group), clopidogrel 150mg per day (IM group) and ticagrelor 90 mg twice daily (PM group) from day 2 to day 21, further adjusted in combination with clinical features

Patients will be randomized to treatment within 72 hours of the start of a minor ischemic stroke or TIA event

R

pharmacogenetic group

1month — 3months — 6months — 12months

1-month Phone Follow-Up (30 ± 2 Days)	3-month Phone Contact/Follow-Up (90 ± 7 Days)	6-month Phone Contact/Follow-Up (180 ± 14 Days)	12-month Phone Contact/Follow-Up (360 ± 14 Days)

standard group

1month — 3months — 6months — 12months

Aspirin 300mg	Aspirin 100 mg from day 2 to day 365
Clopidogrel 300mg	P2Y12 receptor antagonist is selected by the clinician according to the clinical characteristics of the patients

BMJ Open

Personalized antiplatelet therapy based on pharmacogenomics in acute ischemic minor stroke and transient ischemic attack: study protocol for a randomised controlled trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-028595.R2
Article Type:	Protocol
Date Submitted by the Author:	06-Apr-2019
Complete List of Authors:	Zhang, Xiao-guang ; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Zhu, Xiao-qiong; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Xue, Jie; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Li, Zhi-zhang; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Jiang, Hua-yu; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Hu, Liang; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine Yue, Yun-hua; Department of Neurology, Yangpu Hospital, Tongji University School of Medicine
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Neurology
Keywords:	personlized antiplatelet therapy, clopidogrel, pharmacogenomics, acute ischemic minor stroke, transient ischemic attack

Personalized antiplatelet therapy based on pharmacogenomics in acute ischemic minor stroke and transient ischemic attack: study protocol for a randomised controlled trial

Xiao-Guang Zhang, Xiao-Qiong Zhu, Jie Xue, Zhi-Zhang Li, Hua-Yu Jiang, Liang Hu, Yun-Hua Yue

Department of Neurology, Yangpu Hospital, Tongji University School of Medicine, No. 450 Tengyue Road, Shanghai 200090, China

X-GZ and X-QZ contributed equally to this work.

Correspondence to Dr. Liang Hu; 13585696649@163.com Dr. Yun-Hua Yue; 447879206@qq.com

ABSTRACT

Introduction: Antiplatelet therapy combining aspirin and clopidogrel is considered to be a key intervention for acute ischemic minor stroke (AIMS) and transient ischemic attack (TIA). However, the interindividual variability in response to clopidogrel resulting from the polymorphisms in clopidogrel metabolism-related genes has greatly limited its efficacy. To date, there are no reports on individualized antiplatelet therapy for AIMS and TIA based on the genetic testing and clinical features. Therefore, we conduct this randomized controlled trial (RCT) to validate the hypothesis that the individualized antiplatelet therapy selected on the basis of a combination of genetic information and clinical features would lead to better clinical outcomes compared to the standard care based only on clinical features in patients with AIMS or TIA.

Methods and analysis: This trial will recruit 2382 patients with AIMS or TIA who meet eligibility criteria. Patients are randomly assigned in a 1:1 ratio to pharmacogenetic group and standard group. Both groups receive a loading dose of 300 mg aspirin and 300 mg clopidogrel on day 1, followed by 100 mg aspirin per day on days 2 to 365. The P2Y12 receptor antagonist is selected by the clinician according to the genetic information and clinical features for pharmacogenetic group and clinical features for the standard group on days 2 to 21. The primary efficacy endpoint is a new stroke event (ischemic or hemorrhagic) that happens within one year. The secondary efficacy endpoint is analyzed as the individual or composite outcomes of the new

clinical vascular event (ischemic stroke, hemorrhagic stroke, myocardial infarction, or vascular death). Baseline characteristics and outcomes after treatment will be evaluated.

Ethics and dissemination: This protocol has been approved by the ethics committee of Yangpu Hospital, Tongji University School of Medicine (No. LL-2018-KY-012). We will submit the results of this trial for publication in a peer-reviewed journal.

Trial registration number: ChiCTR1800019911

Strengths and limitations of this study

This is a study to assess the efficacy and safety of individualized antiplatelet therapy selected on the basis of a combination of genetic information and a patient's clinical features in patients with AIMS or TIA.

The results from this RCT will provide new evidence of the efficacy of individualized antiplatelet therapy on the basis of genetic information for AIMS or TIA.

This study will help clinicians predict and estimate the risk of clopidogrel resistance in patients with AIMS or TIA, and then take corresponding measures to reduce the risk of stroke recurrence.

The study is mainly focused on the CYP2C19 genotype (*2, *3 and *17 alleles), which might neglect the potential impact of other alleles.

The trial implementation is not multi-centered and only Chinese population are included, which might limit its generalisability.

INTRODUCTION

Acute ischemic minor stroke (AIMS) and transient ischemic attack (TIA) are common cerebrovascular events with a high tendency to cause disability. Patients with AIMS or TIA have a high risk of subsequent ischemic events, especially during the first 90 days after the cerebrovascular event.[1] During this acute phase, there is still a risk of previous ischemic tissue, with ruptured plaques in a state of high thrombus formation and high platelet activation, which may lead to more severe acute instability events.[2] Therefore, antiplatelet therapy is considered to be a key intervention for AIMS and TIA at present.

Clopidogrel is one of the commonly used anti-platelet drugs in clinic, which is recommended for the secondary prevention of ischemic stroke. The Clopidogrel in High-Risk Patients with Acute Nondisabling Cerebrovascular Events (CHANCE) trial reported that compared with aspirin alone, the combination of clopidogrel with aspirin decreased the risk of stroke among patients with AIMS or TIA who can be treated within 24 hours after the onset of symptoms.[3] However, there is interindividual variability in response to clopidogrel, which leads to the result that a large number of subsequent strokes still occur despite clopidogrel treatment and even among those treated with dual-antiplatelet agents.[4] Compared with the inhibition of platelet aggregation expected, poor inhibition using antiplatelet therapy is referred to high on-treatment platelet reactivity (HPR).[5] Those patients who show HPR to clopidogrel and are therefore at greater risk of ischemic events are suffering from clopidogrel resistance.[5, 6] Clopidogrel resistance is an important cause of failure in the prevention and treatment of patients with partial ischemic stroke, and will greatly increase the recurrence of ischemic stroke. To date, the mechanisms related to variability in clopidogrel responsiveness are not fully elucidated.[7]

Mounting evidence have shown that genetic factors may play a crucial role in mediating clopidogrel resistance.[8] As clopidogrel is a prodrug that requires hepatic cytochrome P450 (CYP) for its conversion into an active metabolite[9], polymorphisms of its encoded gene CYP2C19 have been identified as strong predictors of clopidogrel nonresponsiveness.[10] Among them, CYP2C19 loss-of-function genotype (*2 and/or *3 alleles) is found to be related to low responsiveness to clopidogrel, which is a risk factor for ischemic events, whereas the presence of gain-of function CYP2C19 allele (*17) is associated with a high platelet inhibition and increased risk of bleeding.[11] The genetic

substudy of the CHANCE trial also showed that only in patients with AIMS or TIA who did not carry the CYP2C19 loss-of-function alleles, the combined treatment of clopidogrel and aspirin could reduce the risk of a new stroke in comparison with aspirin alone.[12] This study provided evidence to support the genetic testing that may allow clinicians to personalize antiplatelet therapy, especially in East Asian patient populations for whom the prevalence of CYP2C19 loss-of-function allele is high.[12, 13]

To date, the clinical factors and genetic factors affecting clopidogrel responses have not reached a consistent conclusion. Besides, most of the study endpoints are cardiovascular events, and the research on cerebrovascular events is lacking. For example, Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines recommend that non-carriers of CYP2C19 loss-of-function alleles patients with acute coronary syndrome (ACS) continue to take clopidogrel 75 mg daily, and carriers are advised to increase the dose of clopidogrel or switch to other antiplatelet agents such as ticagrelor.[14] Wallentin et al. researched on the genetics of the CYP2C19 gene polymorphism in patients with ACS and found that compared with clopidogrel, treatment with ticagrelor significantly reduced the death rate from vascular causes in patients with CYP2C19 loss of function alleles.[11] However, for patients with AIMS or TIA with clopidogrel resistance, it is unclear whether there will be more clinical benefit when switching to ticagrelor. Based on the above, we conduct this randomized controlled trial to validate the hypothesis that the individualized antiplatelet therapy selected on the basis of a combination of genetic information and a patient's clinical features would lead to better clinical outcomes compared to the standard care based only on clinical features in patients with AIMS or TIA.

METHOD

Design

The design of study is shown in Fig. 1. The Pharmacogenetics of clopidogrel in patients with AIMS or TIA study is a prospective, open-label RCT, aiming to evaluate whether selecting antiplatelet therapy (label-recommended or doubled dosage of clopidogrel or ticagrelor) on the basis of a patient's both genetic and clinical features leads to more clinical benefits compared to the standard care which bases selection only on clinical features. Collection and genetic analysis of samples are subjected to

informed consent from all patients and approval by ethics committee of Yangpu Hospital Tongji University School of medicine.

Patient population

Patients are included into this study if they meet all the following criteria: 1). age of 18 years or older; 2). diagnosis of an AIMS or TIA; AIMS is defined as a sudden focal neurological dysfunction caused by vascular causes, and score of 3 or less at the time of randomization on the National Institutes of Health Stroke Scale (NIHSS; scores range from 0 to 42, with higher scores indicating greater deficits).[3] TIA is defined as a transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia, without acute infarction;[15] 3). onset of the AIMS or TIA symptoms less than 72 h.

Patients are excluded from study participation if one of the following criteria is met: 1) hemorrhage; other conditions, such as vascular malformation, trauma, tumor, abscess, degenerative neurologic disease or other major nonischemic brain disease; 2) systemic infectious diseases, autoimmune diseases, severe heart, liver and kidney diseases; 3) any contraindication to the use of aspirin or P2Y12 receptor antagonists; 4) prior knowledge of the patients' CYP2C19*2, CYP2C19*3 or CYP2C19*17 genotype; 5) ongoing treatment in another observational or registry randomized trial; 6) an inability to provide informed consent or unavailability for follow-up.

Based on the PLATO trial and PHARMCLO trial exclusion criteria, ticagrelor is contraindicated in patients: 1) with active pathological bleeding; 2) with a history of intracranial bleeding; 3) requiring dialysis, 4) taking oral anticoagulant therapy that could not be stopped; 5) with known clinically important thrombocytopenia; 6) receiving fibrinolytic therapy within the previous 24 hours; and 7) taking concomitant therapy with strong CYP3A inhibitors or inducers.[16, 17]

Patients and public involvement

Patients in this trial will not be involved in the design, recruitment and conduction of the study. Clopidogrel genes of patients in pharmacogenetic group will be detected as soon as possible after the random assignment. The individual genetic information and the corresponding anti-platelet aggregation drug adjustment regimen will be disseminated to study participants as soon as possible after the gene detection.

Satisfaction of the intervention and the burden of involvement in this RCT will be assessed as part of the evaluation.

Randomization and treatments

Patients are consented and randomized in a 1:1 ratio to pharmacogenetic or standard group as soon as feasible after the diagnosis of AIMS or TIA is made and no later 72 hours after initial symptom onset. A blood sample is obtained from every participant right after randomisation. Three single-nucleotide polymorphisms (SNPs) for CYP2C19 (National Center for Biotechnology Information [NCBI] Genome build 37.1, GenBank NG_008384), including CYP2C19*2 (681G>A, dbSNP rs4244285), CYP2C19*3 (636G>A, dbSNP rs4986893), and CYP2C19*17 (−806C>T, dbSNP rs12248560), are genotyped in the participants assigned to the Pharmacogenetical strategy. Genotyping of the 3 SNPs is done at the Central laboratory of Yangpu Hospital Tongji University School of Medicine with microarray-based metho (CapitalBio Technology). Patients are categorized by CYP2C19 metabolizer status based on *2, *3, and *17 genotypes within 24 hours of admission. And they are divided into four metabolite types: ultra-metabolizers (UM, *1/*17, *17/*17), extensive metabolizers (EM, *1/*1), intermediate metabolizers (IM, *1/*2, *1/*3, *17/*2, *17/*3), poor metabolizers (PM, *2/*2, *2/*3, *3/ *3).

Both pharmacogenetic group and standard group receive a loading dose of 300 mg aspirin and 300mg clopidogrel on day 1, followed by a dose of 100 mg of aspirin per day on days 2 to 365. Patients randomly assigned to the pharmacogenetic group receive a dose of 75 mg clopidogrel per day (UM and EM group), 150 mg clopidogrel per day (IM group) or ticagrelor 90 mg twice daily (PM group) on days 2 to 21, which can be further adjusted in combination with clinical features. While for standard group, the P2Y12 receptor antagonist is selected by the clinician according to the clinical features of the patients. The clinical features include age, weight, ischemic risk, prior history of stroke/TIA, bleeding risk, intracranial bleeding, active bleeding, history of bleeding, anemia, diabetes or chronic kidney disease.

Primary efficacy endpoint

The primary efficacy endpoint for this trial is a new stroke event (ischemic or hemorrhagic) that happens within one year. Ischemic stroke is defined as a sudden focal

neurological dysfunction caused by vascular causes, duration ≥ 24 hours, or neurological dysfunction due to imaging and clinical symptoms caused by bloody infarction rather than cerebral hemorrhage found by imaging examination. Hemorrhagic stroke is defined as acute extravasation of blood into the brain parenchyma or subarachnoid space with associated neurologic symptoms.

Secondary efficacy endpoint

The secondary efficacy endpoint is analyzed as the individual or composite outcomes of the new clinical vascular event (ischemic stroke, hemorrhagic stroke, myocardial infarction, or vascular death). The definition of vascular death is adapted from the CHANCE trial.[3] Briefly, vascular death is defined as death resulting from stroke (ischemic or hemorrhagic), systemic hemorrhage, myocardial infarction, congestive heart failure, pulmonary embolism, sudden death, or arrhythmia.

Safety assessments

Safety endpoint is a major bleeding event, according to the definitions in International Society on Thrombosis and Hemostasis[18] and Platelet-Oriented Inhibition in New TIA and Minor Ischemic Stroke (POINT) Trial[19]. Major hemorrhage is defined as symptomatic intracranial hemorrhage or intraocular bleeding causing loss of vision, requiring two or more units of red cells or equivalent amount of whole blood replacement, or requiring hospitalization or prolongation of an existing hospitalization, surgical intervention or death. An independent Clinical Event Adjudication Committee evaluates all components of the primary and secondary outcomes and safety endpoint.

Sample size

Based on data from the One-year outcomes of the CHANCE trial with a primary endpoint (ischemic or hemorrhagic stroke) rate of 10.6%[20], and considering that the time point when patients start to receive treatments in our study is later than that in the CHANCE trial, we estimate a higher incidence of primary efficacy endpoint in the standard group and define it as 15%. As we expect an absolute risk reduction of 5% in the pharmacogenomic group, so we define it as 10%. Given a 5% missed follow-up rate, 95% power and a two-sided type I error of 0.05, the calculated sample size is 1,191 patients in each group.

Statistical analyses

The distributions of baseline characteristics are compared between two study groups using T test. Proportions and $\chi 2$ test are used for categorical variables and continuous variables will be reported as median (inter-quartile range). Cox proportional-hazard model is used to estimate the hazard ratio and 95% confidence intervals relating to the primary and secondary outcomes. Schoenfeld residuals test is used to confirm the proportional hazards assumption for the Cox regression model. For estimating the cumulative incidence of endpoints during the 1-year follow-up, we perform the Kaplan–Meier analyses by means of Aalen-Johansen estimator. And Fine-Gray model is used to test the significance of the differences between the sub-distribution of the hazards. Values of $P < 0.05$ are considered statistically significant.

DISCUSSION

Currently, clopidogrel combined with aspirin has become the preferred short-term treatment for patients with AIMS or TIA for many clinicians.[21] However, the pharmacokinetics of clopidogrel could be influenced by metabolic status. Poor metabolizers will cause insufficient anti-platelet effect and impaired clinical benefit.[22] At present, the mechanisms of clopidogrel resistence are not fully elucidated and evidences from the genetic substudy of CHANCE trail showed the correlation between CYP2C19 polymorphisms with clopidogrel nonresponsiveness. For patients with AIMS or TIA treated with clopidogrel and aspirin, CYP2C19 loss-of-function alleles carriers were prone to have increased risk for subsequent stroke and composite vascular events compared with noncarriers.[12] Although these genetic associations with clinical benefits have been widely replicated and the sample sizes are large enough to be predictive in the clinical setting, there are few examples using pharmacogenetic data concerning clopidogrel metabolism to guide clinical practice.[23-25] Regarding to cardiovascular diseases, mounting evidence have shown that for patients with CYP2C19 loss-of-function alleles, obtaining genotype data early after percutaneous coronary intervention (PCI) and thus making genotype-guided personalized antiplatelet therapeutic regimen could reduce risks for major adverse cardiovascular events (MACE).[26, 27] Thus, genotype-guided antiplatelet therapy may be regarded as a prospective alternative approach to personalized treatment in AIMS or TIA.

Given the fact that clopidogrel is currently the most widely used antiplatelet agent for AIMS or TIA with aspirin and there are indeed differences in reactivity among individuals, varying the dose of clopidogrel or shifting to new antiplatelet agents based on genetic datas may be alternatives, but it has not been adequately evaluated.[28] The ongoing Platelet Reactivity in Acute Non-disabling Cerebrovascular Events (PRINCE) study intends to investigate whether the combination of ticagrelor and aspirin is superior to the combination of clopidogrel and aspirin in reducing the 90-day HPR for AIMS or TIA, especially for carriers of CYP2C19 loss-of-function allele.[29] The interim results of PRINCE trial have shown that although ticagrelor could significantly reduce HPR better than clopidogrel, there were no significant differences between the two groups in reducing stroke and the composite endpoint events.[30] More future randomized studies of genotype-guided antiplatelet therapy may be of value. Thus, this RCT will provide evidence for the assumption that using pharmacogenetic data to select P2Y12 receptor antagonists can be successfully incorporated into the clinical care of patients with AIMS or TIA. The selection of a P2Y12 receptor antagonist in our trial (label-recommended or doubled Dosage of clopidogrel or ticagrelor) is based on their pharmacogenetic data and individual clinical features in order to acquire the best trade-off between ischemic events and bleeding complications. Furthermore, for the sake of racial and geographical differences in genetic factors, we will collect patients of AIMS or TIA from both Han and Uygur population in different regions of Shanghai and Xinjiang of China. The North-South differences make the participants more representative, ensuring the effectiveness of clinical trial. In addition, due to the fact that the clinical course of most patients in clinical practice is long, we appropriately expand the time of patient enrollment, so that it can be applied to more people in the future.

Acknowledgements We thank Chang Shan (Department of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Rui-jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai, China) for her help in revising the manuscript.

Contributors LH and Y-hY conceived and designed this study. X-gZ, X-qZ, JX and Z-zL wrote the manuscript with contributions from all authors. H-yJ and Y-hY refined the protocol. All authors read and approved the final manuscript.

Funding This project is supported by Science and Technology Commission of Shanghai Municipality (18411970100).

Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethics committee, Yangpu Hospital, Tongji University School of Medicine, China.

Data sharing statement The results of this study will be available after publication in a peer-reviewed medical journal.

REFERENCES

1 Coull AJ, Lovett JK, Rothwell PM. Population based study of early risk of stroke after transient ischaemic attack or minor stroke: implications for public education and organisation of services. *BMJ* 2004; 328:326.

2 Johnston SC. Clinical practice. Transient ischemic attack. *N Engl J Med* 2002; 347:1687-1692.

3 Wang Y, Wang Y, Zhao X, *et al*. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. *N Engl J Med* 2013; 369:11-19.

4 Mijajlovic MD, Shulga O, Bloch S, *et al*. Clinical consequences of aspirin and clopidogrel resistance: an overview. *Acta Neurol Scand* 2013; 128:213-219.

5 Tantry US, Bonello L, Aradi D, *et al*. Consensus and update on the definition of on-treatment platelet reactivity to adenosine diphosphate associated with ischemia and bleeding. *J Am Coll Cardiol* 2013; 62:2261-2273.

6 Guirgis M, Thompson P,Jansen S. Review of aspirin and clopidogrel resistance in peripheral arterial disease. *J Vasc Surg* 2017; 66:1576-1586.

7 Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, *et al*. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J Am Coll Cardiol* 2007; 49:1505-1516.

8 Juth V, Holman EA, Chan MK, *et al*. Genetics as a molecular window into recovery, its treatment, and stress responses after stroke. *J Investig Med* 2016; 64:983-988.

9 Kazui M, Nishiya Y, Ishizuka T, *et al*. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. *Drug Metab Dispos* 2010; 38:92-99.

10 Mega JL, Close SL, Wiviott SD, *et al*. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009; 360:354-362.

11 Wallentin L, James S, Storey RF, *et al*. Effect of CYP2C19 and ABCB1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial. *Lancet* 2010; 376:1320-1328.

12 Wang Y, Zhao X, Lin J, *et al*. Association Between CYP2C19 Loss-of-Function Allele Status and Efficacy of Clopidogrel for Risk Reduction Among Patients With Minor Stroke or Transient Ischemic Attack. *JAMA* 2016; 316:70-78.

13 Kitzmiller JP, Groen DK, Phelps MA, *et al*. Pharmacogenomic testing: relevance in medical practice: why drugs work in some patients but not in others. *Cleve Clin J Med* 2011; 78:243-257.

14 Scott SA, Sangkuhl K, Stein CM, *et al*. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. *Clin Pharmacol Ther* 2013; 94:317-323.

15 Easton JD, Saver JL, Albers GW, *et al*. Definition and evaluation of transient ischemic attack: a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association Stroke Council; Council on Cardiovascular Surgery and Anesthesia; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; and the Interdisciplinary Council on Peripheral Vascular Disease. The American Academy of Neurology affirms the value of this statement as an educational tool for neurologists. *Stroke* 2009; 40:2276-2293.

16 Shuldiner AR, Palmer K, Pakyz RE, *et al*. Implementation of pharmacogenetics: the University of Maryland Personalized Anti-platelet Pharmacogenetics Program. *Am J Med Genet C Semin Med Genet* 2014; 166c:76-84.

17 Notarangelo FM, Maglietta G, Bevilacqua P, *et al*. Pharmacogenomic Approach to Selecting Antiplatelet Therapy in Patients With Acute Coronary Syndromes: The PHARMCLO Trial. *J Am Coll Cardiol* 2018; 71:1869-1877.

18 Schulman S,Kearon C. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. *J Thromb Haemost* 2005; 3:692-694.

19 Johnston SC, Easton JD, Farrant M, *et al*. Platelet-oriented inhibition in new TIA and minor ischemic stroke (POINT) trial: rationale and design. *Int J Stroke* 2013; 8:479-483.

20 Wang Y, Pan Y, Zhao X, *et al*. Clopidogrel With Aspirin in Acute Minor Stroke or Transient Ischemic Attack (CHANCE) Trial: One-Year Outcomes. *Circulation* 2015; 132:40-46.

21 Kernan WN, Ovbiagele B, Black HR, *et al*. Guidelines for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2014; 45:2160-2236.

22 Holmes MV, Perel P, Shah T, *et al*. CYP2C19 genotype, clopidogrel metabolism, platelet function, and cardiovascular events: a systematic review and meta-analysis. *JAMA* 2011; 306:2704-2714.

23 Peterson JF, Field JR, Unertl KM, *et al*. Physician response to implementation of genotype-tailored antiplatelet therapy. *Clin Pharmacol Ther* 2016; 100:67-74.

24 Price MJ, Berger PB, Teirstein PS, *et al*. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. *JAMA* 2011; 305:1097-1105.

25 Cavallari LH, Weitzel KW, Elsey AR, *et al*. Institutional profile: University of Florida Health Personalized Medicine Program. *Pharmacogenomics* 2017; 18:421-426.

26 Cavallari LH, Lee CR, Beitelshees AL, *et al*. Multisite Investigation of Outcomes With Implementation of CYP2C19 Genotype-Guided Antiplatelet Therapy After Percutaneous Coronary Intervention. *JACC Cardiovasc Interv* 2018; 11:181-191.

27 Kheiri B, Abdalla A, Osman M, *et al*. Personalized antiplatelet therapy in patients with coronary artery disease undergoing percutaneous coronary intervention: A network meta-analysis of randomized clinical trials. *Catheter Cardiovasc Interv* 2019.

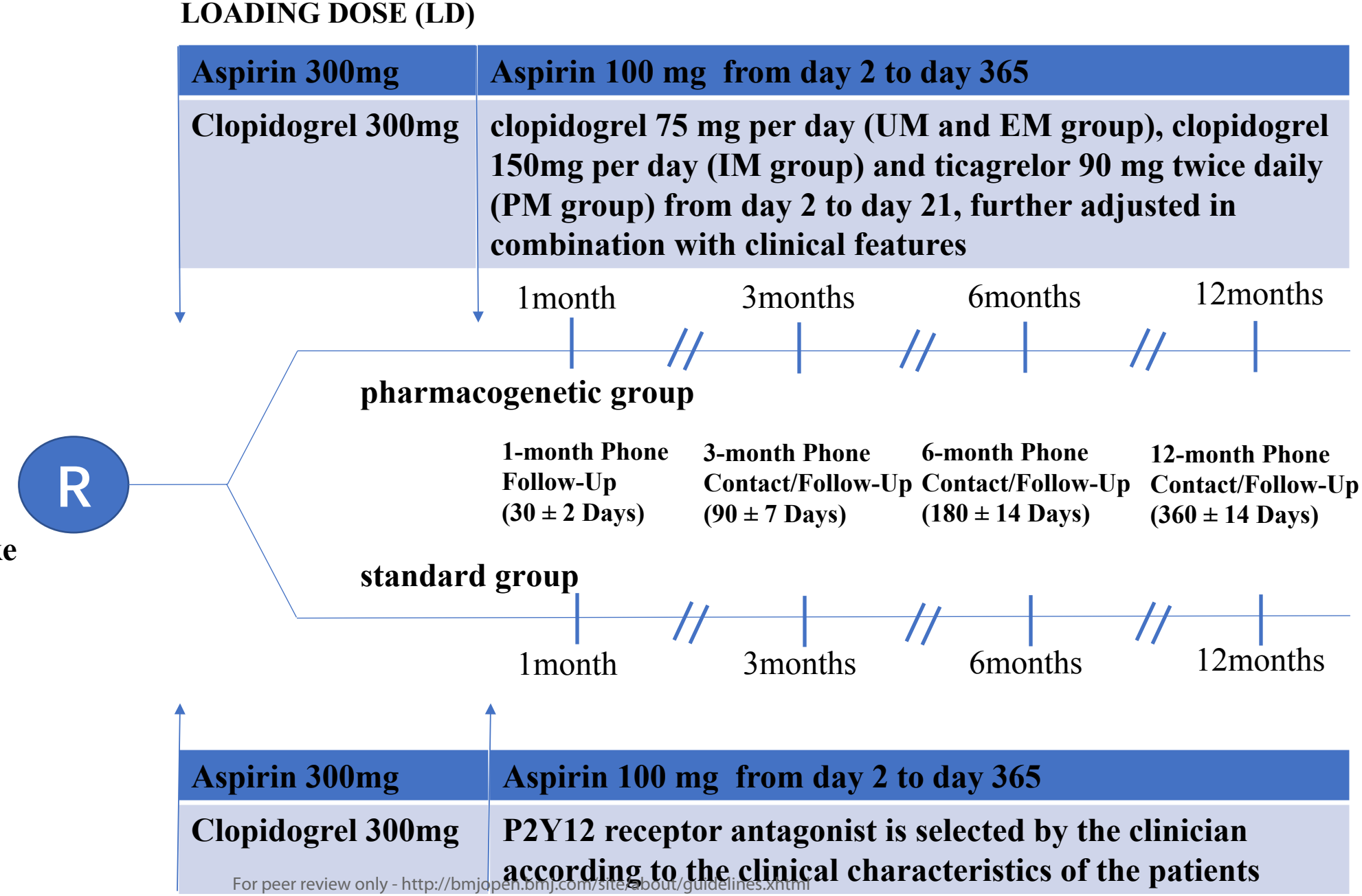
28 Roberts JD, Wells GA, Le May MR, *et al*. Point-of-care genetic testing for personalisation of antiplatelet treatment (RAPID GENE): a prospective, randomised, proof-of-concept trial. *Lancet* 2012; 379:1705-1711.

29 Wang Y, Lin Y, Meng X, *et al*. Effect of ticagrelor with clopidogrel on high on-treatment platelet reactivity in acute stroke or transient ischemic attack (PRINCE) trial: Rationale and design. *Int J Stroke* 2017; 12:321-325.

30 Wang Y, Meng X, Chen W, *et al*. Ticagrelor with Aspirin on Platelet Reactivity in Acute Nondisabling Cerebrovascular Events (PRINCE) Trial--Final Analysis. https://www.wesrch.com/medical/pdfME1XXF000PAZG. *International Stroke Conference* 2018.

Figure legend

Fig. 1. Study flowchart. TIA, transient ischemic attack; UM, ultra-metabolizers; EM, extensive metabolizers; IM, intermediate metabolizers; PM, poor metabolizers.



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
Administrative information		
Title	1 (P1)	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a (P2)	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4 (P10)	Sources and types of financial, material, and other support
Roles and responsibilities	5a (P1)	Names, affiliations, and roles of protocol contributors
	5b (P1)	Name and contact information for the trial sponsor
	5c (P10)	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
	5d (P7)	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)
Introduction		
Background and rationale	6a (P3-P4)	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
	6b (P3-P4)	Explanation for choice of comparators
Objectives	7 (P4)	Specific objectives or hypotheses
Trial design	8 (P4)	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

Study setting	9 (P5)	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
Eligibility criteria	10 (P5)	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a （P6）	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	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)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12 (P6-P7)	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
Participant timeline	13 (P4,P6)	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14 (P7)	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a (P6)	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

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
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a (P4)	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a (P4-P7)	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
	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
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
Statistical methods	20a (P8)	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
	20b (P8)	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	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)
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

	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
Harms	22 (P7)	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

Ethics and dissemination

Research ethics approval	24 (P2)	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
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)
Consent or assent	26a (P6)	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b (P5)	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27 (P5)	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
Declaration of interests	28 (P10)	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29 (P10)	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
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
Dissemination policy	31a (P5)	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
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c (P2)	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
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

*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.