

# Effects of precision antiplatelet therapy based on CYP2C19/11-dhTxB2 variability in prognosis of ischemic stroke/TIA patients: study protocol for a multicenter randomized controlled trial

**JiaJing Wang**

Nanchang University <https://orcid.org/0000-0001-9751-2376>

**Jianglong Tu**

The Second Affiliated Hospital of Nanchang University

**Jie kuang**

Nanchang University

**Katherine Starceвич**

University of Nevada

**Yanqiu Ge**

Nanchang University

**Chen Peng**

Nanchang University

**Yingping Yi** (✉ [13807089678@126.com](mailto:13807089678@126.com))

Department of Information, The Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi Province, China

**Shujuan Yin**

Nanchang University

**Xiaolin Zhang**

Nanchang University

**Jibiao Chen**

Nanchang University

---

**Study protocol**

**Keywords:** Clopidogrel, aspirin, TIA, IS, clopidogrel resistance, cytochrome P450 2C19

**Posted Date:** April 9th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-21845/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

## Introduction

Clopidogrel and aspirin are routine drugs for the treatment of ischemic stroke (IS) and transient ischemic attack (TIA). However, with the increase of clinical application, a large number of patients have displayed clopidogrel resistance and/or aspirin resistance. At present, the effect of genetically tailored medication has not been prospectively evaluated in a large sample.

## Methods and analysis

This study is an investigator-initiated, multicentre, large sample, prospectively, randomized controlled study evaluating the effects of precision antiplatelet medication based on the cytochrome P450 2C19 (CYP2C19) genetic test and the 11-dehydroxetane B2 (11-dhTxB2) test on IS/TIA patients over a duration of 12 months. Outcomes of interest including stroke recurrence, neurologic disabilities defined by the Modified Rankin Scale (mRS), bleeding events, other adverse events, and all-cause mortality will be assessed at the 1st, 3rd, 6th and 12th month post discharge. Demographics, risk factors, laboratory investigations, medications, physiological tests, and brain imaging will be assessed as well.

## Ethics and dissemination

The study was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Nanchang University: (2018) Medical Research Review No.05. In this study, a pre-experiment was carried out in April 2019. Formal recruitment of patients began in June 2019 and will continue until December 2020. Participants will be followed for one-year and the study will end in December 2021. The results of the study will be disseminated through peer-reviewed publications and conference reports.

## Trial registration number

ChiCTR1900026492.

## Trial registration date

2019.10.12

## Trial registry name

“Establishment of intelligent decision-making system for treatment of neurological impairment in cerebrovascular disease”. It is a prospectively study.

## Protocol version

V5.0, 2019/02/19.

# Strengths And Limitations Of This Study

1. This study is the first multicentre, large sample, randomized controlled study design to assess the effect of individualized medication based on the CYP2C19 genetic test and the 11-dhTxB2 test for IS/TIA patients.
2. The use of a definite protocol, a prospective collection of data, and an adequate number of patients assures statistically powered data.
3. No prescribing guides are available for patients with both clopidogrel and aspirin resistance. Due to the nature of these anti-platelet drugs, an increase in dosage may lead to a higher risk of bleeding, thus, medication dosage for participants was not adjusted.
4. There is the possibility of unrecognised confounders despite the multicentre design.

## Introduction

Stroke is a leading cause of disability and mortality affecting 200 per 100,000 people globally<sup>[1]</sup>. There are 13.7 million new cases of stroke occur globally each year<sup>[2]</sup>. Acute ischemic stroke is the most common type of stroke, accounting for 60%-80% of all strokes<sup>[3]</sup>. A dual anti-platelet therapy of clopidogrel and aspirin has been recommended for stroke and TIA patients by the American Heart Association/American Stroke Association guidelines<sup>[4,5]</sup>.

Clopidogrel is a common anti-platelet aggregation drug in clinical practice and plays an important role in the treatment of ischemic stroke<sup>[6]</sup>. However, with the increase of clinical application, the efficacy of clopidogrel varies with some patients displaying resistance. Clopidogrel resistance is characterized by no anti-platelet effect after usage of clopidogrel<sup>[7-10]</sup>. Studies have shown that the CYP2C19 gene polymorphism is closely related to the degree of platelet inhibition by clopidogrel. This has been used to categorize patients by CYP2C19 metabolizer status based on \*2, \*3, \*17 genotypes: poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), ultra-metabolizers (UM)<sup>[11,12]</sup>. Related studies have shown that there is a high probability of clopidogrel resistance in Asian populations, due to individual differences in pharmacodynamic response to clopidogrel<sup>[13,14]</sup>. In recent years, the most common CYP2C19\*2, \*3 mutation sites in the human cytochrome P450 allele have attracted much attention<sup>[15-18]</sup>. Aspirin is widely used in secondary prevention of stroke, however, clinical trials have shown that 5%-65% of patients are aspirin resistant therefore not sensitive to aspirin treatment<sup>[19]</sup>. In recent years, the relationship between aspirin resistance and stroke recurrence has attracted increasing attention and related research<sup>[20]</sup>.

Wang et al. found that compared to aspirin only use, the use of clopidogrel plus aspirin reduced the risk of a new stroke only in the subgroup of patients who were not carriers of the CYP2C19 loss-of-function alleles<sup>[21]</sup>. The impact of CYP2C19 polymorphisms on clopidogrel pharmacodynamics and clinical outcomes (stroke recurrence, composite vascular events, bleeding) of patients with stroke or transient

ischemic attack has been well established<sup>[22-24]</sup>. However, the influence of aspirin-resistance was not included in prior research. At present, there are no research reported genetically tailored therapy guidelines for patients with clopidogrel resistance and/or aspirin resistance.

In the present study, we are establishing a prospective disease cohort study to investigate the effect of precise anti-platelet drug treatment based on CYP2C19 genotyping and 11-dhTxB2 testing in patients with ischemic stroke/TIA and build an intelligent decision-making platform to provide a reference for clinical treatment.

## Methods

### Study design

This study is a multicentre, large sample, randomized controlled study with a 22 months study case recruitment and a one-year post enrollment follow-up. Written informed consent is required for all participants. The trial is registered in clinicaltrials.gov (ChiCTR1900026492). This is the first version of the protocol to be activated.

The study is being conducted at 14 comprehensive first-class hospitals in Jiangxi Province. Participation by eligible patients is voluntary and assignment into control and intervention groups is random. Patients in the control group are treated routinely according to the current clinical guidelines. Patients in the intervention group are tested for CYP2C19 polymorphisms and urine 11-dhTxB2. Finally, personalized medication is developed for intervention group patients according to the test results.

### Participants and recruitment

Inpatients are screen by a researcher in the neurology department of the participating hospital. Eligible patients are then approached in the ward by a member of the research staff, who thoroughly explains the study and consents patients who choose to participate. Patients who voluntarily participate must sign the informed consent document and are asked to leave one to two phone numbers for a one-year follow-up. Researchers then include the participant in the study cohort and fill in the visit information.

### Eligibility criteria

Patient eligibility criteria include: (1) age 18 to 85 years, (2) diagnosis of ischemic stroke (atherosclerotic cerebral infarction) or transient ischemic attack (diagnosis meets the criteria established by the Cerebrovascular Diseases Group of the Chinese Medical Association Neurology Society in 2014)<sup>[25]</sup>, (3) acute period of onset (2 weeks post event), (4) NIHSS score  $\leq$  15 points, (5) voluntarily participation and informed consent.

Patient exclusion criteria include: (1) cancer, (2) cardiac infarction, cerebral infarction with small vessel disease, other causes of cerebral infarction or unexplained cerebral infarction, (3) active stomach disease, (4) Alteplase thrombolytic usage, as well as some cases requiring anticoagulation or dual antiplatelet

(PCI), (5) hemorrhagic stroke, mixed stroke, or tumor stroke, (6) whole blood or platelet transfusion in the past two weeks, coagulopathy or other blood diseases, (7) renal dysfunction (24-hour urine volume <1000ml, serum creatinine >180MMOL/L, urine creatinine <5mmol/L), (8) are combined with serious heart, lung and liver system diseases, (9) poor adherence or inability to complete long-term follow-up, (10) current involvement in any clinical trial of research drugs or medical devices (Figure 1).

Termination of research standards are (1) misdiagnosed or misrepresented cases that do not meet the inclusion criteria and meet the exclusion criteria, (2) patients who voluntarily withdraw from the study, (3) the investigator determines that an effective clinical observer cannot be continued for medical or other reasons, (4) patients whose test results are double resistance (clopidogrel resistance and aspirin resistance), (5) those who switched to other antiplatelet drugs and stopped taking drugs in the middle of the study, or switched to anticoagulants in the middle.

## **Randomization**

Once eligible patients sign informed consent form voluntarily researchers then register each patient in the queue and perform simple randomization. The proportion of control group to intervention group is 1:1.

## **Participant timeline**

During the study period, patients who meet the criteria are required to voluntarily sign the informed consent document in order to participate. Participants are randomly assigned into the control group or the intervention group by the researchers. Baseline data such as BMI, NIHSS, mRS, imaging examination, and laboratory test results is collected during hospitalization. When discharged the NIHSS and mRS scores for each participant is assessed and recorded in addition to individualized medications and medical expenses. All patients are asked to attend follow-up visits at the first, third, sixth, and twelfth month post discharge and to report medication adherence and prognosis (including stroke recurrence, adverse events, neurological impairment, etc.). A participant timeline of the study is detailed in Table 1.

## **CYP2C19 Genotyping and 11-dhTxB2 testing**

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) [26-29], will be genotyped in 3000 participants. Genotyping of the 3 SNPs is performed using the PCR+MassARRAY (flight mass spectrometry). Individuals with complete information for each of the 3 SNPs will be included in the current analyses. Patients are categorized by CYP2C19 metabolizer status based on \*2, \*3, and \*17 genotypes using the common consensus star allele nomenclature.

Patients with at least two \*2 or \*3 alleles (\*2/\*2, \*2/\*3, or \*3/\*3) are classified as poor metabolizers, those with one \*2 or \*3 allele (\*1/\*2 or \*1/\*3) are classified as intermediate metabolizers, and those without a \*2, \*3, or \*17 allele (\*1/\*1) are classified as extensive metabolizers. Individuals carrying at least

one \*17 allele (\*1/\*17 or \*17/\*17) are classified as ultra-metabolizers. Because the clinical consequences of carrying both \*17 and one loss-of function allele (\*2 or \*3) still remain unclear, these individuals (\*2/\*17 or \*3/\*17) are classified as unknown metabolizers<sup>[30,31]</sup>. Those with at least 1 loss-of-function allele (\*2 or \*3) are classified as loss-of-function allele carriers and those with at least 1 gain-of-function allele (\*17) are classified as gain-of-function allele carriers<sup>[32]</sup>.

In addition, aspirin is widely used in secondary prevention of stroke because it inhibits cyclooxygenase (cox) and thus reduces thromboxane A (TXA<sub>2</sub>) production. Urine 11-dhTxB<sub>2</sub> is a stable metabolite of TXA<sub>2</sub>, which reflects the activation state of platelets in the body and the function of aspirin. In this study, aspirin resistance is tested for using 11-dhTxB<sub>2</sub> concentration in urine with 11-dhTxB<sub>2</sub>/urinary creatinine  $\geq 1500$  pg/mg used to characterize the presence of aspirin resistance<sup>[34]</sup>. Once available, results from the genetic and urine testing are used to develop individualized medication options.

## **Treatments**

In 1-8th day, patients in the experimental group are taken 3-5 ml whole blood samples and 3-10 ml urine samples for CYP2C19 genotype and 11-dhTxB<sub>2</sub> test, the results are usually obtained within 7-9th day. After testing, doctors adjust the anti-platelet medication according to the results. If the patient is only resistant aspirin, then only clopidogrel is prescribed; if the patient is only resistant to clopidogrel, then only aspirin is prescribed. If the patient displays no resistance to aspirin and clopidogrel, we will not change the patient's treatment plan. If the patient has dual resistance to clopidogrel and aspirin, we do not make medication interventions due to the risk of bleeding, and only recommend that the doctor increase the dose or use substitutive drugs. Patients in the control group do not receive anti-platelet testing. There are no blinding in this study. The harm in this study is to extract a small amount of blood samples from patients. If adverse events occur during follow-up, researchers will record, report, and dispose them.

## **Endpoints**

The primary outcome of interest is stroke recurrence. Secondary outcomes include disabilities, bleeding events, other adverse events, and all-cause mortality.

## **Clinical assessment**

Baseline assessment includes: (1) demographics (age, sex, nation, address, vital sign, average annual income, educational level, work, medical payment method); (2) risk factors (smoking, alcohol, hypertension, diabetes, heart diseases, hyperlipemia, dementia, hypohepatia, renal insufficiency, obesity, stroke/malignancy/trauma/operation history, r-tPA intravenous thrombolysis); (3) family/personal medical history; (4) medications; (5) laboratory findings (blood, urine, biomarkers); (6) physiological tests (BMI, blood pressure, pulse, respiratory rate, cardioechography); (7) brain imaging (MRI, CT, CTA/MRA/DSA); (8) comprehensive neurological assessment (TOAST subtypes, NIHSS, mRS, GCS); (9) symptoms and duration at admission.

## Sample size

In Wang et al.'s study population 58.8% of participants were carriers of CYP2C19 loss-of-function alleles. Stroke recurrence in Non-carriers only : 6.7% in treatment (clopidogrel plus aspirin) vs. 12.4% control (aspirin only). Based on above results, 417 cases are needed for each group, for a total of 834 cases. We assume a 15% loss to follow-up rate and a design effect of 1.25<sup>[34]</sup>, therefore our final calculated sample size is 1199, approximately 1200. Our goal is to recruit 1,200 to 6,000 patients.

## Data collection and management

We have designed the data collection table on the Electronic Data Capture (EDC) platform according to the project requirements, and clarified the research process, the data table name, the collected data items, and formed the corresponding data collection guide. Finally, eCRF is constructed based on the research of medical records. Once all participants complete the trial, all medical records will be entered into the system. After the primary investigator, sponsor, statistical analyst, and data stewards confirm that the data in the database are correct, the data stewards will check the data integrity. Finally, all the data will be locked, the data administrator will import it into the specified database and send it to the statisticians for statistical analysis. The locked data can no longer be edited, and the problems found after the data is locked can be corrected in the statistical analysis program after confirmation. After the trial is over, the data administrator will file an EDC closure request and cancel all account access rights after obtaining the permission of the sponsor. Finally, when the data is completely backed up, the EDC will be turned off.

## Statistical analysis

We will describe general characteristics of the study population using the mean, median, standard deviation (SD), range and interquartile range (IQR) for continuous and ordinal data, and counts or percentages for categorical data. The normality of variable distribution will be assessed prior to data presentation. For tests of independence the Pearson's  $\chi^2$  test will be used for categorical variables, and for the analysis of variance and Mann-Whitney U test will be used for numeric variables, as appropriate. Multiple imputation will be used to impute missing values, under a missing at random assumption, so as to reduce bias and avoid excluding participants from the analysis. Comparison of endpoint incidence between intervention and control groups will be assessed by risk rate (RR). Multivariate Cox regression analysis will be used for the time-to-event data with adjustment of known confounders. In this study, the estimates of missing values for the primary variables are carried-forward to the absence of test data using the results of the closest observation. All statistical tests will be two-sided and  $P < 0.05$  is considered statistically significant. All of the above statistical analysis will use Statistical Analysis System (SAS Institute, Inc., Cary, NC) V.9.3.

## Quality control measures

This study consists of sponsor commissions and contract research organization to complete the audit work, to ensure that the rights and interests of the subjects in the clinical trials are guaranteed, the

recorded data are accurate and complete, and to ensure the test follows the approved program, "Quality Management Standards for Drug Clinical Trials"<sup>[35]</sup> and related regulations.

If the program has problems during the actual implementation of the clinical trial, the program needs to be revised and will be presented to the sponsor. After consultation and discussion by the multi-center coordination committee, the clinical research unit will revise the plan, then submit to the sponsor and the participating units for signature approval. Finally, it is submitted to the ethics committee for approval and implementation. If important new information related to the test drug is found, the informed consent form must be modified in writing and sent to the ethics committee for approval.

## **Objectives**

In order to verify the effect of genetically tailored medication based on CYP2C19 genotyping and 11-dhTxB2 testing, our study is establishing a prospective cohort study of 1200-6000 patients to collecting data and exploring potential risk factors of neurological impairment. Finally, we will establish individualized treatment standards for stroke/TIA patients, and build an intelligent decision-making platform to provide a reference for clinical treatment.

# **Ethics And Dissemination**

## **Ethics and informed consent**

Medical Research Ethics Committee in the Second Affiliated Hospital of Nanchang University approved the final agreement. The study is conducted in strict accordance with the relevant regulations and the requirements of the committee. All patients are voluntarily sign the informed consent form approved by the committee, indicating that they agree to participate. Consent includes the nature, objectives, and potential benefits, risks, and consequences of the study. In addition, the consent details the research background, the required follow-up time, privacy issues, and the patient's right to withdraw from the study at any time during the treatment period. All collected data will remain private and will not reveal any information about the patient.

## **Dissemination**

The study findings will be disseminated in national and international conferences, and peer-reviewed publications.

# **Discussion**

Stroke is the second most common cause of death, and the leading cause of long-term disability worldwide<sup>[36]</sup>. Clopidogrel and aspirin are routine drugs for the treatment of ischemic stroke and TIA patients<sup>[37]</sup> however a large number of patients have a genetic predisposition to clopidogrel resistance and/or aspirin resistance<sup>[38,39]</sup>. The effects of individualized medication based on CYP2C19 genetic test

and 11-dhTxB2 test is still unknown. Currently, there is a lack of large-scale study for the evaluation of the clinically accurate dual antiplatelet therapy standards based on genetic and drug metabolite tests.

The CYP2C19 genetic test is expensive and not widely available in Chinese hospitals. Our study collects economic information such as family annual income, hospitalization expenses, and drug costs for the enrolled patients. Using this information, we will conduct economic analysis to evaluate the long-term benefits of CYP2C19 genetic/11-dhTxB2 testing for IS/TIA patients, to provide evidence for general genetic/11-dhTxB2 test.

Our proposed study has several strengths. First, this is the first study to assess the effect of individualized medication based on the CYP2C19 genetic test and 11-dhTxB2 test for IS/TIA patients. Additionally, this is a multicentre prospective cohort study with multilateral evaluations and analyses of gene polymorphism, and prognosis of IS/TIA.

There are several potential limitations. First, no prescribing guidelines standards for patients resistant to both clopidogrel and aspirin currently exist. Since forcibly increasing antiplatelet dosage may lead to a higher risk of bleeding this study did not adjust the medication for this group of patients, but only provided a reference for clinicians. Second, there is still the possibility of unrecognised confounders despite the multicentre design. Inconsistent levels of investigators and patient medication compliance in different hospitals may affect the effectiveness of personalized medicine.

Developing personalized antiplatelet medications and reducing stroke recurrence rates are pressing issues for Chinese society. We hope that our study will contribute to a better understanding of the association between personalized antiplatelet medications and stroke prognosis, and develop a standardized stratified treatment plan for IS/TIA patients.

## **Conclusions**

This study will evaluate the impact of individualized medications on stroke/TIA prognosis and provide a reference for clinical medications. The results of this study, whether positive or negative, will be of great interest to both stroke/TIA patients and clinicians.

## **Trial status**

Protocol version: V5.0, 2019/02/19. The recruitment of patients began in June 2019 and will continue until December 2020. Participants will be followed for one-year and the study will end in December 2021.

## **List Of Abbreviations**

IS: ischemic stroke; TIA: transient ischemic attack; 11-dhTxB2: 11-dehydroxetane B2; CYP2C19: cytochrome P450 2C19; mRS: Modified Rankin Scale; PM: poor metabolizers; IM: intermediate metabolizers; EM: extensive metabolizers; UM: ultra-metabolizers; NIHSS: National Institute of Health stroke scale; PCI: percutaneous coronary intervention; BMI: Body Mass Index; NCBI: National Center for

Biotechnology Information; cox: cyclooxygenase; TXA2: thromboxane A; TOAST: Trial of Org 10 172 in acute stroke treatment; EDC: Electronic Data Capture; eCRF: Electronic case report form; SD: standard deviation; IQR: interquartile range; RR: risk rate.

## **Declarations**

### **Consent for publication**

Not applicable.

### **Availability of data and materials**

Only members of the Trial Steering Committee and the supporting Research Assistant will have access to the final dataset and material. The results of the study will be disseminated through peer-reviewed publications and conference reports. After the research results are published, the data and materials will be made public.

### **Acknowledgements**

We thank Lijun Xu for his input to parts of the study design, and Lu Shi, Zhaohui Zhang, Yitong He, Linli Lu, Jianwei Luo for helping with the one-year follow-up. We would like to thank these doctors for their contributions in organisation, recruitment, data collection, sample collection and medication adjustment: Daojun Hong, Ping Fan, Dan Ding, Xiangjun Zeng, Qianxi Chen, Zhihong Huang, Xing Huang, Fangmei Chen, Zefei Lai, Ying Zeng, Yumei Li.

### **Authors' contributions**

JJW: drafting and revising the protocol. YPY, JK: principle investigator and contributed to the concept and design of the protocol. JLT, SK, YQG, CP, SJY, XLZ and JBC: critical revising of the protocol. YPY, JK, JLT: overseeing and auditing the trial.

### **Funding**

This work is supported by the National Key R&D Program of China (grant numbers 2018YFC1312902). Yingping Yi and her team are responsible for study design, data collection, analysis, interpretation, and manuscript writing.

### **Competing interests**

On behalf of all authors, the corresponding author states that there is no conflict of interest.

### **Ethics approval and consent**

This study received ethical approval from the Medical Ethics Committee of the Second Affiliated Hospital of Nanchang University: (2018) Medical Research Review No.05. Informed consent is obtained from all

study participants. All patients are voluntarily sign the informed consent form, indicating that they agree to participate.

## **Confidentiality**

All researchers have been signing a confidentiality agreement with the project responsible unit to keep the relevant documents confidential and not leaked. Data collection is conducted by designated investigators, and data will not be leaked or shared during the study. After the study is over, the researcher needs to keep all relevant documents. Includes subjects' records, original informed consent, original documents, eCRF's PDF electronic documents, ethics committees, and research-related materials between researchers. The research unit is required to keep the above materials for at least 5 years after the end of the clinical trial.

## **Peer review and dissemination policy**

Not commissioned; externally peer reviewed. After the publication of research results such as articles and patents, research data other than patient privacy can be disseminated through inational and international conferences, and peer-reviewed publications.

## **References**

- [1] Benjamin EJ, Blaha MJ, Chiuve SE, Cushman Mary, Das Sandeep R, Deo Rajat, et al, American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics –2017 update: a report from the American Heart Association. *Circulation* 135(10): e146-e603.
- [2] GBD 2016 Stroke Collaborators. Global, regional, and national burden of stroke, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *ARTICLES*. 2019 MAY; 18(5): P439-458.
- [3] Zhao D, Liu J, Wang W, et al. Epidemiological transition of stroke in China: 21-year observational study from the Sino-MONICA-Beijing Project. *Stroke*. 2008;39(6):1668-1674.
- [4] Powers WJ, Rabinstein AA, Ackerson T, et al. 2018 guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/- American Stroke Association. *Stroke* 2018;49:e46-e110.
- [5] 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.
- [6] Xu J, Wang A, Wangqin R, Mo J, Chen Z, Dai L, Meng X, Zhao X, Wang Y, Li H4, Chen W, Xian Y, Wang Y, CHANCE investigators. Efficacy of clopidogrel for stroke depends on CYP2C19 genotype and risk profile. *Ann Neurol*. 2019 Sep;86(3):419-426

- [7]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(7):2160-2236.
- [8]Zhao D, Liu J, Wang W, Zeng Z, Cheng J, Liu J, Sun J, Wu Z, Epidemiological transition of stroke in China: twenty-one-year observational study from the Sino-MONICA-Beijing Project. *Stroke*. 39 (2008) 1668-1674.
- [9]Yi X, Han Z, Zhou Q, Lin J, Liu P (2016) 20-hydroxyeicosatetraenoic acid as a predictor of neurological deterioration in acute minor ischemic stroke. *Stroke*. 47:3045-3047.
- [10]Spokoyny I, Barazangi N, Jaramillo V, Rose J, Chen C, Wong C, Tong D, Reduced clopidogrel metabolism in a multiethnic population: prevalence and rates of recurrent cerebrovascular events. *J Stroke Cerebrovasc Dis*. 23 (2014) 694-698.
- [11]Hoh BL, Gong Y, McDonough CW, Waters MF, Royster AJ, Sheehan TO, Burkley B, Langaee TY, Mocco J, Zuckerman SL, Mummareddy N, Stephens ML, Ingram C, Shaffer CM, Denny JC, Brilliant MH, Kitchner TE, Linneman JG, Roden DM, Johnson JA, CYP2C19 and CES1 polymorphisms and efficacy of clopidogrel and aspirin dual antiplatelet therapy in patients with symptomatic intracranial atherosclerotic disease. *J Neurosurg*. 124 (2016) 1746-1751.
- [12]Wang Yilong, Zhao Xingquan, Lin Jinxi, Li Hao, Johnston S. Claiborne, Lin Yi, Pan Yuesong, Liu Liping, Wang David, Wang Chunxue, Meng Xia, Xu Jianfeng, Wang Yongjun, 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*. 316(1):70-78.
- [13]Kwon HM, Lee YS, Bae HJ, Kang DW, Homocysteine as a predictor of early neurological deterioration in acute ischemic stroke. *Stroke* 45:871-873.
- [14]Yi X, Lin J, Wang Y, Zhou Q, Wang C, Cheng W, Chi L, Association of cytochrome P450 genetic variants with clopidogrel resistance and outcomes in acute ischemic stroke. *J Atheroscler Thromb*. 23 (2016) 1188-1200.
- [15]Uematsu T, Takasaki W, Kosuge K, Wada K, Matsuno H, Tanaka Y, Yamamura N, Nakashima M. Changes in plasma and urinary 11-dehydrothromboxane B2 in healthy subjects produced by oral CS-518, a novel thromboxane synthase inhibitor. *Journal of Clinical Pharmacology*. 1993;45(3):283-6.
- [16]Fang L, Zhao Y, Wang N, Yang Z, Huang H, Lin M, Association of CYP2C19 gene polymorphisms with long-term recurrent risk of ischemic stroke among ethnic Han Chinese from Fujian. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. 32 (2015) 871-876.
- [17]Zhu WY, Zhao T, Xiong XY, Li J, Wang L, Zhou Y, Gong ZL, Cheng SY, Liu Y, Shuai J, Yang QW, Association of CYP2C19 polymorphisms with the clinical efficacy of clopidogrel therapy in patients

undergoing carotid artery stenting in Asia. *Sci Rep.* 3 (2016) 6-25478.

[18]Lin J, Han Z, Wang C, Yi X, Chai Z, Zhou Q, Huang R, Dual therapy with clopidogrel and aspirin prevents early neurological deterioration in ischemic stroke patients carrying CYP2C19 \*2 reduced-function alleles. *Eur J Clin Pharmacol.* 74 (2018)1131-1140.

[19] Lin Weibin, Tang Lingzhi, Liu Xiaoxia, Liu Shan, Peng Haiying, Peng Lingling. Status and Prospect of Aspirin Resistance [J]. *Medical Herald*, 2015, 34 (12): 1618-1622.

[20]Xu K, Ye S, Zhang S, et al. Impact of Platelet Endothelial Aggregation Receptor-1 Genotypes on Platelet Reactivity and Early Cardiovascular Outcomes in Patients Undergoing Percutaneous Coronary Intervention and Treated With Aspirin and Clopidogrel. *Circulation: Cardiovascular Interventions.* 2019; 12:e007019

[21]Wang Yongjun, Wang Yilong, Zhao Xingquan, Liu Liping, Wang David, Wang Chunxue, Wang Chen, Li Hao, Meng Xia, Cui Liying, Jia Jianping, Dong Qiang, Xu Anding, Zeng Jinsheng, Li Yansheng, Wang Zhimin, Xia Haiqin, Johnston S. Claiborne. CHANCE Investigators. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. *N Engl J Med* 369(1): 11-19.

[22]Lewis JP, Backman JD, Reny JL, et al. Pharmacogenomic Polygenic Response Score Predicts Ischemic Events and Cardiovascular Mortality in Clopidogrel-Treated Patients. *Eur Heart J Cardiovasc Pharmacother.* 2019 Sep 3.

[23]Xu J, Wang A, Wangqin R, Mo J, Chen Z, Dai L, Meng X, Zhao X, Wang Y, Li H, Chen W, Xian Y, Wang Y, Efficacy of clopidogrel for stroke depends on CYP2C19 genotype and risk profile. *Ann Neurol.* 2019 Sep;86(3):419-426.

[24]Wang T, Pan Y, Lin J, Anand R, Wang D, Johnston SC, Meng X, Li H, Zhao X, Liu L, Wang Y, Wang Y, Influence of smoking on CYP2C19 genetic variants and clopidogrel efficacy in patients with minorstroke or transient ischaemic attack. *Eur J Neurol.* 2019 Sep;26(9):1175-1182.

[25]Chinese Medical Association Neurology Branch, Chinese Medical Association Neurology Branch Cerebrovascular Disease Group. Guidelines for the diagnosis and treatment of acute ischemic stroke in China 2014. *Chinese Journal of Neurology*, 2015, 48(4):246-257.

[26]Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvallé C, Aiach M, Lechat P, Gaussem P, Cytochrome P4502C19 loss-of-function polymorphisms is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood.* 108 (2006) 2244-2247.

[27]Kitzmiller JP, Groen DK, Phelps MA, Sadee W, Pharmacogenomic testing: relevance in medical practice: why drugs work in some patients but not in others. *Cleve Clin J Med.* 78 (2011) 243-257.

[28]Wang Y, Cai H, Zhou G, Zhang Z, Liu X, Effect of CYP2C19\*2 and \*3 on clinical outcome in ischemic stroke patients treated with clopidogrel. *Journal of the Neurological Sciences.* 369 (2016) 216-219.

- [29]Chen YB, Zhou ZY, Li GM, Xiao GX, Yu WB, Zhong SL, Cai YF, Jin J, Huang M, Influences of an NR1I2 polymorphisms on heterogeneous antiplatelet reactivity responses to clopidogrel and clinical outcomes in acute ischemic stroke patients. *Acta Pharmacologica Sinica*. 40 (2019) 762-768.
- [30]Yi X, Zhou Q, Wang C, Lin J, Cheng W, Chi L, Concomitant Use of Proton Pump Inhibitors and Clopidogrel Is Not Associated with Adverse Outcomes after Ischemic Stroke in Chinese Population. *Stroke Cerebrovasc Dis*. 25 (2016) 2859-2867.
- [31]K. Fukuma, H. Yamagami, K. Kamiyama, Association Between CYP2C19 Genetic Polymorphisms and Clinical Outcome in Acute Atherothrombotic Stroke: A Sub-Analysis of The Praise Study. *European Stroke Journal*. 2(IS) 98-476:AS02-022 (2017).
- [32]Jeong TD, Kim SM, Kim HJ, Lee W, Kwon SU, Min WK, Kang DW, Chun S, CYP2C19 genotype and early ischemic lesion recurrence in stroke patients treated with clopidogrel. *J Stroke Cerebrovasc Dis*. 24 (2015) 440-446.
- [33]Takasaki W, Nakagawa A, Tanaka Y, Nakamura K, Shindo H, Hayashi Y, Yamamoto S. Enzyme immunoassay of human plasma 11-dehydrothromboxane B2. *Thrombosis Research*. 1991 Aug 1;63(3):331-41.
- [34]Luo Wei. Study on Sample Allocation Based on Hierarchical Design Effect [J]. *Statistics and Decision*, 2017 (16): 20-23.
- [35]Zhao Yingpan, Lu Fang, Yan Mingjie, et al. Interpretation of the "Quality Management Standards for Drug Clinical Trials" (Revised Draft). *Chinese Journal of New Drugs* (15):75-77+113.
- [36]GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the global burden of disease study 2015. *Lancet*. 2016;388:1459–1544.
- [37]Sun W, Li Y, Li J, Zhang Z, Zhu W, Liu W, Cai Q, Wang X, Cao L, Bai W, Fan X, Ma M, Guo R, Variant recurrent risk among stroke patients with different CYP2C19 phenotypes and treated with clopidogrel. *Platelets* 26(6):558-562.
- [38]Kwon HM, Lee YS, Bae HJ, Kang DW, Homocysteine as a predictor of early neurological deterioration in acute ischemic stroke. *Stroke* 45:871-873.
- [39]Vahidy FS, Hicks WJ, Acosta I, Halleivi H, Peng H, Pandurengan R, Gonzales NR, Barreto AD, MartinSchild S, Wu TC, Rahbar MH, Bambhroliya AB, Grotta JC, Savitz SI, Neurofluctuation in patients with subcortical ischemic stroke. *Neurology* 83:398-405.

## Table

**Table 1. Schedule of assessments for the strategy to obtain CYP2C19/11-dhTxB2 testing's benefit by evaluating registry study**

Study period	Baseline assessment		Follow-up	Follow-up complete/early termination		
Visit	V1	V2	V3	V4	V5	V6
Timing	1-7d	8d(±2d)	1m(±7d)	3m(±7d)	6m(±7d)	12m(±7d)
Informed consent	●					
Screening/eligibility	●					
Basic informations*	●					
Laboratory tests**	●	●				
Physiological tests***	●	●				
Brain imagings****	●	●	●	●	●	●
NIHSS	●	●				
mRS	●	●	●	●	●	●
GCS	●					
CYP2C19 genetic test	●	●	●	●	●	
11-dhTxB2 test	●	●	●	●	●	
Medications	●	●	●	●	●	●
Clinical outcomes/endpoints†			●	●	●	●

● : required, ●: optional.

\*Basic information include: demographics, risk factors, family/personal medical history, symptoms and duration at admission, etc.

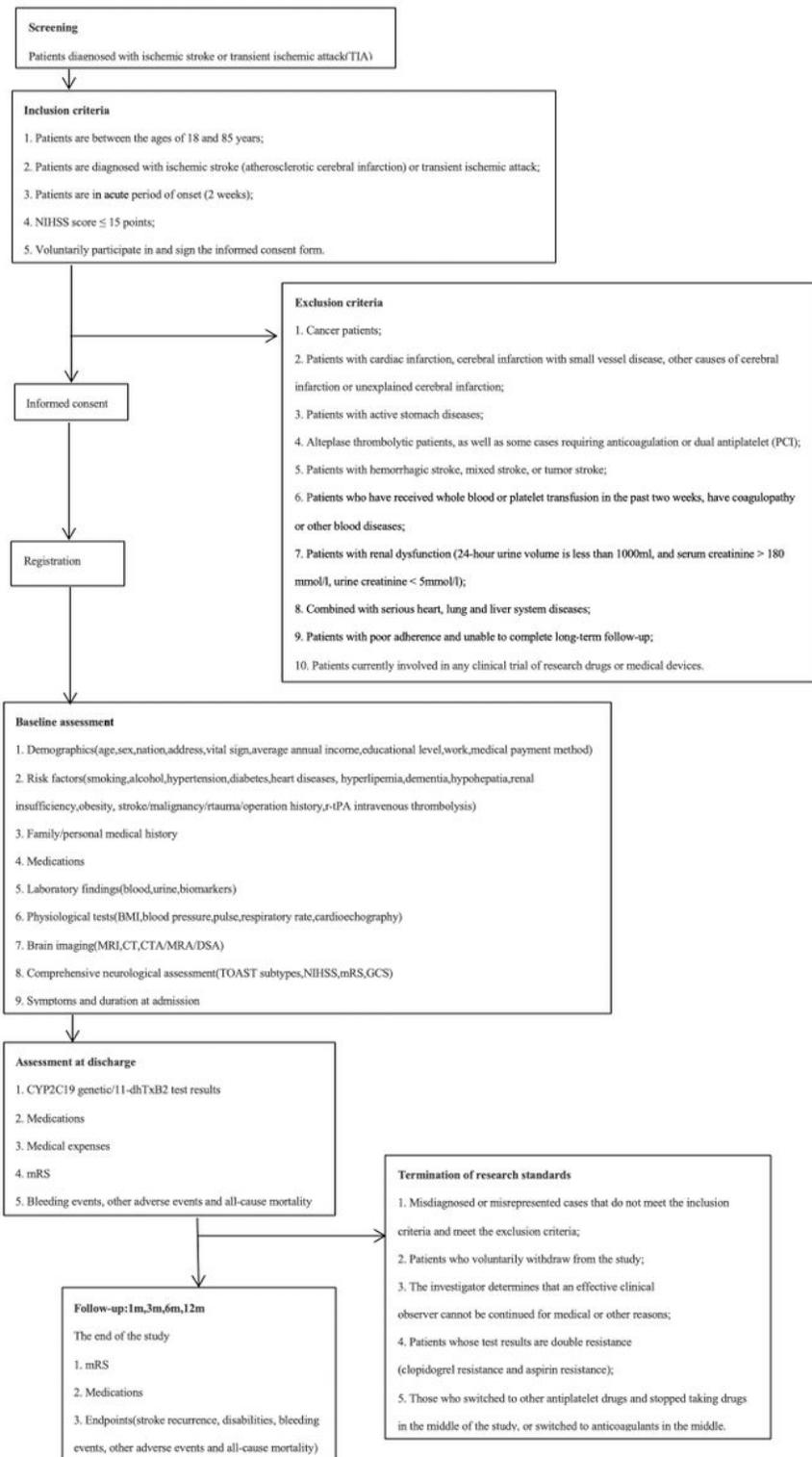
\*\*Laboratory tests include: blood, urine, biomarkers, etc.

\*\*\*Physiological tests include: BMI, blood pressure, pulse, respiratory rate, cardioechography.

\*\*\*\*Brain imaging include: MRI,CT,CTA/MRA/DSA.

†Clinical outcomes/endpoints include: stroke recurrence, safety/fatal bleeding events, adverse events, neurological impairment and all-cause mortality.

## Figures



**Figure 1**

Flow chart of the study design. NIHSS, National Institutes of Health Stroke Scale; mRS, Modified Rankin Scale; r-tPA, recombinant tissue plasminogen activator; BMI, body mass index; MRI, magnetic resonance imaging; CT, computed tomography; CTA, CT angiography; MRA, magnetic resonance angiography; DSA, digital subtraction angiography; TOAST, trial of org 10172 in acute stroke treatment; GCS, Glasgow Coma Scale; m, month.