Clinical Research Protocol

A Multicenter Cohort Study on Precision Antithrombotic Therapy

Applying Institution: Xiangya Third Hospital, Central South University

Lead Unit: Xiangya Third Hospital, Central South University

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Catalogue

Research Plan Summary 3
Research Body 5
1. Research Background 5
2. Purpose of Research 7
3. Research Design 7
4. Research Contents 8
5. Main Evaluation Indicators 12
6. Security Consideration 13
7. Statistical Analysis 14
8. Quality Control 14
9. Explanation of the Format for Releasing Research Findings 14
10. Ethical Statement 14
11. References 15

Research Plan Summary

Research topic：

A multicenter cohort study on precision antithrombotic therapy

Purpose of research：

A systematic analysis of the relationship between trough/peak concentrations and clinical events for novel oral anticoagulants and antiplatelet agents aims to provide high-level evidence-based medical data for therapeutic drug monitoring (TDM) and individualized dosing.

Research design：

This study is a prospective observational study aimed at determining the threshold ranges of trough concentration (C_{trough}) and peak concentration (C_{max}) for NOACs (rivaroxaban, apixaban, dabigatran etexilate, edoxaban) in the prevention and treatment of thrombotic diseases and ischemic stroke patients receiving antiplatelet agents (aspirin, indobutafen).

Research Process:

Determine the threshold ranges for trough concentrations (C_{trough}) and peak concentrations (C_{max}) of NOACs for the prevention and treatment of thrombotic diseases, as well as for antiplatelet agents in patients with ischemic stroke.

（1）Explore the optimal preventive and therapeutic concentration ranges for different NOACs and antiplatelet agents.

（2）Analyze the key factors influencing plasma drug concentrations of NOACs and antiplatelet agents (such as liver and kidney function, age, body weight, and concomitant medications).

（3）Establish a model to predict plasma concentrations of NOACs and antiplatelet agents and their associated clinical events.

Study subjects and inclusion/exclusion criteria:

（1）Patients requiring NOACs for the prevention and treatment of thrombotic diseases

Inclusion criteria:① Age ≥ 8 years; ② Diagnosed with one of the following conditions requiring long-term anticoagulation therapy: non-valvular atrial fibrillation (CHA2DS2-VA score ≥ 1); venous thromboembolism (deep vein thrombosis or pulmonary embolism); or other indications for NOAC use in thrombotic disease prevention or treatment; ③ Currently receiving NOAC therapy (rivaroxaban, apixaban, dabigatran etexilate, or edoxaban); ④ Willing and able to comply with the study protocol requirements and follow-up schedule; ⑤ Signed an informed consent form.

Exclusion criteria:① Contraindications to NOAC use or allergy to NOACs; ② Already enrolled in other clinical trials.

（2）Ischemic stroke patients requiring monitoring of antiplatelet drug plasma concentrations

Inclusion criteria:① Age ≥ 18 years; ② Classified as having high bleeding risk within 72 hours after symptom onset; ③ Receiving first treatment with indobutafen upon hospital admission; ④ Willing and able to comply with the study protocol requirements and follow-up schedule; ⑤ Signing an informed consent form.

Exclusion criteria:① Allergy or contraindication to indobutafen; ② Need for change of antithrombotic medication or concomitant antiplatelet/anticoagulant therapy (e.g., clopidogrel, ticagrelor, warfarin, heparin) during the study period; ③ Cardiogenic stroke or stroke induced by angioplasty/vascular surgery; ④ History of aneurysm (intracranial or peripheral); ⑤ Severe hepatic or renal dysfunction; ⑥ Pregnancy, lactation, or women of childbearing age unwilling to use effective contraceptive measures; ⑦anticipated life span <1 year; ⑧ Mental or emotional disorders preventing participation in follow-up; ⑨ Participation in other clinical trials within 30 days prior to enrollment; ⑩ Inability to complete blood concentration monitoring (e.g., difficult intravenous access).

Statistical method：

(1) Descriptive statistics: Measurement data were expressed as mean ± standard deviation or median (interquartile range), while categorical data were presented as frequency (percentage). Baseline data comparisons were performed using the chi-square test or Fisher's exact test (for categorical variables) and the independent samples t-test or Wilcoxon rank-sum test (for continuous variables).

(2) Concentration-Event Association Analysis: The incidence of endpoint events (ischemic/hemorrhagic events, major adverse cardiovascular events/MACE) was assessed using the paired chi-square test or the Cochran Q test. Independent risk factors for endpoint events were analyzed using a multivariate logistic regression model (including covariates such as age, sex, and baseline blood pressure). The Cox proportional hazards regression model was employed to evaluate the independent association between plasma drug concentrations (as continuous or categorical variables) and clinical endpoint events, after adjusting for potential confounding factors (e.g., age, sex, renal function, comorbidities), calculating hazard ratios (HR) and their 95% confidence intervals (CI). A receiver operating characteristic (ROC) curve analysis was used to determine the optimal plasma concentration thresholds (Ctrough and Cmax) for predicting clinical endpoints. Missing data were handled using multiple imputation (when the missing rate was <5%). All tests were conducted as two-sided analyses with an alpha level of 0.05; a $P<0.05$ was considered statistically significant.

Research Body

1. Research Background

1.1 Thrombotic diseases are characterized by high incidence, high disability rates, high mortality, and significant economic burdens. Novel oral anticoagulants (NOACs), with their superior efficacy and lower bleeding risks, have represented a major breakthrough in the clinical management of thrombotic diseases.

Thrombotic diseases refer to disorders caused by abnormal blood coagulation or intravascular thrombus formation, including thromboembolic diseases and ischemic stroke. The hallmark of these conditions is abnormal blood clotting within blood vessels, leading to thrombus formation that obstructs vascular patency, disrupts normal blood flow, and results in tissue hypoxia and injury. Common thrombotic diseases include deep vein thrombosis (DVT), pulmonary embolism (PE), ischemic stroke, and embolisms triggered by atrial fibrillation. With socioeconomic development, population aging, and accelerated urbanization, unhealthy lifestyle habits have become increasingly prevalent, driving a year-on-year rise in the incidence of thromboembolic diseases[1]. These conditions are characterized by high prevalence, disability rates, and mortality—increasing patients' mortality risk by 5–6-fold and elevating total healthcare costs by 60%, thereby becoming a major constraint on improving healthy life expectancy. While traditional anticoagulation therapy remains effective to some extent, its complex management and elevated bleeding risks pose clinical challenges. The advent of novel oral anticoagulants (NOACs) has transformed this landscape; their superior pharmacokinetic profiles and lower bleeding risks have established them as a pivotal treatment option for thrombotic diseases. NOACs not only enhance medication adherence but also demonstrate significant advantages in both prevention and management of thrombotic conditions.

1.2 Ischemic Stroke Is the Leading Cause of Mortality and Disability in China. Indobutafen Serves as a Potential Alternative Drug for Secondary Prevention of Ischemic Stroke With High Bleeding Risk.

Ischemic stroke refers to a clinical syndrome caused by sudden occlusion or narrowing of cerebral blood vessels, leading to interruption of local cerebral tissue blood flow and subsequent hypoxia, injury, or even necrosis. Stroke is the leading cause of death and acquired disability among adults in China, with approximately 2.3 million stroke-related deaths annually—accounting for over 20% of total fatalities—and ranking as the primary cause of mortality. Among current stroke patients, about 75% suffer varying degrees of disability, including 40% with severe disability[2]. Ischemic stroke is primarily caused by atherosclerotic plaques or vascular stenosis; antiplatelet therapy reduces the risk of thrombus formation following plaque rupture, making it the cornerstone of secondary prevention for ischemic stroke[3]. Guidelines worldwide universally recommend aspirin as the standard treatment for secondary prevention of ischemic stroke, serving as the foundational agent in antiplatelet therapy [3,4]. Aspirin inhibits thromboxane A2 production by irreversibly suppressing COX-1, thereby exerting its antiplatelet effect while simultaneously suppressing Prostaglandin

synthesis in the gastric mucosa disrupts the protective barrier of the gastric mucosa, leading to erosion and ulcer formation, with a significant risk of gastrointestinal bleeding[5]. Although antiplatelet therapy has demonstrated remarkable efficacy in reducing the risk of stroke recurrence, hemorrhagic complications remain a major clinical challenge.

Indobufen is a novel reversible platelet cyclooxygenase inhibitor that reduces thromboxane A2 (TXA2) production by selectively inhibiting COX-1, thereby blocking platelet aggregation[6]. Indobufen exhibits weak inhibition of prostaglandin synthesis and fewer gastrointestinal side effects, resulting in a lower bleeding risk. Given its advantages—including potent antiplatelet activity, minimal gastrointestinal adverse effects, and low bleeding risk—guidelines such as the "China Guidelines for Secondary Prevention of Ischemic Stroke and Transient Ischemic Attack 2022" and the "Guidelines for Primary Care Diagnosis and Treatment of Ischemic Stroke 2021" recommend indobufen as an alternative to aspirin for patients with aspirin intolerance or high bleeding risk[3,7].

1.3 Clarifying the Concentration-Effect Relationship Between NOACs and Antiplatelet Agents Can Reduce the Risk of Adverse Reactions in Patients With High Bleeding Risk.

NOACs and antiplatelet agents, as commonly used medications for preventing thrombotic diseases and ischemic stroke, have demonstrated significant efficacy. However, striking a balance between their therapeutic benefits and bleeding risks remains a major challenge in clinical practice. Although NOACs and antiplatelet agents carry lower bleeding risks and are easier to administer, their plasma concentrations can vary substantially among individuals, thereby affecting both efficacy and safety. Individual differences—such as renal function, age, body weight, comorbidities, and drug-drug interactions—are key factors influencing NOAC and antiplatelet drug concentrations. Studies indicate that excessively low concentrations may result in suboptimal efficacy and increased thrombosis risk, while excessively high concentrations can lead to hemorrhagic adverse events, particularly in high-risk populations like patients with renal impairment or the elderly[8,9]. Therefore, precise monitoring of NOAC and antiplatelet drug concentrations is crucial for achieving personalized treatment. By monitoring trough concentrations (C_{trough}) and peak concentrations (C_{max}), clinicians can optimize dosing regimens, maximize therapeutic outcomes, and reduce the incidence of adverse reactions such as bleeding.**Currently, the quantitative relationship between NOAC and antiplatelet drug concentrations and their efficacy and adverse effects remains incompletely understood. Accurate concentration monitoring enables tailored treatment plans and precise dosing, thereby maximizing efficacy and minimizing adverse reactions.[10]**。

Therefore, this study aims to determine, through a multicenter prospective cohort study, the threshold ranges of trough and peak plasma concentrations for NOACs (rivaroxaban, apixaban, dabigatran etexilate, edoxaban) in the prevention and treatment of thrombotic diseases, as well as for antiplatelet agents in patients with ischemic stroke. First, the optimal preventive and therapeutic concentration ranges for different NOACs and antiplatelet drugs will be investigated.Subsequently, the factors influencing the pharmacokinetic profiles of NOACs and antiplatelet agents will be analyzed.

Key factors influencing platelet-targeted drug plasma concentrations (e.g., hepatic and renal function, age, body weight, concomitant medications, etc.).**Ultimately,**this study aims to establish and validate models capable of predicting NOAC and antiplatelet drug plasma concentrations as well as their associated clinical events. The implementation of this research will help address current gaps in clinical evidence, providing robust evidence-based medical data for therapeutic drug monitoring (TDM) and personalized medication management in specific high-risk populations.

2. Purpose of Research

This study aims to systematically analyze the relationship between trough/peak concentrations of novel oral anticoagulants and antiplatelet agents and clinical events, providing high-level evidence-based medical data for therapeutic drug monitoring (TDM) and individualized dosing.

3. Research Design

This study is a **prospective observational study** aimed at determining the threshold ranges for trough concentrations (C_{trough}) and peak concentrations (C_{max}) of NOACs (rivaroxaban, apixaban, dabigatran etexilate, edoxaban) in the prevention and treatment of thrombotic diseases, as well as for antiplatelet medications in patients with ischemic stroke.

(1) Explore the optimal preventive and therapeutic concentration ranges for different NOACs and antiplatelet agents.

(2) Analyze the key factors influencing plasma drug concentrations of NOACs and antiplatelet agents (such as hepatic and renal function, age, body weight, and concomitant medications).

(3) Establish a model to predict plasma concentrations of NOACs and antiplatelet agents and their associated clinical events.

3.1 Sample Size Calculation

According to the general principles of predictive model research, the sample size must ensure that each target predictive variable corresponds to at least 10–20 events per variable (EPV). The key predictive variables planned for inclusion in this study include plasma drug concentration, age, renal function (eGFR), body mass index, and major concomitant medications (e.g., potent P-gp inhibitors/inducers), with an initial set of approximately 10–15 variables. Based on similar studies, the estimated incidence of the primary composite endpoint events (clinically relevant bleeding or thromboembolism) is 1.5%–2.5% per year. To obtain reliable model parameters and ensure robust extrapolation performance, we employed a high level of statistical rigor.

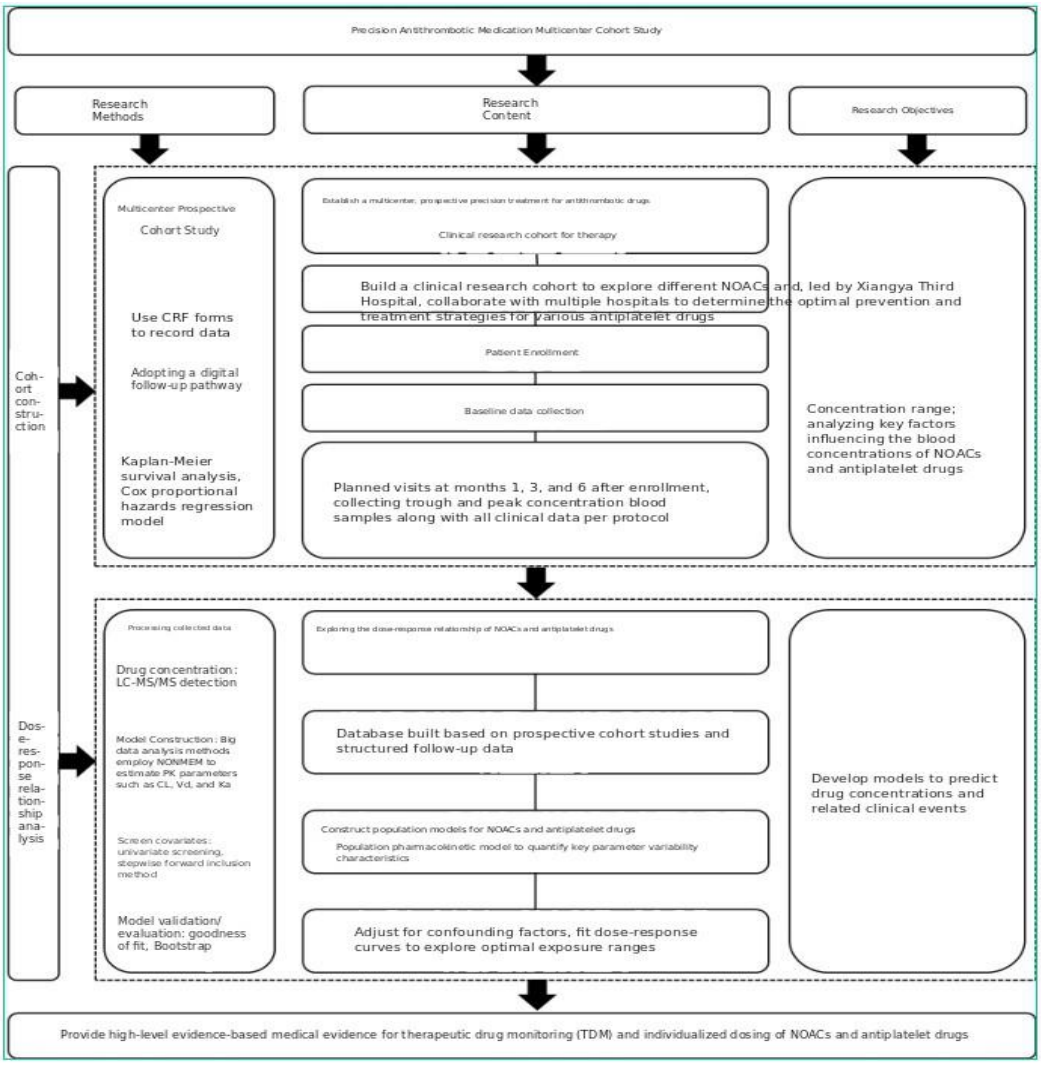
The statistical power (1-**β**) is set at 90% to reduce the risk of Type II error.

The significance level (**α**) was set at 0.05 (two-sided).

Based on the aforementioned EPV principles (with EPV = 15) and reserving approximately 30% of the sample for internal validation (e.g., the Bootstrap method), the total required number of events was calculated. The calculations indicate that each drug group should include approximately 1,000 patients to ensure sufficient statistical power for model construction at the anticipated event incidence rate. In conclusion,

To ensure that all research objectives are achieved under stringent statistical criteria and to fully account for practical considerations such as data missing values and lost-to-follow-up cases, the total planned sample size is 5,000 cases.

3.2 Tree



4. Research Contents

4.1 Research Period:

Enrollment phase: May 2026 – May 2027.

Follow-up period: May 2026 – December 2027 (for patient follow-up and data collection).

4.2 Number of Enrolled Cases:

Based on sample size calculations, this study plans to enroll at least 5,000 patients with thrombotic diseases, including approximately 4,000 patients receiving different NOACs (rivaroxaban, apixaban, dabigatran etexilate, edoxaban) and approximately 1,000 ischemic stroke patients treated with antiplatelet agents. The total sample size is estimated to be around 5,000 cases.

4.3 Study Subjects and Inclusion/exclusion Criteria:

(1) Patients Requiring NOACs for the Prevention and Treatment of Thrombotic Diseases

Inclusion criteria:① Age ≥ 8 years; ② Diagnosed with one of the following conditions requiring long-term anticoagulation therapy: non-valvular atrial fibrillation (CHA2DS2-VA score ≥ 1); venous thromboembolism (deep vein thrombosis or pulmonary embolism); or other indications for NOAC use in thrombotic disease prevention or treatment; ③ Currently receiving NOAC therapy (rivaroxaban, apixaban, dabigatran etexilate, or edoxaban); ④ Willing and able to comply with the study protocol requirements and follow-up schedule; ⑤ Signed an informed consent form.

Exclusion criteria:① Contraindications to NOAC use or allergy to NOACs; ② Already enrolled in other clinical trials.

(2) Ischemic Stroke Patients Requiring Monitoring of Ind 哚 fenacin Blood Concentration

Inclusion criteria:① Age ≥ 18 years; ② Classified as having high bleeding risk within 72 hours after symptom onset; ③ Received first treatment with indobutafen upon hospital admission; ④ Willing and able to comply with the study protocol requirements and follow-up schedule; ⑤ Signed an informed consent form.

Exclusion criteria:① Allergy or contraindication to indobutafen; ② Need for change of antithrombotic medication or concomitant antiplatelet/anticoagulant therapy (e.g., clopidogrel, ticagrelor, warfarin, heparin) during the study period; ③ Cardiogenic stroke or stroke induced by angioplasty/vascular surgery; ④ History of aneurysm (intracranial or peripheral); ⑤ Severe hepatic or renal dysfunction; ⑥ Pregnancy, lactation, or women of childbearing age unwilling to use effective contraceptive measures; ⑦ anticipated life span <1 year; ⑧ Mental or emotional disorders preventing participation in follow-up; ⑨ Participation in other clinical trials within 30 days prior to enrollment; ⑩ Inability to complete blood concentration monitoring (e. g., difficult intravenous access).

4.4 Exit/Termination Criteria:

(1) The subject voluntarily withdrew their informed consent.

(2) After obtaining informed consent and screening qualified subjects, those who did not complete the visits were considered as dropped subjects.

(3) A serious adverse event occurred during the study.

(4) Research terminated.

4.5 Stages of Research：

(1) Screening and Enrollment: Patients meeting the inclusion/exclusion criteria formally enrolled after signing the informed consent form and completed baseline data collection.

(2) Baseline assessment: Collect demographic data, medical history, medication history, physical examination, laboratory tests (complete blood count, liver and kidney function tests, coagulation function, etc.), ischemic/haemorrhagic scores, and imaging data.

(3) Treatment and Follow-up: All patients received routine clinical management. During treatment, blood samples were collected periodically to measure plasma drug concentrations (C_{trough} and C_{max}), and clinical responses and adverse events were recorded. The study established fixed follow-up visit dates (months 1, 3, and 6).

(4) Collection of endpoint events: Throughout the study period, any suspected bleeding or thromboembolic event was immediately recorded, verified, and graded.

(5) Study completion: Completion of at least 6 months of follow-up or early withdrawal.

4.6 Research Technique：

This study is a prospective, non-interventional observational study without randomization. The selection of medications and dosages for patients was determined solely by the attending physicians. The core of the study lies in systematic observation and data collection.

4.7 Follow-up Status:

(1) Follow-up time points: Planned visits were conducted at 1 month, 3 months, and 6 months after enrollment, with any clinical events recorded promptly during the period.

(2) Follow-up content:

① Clinical evaluation: Record adverse events, changes in concomitant medications, and readmissions.

② Laboratory tests: Repeat complete blood count (CBC), coagulation function, D-dimer, liver and kidney function tests, etc.

③ Blood concentration sampling: Blood samples for trough concentration (C_{trough}, within 30 minutes before the next dose) and peak concentration (C_{max}, 2–4 hours after administration, determined according to the specific pharmacokinetic characteristics of the drug) were collected at at least one visit point after steady-state dosing (e.g., month 3 or month 6).

④ End-point event determination and recording.

4.8 Biological Sample Collection and Management:

The blood samples collected in this study were **additional research-specific specimens**. The collection process was clearly explained in the informed consent forms signed by the patients and strictly adhered to ethical guidelines.

Project	**Explain**
Sample Type	Whole blood from a vein
Collection Source	Specialized blood samples will be collected from the peripheral veins of enrolled patients.

Project	Explain
Number of acquisitions and time	To achieve steady-state plasma drug concentrations, additional venous blood samples will be collected at two time points after the patient reaches steady-state following continuous administration (typically after at least five half-lives, approximately during the third or sixth month post-enrollment at the follow-up visit): Field concentration sample: Collect within 30 minutes prior to the next scheduled administration. Peak concentration samples: Collected within a specific time window after administration (determined based on the pharmacokinetic characteristics of the drug and the influence of factors such as food on plasma drug concentrations; for example, rivaroxaban, apixaban, and edoxaban are collected 2–4 hours post-administration; dabigatran is collected 1–3 hours post-administration; indobutafen is collected 2–3 hours post-administration). Supplementary/Repeat Sampling: If core sampling fails (e.g., the patient misses the time window) or a primary study endpoint event (hemorrhage or thrombosis) occurs during follow-up, a supplementary sampling will be arranged as soon as possible within 24 hours after the event (at any time point, with recording of actual medication administration time) to obtain the event-associated concentration.
Collection Volume	At each time point, collect 4–6 mL of whole blood into pre-designed anticoagulation tubes (e.g., 3.2% sodium citrate vacuum blood collection tubes). A maximum of three study-specific blood samples are planned for each patient (i.e., two core samples + one potential supplementary sample).
Acquisition and processing	Use standardized blood collection tubes (e.g., anticoagulation tubes containing sodium citrate) to collect blood samples. Centrifuge and isolate plasma within the specified time frame after collection, then aliquot and code the samples.
Storage and Transportation	The plasma samples were immediately stored in an ultra-low temperature freezer at-80 °C and transported in batches via cold chain to the central laboratory as scheduled.
Test method	LC-MS/MS (the laboratory is ISO 15189 certified; each batch contains quality control samples)

4.9 Data Sources：

（1）Clinical data: Derived from the hospital electronic medical record system, case report forms developed for research purposes, patient interviews, and follow-up records.

（2）Laboratory data: Blood drug concentration data were obtained from central laboratory reports; routine laboratory test data were sourced from the laboratory information systems of each research center.

4.10 Data Management：

（1）data logging：

This study utilized electronic case report forms (eCRFs) to record research data. The requirements for completing an eCRF are as follows:

① For all subjects who have signed the informed consent form and been enrolled, all items in the eCRF must be recorded in detail without leaving any fields blank or omitted; ② All data in the eCRF must be verified against the subjects' original medical records to ensure accuracy; ③ The eCRF serves as the original data record, signed and dated by the investigator; ④ For data significantly elevated or outside the clinically acceptable range, the investigator shall assess their clinical significance; ⑤ Specific requirements refer to the eCRF completion instructions.

（2）Create a database:

This study employed EDC for data management. The data administrator established the database in accordance with the research protocol and the eCRF, conducted logical validation checks on data validity, and granted researchers access to the system to verify data and address inquiries.

（3）Data Entry and Verification:

During clinical trials, data administrators should independently enter data into the eCRF in duplicate in real-time and perform dual verification. For any inconsistent entries, they must review and correct each item against the eCRF.

（4）Database Lock:

After data review and confirmation that the established database is correct, the data administrator, principal investigator, and statistician jointly review the data and determine the analytical set. Subsequently, the data administrator locks the database. The locked database or file generally shall not be modified thereafter.

5. Main Evaluation Indicators

The evaluation criteria of this study include primary efficacy endpoints and safety endpoints.

5.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the composite thromboembolic event occurring during medication administration, which includes any of the following:

（1）Ischemic stroke:A newly onset, acute focal neurological deficit (lasting ≥ 24 hours or resulting in death) confirmed by cranial CT or MRI to exclude hemorrhage and to demonstrate an acute infarct consistent with the clinical symptoms. Transient ischemic attack (TIA) is excluded.

（2）Systemic embolism: Acute arterial vascular occlusion occurring outside the central nervous system, leading to ischemia in the affected organ or limb, and requiring confirmation by vascular imaging (e.g., CTA, MRA, angiography) or surgical/pathological evidence.

(3) Myocardial Infarction: Diagnosis is made according to the standards of the "General Definition of Myocardial Infarction" in the fourth edition. It requires clinical evidence of myocardial necrosis (such as typical symptoms, dynamic changes in electrocardiogram) and the elevation and/or decline of cardiac biomarkers (mainly troponin), and at least one measurement value must be higher than the 99th percentile of the upper limit of the normal range.

(4) Deep vein thrombosis: Symptomatic new thrombosis in the proximal lower extremity (patellar vein and above) or visceral veins, confirmed by ultrasound, CT venography, or venography.

(5) Pulmonary embolism:A new embolism in the pulmonary artery and its branches confirmed by CT pulmonary angiography, pulmonary ventilation/perfusion scanning, or pulmonary angiography.

5.2 Safety Endpoint Indicator

The primary safety endpoint was bleeding events occurring during medication administration, classified and recorded according to the standards of the International Thrombosis and Hematology Society.

(1) Major hemorrhage: Meets any of the following criteria:

Lethal hemorrhage: Bleeding that directly causes death.

Hemorrhage in critical sites: intracranial (including brain parenchyma, ventricles, subarachnoid space, subdural/extradural space), spinal cord, intraocular, pericardial, intra-articular, intramuscular with compartment syndrome, or retroperitoneal hemorrhage.

Hemorrhage resulting in a decrease in hemoglobin of ≥2 g/dL or requiring the infusion of ≥2 units of whole blood/concentrated red blood cells (the site of bleeding is not restricted, provided that the hemoglobin decline or transfusion can be definitively attributed to other causes such as surgery or vascular puncture).

(2) Clinically Relevant Non-Major Bleeding:A Bleeding Event That Does Not Meet the Above Major Bleeding Criteria but Meets Any of the Following Conditions:

This leads to patients seeking medical care (outpatient, emergency, or hospitalization).

This leads to the suspension, interruption, or dose adjustment of the target drug.

Local hemostatic treatment (such as compression, suturing, or endoscopic hemostasis) or the use of hemostatic agents is required.

The patient or physician deems it clinically significant, causing substantial disruption to daily life (e.g., recurrent and extensive skin ecchymoses, severe epistaxis, gingival bleeding, etc.).

6. Security Consideration

The potential risks during the implementation of this study primarily include risks associated with biological sample collection, data security management, and limitations in study design. Tiered prevention and control plans have been established for each category of risk. Regarding biological sample management, in the event of sample labeling errors or contamination (low probability/medium severity), backup samples will be activated or samples will be re-collected with patient consent. For data security, information desensitization (retaining only study identifiers) and database encryption (AES-256) technologies are employed to prevent privacy breaches (low probability/severe consequences); should a breach occur Lu will

immediately suspend the account privileges and report the incident to the regulatory authorities. At the study design level, to address potential temporal bias introduced by the before-and-after control (e.g., seasonal blood pressure fluctuations), seasonal variables will be incorporated as covariates for adjustment during statistical analysis, and the robustness of the results will be validated through sensitivity analyses. Additionally, all serious adverse events (SAEs) must be reported to the ethics committee within 24 hours, and the independent Data Safety Monitoring Board (DSMB) will review the study progress quarterly. Researchers undergo annual GCP training and emergency response drills to ensure the effective implementation of risk mitigation measures, thereby maximizing the protection of participants' rights and the quality of the study.

7. Statistical Analysis

(1) Descriptive statistics: Measurement data were expressed as mean ± standard deviation or median (interquartile range), while categorical data were presented as frequency (percentage). Baseline data comparisons were performed using the chi-square test or Fisher's exact test (for categorical variables) and the independent samples t-test or Wilcoxon rank-sum test (for continuous variables).

(2) Concentration-Event Association Analysis: The incidence of endpoint events (ischemic/hemorrhagic events, major adverse cardiovascular events/MACE) was assessed using the paired chi-square test or the Cochran Q test. Independent risk factors for endpoint events were analyzed using a multivariate logistic regression model (including covariates such as age, sex, and baseline blood pressure). The Cox proportional hazards regression model was employed to evaluate the independent association between plasma drug concentrations (as continuous or categorical variables) and clinical endpoint events, after adjusting for potential confounding factors (e.g., age, sex, renal function, comorbidities), calculating hazard ratios (HR) and their 95% confidence intervals (CI). A receiver operating characteristic (ROC) curve analysis was used to determine the optimal plasma concentration thresholds (Ctrough and Cmax) for predicting clinical endpoints. Missing data were handled using multiple imputation (when the missing rate was <5%). All tests were conducted as two-sided analyses with an alpha level of 0.05; a $P<0.05$ was considered statistically significant.

8. Quality Control

Researchers shall ensure that the data are authentic, accurate, complete, and traceable, and shall guarantee the integrity of the fundamental clinical research documents throughout their retention period to prevent intentional or unintentional alterations or loss.

9. Explanation of the Format for Releasing Research Findings

The research findings will be published in the form of a clinical research report.

10. Ethical Statement

The clinical research will comply with the Declaration of Helsinki issued by the World Medical Assembly and the guidelines for biomedical research involving humans.

Relevant regulations include the "Ethical Review Measures," the "Ethical Review Measures for Life Science and Medical Research Involving Human Subjects," and the "Management Measures for Investigator-Initiated Clinical Research Conducted by Healthcare Institutions." Prior to the commencement of any study, the clinical research protocol must be approved by the Ethics Committee before implementation. During the study, the personal privacy and data confidentiality of participants will be strictly protected. I hereby pledge to comply with all scientific research standards and integrity-related regulations.

11. References

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