(Assessing Early Prognosis of Heart Failure After Acute Myocardial Infarction Using Left Ventricular Pressure-Strain Loop: a prospective randomized controlled clinical study)
Clinical study protocol

Subject source: Jiangsu Health Vocational College
Clinical Trial Group Leader Unit: Pukou District Hospital of Traditional Chinese Medicine, Nanjing
PI: Dr. Mo Yanfei, Associate Chief Physician
Department: Cardiology
Contact: Mo Yanfei

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# Summary

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| **Project name** | Assessing Early Prognosis of Heart Failure After Acute Myocardial Infarction Using Left Ventricular Pressure-Strain Loop: a prospective randomized controlled clinical study |
| **Objective** | Main objective:<br>To assess the cardiac functional status of patients with heart failure after acute myocardial infarction using the left ventricular pressure-strain loop technique in echocardiography.<br>Secondary objectives:<br>1) Analyze the impact of sacubitril/valsartan combined with dapagliflozin on cardiac function in patients with heart failure after acute myocardial infarction.<br>2) Analyze the advantages of sacubitril/valsartan combined with dapagliflozin over sacubitril/valsartan monotherapy.<br>3) Analyze the advantages of the left ventricular pressure-strain loop technique over cardiac functional indicators such as left ventricular ejection fraction and longitudinal strain.; |
| **Research Design** | Multicentre, prospective, open-label, randomised controlled trial |
| **Total number of cases** | Based on previous research, it is expected that the use of sacubitril/valsartan alongside conventional treatment will result in an approximate 26% improvement in GWI, with a standard deviation of 2 and 0.5%, $\alpha$=0.05, power=0.9. A sample size of 28 cases per group is required. Anticipating a dropout rate of 20%, this study plans to include 70 patients, with 35 in each group. |

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| **Case Selection** | Inclusion criteria<br>1. Age > 18 years old and < 80 years old;<br>2. The time from onset of symptoms to emergency coronary angiography (CAG) is <12h, with obvious chest pain before surgery, and the angiography shows myocardial infarction related vessel with thrombolysis in myocardial infarction (TIMI) grade 0 and no collateral circulation retrograde perfusion at its distal end;<br>3. Left ventricular ejection fraction after surgery is less than 40%;<br>4. No history of heart failure in the past;<br>5. Patients who have signed an informed consent form.<br>Exclusion criteria:<br>1. Poor acoustic window in patients, unable to undergo transthoracic echocardiography;<br>2. Acute non-ST-elevation myocardial infarction;<br>3. Complications such as old myocardial infarction, cardiogenic shock, ventricular septal perforation, papillary muscle or chordae tendineae rupture;<br>4. Heavy thrombus burden, anatomical structures unsuitable for PPCI treatment.<br>5. CAG shows vascular lesions with TIMI≥1 or retrograde filling of collateral circulation in the distal vessel;<br>6. Allergic to drugs such as sacubitril/valsartan or dapagliflozin;<br>7. Unable to attend follow-up for various reasons;<br>8. Known significant gastrointestinal functional impairment or gastrointestinal diseases that may affect the absorption of the investigational drug, such as diagnosed active ulcers (Forrest grade II and below), inflammatory bowel disease, absorption-related disorders, and uncontrollable diarrhea, post-gastrointestinal surgery (e.g. bariatric surgery);<br>9. Patients diagnosed with malignant tumors;<br>10. Patients who have participated in other clinical trials within the last 3 months;<br>11. In addition to the above, patients deemed unsuitable for participation in this clinical trial by the investigator. |

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| **Study Period and Visit** | A total of 26 weeks, including a baseline period (-2 to 0) and 3 follow-up visits (4 weeks, 12 weeks, 24 weeks). |
| **Treatment Protocol** | Following surgery, the observation group was given a combination of Sacubitril/Valsartan (trade name: Entresto, 100 mg (49 mg/51 mg), National Drug Approval Number HJ20170362), administered orally. The initial dose was 50 mg per time, taken twice a day. Depending on the specific situation, the dose was increased by 50 mg after a 2-week interval, with a maximum dose of 200 mg per time, taken twice a day. Dapagliflozin (AstraZeneca Pharmaceuticals LP, 10 mg; National Drug Approval Number J20170040) was initially given at a single dose of 5 mg, once a day, and the dosage was increased to 10 mg once a day based on the specific situation. |
| **Assessment of therapeutic efficacy** | Main efficacy indicators mainly include:<br>Using the two-dimensional speckle tracking echocardiography left ventricular pressure-strain loop (LV-PSL) to compare the baseline and 24-week left ventricular myocardial work index, mainly including global work index (GWI), global constructive work (GCW), global wasted work (GWW), and global work efficiency (GWE) changes;<br>Secondary efficacy indicators:<br>1. Changes in the following parameters of echocardiography at 24 weeks: left ventricular ejection fraction (LVEF), left ventricular mass index (LVMI), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), the ratio of early diastolic mitral inflow velocity to early diastolic mitral annular velocity (E/E'), and the ratio of early diastolic to late diastolic filling velocity (E/A);<br>2. 4 weeks, 12 weeks, 24 weeks NT-proBNP, soluble suppression of tumorigenicity 2 protein (sST2), 6-minute walk test (6MWT);<br>3. Changes in left ventricular longitudinal strain values at 4 weeks, 12 weeks, and 24 weeks (LV-GSL);<br>4. Changes in SBP and DBP at 4 weeks, 12 weeks, and 24 weeks. |
| | Safety Assessment Indicators:<br>Incidence of major adverse cardiovascular events (MACE) in two patient groups. |

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| **Statistical methods** | Using SAS 9.4 software for statistical analysis, the efficacy evaluation analysis is based on FAS and PPS, and the safety evaluation is based on SS. Assuming a two-sided test (except for special instructions), a difference is considered statistically significant at $P<0.05$.<br>Statistical description: Summary analysis of qualitative variables includes N (based on non-missing sample observations), frequency, and percentage. For quantitative variables, summary analysis includes N (based on non-missing sample observations), arithmetic mean, median, standard deviation, and interquartile range based on data distribution characteristics. Basic characteristics of the study population, current medical history, and past medical history will be analysed using descriptive statistical methods.<br>Statistical inference: In general, all data will be observed for changes between visits based on patient characteristics. Paired t-tests or Wilcoxon signed-rank tests will be used for within-group comparisons of quantitative data before and after treatment, and grouped t-tests (or t' tests) will be used for between-group comparisons. Chi-square tests will be used for between-group comparisons of count data, and Fisher's exact test will be used if necessary. Wilcoxon rank-sum tests will be used for between-group comparisons of ordinal data. In addition to descriptive analysis of time point measurements for laboratory repeated measurements, a model analysis of longitudinal study results (Mixed Model) will also be conducted. |
| **Research Period** | This study is divided into the following three stages:<br>1. Initiation Phase (Research Preparation): Expected to take 6 months, including the development of Case Report Forms (CRF), Informed Consent Forms (ICF), Electronic Data Capture System (EDC) materials, selection and evaluation of research centres, ethical committee approval, and the initiation of research centres.<br>2. Research Phase (Patient Enrollment and Follow-up): Patient enrollment is expected to take 12 months, followed by a 6-month follow-up period.<br>3. Analysis and Statistical Phase: Data entry, statistical analysis of research results, and conclusion, expected to take 2 months.<br>The project's overall duration is estimated at 26 months (6 months for initiation, 18 months for patient enrollment and follow-up, and 2 months for statistical analysis and conclusion). |

# Contents

## 1. Research Background

Acute myocardial infarction (AMI) is a common critical condition in the emergency room. In recent years, with the establishment of "chest pain centres", the concept that "time is life, time is myocardium" has become deeply ingrained. However, some patients still experience serious complications due to the delayed opening of blocked blood vessels, leading to post-myocardial infarction heart failure (P-MI-HF), including factors such as recurrent MI, ventricular remodeling, mechanical MI complications, and stunned or hibernating myocardium [1]. Sacubitril/valsartan is an angiotensin receptor neprilysin inhibitor (ARNI). Studies have shown [2-5] that sacubitril/valsartan has a positive effect on improving adverse ventricular remodeling in patients with reduced ejection fraction heart failure, hypertensive patients, and animal models of acute myocardial infarction. Although a recent study, PARADISE-MI, did not achieve the expected results for the treatment of acute myocardial infarction patients with sacubitril/valsartan [6], there is still a large body of research showing that sacubitril/valsartan can improve cardiac function in patients with heart failure after AMI [7], and the European Society of Cardiology recommends the use of sacubitril/valsartan in stable patients with acute HFrEF [8]. Dapagliflozin was initially used to control blood sugar in diabetic patients [9], but increasingly, research indicates that dapagliflozin can bring benefits in cardiovascular and renal diseases [10-12]. In the treatment of HFrEF, dapagliflozin and sacubitril/valsartan can effectively reduce the risk of cardiovascular events for patients [13], but there is limited research on the combined treatment of P-MI-HF with both medications.

How to choose a suitable examination to assess the early effects of combined therapy? Russel et al. proposed a non-invasive method to measure left ventricular myocardial work by tracking the change in myocardium relative to the initial length during the cardiac cycle (i.e., strain curve) instead of segment length change. This method replaces invasively measured left ventricular pressure with estimated non-invasive left ventricular pressure curve, combining segmental myocardial strain obtained from two-dimensional speckle tracking echocardiography (STE) with estimated left ventricular pressure curve to measure left ventricular myocardial work. This overcomes the load dependency of left ventricular ejection fraction (LVEF) and STE, providing a more accurate assessment of myocardial function [14]. The non-invasive left ventricular pressure-strain loop (LV-PSL) derived myocardial work shows good correlation with invasively measured myocardial work using catheterization, which has been demonstrated in both animal and human experiments [14, 15]. LV-PSL is superior to other echocardiographic parameters for predicting coronary heart disease, including global longitudinal strain (GLS) and LVEF, with good reproducibility [16]. This technology has become a hot topic in recent years for research by cardiac ultrasonographers [17].

This study will use LV-PSL technology to evaluate the early prognosis of patients with acute heart failure after myocardial infarction treated with sacubitril/valsartan combined with dapagliflozin.

## 2. Research Objectives

2.1. Primary Objective:

To assess the cardiac function status of patients with heart failure after acute myocardial infarction using the left ventricular pressure-strain loop technique of echocardiography.

2.2. Secondary Objectives:

1) To analyze the impact of sacubitril/valsartan combined with dapagliflozin on the cardiac function of patients with heart failure after acute myocardial infarction;

2) To analyze the advantages of sacubitril/valsartan combined with dapagliflozin over sacubitril/valsartan monotherapy;

3) To analyze the advantages of the left ventricular pressure-strain loop technique over other cardiac function indicators such as left ventricular ejection fraction and longitudinal strain.

**3. Types and Steps of Research Design**

3.1. Research Design

This study is a multi-centre, prospective, open-label, randomised controlled trial. The study will stratify patients with heart failure after acute myocardial infarction in a 1:1 randomisation by centre. All patients will receive a loading dose of oral aspirin 300 mg (Bayer AG, Germany, 0.1 g/tablet; National Drug Approval Number HJ20160685) and ticagrelor 180 mg (AstraZeneca Pharmaceutical Co., Ltd., 90 mg/tablet; National Drug Approval Number H20130058) before emergency intervention, and anticoagulation therapy during and after the procedure. Standard medication will be administered postoperatively, and emergency intervention surgery will be performed by experienced senior physicians in the department. In addition to standard heart failure treatment (beta-blockers, aldosterone receptor antagonists), sacubitril/valsartan and SGLT-2 inhibitors will be added. The control group will receive standard heart failure treatment (beta-blockers, aldosterone receptor antagonists) with the addition of sacubitril/valsartan. The specific medication regimen is as follows: the observation group will receive postoperative sacubitril/valsartan (sacubitril/valsartan, 100 mg (49 mg/51 mg), National Drug Approval Number HJ20170362) orally, with an initial dose of 50 mg per dose, twice a day. The dose may be increased by 50 mg every 2 weeks based on specific conditions, with a maximum dose of 200 mg per dose, twice a day. Dapagliflozin (AstraZeneca Pharmaceutical Co., Ltd., 10 mg; National Drug Approval Number J20170040) will be administered at an initial dose of 5 mg once daily, with the possibility of increasing the dose to 10 mg once daily based on specific conditions. Both groups will receive medication for 6 months.

Blinding: Considering the different quantities of the two drugs, and both being recommended heart failure medications, the study is designed as non-blinded, open-label.

Research Centres: 3

Sample Size Calculation: Based on previous studies, it is expected that the use of sacubitril/valsartan alongside conventional treatment will result in an approximate 26% improvement in GWI, with SD of 2 and

0.5%, α=0.05, power=0.9. Each group is estimated to require 28 cases. Anticipating a dropout rate of 20%, this study aims to include 70 patients, with 35 in each group.

3.2 Research Procedure

The research program consists of a 2-week enrollment period, baseline data collection, randomization, and 3 follow-up visits (4 weeks, 12 weeks, 24 weeks).

Enrollment Period (-2 weeks)

Inform about the research details, obtain informed consent, conduct preliminary assessment for inclusion criteria, and record the following data: demographic information, medical and treatment history, physical examination and vital signs, NT-proBNP, sST2, echocardiogram results (EF value), left ventricular global longitudinal strain (LV-GLS), 6-minute walk test (6MWT), 12-lead electrocardiogram results, and medication status.

Patients with poor medication compliance or inability to maintain a stable dose of heart failure treatment drugs will be excluded.

Baseline (-2 to 0 days)

Evaluate specific inclusion criteria, record the following data: physical examination and vital signs, laboratory tests (complete blood count, urinalysis, lipid profile, liver function, kidney function, ACR, electrolytes, fasting blood glucose), cardiac function parameters (NT-proBNP, sST2, 6MWT), two-dimensional speckle tracking echocardiogram results, and concomitant medication status.

Randomize eligible participants and dispense investigational drugs. Visit 1 (4 weeks) and Visit 2 (12 weeks)

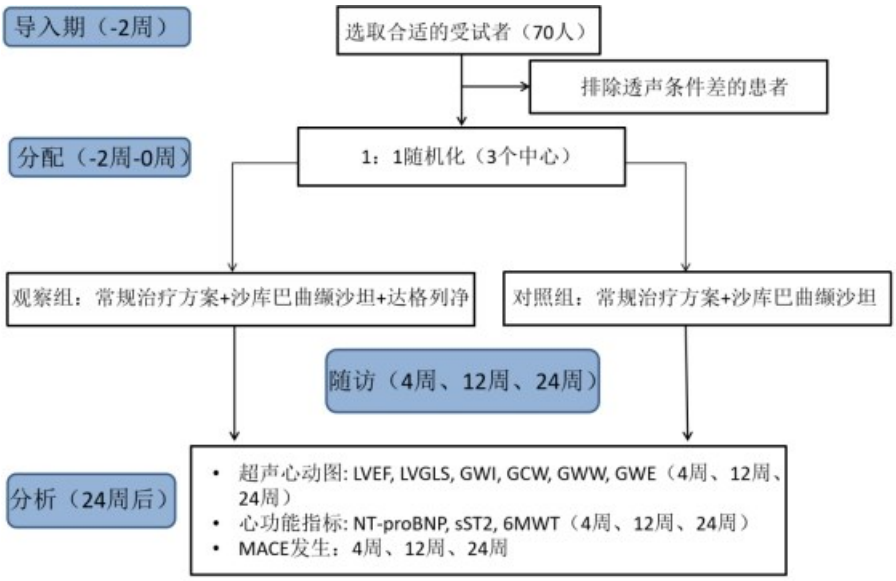
Evaluate and record the following data: physical examination and vital signs, kidney function, electrolytes, fasting blood glucose, medication status, LVEDD, LVESD, LVEF, LVGLS, GWI, GCW, GWW, GWE, occurrence of MACE, etc.

Inventory investigational drugs, dispense and retrieve medications. Visit 3 (24 weeks)

Evaluate and record the following data: physical examination and vital signs, kidney function, electrolytes, fasting blood glucose, medication status, LVEDD, LVESD, LVEF, LVGLS, GWI, GCW, GWW, GWE, occurrence of MACE, etc.

Inventory investigational drugs, dispense and retrieve medications.

Research Implementation Process Diagram



## Process Diagram

| **Project** | **Import Period** | **Baseline** | **Treatment and Follow-up** | | |
|---|---|---|---|---|---|
| **Time** | **2 weeks before enrollment** | **Week 0** | **Week 4** | **Week 12** | **Week 24** |
| **Window period (days)[a]** | | **-7 day** | **±7 day** | **±7 day** | **±7 day** |
| Sign Informed Consent Form | × | | | | |
| Demographic data | × | | | | |
| Medical history[b] and treatment history | × | | | | |
| Examination and Vital Sign[c] | × | × | × | × | × |
| Complete Blood Count | | × | | | × |
| Urinalysis | | × | | | × |
| Lipids[d] | | × | | | × |
| NT-proBNP | | × | × | × | × |
| sST2 | | × | × | × | × |
| Liver Function | | × | × | × | × |
| Kidney Function | | × | × | × | × |
| Electrolyte | | × | | | × |
| 6-Minute Walk Test | | × | × | × | × |
| Echocardiogram | | × | × | × | × |
| 12-lead electrocardiogram | | × | | | × |
| Two-dimensional speckle tracking echocardiography（2D-STE） | | × | × | × | × |
| Fasting Blood Sugar | | × | | | × |
| **Other assessments** | | | | | |
| Medication Usage[e] | × | × | × | × | × |
| **Clinical Trial Medication Dispensing** | | | | | |
| Randomization | | × | | | |
| Drug dispensing and collection experimentation | | × | × | × | × |

Note:

a. Allow a window period of ±1 week before and after.

b. Medical history: including present illness and past medical history.

c. In vital signs, the baseline blood pressure is the average of two measurements on the day of the 0-week visit; the follow-up blood pressure is the average of two measurements on the day of the visit.

d. Blood lipids: including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C).

e. Record concomitant medications: generic name of the medication, duration of use, dosage, route of administration, and indications. If there are any adjustments during the treatment period, record the time of addition and the reasons for adjustment.

**4. Case Selection**

4.1 Inclusion Criteria

1) Age > 18 years old and < 80 years old;

2) Onset of symptoms to emergency coronary angiography (CAG) <12 hours, significant chest pain before surgery, and CAG showing thrombolysis in myocardial infarction (TIMI) grade 0 with no collateral circulation retrograde perfusion at its distal end;

3) Left ventricular ejection fraction after surgery is less than 40%;

4) No history of heart failure in the past;

5) Patients who have signed informed consent forms.

4.2 Exclusion Criteria

1) Patients with poor acoustic conditions that do not meet the requirements for transthoracic spot tracking imaging;

2) Acute non-ST-segment elevation myocardial infarction;

3) Complications such as old myocardial infarction, cardiogenic shock, ventricular septal perforation, mitral valve chordae tendineae or papillary muscle rupture;

4) Heavy thrombus load, anatomical structures unsuitable for PPCI treatment;

5) CAG showing TIMI≥1 grade in the diseased vessel or collateral circulation retrograde perfusion at the distal end;

6) Allergies to drugs such as ticagrelor or valsartan;

7) Unable to follow up for various reasons;

8) Known gastrointestinal functional impairment or gastrointestinal diseases that may significantly affect the absorption of the investigational drug, such as diagnosed active ulcers (Forrest grade II and below), inflammatory bowel disease, absorption-related diseases, and uncontrollable diarrhea, post-gastrointestinal surgery (e.g., weight loss surgery);

9) Patients diagnosed with malignant tumors;

10) Patients who have participated in other clinical trials within 3 months;

11) Patients whom the investigator deems unsuitable to participate in this clinical trial, in addition to the above.

4.3 Assessment criteria:

1）Proteinuria: Judged by a clinical doctor, or albumin/creatinine ratio (ACR) ≥ 30 mg/g (3.5 mg/mmol).

2）Dyslipidemia: TC≥5.2mmol/L or HDL-C＜1mmol/L or LDL-C≥3.4mmol/L or TG≥1.7 mmol/L.

3）Left ventricular hypertrophy: Echocardiography shows left ventricular hypertrophy.

4）Brinkman index: The daily number of cigarettes smoked multiplied by the number of years of smoking.

5）Hypovolemic state: Judged by the clinical doctor combining examination results and clinical manifestations, such as systolic blood pressure <100 mmHg, heart rate >90 times/min, capillary refill time >2 s, respiratory rate >20 times/min, positive response to passive leg raising test[18].

4.4 Exclusion criteria

Patients who do not meet the inclusion criteria but were mistakenly included;

Patients who meet the inclusion criteria but have not used the medication after inclusion or have poor medication compliance.

4.5 Termination criteria

1) The investigator judges that the subject will not benefit from continuing the study medication treatment;

2) the occurrence of adverse events that are intolerable, unrelieved, or may progress to serious adverse events, such as infections in the lower limbs, new-onset pain or tenderness in the lower limbs, sores, or ulcers;

3) the occurrence of serious adverse events (SAE) that the investigator judges unsuitable for continued participation in the study;

4) serious deviations or violations of the protocol that affect the evaluation of drug safety or efficacy;

5) subjects withdraw informed consent;

6) inability to continue follow-up for various reasons.

## 5. Research Methods and Technical Route

5.1 Experimental Drug Name and Specifications

Drug name: Dagliflozin Tablets

Dosage form: Tablets, 10mg

Manufacturer: AstraZeneca Pharmaceuticals Ltd.

Shelf life: 24 months

Storage and Management of Experimental Drugs

Experimental drugs should be stored away from light at room temperature. If the hospital pharmacy has the experimental drugs, they will be obtained from there; otherwise, they will be procured from an external pharmacy.

Each research center must designate a person in charge of managing the experimental drugs. The quantity of drugs distributed must be recorded during each follow-up visit, and the remaining quantity must be verified and documented in the drug distribution register. At the end of the study, all remaining drugs and empty bottles should be returned to the researcher, and an experimental drug recovery form should be completed. Any undistributed drugs must be sealed upon return.

The designated person in charge of managing the experimental drugs at each research center must provide a copy of the drug distribution/recovery register after the study concludes.

5.2 Treatment Plan

1）Observation group:

Based on the standard anti-heart failure treatment (beta-blockers, aldosterone receptor antagonists), sacubitril/valsartan and SGLT-2 inhibitors are added. The specific medication plan is as follows: After the operation, the observation group adds sacubitril/valsartan (Entresto, 100 mg (49 mg/51 mg), National Drug Approval No. HJ20170362), taken orally. The initial dose is 50 mg per time, taken twice a day. After 2 weeks, the dose is increased by 50 mg based on specific conditions, with a maximum dose of 200 mg per time, taken twice a day; Dapagliflozin (AstraZeneca Pharmaceuticals, 10 mg, National Drug Approval No. J20170040), the initial dose is 5 mg once daily, and the dose is increased to 10 mg once daily based on specific conditions. Oral medication is taken before the first meal of the day. The medication period lasts for 6 months.

2）Control group:

Based on the standard anti-heart failure treatment (beta-blockers, aldosterone receptor antagonists), sacubitril/valsartan is added. Oral medication. The medication period lasts for 6 months.

5.3 Medication Combination

After enrollment, the basic treatment plan including lifestyle interventions remains unchanged. Lifestyle interventions, including dietary control, physical exercise, and other health education, are uniformly implemented for both groups of patients. For comorbid conditions such as hypertension, dyslipidemia, and proteinuria, medication should adhere to the relevant diagnosis and treatment guidelines and norms of our country. After enrollment, the previous medication regimen will be maintained, including the use of RAAS inhibitors, diuretics, statins, and other medications. For other comorbid conditions, symptomatic treatment is permissible as long as it does not affect the evaluation of the investigational drug's efficacy. All previously used concomitant medications (by generic name) must be documented in the original medical records and electronic case report forms (eCRF). In the event of unavoidable use of other medications or changes in dosage, the name (generic name), reason for use, administration method, dosage, and treatment duration must be recorded in the original medical records. Treatment regimens for comorbid conditions other than diabetes must not be altered after enrollment.

5.4 Research Process

| **Project** | **Import Period** | **Baseline** | **Treatment and Follow-up** | | |
|---|---|---|---|---|---|
| **Time** | **2 weeks before enrollment** | **Week 0** | **Week 4** | **Week 12** | **Week 24** |
| **Window period (days)[a]** | | **-7 day** | **±7 day** | **±7 day** | **±7 day** |
| Sign Informed Consent Form | × | | | | |
| Demographic data | × | | | | |
| Medical history[b] and treatment history | × | | | | |
| Examination and Vital Sign [c] | × | × | × | × | × |
| Complete Blood Count | | × | | | × |
| Urinalysis | | × | | | × |
| Lipids[d] | | × | | | × |
| NT-proBNP | | × | × | × | × |
| sST2 | | × | × | × | × |
| Liver Function | | × | × | × | × |
| Kidney Function | | × | × | × | × |
| Electrolyte | | × | | | × |
| 6-Minute Walk Test | | × | × | × | × |
| Echocardiogram | | × | × | × | × |
| 12-lead electrocardiogram | | × | | | × |
| Two-dimensional speckle tracking echocardiography（2D-STE） | | × | × | × | × |
| Fasting Blood Sugar | | × | | | × |
| **Other assessments** | | | | | |
| Medication Usage[e] | × | × | × | × | × |
| **Clinical Trial Medication Dispensing** | | | | | |
| Randomization | | × | | | |
| Drug dispensing and collection experimentation | | × | × | × | × |

Note:

a. Allow a window period of ±1 week before and after.

b. Medical history: including present illness and past medical history.

c. In vital signs, the baseline blood pressure is the average of two measurements on the day of the 0-week visit; the follow-up blood pressure is the average of two measurements on the day of the visit.

d. Blood lipids: including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C).

e. Record concomitant medications: generic name of the medication, duration of use, dosage, route of administration, and indications. If there are any adjustments during the treatment period, record the time of addition and the reasons for adjustment.

## 6. Observation Items and Timing of Testing

6.1 Import Period (-2 weeks)

During the import period, the following items should be collected:

- Signing of informed consent. The signing of informed consent should take place before any required examinations for the study.

- Demographic data: age, gender, ethnicity, occupation, education level;

- Past medical history and comorbidities: history of hypertension, hyperlipidemia, kidney disease, cardiovascular diseases, respiratory system diseases, infectious diseases such as hepatitis B, hepatitis C, etc.;

- Present medical history;

- Physical examination: weight, height, BMI, and examination of the patient's major body systems, including head and face, skin system, lymph nodes, eyes, ears, nose and throat, oral cavity, respiratory system, cardiovascular system, abdomen, reproductive and urinary system, musculoskeletal system, nervous system, and the patient's mental status;

- Vital signs: heart rate, blood pressure (systolic/diastolic), body temperature;

- Medication status;

- 12-lead electrocardiogram;

- Routine echocardiography: ejection fraction (EF), presence of left ventricular hypertrophy.

6.2 Baseline period (Week 0) (Window period: -7 days)

The following tasks should be completed within 7 days before the start of the study:

- Physical examination: weight, height, BMI, and examination of the patient's major body systems, including the head and face, skin, lymph nodes, eyes, ears, nose and throat, mouth, respiratory system, cardiovascular system, abdomen, reproductive and urinary system, musculoskeletal system, nervous system, and the patient's mental status;

- Vital signs: heart rate, blood pressure (systolic/diastolic), body temperature;

- Laboratory tests:

  - Blood routine: red blood cell count (RBC), hemoglobin (Hb), white blood cell count (WBC), platelets (PLT);

  - Urine routine: red blood cells, white blood cells, specific gravity of urine (SG);

  - Liver function: alanine aminotransferase (ALT), aspartate aminotransferase (AST);

- Kidney function: serum creatinine (SCr), blood uric acid (BUA);

- Urine albumin-to-creatinine ratio (ACR);

- Blood electrolytes: sodium, potassium, chloride;

- Blood glucose-related indicators: fasting blood glucose (FBG), glycated hemoglobin (HbA1c), insulin resistance index;

- Blood lipids: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C);

- Pregnancy test (only for women of childbearing age);

- Two-dimensional speckle tracking echocardiography (2D-STE): left ventricular global longitudinal strain (LVGLS), left ventricular ejection fraction (LVEF), left ventricular mass index (LVMI), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), ratio of early diastolic mitral inflow velocity to early diastolic mitral annular velocity (E/E'), and ratio of early and late diastolic filling velocities (E/A).

6.3 Treatment Period (0-24 weeks)

Visits and examinations during the treatment period allow a window period of ±7 days.

(1) First visit, second visit (4w±7d, 12w±7d)

- Physical examination

- Vital signs: heart rate, blood pressure (systolic/diastolic), body temperature

- Laboratory tests: fasting blood glucose, renal function, blood electrolytes

- Medication status: inventory of remaining medications to assess compliance, recording of concomitant medications including generic names, timing, dosage, route of administration, and indications

- Echocardiography (LVEF, LVGLS, LVPSL)

- Cardiac function tests (NT-proBNP, sST2, 6-minute walk test)

- Adverse events and serious adverse events

(2) Third visit (24w±7d)

- Physical examination

- Vital signs: heart rate, blood pressure (systolic/diastolic), body temperature

- Echocardiography (LVEF, LVGLS, LVPSL)

- Cardiac function tests (NT-proBNP, sST2, 6-minute walk test)

- Medication status: inventory of remaining medications to assess compliance, recording of concomitant medications including generic names, adverse events, and serious adverse events.

**7. Criteria for Evaluating Therapeutic Effects**

7.1 Primary Therapeutic Endpoints

Using two-dimensional speckle tracking echocardiography (LV-PSL) to compare the changes in left ventricular myocardial work index-related indicators at baseline, 4 weeks, 12 weeks, and 24 weeks.

7.2 Secondary Therapeutic Endpoints

1) Changes in the following parameters of echocardiography at 24 weeks: left ventricular ejection fraction (LVEF), left ventricular mass index (LVMI), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), ratio of early diastolic mitral inflow velocity to early diastolic mitral annular velocity (E/E'), and ratio of early to late diastolic filling velocities (E/A).

2) Changes in NT-proBNP and sST2 levels at 24 weeks.

3) Changes in the 6-minute walk test at 24 weeks.

4) Occurrence of MACE at 24 weeks.

7.3 Safety Endpoints

Incidence of adverse events (AEs) and serious adverse events (SAEs).

**8. Adverse Events**

8.1 Definition of Adverse Events

Adverse Events (AE) refer to all adverse medical events that occur in subjects after receiving investigational drugs, which may manifest as symptoms, signs, diseases, or abnormal laboratory findings, but are not necessarily causally related to the investigational drugs.

8.2 Recording of Adverse Events

All adverse events occurring after the patient has signed the informed consent form and received the investigational drug must be fully documented in the original medical records. Clinically relevant abnormal laboratory findings (e.g., leading to early withdrawal from the study, requiring treatment, or causing significant clinical manifestations, or deemed clinically relevant by the investigator) should be reported as adverse events. Adverse events should be followed up until their resolution or stabilization, unless the investigator considers that the adverse event cannot be improved due to the subject's pre-existing condition. Adverse events that persist at the end of treatment, as well as those occurring after the end of treatment and considered possibly related to the investigational drug, should be followed up by telephone within 30 days

after the end of treatment to obtain their outcomes.

Any adverse events discovered by the investigator during the clinical trial, regardless of whether they are related to the study drug, should be recorded. The recording of adverse events includes: ① description of all relevant symptoms of adverse events; ② the time of occurrence and duration of adverse events; ③ the severity of adverse events; ④ examinations and treatments performed due to adverse events; ⑤ the results and basis of the investigator's judgment on whether the adverse event is related to the investigational drug.

The investigator should inform the subjects to truthfully report any changes in their condition after drug administration, but should avoid leading questions. While observing the efficacy, close attention should be paid to adverse events or unforeseen toxic side effects (including symptoms, signs, laboratory tests), analyzing the causes, making judgments, and conducting follow-up observations and recording, as well as calculating the incidence of adverse events.

Adverse Event Rate=Adverse Event Cases/Total number of cases ×%

8.3 Adverse Event Assessment

1) Severity assessment

During the study, researchers can assess the severity of adverse events and serious adverse events according to the following criteria:

Grade 1 (Mild: asymptomatic or minor; only clinical or diagnostic findings; no treatment required.)

Grade 2 (Moderate: requires minor, local, or non-invasive treatment; restricts instrumental activities of daily living equivalent to the patient's age.)

Grade 3 (Severe or of major medical importance but not immediately life-threatening; results in hospitalization or prolongation of hospitalization; disables: restricts self-care activities of daily living.)

Grade 4 (Life-threatening; requires urgent treatment.)

Grade 5 (Death related to the adverse event.)

Note the distinction between severe adverse events and serious adverse events: severity is a category used to measure the severity of an event, and both adverse events and serious adverse events can be classified as severe. Any event meeting the definition of "serious adverse event" in section 14.5 should be classified as a serious adverse event.

2) Assessment of Cause and Effect

The causal relationship of (serious) adverse events (to all study treatments/procedures) is assessed by the investigators.

**Criteria for the evaluation of the correlation between adverse events and investigational drugs**

| Indicators | Certainly related | Certainly related | Certainly related | Definitely irrelevant | Definitely irrelevant |
|---|---|---|---|---|---|
| Reasonable time sequence | Yes | Yes | Yes | Yes | No |
| Known reaction types of the test drug | Yes | Yes | Yes | No | No |
| Discontinuing the study drug can lead to improvement. | Yes | Yes | Yes or No | Yes or No | No |
| The use of the investigational drug may lead to recurring occurrences. | Yes | Uncertain | Uncertain | Uncertain | No |
| Reactions can be interpreted differently. | No | No | Yes | Yes | Yes |

3) Definition and Reporting of Serious Adverse Events

Serious Adverse Event (SAE) refers to adverse medical events that occur in subjects after receiving investigational drugs, including death, life-threatening conditions, permanent or severe disabilities or functional loss, the need for hospitalization or prolonged hospital stay, as well as congenital anomalies or birth defects.

In the event of any SAE during the study, the physician should follow the standard operating procedures for emergency treatment in the hospital, record the incident, report to the sponsor within 24 hours of awareness, and promptly report to the ethics committee.

4) Handling of adverse events:

When adverse events occur, the investigator may decide on measures based on the condition, including: (1) observation without discontinuing the investigational drug; (2) observation and discontinuation of the investigational drug without corresponding treatment; (3) discontinuation of the investigational drug and administration of corresponding treatment.

All adverse events should be thoroughly investigated, with detailed records of the handling process and outcomes, until the subject is properly resolved or the condition stabilizes. Those with abnormal laboratory results should be tracked until they recover to normal or to the pre-medication levels. The tracking method can be chosen based on the severity of the adverse event, including hospitalization, outpatient visits, phone follow-ups, and other forms.

**Adverse Reactions and Management Measures of Dacarbazine**

| Number | Adverse Reactions | Disposal Measures |
|---|---|---|
| 1 | Low Blood Pressure | After using Dapagliflozin, blood volume may decrease, leading to symptomatic hypotension, especially in patients with renal impairment (eGFR less than 60mL/min/1.73m2), elderly patients, those receiving diuretics or RAAS inhibitors, or patients with lower baseline systolic blood pressure. Patients with these characteristics should be thoroughly assessed for blood volume status and promptly corrected before starting treatment with Dapagliflozin. Symptoms and signs of hypotension should also be monitored after initiating treatment. |
| 2 | Urinary system and reproductive system infections | The incidence of urinary system infections with dapagliflozin is about 4%, which does not increase the risk of urinary tract infections compared to a placebo. The incidence of reproductive system infections is about 6.8%, mostly mild to moderate, and the 10mg dose does not significantly increase the infection rate compared to the 5mg dose. The incidence of UTIs caused by SGLT2 inhibitors is similar to that of other antidiabetic drugs. Some scholars speculate that the reason why SGLT2 inhibitors do not increase the chance of urinary tract infections may be due to increased excretion of urinary glucose and sodium, increased urine volume through osmotic diuresis, making it difficult for bacteria to thrive in the urethra, and reducing the chances of bacteria traveling retrograde from bottom to top.<br><br>According to the "Expert Consensus on the Rational Clinical Application of SGLT-2 Inhibitors in China" in 2017:<br><br>Before use: Patients with recurrent urinary and reproductive infections within the past six months are not recommended for use; During use: Patients using SGLT2 inhibitors are advised to pay attention to personal hygiene of the external genitalia, drink water in moderation, and maintain smooth urination. During the use of SGLT2 inhibitors, especially in the first month of use, attention should be paid to whether the patient exhibits symptoms and signs of infection. It is worth noting that many patients with urinary tract infections do not have clinical symptoms and can undergo regular urine tests. Patients are advised to pay attention to personal hygiene of the external genitalia, drink water in moderation, maintain smooth urination, change underwear frequently, which can reduce the occurrence of infections. Prophylactic use of antibiotics is not recommended. If an infection occurs, it is necessary to suspend the use of SGLT2 inhibitors, administer anti-infective treatment, and drink plenty of water. After the infection is cured, the use of SGLT2 inhibitors can be continued. |

| | | |
|---|---|---|
| 3 | Ketosis Acidosis | Prevention and management: The risk factors for DKA in T2DM patients treated with SGLT2 inhibitors are basically the same as those in the general diabetic population, including acute illnesses (such as urinary tract infections, gastroenteritis, flu, or trauma), reduced calorie or fluid intake, or significantly reduced daily total insulin dose. Before using dapagliflozin for treatment, make sure the patient does not have the above conditions. If the known triggering factors occur during medication, consider monitoring ketones and blood gases, and follow the listed factors and corresponding treatments. If ketoacidosis is suspected, discontinue the medication, evaluate the patient, and immediately initiate DKA treatment. |
| 4 | Acute Kidney Injury | Before combining dapagliflozin with diuretics, attention should be paid to the potential risk of AKI (acute kidney injury).<br>Measures to be taken: Before starting dapagliflozin treatment, factors that may lead to the patient developing acute kidney injury should be considered, including low blood volume, chronic renal insufficiency, congestive heart failure, concomitant use of medications (diuretics, ACEI, ARB, NSAID), reduced oral intake (such as acute illness or fasting), or fluid loss (such as gastrointestinal diseases or exposure to high temperatures). For patients at risk prior to medication, monitor blood pressure, volume status, and renal function. During medication, monitor patients for signs and symptoms of acute kidney injury. If acute kidney injury occurs, discontinue the medication immediately and initiate appropriate treatment (often volume replacement for prerenal AKI). |
| 5 | Amputation | Lower limb infections, gangrene, and diabetic foot ulcers are common causes of amputation. Patients with a history of amputation, peripheral vascular disease, and neuropathy are at the highest risk of amputation. For safety reasons, the use of canagliflozin should be avoided in diabetic patients with amputation risk factors. Patients taking canagliflozin should be informed of the importance of routine preventive foot care. If new onset of pain or tenderness, ulcers, or infection (including osteomyelitis) occurs in the lower limbs, the medication should be discontinued, and prompt medical attention should be sought. |

## 9. Quality Control and Quality Assurance of Research

Researchers should strictly adhere to the clinical trial protocol and use standard operating procedures to ensure the implementation of quality control and quality assurance systems for clinical trials. The project will proactively address potential quality issues in the research. The potential quality issues in this study involve multiple aspects of the project, and the main quality control plan is as follows.

Project Implementation Preparation Phase

It is essential to carefully review and improve the formulation of the research plan, ensuring alignment between the plan design and research objectives. The research team should supervise and ensure the planned completion of team building, ethical review, researcher training, medical institution assessment, and preparation. Furthermore, based on the finalization of the research plan, it is important to develop project management plans, monitoring plans, quality management systems, and unified quality control measures.

Project Implementation Phase

During the project implementation phase, regular monitoring and auditing will be conducted according to the project SOP. The implementation will strictly follow the SOP, and any deviations from the protocol or SOP requirements identified during monitoring and auditing will result in corrective actions for the center, followed by retraining.

To minimize measurement bias, the same radiologist with 5 years or more of imaging experience will be responsible for the measurement and evaluation of key indicators within the same center during both the baseline and follow-up assessments.

Research Completion and Acceptance Phase

Inspectors are responsible for monitoring the relevant procedural documents required for the research and conducting professional and technical reviews. The data administrator collaborates in resolving issues related to data management and conducts audits of the database.

Verification of Raw Data

Data management personnel strictly adhere to the company's SOP requirements and pre-established data management plans. The project statistician develops a statistical analysis plan and carries out data statistical analysis in accordance with the final statistical analysis plan to produce a statistical analysis report. Medical specialists are responsible for writing a research summary report based on the statistical analysis report, which will be finalized internally at the medical research center and then reviewed by experts.

**Supervision**

Before the study begins, the inspector should develop a comprehensive inspection plan and conduct inspections of the research according to the plan. The main responsibility of the inspection team is to assist

researchers in maintaining high-quality standards in ethics, science, technology, and law aspects of the research. In addition, the inspection team must ensure that the clinical trial is conducted in accordance with the trial protocol and China GCP implementation.

During the monitoring of this study, it is necessary to confirm the completeness and accuracy of medical records, and to conduct a comparative review of the original records in the presence of the researcher. During the monitoring visit, the monitoring group and the researcher review the following items: subject informed consent forms, subject recruitment and follow-up status, recording and reporting of serious adverse events, allocation of investigational drugs, adherence to the drug administration protocol and dosage, quantity of investigational drugs, concomitant therapy, and data quality.

**Inspection**

Inspection personnel systematically examine the activities and documents related to clinical trials to evaluate whether the research is conducted in accordance with the trial protocol, standard operating procedures, and relevant regulatory requirements, and whether the research data is recorded in a timely, truthful, accurate, and complete manner.

## 10. Data Management and Security Monitoring

### Data Management

This research adopts an Electronic Data Capture (EDC) system for data management. The data management plan is drafted by the project data administrator according to the clinical trial protocol and research medical records/CRF. It describes the data management process, personnel allocation, timetable, and document archiving to ensure the consistency, effectiveness, and standardization of data management. This promotes communication and exchange among various clinical research departments to establish a high-quality database for statistical analysis. During the study, if users need to modify the data management plan, they should notify the data administrator for real-time revision, release it to all relevant personnel, and obtain their signature confirmation.

### Database Management

The design and testing of the database are completed by the data management personnel based on the final plan and CRF. Data management personnel should annotate the CRF and develop variable descriptions. For fields with logical relationships, logical checks are set according to the data verification plan.

After the database is established, it should be tested and verified, fully tested by relevant personnel, and entered after review and confirmation by the head of the data management department.

### Data Verification, Cleaning, and Quality Control

Under the premise of fully understanding the design plan, the data management statistics department, medical department, and project manager formulate a specific data verification plan for all verification points based on the data management content of the plan. If modifications are needed during the execution, the data

administrator revises and updates the version.

During the data verification process, questions arise in two forms: those generated by the online system and those raised manually. Researchers or authorized CRC address these questions until the data is cleaned. The data management department keeps an electronic version of the question log.

Real-time editing and verification require action to be taken and tracked for any non-compliance with quality control or quality inspection requirements. During the implementation of quality control, a list of non-compliant data should be maintained. This dynamic record needs to be summarized and communicated by data administrators, monitors, or auditors based on the actual error rates found.

Data review meetings are held after the data cleaning is completed. The principal investigator, data management personnel, and statisticians conduct a final review of unresolved data issues.

In clinical research, an appropriate data security monitoring plan is developed based on the size of the risk. All adverse events are documented in detail, handled appropriately, and tracked until resolved or stabilized. Serious adverse events and unexpected events are reported to the ethics committee, regulatory authorities, and drug regulatory authorities in a timely manner. The principal investigator periodically conducts a cumulative review of all adverse events and, if necessary, convenes a researcher meeting to assess the risks and benefits of the study.

**11. Statistical Processing**

Statistical analysis was performed using SAS 9.4 software. Efficacy evaluation analysis was based on FAS and PP, and safety evaluation was based on SS. Assuming a two-sided test (except for special instructions), a difference is considered statistically significant when $P<0.05$.

Statistical description: For qualitative variables, a summary analysis includes N (based on non-missing sample observations), frequency, and percentage. For quantitative variables, a summary analysis includes N (based on non-missing sample observations), arithmetic mean, median, standard deviation, and interquartile range based on data distribution characteristics. Baseline data on demographic characteristics, medical history, and past history of the study population will be analyzed using descriptive statistical methods.

Statistical inference: In general, all data are observed and compared between visits based on patient characteristics. Paired t-tests or Wilcoxon signed-rank tests are used for within-group comparisons of metric data before and after treatment, while grouped t-tests (or t' tests) are used for between-group comparisons. Chi-square tests are used for between-group comparisons of count data, with the possibility of using Fisher's exact probability method if necessary, and Wilcoxon rank-sum tests are used for between-group comparisons of ordinal data. In addition to descriptive analysis of time point measurements for laboratory repeat measurements, a model analysis of longitudinal study results (Mixed Model) will also be conducted.

**Analysis Set Definitions**

The primary efficacy analysis is based on the Full Analysis Set (FAS) and also analyzed using the Per Protocol Set (PPS). Secondary endpoints and safety analysis are based on the Per Protocol Set (PP) and the Safety Set (SS) respectively.

**Full Analysis Set (FAS)**

The FAS includes all randomized subjects who have received at least one dose of the investigational drug, at least one baseline efficacy assessment, and at least one post-baseline efficacy assessment. Analysis is conducted according to the treatment group to which the subject was randomized. The FAS is the primary efficacy analysis set for this study.

**Per Protocol Set (PP)**

The PP analysis set is a subset of the FAS, comprising subjects who have no significant protocol deviations. The PP analysis set is determined prior to database lock and excludes subjects for reasons including but not limited to concomitant use of prohibited medications, significant protocol deviations, or non-compliance. Detailed discussions regarding the exclusion of subjects from the PP analysis set and the rationale will be conducted at the data review meetings prior to database lock.

**Safety Set (SS)**

The Safety Set (SS) is a subset that includes all patients who have received at least one dose of the investigational drug and have safety data recorded in the database; the analysis will only use actual measured values.

**Sample Size Calculation and Basis**

Based on previous studies, it is expected that the use of sacubitril/valsartan in conjunction with conventional treatment will result in an approximately 26% improvement in GWI, with SD of 2 and 0.5%, $\alpha$=0.05, power=0.9. A sample size of 28 cases per group is required. With an estimated dropout rate of 20%, this study plans to include 70 patients, 35 in each group.

**12. Ethics of Clinical Research**

Clinical research will adhere to relevant regulations such as the World Medical Association's "Declaration of Helsinki." Before the research begins, the clinical study will only be implemented after the trial protocol is approved by the ethics committee. Prior to enrolling in this study, researchers have a responsibility to fully and comprehensively inform the subjects or their representatives about the purpose, procedures, and potential risks of the study. They must also sign a written informed consent form, informing the subjects of their right to withdraw from the study at any time. The informed consent should be retained as part of the clinical research documentation. Throughout the research process, the personal privacy and data confidentiality of the subjects will be protected.

**Ethics Committee (EC)**

In accordance with ICH-GCP, local laws, regulations, and requirements of relevant organizations, all participating research centers must obtain approval documents from the appropriate ethics committee before the start of the study. If necessary, any extensions, amendments, or updates must also be approved by the ethics committee.

**Informed Consent Form**

Before the start of the study, researchers must obtain approval from the ethics committee for the written informed consent form and other written materials to be provided to the patients.

The ethics of clinical research require adherence to relevant regulations, such as the World Medical Association's "Declaration of Helsinki." Researchers must obtain approval from the ethics committee before implementing the clinical study, and they have a responsibility to fully inform the subjects or their representatives about the study's purpose, procedures, and potential risks. Subjects must also be informed of their right to withdraw from the study at any time and must sign a written informed consent form. Throughout the research process, the personal privacy and data confidentiality of the subjects will be protected.

Research centers must obtain approval documents from the appropriate ethics committee before the study begins, in accordance with ICH-GCP, local laws, regulations, and requirements of relevant organizations. Any extensions, amendments, or updates must also be approved by the ethics committee.

Researchers must obtain approval from the ethics committee for the written informed consent form and other materials provided to the patients before the study begins.

# Signature Page

Researcher's Declaration:

I agree to abide by the review opinions of the ethics committee and commence the clinical trial after approval, promptly report any changes in the clinical trial activities to the ethics committee, as well as any unexpected issues involving risks to subjects or other individuals, and proceed only after re-obtaining ethics review approval. I will comply with the requirements of the ethics committee for ongoing review and final review in the study.

I agree to strictly follow the design and specific requirements of this trial.

I understand that for the maximum benefit of the subjects, I may interrupt or terminate the clinical trial at any time.

I agree to personally conduct or supervise the clinical trial and ensure that all research personnel assisting me in conducting the trial at my institution understand their responsibilities in the trial.

Throughout the conduct of the clinical trial, I will strictly adhere to the current GCP and Helsinki Declaration. I commit to ensuring that the entire trial process complies with ethical, moral, and scientific principles.

During the conduct of the clinical trial, I will strictly adhere to all laws and regulations related to the clinical trial to protect the rights and interests of the patients.

I agree to maintain sufficient and accurate medical records and ensure that these records are available for inspection and audit in accordance with relevant laws and regulations at any time.

Primary Researcher: Primary Investigator Signature: Date (Year/Month/Day):

# Abbreviations

| Abbreviations and Specialized Terms | Explanation |
|---|---|
| AEs | Adverse Events |
| ALT | Alanine aminotransferase |
| AST | Aspartate Aminotransferase |
| BMI | Body Mass Index |
| LV-PSL | Left ventricular pressure-strain loop |
| LVGSL | Left ventricular global longitudinal strain |
| E/A | Ratio of early diastolic peak velocity to late diastolic peak velocity of the mitral valve |
| E/e’ | Ratio of early diastolic peak flow velocity to early diastolic peak motion velocity of mitral valve |
| eCRF | Electronic Medical Record Report Form |
| EC | Ethics Committee |
| ECG | Electrocardiogram |
| 6MWT | 6-Minute Walk Test |
| sST2 | Soluble Growth Stimulating Expression Gene 2 Protein |
| GCP | Good Clinical Practice for Drug Clinical Trials |
| GLS | Longitudinal Strain Rate |
| GWI | Overall Efficiency Index |
| GCW | Overall effective power |
| GWW | Overall ineffective effort |
| HDL-C | High-density lipoprotein cholesterol |
| HFrEF | Heart failure with reduced ejection fraction |
| GWE | Overall efficiency of work |
| ICF | Informed Consent Form |
| LEVF | Left ventricular ejection fraction |
| LDL-C | Low-density lipoprotein cholesterol |
| LVEDD | Left ventricular end-diastolic diameter |
| LVEDV | Left ventricular end-diastolic volume |

| | |
|---|---|
| LVESD | Left ventricular end-diastolic diameter |
| LVESV | Left ventricular end-systolic volume |
| LVH | Left ventricular hypertrophy |
| LVMI | Left Ventricular Mass Index |
| PP | Compliant Solution Set |
| RAASi | Renin-angiotensin-aldosterone system inhibitors |
| SAEs | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SCr | Blood creatinine |
| SBP | Systolic Blood Pressure |
| SGLT-2 | Sodium-glucose co-transporter 2 |
| SS | Security Analysis Set |
| TC | Total cholesterol |
| TG | Triglycerides |

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Attachment 1: Research Progress

| Number | Events | 计划完成时间 | 备注 |
|---|---|---|---|
| **1** | Research Project Initiation by the team leader unit (Pukou District Traditional Chinese Medicine Hospital, Nanjing City) | April 2020 | Finalize all research materials such as proposals and ICF in electronic format. Upload them to the research system before April 10th. Expect to receive the research department's review results within 1 week. After approval, submit the stamped hard copies to the ethics committee. Simultaneously, complete the procurement of insurance and SMO suppliers. |
| **2** | Obtaining the approval from the Ethics Committee of the team leader unit (Nanjing Pukou District TCM Hospital). | End of April 2020 | After the research department approves on 4.10, submit the stamped paper materials to the ethics committee for review. |
| **3** | The head unit (Pukou District Traditional Chinese Medicine Hospital, Nanjing) has completed the signing of the main agreement and SMO agreement. | June 2020 | The Pukou District Traditional Chinese Medicine Hospital, Nanjing City, needs to first send the electronic version of the agreement to the Technology Department before scheduling the contract review meeting with the Ministry of Science and Technology (once a month, in the second half of the month). The person in charge of the applicant needs to attend the meeting, and the process of submission, review, and stamping is expected to take approximately 2 months. |
| **4** | Two research centers (Nanjing Brain Hospital, Lujiang County People's Hospital) have completed the research project establishment in the field of neuroscience. | June 2020 | Principal investigators must present ethical reports on site for academic review, otherwise they will not be allowed to attend the meeting. |
| **5** | The first center is launched, and the first participant is enrolled. | July 2020 | CRC and CRA registration completed, center kick-off meeting held, researchers authorized for their respective roles, and the first subject |

| | | | |
|---|---|---|---|
| | | | underwent assessment. |
| **6** | Obtain the main clinical trial protocols and SMO agreements signed by 2 participating research centers (Nanjing Brain Hospital, Lujiang County People's Hospital). | August 2020 | The signing progress of the agreement is based on the actual situation of each center. The main agreement requires signatures and seals from the sponsor, hospital, and CRO. The SMO agreement requires signatures and seals from the hospital, CRO, and SMO. |
| **7** | Two clinical research centers (Nanjing Brain Hospital, Lujiang County People's Hospital) have been initiated, and participants have been enrolled. | September 2020 | The training for center activation was completed at two locations, with researchers assigning responsibilities and recruiting participants for the study. |
| **8** | The final participant has been enrolled. | February 2021 | At the end of the 6-month enrollment period, the final participant will undergo evaluation. |
| **9** | First test subject dropped out. | August 2021 | The study period is 24 weeks, and the telephone follow-up is completed. |
| **10** | The last participant has left the group. | September 2021 | The study period is 24 weeks, and the telephone follow-up is completed. |
| **11** | Quality control at each center has been completed. | October 2021 | Quality control issue resolved. |
| **12** | Database lock | November 2021 | After the data Q&A session is completed, the EDC will lock the database. |
| **13** | Research Center Closed | December 2021 | Quality control completed, material retrieval completed, final payment settled, ISF institution archived, and materials, consumables, and drugs retrieved. |
| **14** | Finalize Statistical Analysis | January 2022 | Review and submit data report. |
| **15** | Final Draft of Clinical Trial Summary Report | February 2022 | Clinical Trial Summary Meeting |
| **16** | Clinical Trial Conclusion | March 2022 | Complete paper writing and submission. |

Attachment 2: List of Research Centers

| Number | Research Center Name | Principal Investigator | Participants |
|---|---|---|---|
| 1 | Pukou District TCM Hospital, Nanjing | Yanfei Mo | 25 |
| 2 | Nanjing Brain Hospital | Ming Wang | 20 |
| 3 | People's Hospital of Lujiang County | Zheng Liang | 25 |

Attachment 3, Leader's Unit Participants

| Name | Title/Professional expertise | Task | GCP Training |
|---|---|---|---|
| Yanfei Mo | Associate Chief Physician/Cardiology | Principal Investigator | 2019 |
| Feng Wang | Attending Physician/Cardiology | Investigator | 2020 |
| Yaoyao Yang | Attending Physician/Cardiology | Investigator | 2020 |
| Juan Gao | Head Nurse | Drug Administrator | 2020 |