# Protection of Cardiovascular Function with Crocin in BrEast Cancer patients undergoing Radiotherapy and Chemotherapy （ProtECtion Study）

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# Research Protocol

## BACKGROUND

Cardiovascular disease (CVD) and tumors lead to more than 2/3 of the world's death population each year [1]. Because CVD and tumors often share common risk factors, such as diabetes, they often coexist. At the same time, in the course of tumor drug therapy, radiotherapy and related adjuvant therapy, different degrees of cardiovascular function impairment can also occur [2]. In recent years, with the continuous development of medical technology, great progress has been made in the diagnosis and treatment of patients with malignant tumors, and the survival time has been significantly prolonged. Subsequently, the potential cardiovascular toxicity of tumor treatment and the resulting cardiovascular events have gradually become an important health risk for tumor survivors, and the death caused by cardiovascular events in tumor patients during the treatment process ranks first among all causes of death [3].

Traditional chemotherapeutic drugs, newly emerging biological agents, and cardiothoracic radiotherapy can all act on the cardiovascular system, causing structural and functional damage to the corresponding organs. Traditional cytotoxic drugs such as anthracyclines and their derivatives, antimetabolites, paclitaxels, alkylating agents, platinums, and alkaloids can cause irreversible cardiovascular damage with increasing cumulative doses [2]. Among them, doxorubicin and 5-fluorouracil (5-FU) are reported more. It has been confirmed that doxorubicin can produce cardiotoxicity by inhibiting mitochondrial and sarcoplasmic reticulase activities, causing myocardial cell necrosis or apoptosis, resulting in cardiac insufficiency [4-6]; 5-FU can damage endothelial cell function, interfere with Smooth muscle cell signal transduction triggers abnormal vasoactive responses that ultimately lead to coronary spasm and myocardial ischemia. Biologics, including monoclonal antibodies, tyrosine kinase inhibitors (TKIs), and endocrine agents have the same potential for cardiovascular toxicity, especially vascular endothelial growth factor (VEGF) inhibitors. VEGF inhibitors can increase the risk of heart failure, coronary

heart disease, hypertension and thromboembolic diseases through mechanisms such as endothelial damage, vasoconstriction and remodeling, inflammation and platelet activation [7-9]. Different from cytotoxic drugs, cardiovascular injury caused by biological agents can be partially or completely alleviated after timely intervention (such as adding cardioprotective drugs, adjusting anti-tumor regimen, etc.) [10]. In addition, the cardiovascular effects of radiotherapy are mainly due to radiation-related injury. When the radiation acts on the tumor tissue, it will inevitably cause damage to the normal tissue in and around the radiation field, resulting in radiotherapy-related heart disease such as diffuse myocardial or pericardial fibrosis, pericarditis, coronary artery disease and valvular disease. The incidence rate is as high as 10% to 30% [11-13].

To the above mentioned problems, an emerging discipline--Cardio-Oncology, came into being. Cardio-Oncology is a discipline that studies the development of cardiovascular disease, risk assessment, diagnosis and treatment, and prognosis follow -up in tumor patients, involves all aspects of tertiary prevention of cardiovascular disease in cancer patients. Screening of malignant tumor patients with high cardiovascular risk factors, and then intervening in their early stages, in order to maximize the protection of patients' cardiac function, is undoubtedly important in the secondary prevention of cardiovascular disease in cancer patients. Because of its non-invasive, simple and real-time characteristics, echocardiography is widely used in the follow-up of cardiac function in cancer patients. However, due to the special pathophysiology of tumor patients, traditional conventional ultrasound indicators such as two-dimensional left ventricular ejection fraction (2D LVEF), left ventricular fractional shortening and tissue doppler imaging cannot sensitively diagnose early cardiovascular damage [14]. In recent years, new ultrasound technology based on speckle tracking imaging (STI) has shown superiority in the diagnosis, risk stratification and prognosis evaluation of cardiovascular diseases related to tumor therapy [15]. STI can automatically track the natural acoustic speckle in the myocardial tissue , and calculate the motion trajectory between two points. Through the subsequent processing of the recorded trajectory, it can provide information for

evaluating the systolic and diastolic function of the whole and individual segments of the myocardium. STI tracks myocardial motion well even with very small changes in the position of the myocardium. STI has no Doppler angle dependence, can integrate myocardial motion in longitudinal, radial, and circumferential directions, and has high specificity and sensitivity in evaluating cardiac function [16]. STI-related parameters mainly include: global longitudinal strain (GLS)/strain rate, global circumferential strain, global radial strain, global area strain (3D-GAS), left ventricular torsion, left ventricular rotation/ Untwisting speed, systolic dissynchrony index (SDI) and right ventricular free wall systolic peak strain (RVFWS) and so on [17]. Among them, left ventricular global longitudinal strain (GLS) has been proved to be a more sensitive indicator of myocardial contractile function than LVEF. Studies have reported that GLS decline in the early stage of chemotherapy has a strong predictive value for patients with subsequent LVEF <50%. There is no literature report on the magnitude of the effect of cardioprotective therapy on GLS in cancer patients treated with chemoradiotherapy. A real-world study by our group used STI technology to monitor the changes of cardiac function in breast cancer patients during chemotherapy. The preliminary results showed that GLS in breast cancer patients was significantly lower than the baseline level after 4 cycles of chemotherapy.

The prevention of cardiovascular events has become one of the bottlenecks in improving the prognosis of cancer patients. However, because most classic clinical studies in the cardiovascular field exclude cancer patients, the evidence-based cardiology for first-line doctors is very limited. It is urgent to explore and develop methods to effectively protect cardiovascular function. Crocus, also known as saffron, is the dried stigma of the perennial Iridaceae plant, saffron, and is a precious Chinese medicine. Crocin is a water-soluble carotene compound of saffron. It is a series of ester glycosides composed of saffron acid and different sugars. It is one of the main active components of saffron. It has various effects such as anti-oxidative stress, anti-platelet aggregation, anti-thrombosis, and anti-myocardial ischemia [18]. Studies have shown that crocin has good curative effect on cardiovascular diseases such as hypertension, hyperlipidemia and atherosclerosis, and also has anti-cancer,

anti-inflammatory, anti-oxidative, hepatoprotective, choleretic and anti- diabetes properties etc [19]. A number of basic and small-sample clinical studies have shown that crocin can protect the myocardium from ischemia-hypoxia injury by anti-oxidative stress and reducing the activity of creatine kinase. On the other hand, basic research has also found that crocin can significantly improve doxorubicin-induced cardiac injury and structural changes without affecting the in vitro anticancer activity of doxorubicin [20]. Crocin can reduce the level of malondialdehyde (MDA) and increase the level of glutathione in the heart tissue of rats taking chemotherapy drugs, and by reducing the ratio of Bax/Bcl-2, the release of cytochrome C and activation of caspase-3 attenuates cardiomyocyte apoptosis, thereby ameliorating cardiac histopathological damage [21]. These studies suggest that crocin may help prevent or improve the myocardial injury induced by radiotherapy and chemotherapy in cancer patients and protect cardiovascular function.

The main component of saffron total glucoside tablets is crocin, which has been approved to be used in patients with angina pectoris in China. At present, the protective effect of crocin on cardiovascular mainly focuses on basic research and small sample clinical research, and serological indicators are the main observation indicators. There is still a lack of early application of saffron in patients with cardiac function and vascular function damage. Therefore, the protective effect of crocin on the cardiovascular function of tumor patients needs further research.

## OBJECTIVES:

This study was to observe the effect of crocin on cardiovascular function in patients with early breast cancer undergoing radiotherapy and chemotherapy.

## RESEARCH DESIGN

This is a randomized, double-blind, parallel-controlled, single-center clinical study.

### Participant:

The patients are mainly from breast cancer patients who are hospitalized during

the study period, planned to undergo radiotherapy and chemotherapy, and have high risk factors for cardiovascular disease.

***Inclusion criteria:***

1)Age 25-80 years old, female.

2) Patients diagnosed with breast cancer by histopathology.

3) Patients who plan to receive adjuvant radiotherapy/chemotherapy or combined adjuvant trastuzumab or pertuzumab targeted therapy.

4)Patients who completed at least 6 cycles of treatment after enrollment.

***Exclusion criteria:***

1)pregnant or breastfeeding women.

2)Patients with poor echocardiographic image quality.

3)Persistent atrial fibrillation and severe arrhythmia affect the collection and analysis of ultrasound data.

4)Patients who are participating in other clinical studies.

**Dosing regimen**

***1)Crocin group***: The chemotherapy/radiotherapy protocols are made by oncologists adopted for patients depending on specific conditions, take saffron total glucosides tablets (provided by REYOUNG Pharmaceutical Co., Ltd.) for 8 days during each chemotherapy (started on the 1st day before chemotherapy), 4 tablets/time, 3 times a day.

***2) Placebo group***: Undergoing chemotherapy/radiotherapy protocols as planned, take placebo piece during (the same appearance of saffron total glucosides tablets, production unit:REYOUNG incorporated company) for 8 days during each chemotherapy (started on the 1st day before chemotherapy), 4 tablets/time, 3 times a day.

**Criteria for Subject Discontinuation from Study**

1) Allergic reactions that are clearly related to the study drug.

2) In the event of abnormal bleeding, other adverse symptoms or signs, and abnormal examination results clearly related to the study drug, the investigator judges that the study must be terminated.

3) Women who become pregnant during the study period.

4) Continued research should be terminated at the request of the patient.

**Criteria for withdrawing from the study (dropout)**

1) The patient was lost to follow-up.

2) Poor patient compliance.

3) Blind release or emergency unblinding.

4) There is an adverse event related to the drug.

**Criteria for full suspension of the test**

1) The researchers found serious safety problems.

2) The curative effect is too poor, and there is no need to continue the test.

3) There are major mistakes in the research plan.

4) Unsustainable research funding.

5) The administrative department cancels the test.

A full suspension of the trial can be temporary or permanent. When the test is terminated, all test records shall be retained for future reference

## RESEARCH PROPOSAL

### Estimation of Sample Size

Use PASS 11.0.7 software, bilateral inspections, $\alpha = 0.05$, $\beta = 0.2$ (grasping degree = 80%), and the number of allocations of the test group and the control group should be allocated by 1: 1. It is expected that the LVEF of the crocin group will decrease by no more than 0.17% compared with the baseline at the end of the 6-month observation, and the average LVEF of the placebo control group will decrease by 3.28% compared with the baseline, with a standard deviation of 5.38%. The number of cases should be 48 pairs. Considering the dropout rate of 20%, a total of 120 patients were randomly selected for this study.

Preliminary results from our real-world study showed that breast cancer patients (n=79) had a GLS of -22.6 ± 1.77 before chemotherapy, -16.96 ± 3.65 after 6 cycles of chemotherapy, and -15.94 ± 4.03 after 8 cycles of chemotherapy. Since there is no literature report on the magnitude of the effect of cardioprotective therapy on GLS in

cancer patients undergoing radiotherapy and chemotherapy, the sample content was not calculated based on GLS.

**Random grouping**

Eligible patients were randomly divided into 1:1 group and divided into crocin group and placebo control group. Both groups of subjects received crocin or placebo on the basis of standard anti-tumor treatment. The randomization plan adopts the method of stratified block randomization, and the statistician uses SAS14.0 software to generate a random code table with a block of 4 and the treatment group corresponding to the random number. The randomization table (blind bottom) was sent to the project statistician by the random coders after the database was locked and approved by the sponsor at the end of the study. The drugs were blinded according to the serial number and random number. After the subjects are screened and qualified, the researchers will distribute the drugs in order according to the drug number.

**Research Process**

After the selected patients signed the informed consent form, they were randomly tested in the crocin group and the placebo group according to a randomized, double-blind, placebo-controlled method. Dosage of crocin group or placebo: 3 times a day, 4 tablets each time. The follow-up period was 6 months. Crocin group: take saffron total glucosides tablets for 8 days during each radiotherapy and chemotherapy (started on the 1st day before radiotherapy/chemotherapy), 4 tablets/time, 3 times a day; Placebo group: placebo during radiotherapy and chemotherapy for 8 days, start to take 1 day before radiotherapy/chemotherapy, 4 tablets/time, 3 times a day. Follow-up was performed every 3 months after enrollment, and the follow-up period was 6 months. After all patients were enrolled and followed up for 6 months, the study was terminated and unblinded.

**Chemotherapy regimens**

The chemotherapy regimens are determined by the patient's condition.

***(1) Patients with Her-2 positive breast cancer:***

*(i) A (E) C → PH/TH ± Pertuzumab regimen:*

Doxorubicin (A) 50-60mg/m2 iv on Day 1 or Epirubicin (E) 80-100mg/m2 iv on

Day 1, Cyclophosphamide (C) 600mg/m2 iv on Day 1, Day 21 is 1 cycle, a total of 4 cycles; the sequential A/B scheme is as follows:

A. Sequentially paclitaxel (P) 80 mg/m2 iv on day 1, once a week, for 12 weeks, and trastuzumab (H) at the first dose of 4 mg/kg, then 2 mg/kg, once a week, for a total of 12 weeks; it can be combined with Pertuzumab for the first dose of 840 mg, followed by 420 mg each time, 21 days as a cycle, a total of four cycles.

B. Sequentially docetaxel (T) 100mg/m2 iv on the first day, 21 days as a cycle, a total of 4 cycles. At the same time, the first dose of trastuzumab (H) is 8mg/kg, followed by 6mg/kg, 21 days as a cycle, a total of 4 cycles, can be combined with the first dose of Pertuzumab 840mg, and then 420mg each time, 21 days For 1 cycle, a total of 4 cycles, used concurrently with trastuzumab.

*(ii) TCbH±Pertuzumab regimen:*

Docetaxel (T) 75 mg/m2 iv on day 1, carboplatin (Cb) AUC 6 iv on day 1, 21 days as a cycle, a total of 6 cycles; The first dose is 8mg/kg, followed by 6mg/kg, 21 days as a cycle, a total of 6 cycles, can be combined with the first dose of Pertuzumab 840mg, and then 420mg each time, and trastuzumab can be used at the same time.

A (E) C→PH/TH regimen and TCbH regimen both have a dose of 6 mg/kg of trastuzumab (H) after chemotherapy, and can be combined with a dose of 420 mg of pertuzumab, once every 3 weeks for full 1 year.

***2) Patients with HER-2 negative breast cancer:***

*(i) A (E) C → P/T regimen;*

Doxorubicin (A) 60mg/m2 iv on day 1 or epirubicin (E) 60-100mg/m2 iv on day 1, cyclophosphamide (C) 600mg/m2 iv on day 1, 1 on day 21 cycle, a total of 4 cycles.

Sequentially with paclitaxel (P) 80mg/m2 iv on the 1st day, once a week for a total of 12 weeks; or docetaxel (T) 100mg/m2 iv on the 1st day, 21 days as a cycle, a total of 4 cycle.

*(ii) TC regimen:*

Docetaxel (T) 75 mg/m2 iv on day 1, carboplatin (Cb) AUC 6 iv on day 1, 21 days as a cycle, a total of 6 cycles.

**The main test indicators in the selection and follow-up period**

***(1) The main observation and detection indicators at the time of selection***

(i) Clinical conditions: chest tightness, chest pain, palpitation, fatigue and other clinical symptoms and signs

(ii) ECG examination: use resting ECG and dynamic ECG to evaluate arrhythmia and ST-T changes.

(iii) Laboratory tests: fasting for more than 6 hours, blood routine, urine routine, fasting blood glucose, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, plasma total cholesterol, triglyceride, low-density lipoprotein cholesterol, high-density lipid Protein cholesterol, high-sensitivity C-reactive protein, prothrombin time, serum troponin, NT-proBNP.

(iv) Transthoracic echocardiography:

Two-dimensional dynamic images of left ventricular parasternal long axis, apical four-chamber heart, apical two-chamber heart, apical long axis, left ventricular apex level, papillary muscle level, and mitral valve level short-axis view; measure left ventricular ejection fraction (LVEF ); Pulse wave Doppler was used to obtain the diastolic mitral valve orifice blood flow spectrum, and tissue Doppler was used to obtain the mitral valve annulus motion spectrum, and E/e' was calculated; Using speckle tracking imaging (STI) ultrasound technology to measure global longitudinal strain (GLS)/strain rate, global circumferential strain, global radial strain, global area strain (3D-GAS), left ventricular torsion, left ventricular rotation/untwisting speed, systolic dissynchrony index (SDI) and right ventricular free wall systolic peak strain (RVFWS) and so on. See Annex 1 for details.

***(2) The main observation and detection indicators of the first follow-up (3 months)***

(i) Clinical situation: chest tightness, chest pain, palpitation, fatigue and other clinical symptoms and signs

(ii) ECG examination: use resting ECG and dynamic ECG to evaluate arrhythmia and ST-T changes.

(iii) Laboratory tests: fasting for more than 6 hours, blood routine, urine routine,

fasting blood glucose, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, high-sensitivity C-reactive protein, prothrombin time, serum troponin, NT-proBNP.

(iv) Transthoracic echocardiography:

Two-dimensional dynamic images of left ventricular parasternal long axis, apical four-chamber heart, apical two-chamber heart, apical long axis, left ventricular apex level, papillary muscle level, and mitral valve level short-axis view; measure left ventricular ejection fraction (LVEF )、E/e'； Using speckle tracking imaging (STI) ultrasound technology to measure global longitudinal strain (GLS)/strain rate, global circumferential strain, global radial strain, global area strain (3D-GAS), left ventricular torsion, left ventricular rotation/untwisting speed, systolic dissynchrony index (SDI) and right ventricular free wall systolic peak strain (RVFWS) and so on.

***(3) The second follow-up (6 months, at the end of the study), the main observation and measurement indicators***

After enrollment, the following indicators were detected before each chemotherapy cycle and every 3 months after the end of the chemotherapy cycle:

(i) Clinical situation: chest tightness, chest pain, palpitation, fatigue and other clinical symptoms and signs，etc.

(ii) ECG examination: use resting ECG and Holter ECG to evaluate arrhythmia and ST-T changes.

(iii) Laboratory tests: fasting for more than 6 hours, blood routine, urine routine, fasting blood glucose, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, plasma total cholesterol, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, High-sensitivity C-reactive protein, prothrombin time, serum troponin, NT-proBNP.

(iv) Transthoracic echocardiography:

Two-dimensional dynamic images of left ventricular parasternal long axis, apical four-chamber heart, apical two-chamber heart, apical long axis, left ventricular apex level, papillary muscle level, and mitral valve level short-axis view were obtained;measure the LVEF, E/e’; Using STI ultrasound technology to measure global

longitudinal strain (GLS)/strain rate, global circumferential strain, global radial strain, global area strain (3D-GAS), left ventricular torsion, left ventricular rotation/untwisting speed, systolic dissynchrony index (SDI) and right ventricular free wall systolic peak strain (RVFWS) .etc.

**DATA MONITORING**

(1) Inform the participant that his/her personal study-related data will be used in accordance with local data protection laws. The extent of disclosure must also be explained to participants.

(2) All participant data related to the study will be recorded on the CRF. Researchers are responsible for verifying the accuracy and correctness of data entry.

(3) The investigator must maintain accurate documentation supporting the CRF information.

(4) Investigators must allow study-related monitoring and provide direct access to source data files.

(5) Designate personnel to be responsible for data management for this study, including data quality checks.

(6) Regulatory agency inspectors are responsible for contacting and visiting investigators to inspect facilities and, upon request, to inspect various records of clinical studies (eg, case report forms and other relevant data).

(7) Monitors are responsible for regularly validating case report forms throughout the study to verify compliance with the protocol; completeness, accuracy, and consistency of data; and compliance with local regulations for conducting clinical studies.

(8) Educate patients to promptly report any symptoms and unusual signs. Safety Assessment Physicians record the patient's symptoms and physical examination results. Patients with serious adverse events can discontinue their assigned medication and be closely monitored or receive emergency treatment until the adverse event resolves.

**EFFICACY ASSESSMENT**

**Primary study endpoint:**

1) Differences between groups in the difference in LVEF measured by echocardiography at the end of the experiment compared to baseline.

2) Differences between groups in the difference in GLS measured by echocardiography at the end of the experiment compared to baseline.

**Secondary study endpoints**

1) Differences in the incidence rates of serum troponin exceeding the upper limit of normal value and NT-proBNP higher than the normal age reference value between the two groups during follow-up.

2）The differences in the incidences of chest tightness, chest pain and palpitation between the two groups.

3）Differences between the two groups in the incidences of arrhythmia and ST-T changes displayed by dynamic electrocardiogram.

4） Differences between groups in the difference in the E/e', 2D-STI indexes, 3D-STI indexes measured by echocardiography at the end of the experiment compared to baseline.

5) Differences between groups in the difference in the indexes of left ventricular diastolic function measured by echocardiography at the end of the experiment compared to baseline.

6) Differences between groups in the difference in the right ventricular function monitoring indicators measured by echocardiography at the end of the experiment compared to baseline.

**SAFETY ASSESSMENT**

Including clinical adverse reactions, laboratory indicators, etc.

**ADVERSE EVENT OBSERVATION AND REPORTING**

**Definition of Adverse Events**

Adverse events refer to any adverse medical events that occurred between the time the patient signed the patient's informed consent and were enrolled in the trial

and the last follow-up visit in this clinical trial, regardless of whether the event had a causal relationship with the above-mentioned drugs.

Note: Adverse reactions may be any discomfort and unintentional signs (including abnormal laboratory test results), symptoms, or illnesses (new or worsening) associated with the drug.

*Events that meet the definition of an adverse event include:*

(i) Exacerbation of existing chronic or intermittent disease, including increased frequency and/or intensity.

(ii) Newly detected or diagnosed disease after taking the trial drug, even though it may have existed before the trial started.

(iii) Signs, symptoms or clinical sequelae of suspected interaction.

(iv) Signs, symptoms or clinical sequelae of suspected overdose of investigational drug or concomitant drug (overdose itself is not an adverse event/serious adverse event).

(v) "Lack of efficacy" or "not achieving expected pharmacological effects" by themselves are not reported as adverse events or serious adverse events. However, signs, symptoms and/or clinical sequelae due to lack of efficacy will also be reported as adverse events or serious adverse events if they meet the definition of an adverse event or serious adverse event.

*Events that do not meet the definition of an adverse event include:*

(i) Medical or surgical procedures (eg, endoscopy, appendectomy); events leading to surgery are adverse events.

(ii) Situations where adverse medical events would not occur (social security and/or hospital admissions).

(iii) No worsening of pre-existing disease or conditions present or detected at the start of the trial, only expected day-to-day fluctuations.

(iv) The disease/disorder under study, or the expected progression, signs or symptoms of the disease under study. Unless the situation is more serious than expected.

***Definition of Serious Adverse Events***

A "serious adverse event" is an adverse event that can occur at any dose and will:

(i) Causing death.

(ii) Life-threatening.

Note: "Life-threatening" means that the subject is in danger of dying when the event occurs, not that death could theoretically result if the event were more severe.

(iii) Requiring hospitalization or prolonged hospitalization.

Note: Generally, hospitalization refers to a subject's stay in a hospital or emergency room (usually at least overnight) for observation and/or treatment that cannot be accomplished in a doctor's office or outpatient clinic. Complications that occurred during hospitalization were considered adverse events. An event was classified as a serious adverse event if the complication prolonged hospital stay or met the criteria for any other serious adverse event. Treat as a serious adverse event when it cannot be determined whether "hospitalization" occurred or was necessary.

Selective hospitalization with no worsening of pre-existing disease compared to baseline was not considered an adverse event.

(iv) Causing disability, or affect the ability to work or live.

Note: "Disability" refers to a material that impairs an individual's ability to lead a normal life. Discomforts of insignificant clinical significance, such as common headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle), which may interfere with quality of life but are not substantially disruptive, are excluded.

(v) Causing congenital deformities.

In other cases, medical or scientific judgment should be used to determine the appropriateness of adverse event reporting. For example, some important medical events may not be immediately life-threatening, lead to death or hospitalization, but may harm the subject or require medical or surgical intervention to avoid the occurrence of the serious adverse events listed above. These events should also be considered serious adverse events, such as invasive or malignant cancer, allergic bronchospasm requiring intensive monitoring in the emergency room or at home, blood cachexia, or convulsions that do not result in hospitalization, drug dependence,

or drug abuse.

**Medical side effects**

Definition: Refers to the harmful reactions of qualified drugs that are not related to the purpose of drug use under normal usage and dosage.

Causal judgment indicators for adverse reaction judgment:

Item 1: Whether there is a reasonable relationship between the time when the drug was started and the time when the suspicion appeared.

Item 2: Whether the suspected adverse reaction conforms to the known adverse reaction type of the drug.

Item 3: Whether the suspected adverse reaction can be explained by the patient's pathological condition, concomitant medication, concomitant therapy, or previous therapy.

Item 4: Whether the suspected adverse reaction is alleviated or disappeared after drug discontinuation or dose reduction.

Item 5: Whether the same reaction reappears after the suspected drug is used again.

Criteria for causal judgment: according to the order of the above five judgment indicators.

Possible associations between adverse events, study drug, and concomitant medications were assessed by the investigator according to the table below.

Adverse reaction cause and effect judgment form

| critical result | Judgment index | | | | |
|---|---|---|---|---|---|
| | Item1 | Item 2 | Item 3 | Item 4 | Item 5 |
| Definitely related | ＋ | ＋ | － | ＋ | ＋ |
| Might be related | ＋ | ＋ | － | ＋ | ? |
| May be | ＋ | ＋ | ± | ± | ? |

| | | | | | |
|---|---|---|---|---|---|
| related | | | | | |
| suspicious | ＋ | － | ± | ± | ? |
| Impossible | ＋ | － | ＋ | － | － |

Description: + affirmative, - negative, ± difficult to affirm or deny, ? The situation is unknown.

1) According to the above table, determine the relationship between the adverse events and drugs to the following five grades

(i) definitely related, (ii) likely related, (iii) likely related, (iv) doubtful, (v) unlikely to be related.

2) The incidence of adverse reactions was calculated using the total number of 1+2+3+4 cases as the numerator, and all the selected cases for evaluation of adverse reactions as the denominator.

**Records of adverse event**

For adverse events that occurred during the trial, the type, degree, time of appearance, duration, treatment measures, and treatment process should be recorded in the case report form, and on the basis of comprehensive consideration of comorbidities and concomitant medication, the evaluation of their relationship with the experiment should be made. Dependence of medication and detailed documentation by physician.

If adverse events are found, the observing physician can decide whether to discontinue the observation according to the condition. Cases of discontinuation due to adverse events should be followed up and the results recorded in detail. If abnormal safety test indicators (blood, urine, stool routine, electrocardiogram, liver function, renal function) occur during and after treatment, the adverse event table should be filled in in time, and re-examination should be conducted at an appropriate time, and the subject should be consulted the incidence, treatment, etc. are comprehensively analyzed to determine whether it is related to the test drug.

**Management of serious adverse events**

Any serious adverse events during the trial (including events requiring hospitalization, prolonged hospitalization, disability, affecting work ability, endangering life or death, causing congenital malformations, etc.) In addition to taking urgent measures, the subject must also immediately report to the Ethics Committee of the National Drug Clinical Trial Institution of Qilu Hospita of Shandong University, the main research unit, and REYOUNG Pharmaceutical Co., Ltd. The report of serious adverse events should include : patient's name, random number, length of study participation, start and stop dates of serious adverse events, maximum intensity of serious adverse events, possible relationship between serious adverse events and study drug, due to serious adverse events. Whether the adverse event required a change in study drug, the treatment given to the patient due to the serious adverse event, concomitant medications in the event of a serious adverse event, and the outcome of the serious adverse event.

## DATA MANAGEMENT AND STATISTICAL ANALYSIS

### Data management

(1) Establishment of database: The data administrator establishes the EXCEL database according to the research protocol and CRF, and sets up logical verification according to the data verification plan (DVP). After the test is passed, it will be released for use.

(2) Data entry: The data entry personnel conduct independent double entry and double verification. For inconsistent results, check and correct each item against the CRF until the results are completely consistent.

(3) Data questions and answers: After the data entry is completed, the data administrator conducts question screening according to the manual verification plan of the DVP, opens the database access rights to the researchers, and answers the questions remotely. The data administrator replies to the query, and if necessary, can re-issue the query until the data is "clean".

(4) Database lock: After the principal investigator, sponsor, statistical analyst and data administrator jointly sign the "database lock record", the data administrator locks

the database.

(5) Database submission: The data administrator submits the database to the statistician.

**Statistical analysis dataset**

(1) Full Analysis Set (FAS): A collection of all randomized patients who received at least one study drug.

(2) Per-Protocol Set (PPS): is a data set generated by subjects who are fully compliant with the trial protocol, including the treatment received, the availability of measurements of the primary endpoint, and the absence of major deviations from the trial protocol. PPS analysis was used for the primary efficacy measure.

(3) Safety Data Set (SS): Actual data on at least one treatment with recorded safety indicators after treatment. The incidence of adverse reactions was taken as the denominator of the number of SS cases.

**Statistical methods**

***Subject Distribution Analysis***

(1) The number of subjects enrolled and who completed the trial was listed, and three data sets for analysis (FAS, PPS, SS) were identified.

(2) The reasons for not entering PPS were classified and analyzed, and the number of subjects in different categories was calculated.

(3) List the detailed list of population groups, including the reasons for not being included in PPS/FAS/SS.

(4) Draw the subject distribution flow chart.

***Demographic data and baseline analysis***

Descriptive Statistics Demographics and other baseline characteristic values:

(1) Calculate the number of cases, mean, standard deviation, 95% CI, minimum and maximum value of continuous variables.

(2) Count and grade data to calculate frequency and composition ratio.

(3) Inferential statistical results (P values) are listed as descriptive results.

***Analysis of the compliance and consolidation of medication***

(1) Calculating the percentage of the subjects with the compliance of the

medication in the range of 80%-120%, and the difference between the comparison of the comparison of the χ2 test or the Fisher accurate probability method.

(2) Drug exposure, use T test comparison between group differences.

(3) Calculate the percentage of the subjects with consolidated medication, and the difference between the comparison of the comparison of the χ2 test or the Fisher accurate probability probability method.

***Analysis of efficacy***

Main efficacy index analysis

At the end of treatment, the differences between the LVEF and GLS measured by ultrasound compared with baseline were compared between groups using the t test. PPS and FAS analyses were performed simultaneously.

***Secondary efficacy index analysis***

(1) During the follow-up period, the incidence rates of serum troponin (cTnI)>30ng/L and NT-proBNP higher than the normal age reference value were compared between groups by χ2 test or Fisher's exact test. The actual data in FAS were used for analysis.

(2) The frequency and duration of chest tightness, chest pain and palpitation were compared between groups using t test. The actual data in FAS were used for analysis.

(3) Holter electrocardiogram showed the degree of arrhythmia and ST-T changes, and t-test was used to compare the differences between groups. The actual data in FAS were used for analysis.

(4) Compared with baseline, ultrasound-measured global radial strain, systolic dyssynchrony index (SDI), and t-tests were used to compare differences between groups. The actual data in FAS were used for analysis.

***Security analysis***

(1) Calculate the incidence of adverse events/reactions, serious adverse events/reactions, and adverse events/reactions leading to dropout.

(2) A detailed list of cases of various adverse events/reactions, serious adverse events/reactions, adverse events/reactions leading to dropout.

(3) List the cross-tabulation of laboratory examinations and electrocardiogram examinations before and after medication.

(4) Descriptive statistics of laboratory examinations, electrocardiograms, and changes in vital signs from baseline and measured values.

(5) A detailed list of abnormal values of laboratory tests, electrocardiograms, and physical examinations is listed.

***Statistics software***

(1) SAS software (version 9.4) was used for analysis.

(2) All statistical tests are two-sided, and a P value less than or equal to 0.05 will be considered to be statistically significant for the differences tested.

(3) Detailed statistical methods will be provided in the statistical analysis plan.

**RESEARCH PLAN**

(1) Case entry stage: from March 2021 to December 2022—complete the entry of each group of cases.

(2) Follow-up time: 6 months. For follow-up time points, please refer to the attached trial procedure. All subjects were followed up by June 2023.

(3) Data analysis: June 2023 to September 2023—Data collection and analysis, writing papers .

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# Annex 1. Standard Operating Procedures for Ultrasound Examination

**Personnel and equipment requirements**

In order to ensure the quality of image acquisition and the standardization of measurement data, two experienced sonographers from the central laboratory-Qilu Hospital of Shandong University first conducted a demonstration of standard image acquisition according to the research protocol. In the screening stage, the researchers of each research unit need to submit the sample images to the central laboratory for review, which will be evaluated and fed back by the sonographer. All participants in the collection and analysis have no stake in this research. Each operator needs to have corresponding qualifications and training, and the ultrasound machine that collects images has a clear imaging function, so as to better perform ultrasound examinations and capture images.

**Operation method**

Standardized cardiac anatomical parameters and hemodynamic function parameters were measured using internationally recognized standardized echocardiographic cardiac views. All measured values are determined uniformly to 1 digit after the decimal point. Three cardiac cycle parameters were measured for each parameter, and the mean value was taken.

**Specific implementation methods and steps:**

***Preparation before inspection***

Rest for 5 minutes before the test.

Connect synchronized ECG monitoring electrodes to determine cardiac cycle phases. The end of the T wave of the ECG was used to define the end systole of the ventricle, and the peak of the R wave of the QRS wave was used to define the end of the ventricular end diastole.

***Position***

The left lateral decubitus position was used as the detection position for echocardiographic parasternal and apical views.

***Breathing***

In order to exclude the influence of respiration on the measured value, the respiration should be controlled at the end of expiration and temporarily held before the image acquisition (except when observing the inner diameter of the inferior vena cava).

***Inspection site***

The following sound-transmitting windows are used for detection: the detection area of the left sternum and the left apex of the heart. After the detection area is determined, enough ultrasonic couplant is filled between the skin of the detection area and the ultrasound probe to remove air and reduce gas interference.

***Parameter setting of echocardiography equipment and technical requirements before image acquisition***

(1) It is recommended to use the fundamental wave image for cardiac structure observation and measurement to avoid the distortion effect of tissue harmonic imaging. Optimize the resolution of two-dimensional gray-scale ultrasound images: try to use the smallest detection depth and the highest ultrasonic emission frequency; use standard two-dimensional cardiac slices and appropriate detection sound-transmitting windows to ensure that the observation slices are displayed clearly and completely.

(2) For two-dimensional echocardiographic grayscale images, the frame rate of observed and recorded images should be greater than or equal to 30 frames/second; for color tissue Doppler velocity images, the frame rate of observed and saved images should be greater than or equal to 80 frames/second , in order to facilitate subsequent functional parameter analysis.

(3) In order to avoid the artificial shortening effect of the long axis of the left ventricle when observing the apex, the left lateral position should be used, the use of an overly soft mattress should be avoided, and the position of the apex palpable by palpation should be avoided. The section with the largest inner diameter of the long axis is measured.

(4) In order to accurately determine the end-diastolic and end-systolic phases of

the ventricle, both the mitral valve motion and the chamber diameter change should be referenced, and excessive reliance on ECG to determine the phase should be avoided.

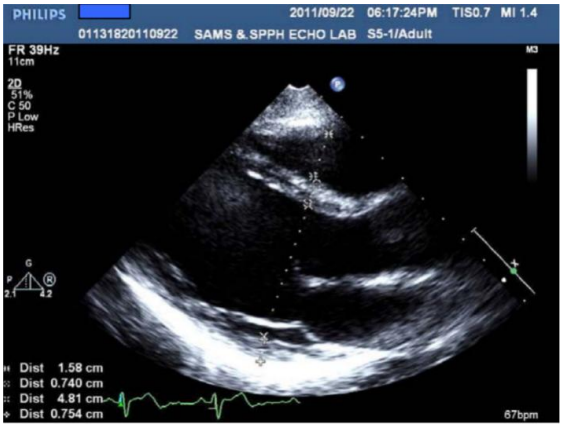
(5) The tissue Doppler sampling frame was set to 1 mm, and color Doppler flow imaging should be canceled during sampling to determine the spatial location of cardiac anatomy. Adjust the filter appropriately to avoid spectral distortion caused by too low or too high (too high filter results in low-speed frequency shift signal filtering, too low filter results in too strong noise signal).

(6) Image acquisition and image storage requirements: use a high-end cardiovascular color Doppler diagnostic instrument to acquire cardiac images, and image storage requires 5 continuous cardiac cycle dynamic images, which are stored in DICOM format. Each patient is a folder, including each image acquisition time and operator .

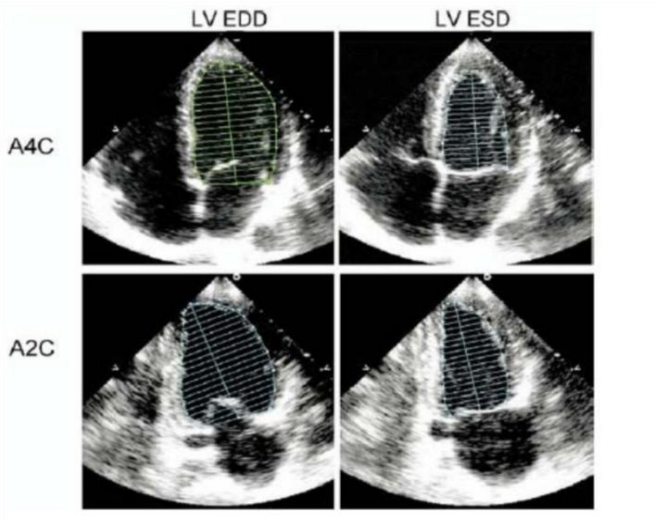
(7) Image analysis and repeatability test: The image information is sent to the central laboratory by the responsible personnel of each center by email for collection and storage, and then two experienced sonographers apply professional multi-center data analysis methods in accordance with the double-blind and random principles. Professional software is used for data reading, and each data is measured three times and averaged. The data with poor image quality will be re-examined by a team of professionals, analyzed multiple times, and finally decided whether to use it or not. In order to test the reproducibility of the measurement, this study randomly selected 50 subjects' images, and repeated the measurement of all key parameters. Inter-observer variability is a comparison of the difference between two sonographers' measurements, and intra-observer variability is a comparison of the difference between two measurements of the same physician at different times. Differences were compared using the Bland-Altman method to analyze and draw scatter plots, and to calculate correlation coefficients.

(8) The specific operation diagram of each measurement parameter is as follows:

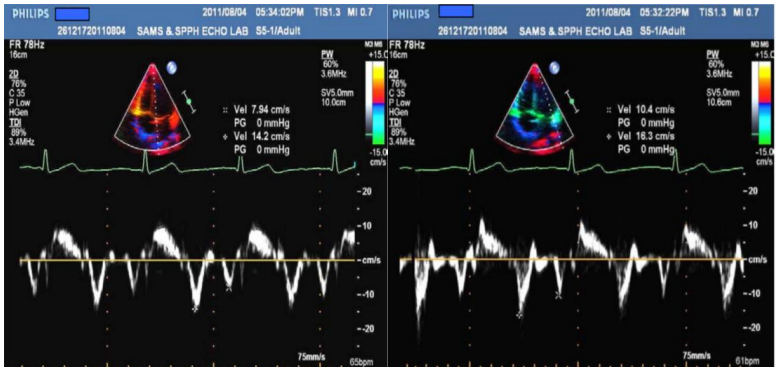
Parasternal long-axis view of the left ventricle showing end-diastolic left ventricular diameter measurement by two-dimensional echocardiography



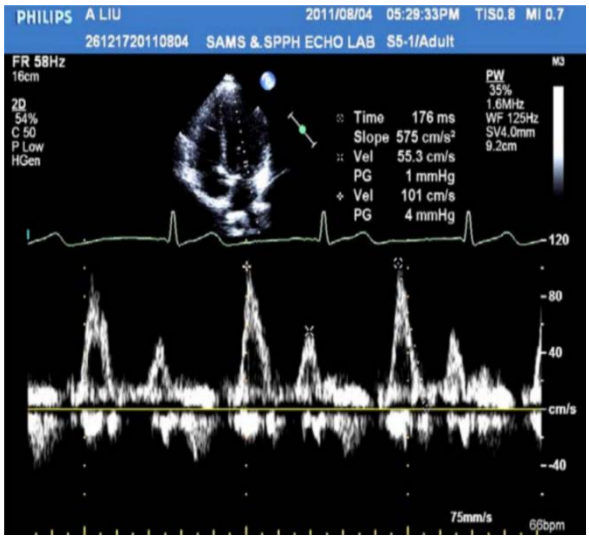

Standard apical four-chamber view for left ventricular end-diastolic and end-systolic volume and LVEF measurements using the biplanar Simpson method



Standard apical four-chamber view-guided pulse wave tissue Doppler sampling of mitral valve sidewall and septal annulus tissue motion e' velocity, and calculating the average.




Standard apical four-chamber view-guided pulse-wave Doppler sampling Peak E-peak velocity across the mitral valve during diastole




**Data storage and analysis**

All data are saved in DVD disc or mobile hard disk in DICOM format. Each patient is a folder, including the time of each image acquisition and the operator. The image information is sent to the central laboratory by the responsible personnel of each center by email for summary and storage, and then analyzed by two experienced sonographers. According to the double-blind and random principle, professional software for multi-center data analysis was used for data reading, and each data was

measured three times to obtain the average value. The data with poor image quality will be re-examined by a team of professionals, analyzed multiple times, and finally decided whether to use it or not.

**Intra-observer variation and inter-observer variation observed**

To test the reproducibility of the measurement, 50 subjects were randomly selected in this study, and all key parameters were repeatedly measured. The interobserver variability compared the difference between two sonographers' measurements, and the intraobserver variability compared the difference between the same physician's two measurements. Differences were compared using the Bland-Altman method to analyze and draw scatter plots, and the correlation coefficient was calculated.