A prospective, single-armed, phase II clinical trial of the efficacy and safety of weekly paclitaxel/cisplatin neoadjuvant chemotherapy combined with dual anti-HER2 therapy of trastuzumab and pyrotinib in patients with HER2-positive locally advanced breast cancer (NeoATP study)

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Responsible Institution: Renji Hospital, School of Medicine Shanghai Jiao Tong University

Principal Investigator: Jinsong Lu, Wenjin Yin

Signature page

As the principal investigator of this research project, I will follow the ethical principles of the Ministry of Health's Measures for Ethical Review of Biomedical Research Involving Human (2016), WMA's Declaration of Helsinki (2013) and CIOMS International Ethical Guidelines for Biomedical Research involving Human (2002) and GCP’s ethical principles, and conduct the research according to the requirements of the plan approved by the Ethics Committee, in order to ensure the scientificness of the research and protect the health and rights of the subjects.

Principal Investigator (print name):

Principal investigator (signature):

Signature date (Day/Month/Year):

TABLES OF CONTENTS

0	Protocol synopsis	4
1	Study purpose	9
2	Study background	10
3	Study rationale	12
4	Study content	20
5	Study design	21
6	Trial procedure	32
7	Start and end of trial	35
8	Clinical criteria for early termination of trials	36
9	Data security and monitoring plan	37
10	Compliance with ethical principles and related regulations	43
11	Statistical analysis plans	45
	Appendix I	47
	Appendix II	48
	Appendix III	51

Protocol synopsis

Study title	A prospective, single-armed, phase II clinical trial of the efficacy and safety of weekly paclitaxel/cisplatin neoadjuvant chemotherapy combined with dual anti-HER2 therapy of trastuzumab and pyrotinib in patients with HER2-positive locally advanced breast cancer (NeoATP study)
Version No./Date	1.3/2019.10.20
Responsible Institution	Renji Hospital , School of Medicine, Shanghai Jiaotong University
Principal Investigator	Jinsong Lu, Wenjin Yin
Study objectives	To investigate the efficacy and safety of pyrotinib and trastuzumab together with paclitaxel/cisplatin neoadjuvant chemotherapy in patients with HER2-positive locally advanced breast cancer.
Sample size	52 subjects
Study subjects	Patients with HER2-positive locally advanced breast cancer
Study methods	A prospective, open-label, single-armed, phase II clinical trial
Inclusion criteria	1) Female, aged ≥18 years old, ≤70 years old; 2) Patients with primary invasive breast adenocarcinoma confirmed by pathology and with clinical stages of stage IIA-III (according to the anatomic staging standard of AJCC Version 8) before neoadjuvant chemotherapy; If bilateral breast cancers are found at the same time, patients with invasive breast cancer on one side and carcinoma in situ on the other side can be enrolled. 3) The patient has at least one measurable lesion before neoadjuvant treatment according to RECIST Version 1.1; 4) The pathology in Renji Hospital, School of Medicine, Shanghai Jiaotong University, clearly shows that HER2 is positive in primary breast lesions or axillary metastasis, and HER2 positivity is defined as HER2(3+) by immunohistochemistry or amplification by fluorescence in situ hybridization (FISH), and the specimens from external hospital must be consulted by the Pathology Department of Renji Hospital,

	School of Medicine, Shanghai Jiaotong University; 5) During the period of neoadjuvant therapy, drug-induced ovarian castration is allowed to be given at the same time; 6) ECOG score 0 to 1 point (see Appendix I for ECOG scores); 7) Hematological and biochemical tests before enrollment should be as follows: WBC ≥ 4.0× 10^9/L, ANC ≥ 1.5× 10^9/L, PLT ≥ 100× 10^9/L; Hemoglobin (Hb) ≥ 90 g/L; Total bilirubin (TBIL)≤1.5× upper limit of normal (ULN), AST (sGOT), ALT (sGPT)≤1.5×ULN, urea nitrogen (BUN)≤1.5×ULN, and creatinine (Cr) ≤ 1.5 × ULN; 8) Creatinine clearance ≥50mL/min (Cockcroft-Gault formula); 9) Echocardiography should meet the requirement of left ventricular ejection fraction (LVEF) ≥ 50%; 10) A 12-lead ECG showed that the Fridericia-corrected QT interval (QTcF) < 470ms; 11) The subjects voluntarily participated in the study and signed informed consent.
Exclusion criteria	1) Pregnant or lactating women, women of childbearing potential who are fertile and have a positive baseline pregnancy test or who are reluctant to use effective contraception throughout the trial; 2) Patients with distant metastasis of breast cancer confirmed by imaging or pathology before randomization; 3) Patients with bilateral invasive breast cancer are found at the same time; 4) Being unable to swallow, chronic diarrhea and intestinal obstruction, and there are many factors that affect the taking and absorption of drugs; 5) Other targeted therapeutic drugs (such as lapatinib, pertuzumab, and T-DM1); 6) Application of anti-tumor therapy other than the research protocol during the experiment; 7) Allergies to study drugs or auxiliary materials; 8) Patients with sensory or motor nerve disease evidence; 9) A history of immunodeficiency, including HIV-positive tests, or other acquired or congenital immunodeficiency diseases, or a history of organ transplantation;

	10) Has suffered from any heart disease, including angina pectoris, arrhythmia requiring medical treatment or with clinical significance, myocardial infarction and heart failure, and any other heart disease judged by the researcher to be unsuitable for participating in the experiment; 11) Has a previous history of other malignancies, except for cured basal cell carcinoma of the skin or squamous cell carcinoma of the skin, and carcinoma in situ of the cervix; 12) In the judgment of the investigator, there are concomitant diseases (including but not limited to severe uncontrollable hypertension, severe diabetes, active infection, and thyroid disease) that seriously jeopardize the safety of the patient or affect the patient's completion of the study; 13) Has a clear history of neurological or psychiatric disorders, including epilepsy or dementia; 14) Any other circumstances in which the investigator concluded the patient was not eligible for participation in the study.
End of the study	1) The last patient enrolled completes the surgery; 2) The study is found to have unexpected, significant, or unacceptable risk to the subject; 3) In the study process, the execution of the scheme is found to have a significant error; 4) The study drug/test treatment is invalid, or is meaningless to continue to test; 5) It is extremely difficult to complete the study due to severe delay in enrollment of subjects or frequent protocol deviations.
Criteria of early withdrawl	1) The subject spontaneously withdrew the informed consent form at any time; 2) Subjects were found to have seriously violated the inclusion criteria; 3) Medical imaging or clinical progress; 4) Use of anti-tumor drugs other than the research protocol during the test; 5) Medications were not given according to the dose, method and course of treatment specified in this study protocol; 6) Any clinical adverse event, laboratory abnormality, or other medical condition that results in the possibility that the subject may no longer benefit from continuing

	to take the drug; 7) Gestation events that occurred in the subject during the study; 8) The research on the influence of medical or ethical reasons continues; 9) The subject is lost to follow-up; 10) Any other circumstances that the investigator deems necessary to withdraw from the study.
Administration route	Weekly chemotherapy regimen of pyrotinib/trastuzumab HER2 combined with paclitaxel/cisplatin; Trastuzumab: the first dose was 4mg/kg, and then 2mg/kg was maintained every week for 16 weeks. Pyrotinib: 400mg once a day, orally within 30 minutes after breakfast, continuously for 16 weeks; Paclitaxel: 80mg/m^2, intravenous drip, on the 1^{st}, 8^{th}, 15^{th} and 22^{nd} days, with a cycle every 28 days, for a total of 4 cycles; Cisplatin: 25mg/m^2, intravenous drip, on the 1^{st}, 8^{th} and 15^{th} day, with a cycle every 28 days, for a total of 4 cycles; 30 minutes before chemotherapy, intravenous dexamethasone 10mg (intravenous dexamethasone 20mg for the first chemotherapy) was given to prevent gastrointestinal adverse reactions and allergic reactions. 15 minutes before chemotherapy, intravenous antiemetics were given according to clinical habits. After cisplatin administration, 500ml of normal saline was given for intravenous hydration. For patients who have completed 4 consecutive treatment cycles, the investigator will decide whether to operate, the specific operation method and the arrangement of operation time. According to the scheme, the investigator can decide whether to adjust the drug dose and administration time according to the adverse reactions of the subjects. The subjects continued to take the drug until disease progression, intolerable toxicity, withdrawn of the consent, or researcher judgement that the drug must be stopped, and the researcher decided whether to advance the operation and the follow-up treatment plan. In case of missed administration of pyrotinib or suspension of administration due to adverse events, the subjects should record in detail the time when the drug should have been taken and the reason why it was not taken, and then continue to take the drug according to the plan, without

	supplementary administration or cycle adjustment.
Primary end point	The rate of total pathological complete response (tpCR, defined as ypT0 ypN0)
Secondary end points	The rate of locoregional pathological complete response (ypT0/is ypN0), the breast mass retraction rate, clinical complete response rate (cCR), portion of residual cancer burden 0 and 1, proportion of Miller-Payne grade 4 and 5, disease-free survival (DFS), invasive disease-free survival (iDFS), distant metastasis-free survival (DDFS), overall survival (OS); safety; comparison with HER2-positive breast cancer patients who have received weekly paclitaxel/cisplatin chemotherapy regimen combined with trastuzumab in the past; comparison with HER2-positive breast cancer patients receiving weekly paclitaxel/cisplatin chemotherapy regimens with either trastuzumab or trastuzumab/pertuzumab in the same period.
Safety criteria	All adverse events evaluated using NCI CTCAE version 5.0 should be documented in medical records and the principal investigator should determine the severity of the adverse event and its relationship to the study protocol.
Study progress	Estimated time for the first enrollment: March, 2019 Estimated time for the last enrollment: March, 2020 Estimated time for the last subject to leave the group: July, 2020 Estimated end time of the study: September, 2020
Data Analysis and Statistical Method	Based on the results of clinical trials such as NeoSphere and NeoALTTO as well as the results of the weekly paclitaxel plus cisplatin for locally advanced breast cancer patients in the Phase II clinical trial published by our center, assuming that tpCR increased from 50% of the trastuzumab/paclitaxel/cisplatin regimen to 70% of this study regimen, $\alpha=0.05$ (bilateral) and $\beta=0.2$ were set and at least 47 subjects needed to be enrolled were calculated according to the power of 80%. If a dropout rate of 10% was considered, 52 subjects needed to be enrolled in each group.

1 Study purpose

This study aimed to investigate the efficacy and safety of dual anti-HER2 regimen pyrotinib and trastuzumab together with weekly paclitaxel/cisplatin neoadjuvant chemotherapy in patients with human epidermal growth factor receptor 2(HER2)-positive locally advanced breast cancer.

The primary endpoint was the rate of total pathological complete response (tpCR), defined as the absence of residual viable tumor (invasive or non-invasive) cells under the microscopic examination of the breast and the axillary lymph nodes at surgery (ypT0 ypN0). Secondary endpoints included: the rate of locoregional pCR, defined as no invasive cancer in the breast and no pathological involvement of axillary lymph nodes (ypT0/is ypN0); the breast mass retraction rate; clinical complete response rate (cCR); proportion of residual cancer burden 0 and 1, proportion of Miller-Payne grade 4 and 5; disease-free survival (DFS), defined as the time from the date of surgery to the first occurrence of local and/or regional recurrence, contralateral breast cancer, distant metastasis, or death from any cause; invasive disease-free survival (iDFS), defined as the time from the date of surgery to the first occurrence of ipsilateral invasive breast cancer, contralateral invasive breast cancer, local and/or regional invasive cancer recurrence, distant metastasis, or death from any cause; distant metastasis-free survival (DDFS), defined as the time from the date of surgery to the first occurrence of distant metastasis; overall survival (OS), defined as the time from the date of surgery to the occurrence of any cause of death; safety; comparison with HER2-positive breast cancer patients who have received weekly paclitaxel/cisplatin chemotherapy regimen combined with trastuzumab in the past; comparison with HER2-positive breast cancer patients receiving weekly paclitaxel/cisplatin chemotherapy regimens with either trastuzumab or trastuzumab/pertuzumab in the same period.

The exploratory endpoints were the predictive and prognostic value of molecular biological indicators, and indicators related to cardiac function.

2 Study background

Currently, cancer has become the most common cause of death worldwide. According to the 2012 Annual Report of China Oncology Registration issued by the National Oncology Registration Center in 2012, there are about 3.12 million new cancer cases and about 2.7 million cancer deaths in China every year. Malignant tumor is the first cause of death in cities (accounting for 25.0% of the total urban deaths) and the second cause of death in rural areas (accounting for 21.0%).

Breast cancer is the most common malignant tumor in women worldwide and also an important disease that seriously endangers women's health. The American Cancer Society report estimates that breast cancer in the United States ranked first (30%) and second (14%) in female malignancies for morbidity and mortality in 2017. According to the authoritative statistics of malignant tumors in China, in 2015, the incidence of breast cancer in China ranked first among female malignant tumors, accounting for 15% of new malignant tumors among women. It is also the most common cause of cancer death among women under the age of 45 in China.

According to the data of a national multi-center study conducted from 1999 to 2008, among the newly diagnosed breast cancers in China, 15.7% were in stage I, 44.9% were in stage II, 18.7% were in stage III, and 2.4% were in stage IV. Although with the development of early breast cancer screening, the popularity of health promotion and the improvement of early diagnosis in China, there are still some patients who presented with a locally advanced disease in the initial treatment. At present, neoadjuvant therapy is a routine treatment for locally advanced breast cancer. With the extensive clinical application and in-depth research, the use of neoadjuvant therapy also has more connotation: For example, for inoperable breast cancer with a large mass or operable breast cancer with breast conservation requirements, the tumor size can be reduced and the clinical stages can be reduced by chemotherapy, so that more patients can have the opportunity to receive surgical treatment or breast-conserving treatment; As a means of detecting the sensitivity of patients to the treatments, to further improve the long-term efficacy of breast cancer, etc.

HER2 molecule is an independent factor indicating a poor prognosis for breast cancer, and about 20–30% of breast cancer patients in China have amplified/overexpressed HER2 gene. According to the NCCN guidelines and the diagnosis and treatment practice in China, anti-HER2 targeted drugs should be recommended for patients with early-stage breast cancer or locally advanced breast cancer in the (neo)adjuvant treatment stage.

The results of the single-arm Phase II clinical trial of weekly paclitaxel plus cisplatin regimen used for neoadjuvant treatment of locally advanced breast cancer published by our center show that the pCR(ypT0 ypN0) rate of HER2-positive breast cancer after receiving the weekly paclitaxel/cisplatin chemotherapy plus trastuzumab can reach 52.4%, which is significantly superior to other similar studies in efficacy and better in safety.

With the continuous emergence of targeted anti-HER2 drugs, more and more clinical trial results show that the combination of dual anti-HER2 targeted therapy and neoadjuvant chemotherapy can further improve the treatment efficacy of patients with HER2 positive breast cancer compared with the combination of trastuzumab single-target therapy. In the NeoALTTO

study, patients with primary HER2-positive breast cancer with a primary lesion > 2cm were randomly divided into three groups: lapatinib single target, trastuzumab single target and lapatinib combined with trastuzumab dual target neoadjuvant therapy. The results showed that dual target therapy (51.3%) could significantly improve the pathological complete response (pCR) rate of patients compared with trastuzumab single target therapy (29.5%) (P=0.0001). NeoSphere study randomly divided HER2-positive locally advanced breast cancer, inflammatory breast cancer or operable breast cancer into four groups: trastuzumab combined with docetaxel (group A), trastuzumab/pertuzumab combined with docetaxel (group B), pertuzumab combined with trastuzumab (group C) and pertuzumab combined with docetaxel (group D). The results showed that the pCR rates of the four groups were 29.0%, 45.8%, 16.8% and 24.0%, respectively. Compared with group A, the pCR rate of patients in group B was significantly improved (P=0.0141). Therefore, optimizing the strategy of dual HER2 blockade might make it possible to promote the efficacy for HER2-positive breast cancer.

Pyrotinib is a small molecule, irreversible, pan-ErbB receptor tyrosine kinase inhibitor (TKI) developed by Jiangsu Hengrui Pharmaceutical Co., Ltd. In the preclinical and in vivo studies, pyrotinib was found to have significant inhibition on HER2-positive breast cancer tumors. As a small molecule irreversible inhibitor of double targets (EGFR and HER2), pyrotinib has different action sites and increased EGFR target compared with HER2 macromolecule trastuzumab, which makes it possible that pyrotinib is still effective in subjects who have used trastuzumab before, and the long-term treatment may not have to be stopped due to cardiotoxicity. Compared with the small molecule inhibitor lapatinib, the pyrotinib is an irreversible inhibitor, and better efficacy is likely to be achieved at a lower human plasma exposure level.

According to the results of clinical studies that have been completed or are ongoing, pyrotinib is safe and well tolerated. Predictable adverse events are clinically controllable, and most of them spontaneously resolve. Similar to other EGFR/HER2-targeting drugs, these drugs mainly cause adverse reactions related to common pharmacological mechanisms, such as diarrhea and rash. The majority of adverse events were mild to moderate and clinically controllable. All adverse events did not result in dosing suspension, dose adjustment, and discontinuation. Except for the reported adverse events, laboratory tests, vital signs, physical examinations, and electrocardiograms (including QTc) showed no clinically significant abnormalities or abnormal changes. In terms of effectiveness, pyrotinib or pyrotinib combined with chemotherapy have shown certain anti-tumor effect in advanced breast cancer and gastric cancer. In particular, in advanced breast cancer, the results of the Ic and I/II phase trials of the pyrotinib monotherapy Ib and the pyrotinib combined with capecitabine have shown that the antitumor effect of pyrotinib is efficient, rapid and sustainable.

In summary, we can thus speculate that the combination of pyrotinib/trastuzumab HER2 dual-target therapy and weekly paclitaxel/cisplatin chemotherapy regimen could further improve the neoadjuvant therapeutic efficacy and even the prognosis of HER2-positive locally advanced breast cancer patients as compared with the combination of trastuzumab single-target therapy.

3 Study rationale

3.1 Animal experiment and literature basis in the early stage of research

3.1.1 Drug name and physicochemical properties

[Generic name] Pyrotinib

[Chemical name] (R,E)-N-(4-(3-chloro-4-(pyridin-2-ylmethoxy)phenylamino)-3cyano-7-ethoxyquinolin-6-yl)-3-(1-methylpyrrolidin-2-yl) acrylamide, maleic acid salt.

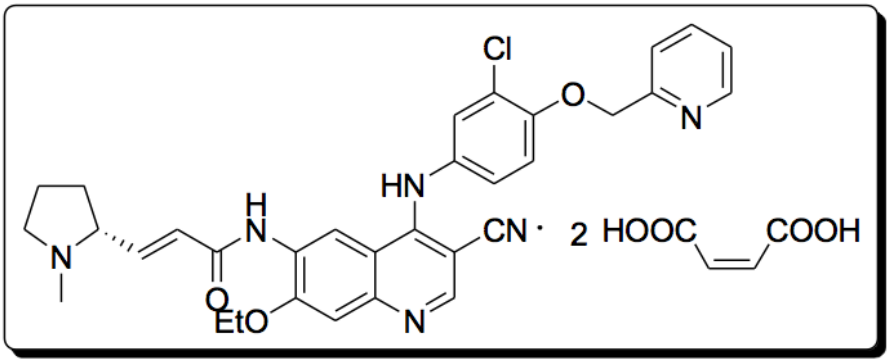

Figure 1 Chemical structural formula of pyrotinib

[Molecular formula] $C_{40}H_{39}ClN_6O_{11}$, see Figure 1 for the molecular structure.

[Molecular weight] 815.22

3.1.2 Pharmacological characteristics and mechanism of action of pyrotinib

Pyrotinib is a small molecular irreversible inhibitor of tyrosine kinase receptor which inhibits epidermal growth factor receptor (EGFR) and human epidermal factor receptor 2 (HER2). Pyrotinib blocks the formation of homodimer and heterodimer of EGFR and HER2 in tumor cells through covalent binding to the ATP binding site in the kinase region of intracellular EGFR and HER2, inhibits its own phosphorylation and blocks the activation of the downstream signal pathway, thereby can inhibit the growth of tumor cells.

3.1.3 Preclinical pharmacodynamic study of pyrotinib

Pyrotinib has obvious inhibition effect on EGFR and HER2 at molecular level, the half inhibitory concentration (IC50) is 5.6 nM and 8.1 nM respectively. It has strong inhibition effect on the proliferation of tumor cells with high expression of HER2, the IC50 ranges from 1~43 nM. And it can significantly inhibit the phosphorylation of EGFR and HER2, and also has an inhibitory effect on the activation of the downstream signal ERK1/2 and Akt, which is irreversible.

Pyrotinib can significantly lead to the stagnation of BT474 cells in G1 phase of cell cycle. Pyrotinib significantly inhibits the proliferation of the tumor model with high expression of HER2, such as the growth of SK-OV-3, Calu-3 and BT-474, the inhibition is obviously dose depending and lead to partial tumor reduction. Antitumor effects in vivo and in vitro are superior or equivalent to the active comparator HKI-272, see Table 1 for the details; thus, the preclinical data support further clinical studies for pyrotinib.

Table 1 Inhibitory effect of pyrotinib on tyrosine kinase

Kinase	IC_{50} (nM, mean±SD)	
	pyrotinib	neratinib（HKI-272）
HER2	8.1 ± 2.3	6.8 ± 0.9
EGFR1	5.6 ± 3.9	4.2± 0.9
c-Src	790.3±190.	1159.1±1036.5
KDR	>3,000	>3,000
c-Kit	>3,000	>3,000
PDGFRβ	>3,000	>3,000
C-Met	>3,000	>3,000

3.1.4 Pre-clinical toxicology study of pyrotinib

Including safety pharmacology, acute toxicity, long-term toxicity, and genetic toxicology studies, detailed in the Manual for Investigators of pyrotinib Maleate.

3.1.5 Pre-clinical pharmacokinetic studies of pyrotinib

A number of studies have been completed, including rat absorption test, in vivo pharmacokinetics test in beagle dogs, plasma protein binding rate test, rat tissue distribution test, and in vitro metabolism test. Please refer to the Handbook for Investigators of pyrotinib maleate.

3.1.6 Clinical research on pyrotinib

To date, five phase I clinical studies and 1 Phase I/II clinical study of pyrotinib have been conducted in China. As of December 31, 2015, two hundred and two Chinese subjects (58 healthy subjects, 120 HER2-positive advanced breast cancer patients and 24 HER2-positive advanced gastric cancer patients) have been exposed to pyrotinib, and the administration time has reached 120 weeks at most. Summary reports have been completed for Phase Ia, Ib, and food pharmacokinetics clinical studies, with the remaining trials ongoing.

In addition, a phase I clinical study with dose escalation of advanced solid tumors in the United States was conducted with pyrotinib.

The following is an overview of the results of current clinical studies of pyrotinib in China.

3.1.6.1 Phase Ia Clinical Trial of Tolerability and Pharmacokinetics in Healthy Subjects

In September 2012, the "Phase I Clinical Trial of Tolerability and Pharmacokinetics of Single Oral Administration of pyrotinib Maleate Tablets in Healthy Subjects" was conducted in the Drug Clinical Facility of Tianjin TEDA International Cardiovascular Hospital. It was a single-center, single-dose, randomized, double-blind, placebo-controlled, dose-escalation designed Phase I clinical study. A primary study objective was to evaluate the tolerability and safety of a single oral administration of pyrotinib maleate tablets in healthy subjects and a secondary study objective was to characterize the pharmacokinetics of pyrotinib maleate in healthy subjects. Each subject was given pyrotinib maleate tablets orally once after breakfast after enrollment.

A total of 56 healthy subjects were enrolled and completed in the study, 46 in the experimental drug group and 10 in the placebo group. Among the 46 subjects in the experimental medication

group, six subjects had seven adverse events, and the incidence rate was 13.0% (6/46), all of which was Grade 1 (CTCAE 3.0). The adverse events were transient and had returned to normal, and no concomitant medication was used. The incidence of adverse events was dose-related. Except for one adverse event, hemorrhoids, that was considered possibly not related to the test drug, other adverse events were considered possibly related to the test drug. The incidence of adverse events by test drug group was as follows: No adverse events occurred in the 20mg, 40mg, 80mg, and 160mg test drug groups; One adverse event (hemorrhoid) occurred in 1 subject in the 240mg experimental drug group, and the incidence rate was 12.5% (1/8). Three adverse events (1 abdominal pain and 2 diarrhea) occurred in three subjects in the 320mg experimental drug group, with the incidence rate of 37.5% (3/8). In the 400mg experimental drug group, two subjects had three adverse events (one for abdominal pain, one for diarrhea, and one for headache), and the incidence rate was 33.3% (2/6). There were no withdrawals due to adverse events, no serious adverse events (SAE), and no deaths from either dose of pyrotinib.

Pharmacokinetic analysis showed a single oral administration of 80mg, 160mg, 240mg, 320mg, and 400 mg pyrotinib maleate tablets at $t_{1/2}$ (mean) of 15.0h, 20.9h, 18.4h, 18.1h, and 18.8h, respectively, with plasma pyrotinib concentrations peaking at 4–5.5 h. Within the dose range of 80–400 mg, the *AUC* and *Cmax* of pyrotinib increased with the increase of the dose, and the increase of *Cmax* was consistent with the dose increase (β=1.0349); the increase of $AUC_{0\text{-}t}$ was consistent with the dose increase (β = 1.2). There was no significant change in *Cl/F* among the dosage groups. The results showed that the pharmacokinetics of pyrotinib basically conformed to the linear kinetic characteristics within the dose range of study (80–400 mg).

3.1.6.2 Food pharmacokinetic of pyrotinib

The food pharmacokinetics and material balance study of single oral administration of pyrotinib maleate tablets in healthy Chinese subjects was initiated in September 2014 at Shengjing Hospital, Shenyang. This study was a randomized, open-label, single-center, and two-stage crossover design Phase I clinical trial. The purpose of the study was to investigate the pharmacokinetic effects of meals on pyrotinib, understand the metabolic pathway, excretion pathway, and excretion of pyrotinib in humans, and further confirm the safety of single administration of pyrotinib maleate tablets 320mg. A total of 12 healthy Chinese men were randomized into group A and group B (n = 6 in each group). Each healthy subject in both groups received an oral dose of pyrotinib maleate 320mg on the first day of both phases, and subjects in Group A were dosed once on an empty stomach on day 1 and once postprandial on day 9; Subjects in Group B were dosed once postprandial on day 1 and once fasted on day 9 of the trial. PK plasma samples were collected before administration and at 0–96 h after administration for all subjects, while urine and feces samples of subjects in Group B were collected for material balance studies before administration and at 0–120 h after administration. Subjects were also closely observed during the trial and safety indicators, data, and adverse events were collected.

Of the 12 subjects included in the safety analysis set, 11 had an adverse event incidence of 92%. The incidence of digestive adverse events was 58% (7 cases). The incidence of adverse laboratory events was 92% (11 cases). The incidence of digestive adverse events was as follows: abdominal distension 42% (5 cases), diarrhea 25% (3 cases), abdominal pain 8% (1

case), loose stool 8% (1 case), dry mouth 17% (2 cases). Adverse events of digestive system were possibly related to the test drugs. The incidence of laboratory-specific adverse events was as follows: increased alanine aminotransferase 8% (1 case), increased aspartate aminotransferase 8% (1 case), increased blood glucose 8% (1 case), fecal occult blood positive 25% (3 cases), increased indirect bilirubin 17% (2 cases), increased lymphocyte count 17% (2 cases), increased leukocyte 8% (1 case), increased triglyceride 33% (4 cases), and urinary protein positive 25% (3 cases). Laboratory adverse events were probably not related to the test drugs.

Of the total adverse events observed in this clinical study, the only adverse event that occurred in Subject B05 was a Grade 2 increase in alanine aminotransferase (NCICCAE 4.0), and the remaining adverse events were Grade 1 in severity. With the exception of Subject B05 who was disenrolled from the group due to adverse events with increased alanine aminotransferase and increased aspartate aminotransferase, no other subject withdrew due to an adverse event. The results of this study demonstrated the safety of a single oral administration of 320mg of pyrotinib maleate tablets in a healthy male population. There were no SAEs and no deaths. Dietary factors had no significant effect on drug safety.

A total of 11 subjects completed the full clinical trial (Subject B05 dropped out of the trial) and 12 subjects were included in the PK data analysis set, but Subject B05 did not participate in the relative bioavailability comparisons. The results showed that the main pharmacokinetic parameters (*AUC* and *Cmax*) of pyrotinib were significantly different between postprandial and fasted administration ($P < 0.01$). Compared to fasted administration, subjects experienced a 43.31% increase in *AUC0-∞* and 78.89% increase in *Cmax* after postprandial oral administration of pyrotinib maleate, with no change in peak arrival time or substantial change in drug elimination half-life. The results of this experiment showed that the bioavailability of pyrotinib after meal administration was increased, and the postprandial administration method was more reasonable in clinic.

To date, there is no clear data on the interaction between pyrotinib and food and other drugs. Only by referring to the regulations of similar drugs that pyrotinib is administrated with food, and in combination with preclinical in vitro data, any drug that may inhibit or induce CYP3A4 or CYP2C9 enzymes should be used with caution during treatment.

3.1.6.3 Phase Ib clinical trials of tolerability and pharmacokinetics in subjects with HER2-positive advanced breast cancer

In February 2013, the "Phase Ib Clinical Trial of pyrotinib maleate" was conducted in Cancer Hospital, Chinese Academy of Medical Sciences. The study was designed as a single center, single arm, open-label, dose-climbing, and the main research purpose was to determine the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of oral administration of pyrotinib maleate tablets in Chinese patients with advanced breast cancer with positive HER2 expression. Secondary study objectives include evaluation of pharmacokinetics and clinical efficacy, and exploration of the relationship between HER2 expression status and efficacy. Dose escalation was designed for 80, 160, 240, 320, and 400mg/d, administered once daily to all subjects orally within 30 minutes of breakfast for 28 consecutive days as one cycle. For patients with complete response (CR), partial response (PR) and stable disease (SD) evaluated as the first efficacy evaluation in cycle 2, if provided that the patient voluntarily agrees and the

investigator judges that continuous dosing is beneficial to the patient, the patients will enter the extended trial of this study and continue dosing until the disease progresses, or the toxicity is intolerable, or the patient withdraws the knowledge, or the investigator judges that dosing must be terminated.

Safety analysis over 2 consecutive dosing cycles in this study showed that the MTD of pyrotinib monotherapy for HER2-positive advanced breast cancer was 400mg. The proportion of subjects with at least one AE in the 80–400 mg dose group was 69.4% (25/36), and the majority of AEs were mild to moderate and clinically controllable. None of the adverse events led to a suspension, dose adjustment, discontinuation, or early withdrawal of pyrotinib from the study. Adverse events with an incidence rate of ≥5% included diarrhea in 15 cases (41.7%), nausea, oral ulcer, decreased white blood cell count, rash, and increased alanine aminotransferase in 3 cases (8.3%), and fatigue, abdominal pain, dyspepsia, and increased aspartate aminotransferase in 2 cases (5.6%). Of all the above adverse events, only 4 subjects (3 with diarrhea and 1 with oral ulcer) were treated with medication. Grade ≥3 adverse events occurred in 11.1% (4/36) of subjects, with 3 cases of Grade 3 diarrhea and 1 case (Grade 5) death due to preexisting disease progression; this SAE was not related to drugs. This was the only 1 SAE reported in this study. The incidence of diarrhea was 41.7% (15/36), and the incidence, severity, duration, and frequency of diarrhea in individual subjects showed a dose-dependent pattern. The majority of subjects had mild to moderate, transient, and clinically controlled diarrhea. The duration of grade 3 diarrhea in the 3 cases did not exceed 3 days. Except for the reported adverse events, laboratory tests, vital signs, physical examinations, and electrocardiograms (including QTc) showed no clinically significant abnormalities, abnormal changes, or abnormal trends. In summary, continuous administration of 80–400 mg pyrotinib maleate tablets in the treatment of HER2-positive breast cancer with two cycles was well tolerated and safe, and its long-term safety needs further observation and confirmation in subsequent studies.

Pharmacokinetic analysis revealed median T_{max} of 80, 160, 240, 320, and 400mg, geometric mean $t_{1/2z}$ of 9.43–16.5 h, and geometric mean C_{max} of 38.8, 80.8, 98.7, 143, and 147ng/ml, respectively, for Chinese breast cancer patients after oral administration of pyrotinib maleate tablets on the first day. For the eighth consecutive day, the blood drug concentration reached a steady state. The median T_{max} at steady state was 2.00–4.00 h, and the geometric mean $t_{1/2z}$ was 11.4–15.9 h, respectively. The geometric mean of the accumulation ratios R, calculated by $AUC_{0\text{-}24h}$, were 1.32, 1.35, 1.57, 1.35, and 1.22 at steady state (Day 28) with continuous administration of 80–400 mg of pyrotinib maleate tablets, suggesting that there was no significant dose-dependent accumulation with continuous administration; The geometric means of C_{max} were 43.0, 102, 156, 175, and 170 ng/ml, respectively, and the geometric means of $AUC_{0\text{-}24h}$ were 549, 1260, 2080, 2660, and 2270, respectively. The increase in C_{max} was consistent with the dose-dependent increase (β=0.8092), and the increase in $AUC_{0\text{-}24h}$ was consistent with the dose-dependent increase (β=0.9066), suggesting that the pharmacokinetics of pyrotinib at steady-state were consistent with linear kinetics.

3.1.6.4 Phase Ic clinical study of pyrotinib tablets combined with capecitabine in the treatment of HER2-positive metastatic breast cancer in stage

In July 2014, a Phase Ic clinical study of pyrotinib maleate tablets combined with capecitabine

in the treatment of HER2-positive advanced breast cancer was launched in the Cancer Hospital, Chinese Academy of Medical Sciences. The study was designed as a single arm, open-label, and climbing study. The primary objectives were to determine the MTD and safety of pyrotinib tablets combined with capecitabine in the treatment of HER2-positive advanced breast cancer patients, and to determine the recommended dose of pyrotinib tablets in Phase II in the combination regimen with capecitabine. Secondary objectives included pharmacokinetics and clinical effectiveness. The dose escalation designs for the pyrotinib maleate tablets were 160, 240, 320, and 400 mg/d (qd) with an initial dose of capecitabine at 1000 mg/m2, twice daily for 1 cycle every 21 days on consecutive days 1 through 14.

By the end of December 2015, 29 subjects had been enrolled in the study, completing all enrollment. Preliminary safety profile up to the end of October 2015: No DLT was observed during the 160–400 mg dose escalation. The main adverse events were diarrhea (46.2%), neutropenia (26.9%), leukopenia (23.1%), hand-foot syndrome (19.2%), nausea (15.4%), oral ulcer (11.5%), and direct bilirubin elevation (11.5%). Only 1 subject had a Grade 3 adverse event, which was 2 Grade 3 diarrhea episodes in 1 subject in the 400mg dose group (Grade 3 diarrhea with a clinically controllable duration of less than or equal to 3 days was not counted as DLT according to the definition of protocol DLT), and the other adverse events were all Grade 1–2. Three subjects resumed dosing after a 7-day suspension due to Grade 2 hand-foot syndrome (1 in each of the 160mg, 320mg, and 400mg dose groups). No subject had a dose suspension, dose adjustment, or early withdrawal from the study due to other adverse events such as diarrhea. Initial efficacy profile as of 31 December 2015: Results from the best efficacy evaluation of confirmed efficacy for subjects in each group were 2 PR and 1 SD in the 160mg group; In the 240mg group, there were 2 cases of PR and 1 case of SD; Nine cases of PR, one case of SD and one case of active request for withdrawal from the study after two weeks of drug administration in the 320mg group. There were 9 cases of PR and 1 case of SD in the 400mg group. The above safety and effectiveness data are preliminary results, which have not been reviewed and questioned by the data management section, and may not be completely consistent with the final summary report.

3.1.6.5 A phase I clinical study on the treatment of HER2-positive metastatic gastric cancer with pyrotinib tablets alone or in combination with docetaxel

In August 2014, a phase I clinical study of pyrotinib maleate tablets monotherapy/combined with docetaxel in the treatment of HER2-positive advanced gastric cancer was initiated. Currently, research centers include Peking University Cancer Hospital and Affiliated Cancer Hospital of Sun Yat-sen University. This study was a single-armed, open-label, dose-escalation design. The primary objectives of the study were to determine the MTD and safety of pyrotinib maleate tablets alone/in combination with docetaxel in HER2-positive advanced gastric cancer patients and to determine the recommended dose of pyrotinib maleate tablets in Phase II of the combination regimen with capecitabine. Secondary objectives included pharmacokinetics and clinical effectiveness. The experiment was divided into two stages. In the first stage, pyrotinib maleate tablets were used alone to climb the hill, and more than one dose group (240, 320, and 400 mg) was designed. In the second stage, docetaxel was combined based on the safety and preliminary efficacy in the first stage. Two recommended doses of pyrotinib maleate tablets were selected according to the results of the first stage. The initial dose of docetaxel was

60mg/m^2, which was instilled once per cycle. One cycle was required for 21 days.

As of December 31, 2015, a phase I clinical study of pyrotinib maleate tablets monotherapy for HER2-positive metastatic gastric cancer has enrolled 24 subjects. The single drug dose escalated to the 480mg group, and the combination dose with docetaxel and dose escalated to the 400mg group. Preliminary safety profile: Part of pyrotinib monotherapy trial: Adverse events were similar to those in the Phase Ib trial, mainly gastrointestinal reactions. DLT occurred in 1 case in the 400mg dose group, indicating grade 3 diarrhea. In the pyrotinib combined with docetaxel group, adverse events were mainly gastrointestinal adverse events such as diarrhea. One subject in the 320mg combined administration group experienced Grade 4 decrease in white blood cells and neutrophils, which was reexamined and improved after one week. In addition, five SAEs including liver function impairment (possibly unrelated), lower gastrointestinal bleeding (possibly unrelated), intestinal obstruction (possibly unrelated), arrhythmia (possibly related) and abdominal pain (possibly unrelated) occurred in the coadministration part, which were all considered by the investigator to be unrelated to the test drug. Results obtained from the first efficacy evaluation (at the end of 2 weeks after dosing): Partial results with pyrotinib alone: 1 PR and 2 PD in the 240mg group; In the 320mg group, there were 1 case of SD, 2 cases of PD, and 1 case could not be evaluated. In the 400mg group, there were 3 PD cases, 2 SD cases, and 1 case could not be evaluated. Two cases of PR in 480mg group. Part of the results of the co-administration were that in the 240mg group, there were 1 case of PR and 2 cases of SD associated with docetaxel. 320mg combined with docetaxel 3 cases of SD. Phase I clinical studies on the safety, efficacy, and pharmacokinetics of pyrotinib maleate tablets alone/in combination with docetaxel in Chinese patients with HER2-positive advanced gastric cancer are still ongoing.

3.1.6.6 Phase I/II clinical study of pyrotinib maleate combine with capecitabine

A randomized, open-label, parallel-controlled, multi-center Phase I/II clinical study of pyrotinib maleate tablets combined with capecitabine versus lapatinib combined with capecitabine in the treatment of HER2-positive metastatic breast cancer was initiated in May 2015 at the team leader's unit, Cancer Hospital, Chinese Academy of Medical Sciences. The objective of the study was to compare the safety and efficacy of the pyrotinib combined with capecitabine regimen and the lapatinib combined with capecitabine regimen in the treatment of HER2-positive metastatic breast cancer. In this study, a multi-centered, randomized, open-label, double-armed, positive-drug parallel control design was used. After 128 breast cancer patients who had failed to receive anthracyclines and taxanes and had no more than 2 lines of chemotherapy after recurrence/metastasis, they were randomized, according to the previous use of macromolecular antibodies targeting HER2 for stratification, to the pyrotinib combined with capecitabine treatment group (experimental group) or the lapatinib combined with capecitabine treatment group (control group) in a 1:1 ratio.

Up to December 30, 2015, a total of 105 subjects were enrolled, including 53 cases in the experimental group and 52 cases in the control group. Preliminary safety profile: common adverse events related to test drugs included diarrhea (60.4%), vomiting (22.6%), nausea (13.2%) and hand-foot syndrome (11.3%) in the test group; The control group had hand-foot syndrome (19.2%), rash (15.4%), diarrhea (11.5%), and elevated total bilirubin (11.5%). Grade 3 adverse events related to test drugs reported: 2 cases of diarrhea, 1 case of hand-foot

syndrome, 1 case of stomachache (test group) and 1 case of hypokalemia (test group) in the test group; In the control group, there was 1 case of leukopenia and 1 case of neutrophil decline. At present, two SAEs occurred in the control group, and one case was hospitalized due to dyspnea, and the symptoms disappeared after drug administration was suspended. The other case was hospitalized due to malignant ascites and disease progression, and our patient died. Initial effectiveness profile: A total of 57 subjects received a confirmed evaluation of efficacy. The objective response rate (ORR) of the experimental group was 66.2%, including 1 case (1.9%) of Cr and 18 cases (64.3%) of PR; In the control group, the ORR was 44.8%, and all cases were PR 13 (44.8%). At present, nine subjects with PD were out of the group, including eight cases as the control group (treatment time: 6–24 weeks) and one case as the experimental group (treatment time: 18 weeks). The above safety and efficacy data are preliminary results and the test is still in progress.

3.2 Subjects Selection Basis

This study included patients with stage IIA-III, HER2-positive primary invasive breast cancer. According to the 2018 NCCN guidelines, patients with stage IIA-III may consider neoadjuvant therapy.

The NeoALTTO study was a phase III, randomized, open-label, multicenter study comparing the efficacy and safety of a neoadjuvant therapy with lapatinib single target, trastuzumab single target, and lapatinib combined with trastuzumab double targets in HER2 positive breast cancer patients enrolled in HER2 positive breast cancer patients with a primary lesion > 2cm.

The NeoSphere study was a phase II, randomized, open, multicenter study comparing the efficacy and safety of neoadjuvant treatments of trastuzumab in combination with docetaxel, trastuzumab/pertuzumab in combination with docetaxel, pertuzumab in combination with trastuzumab, and pertuzumab in combination with docetaxel in HER2-positive breast cancer patients enrolled in HER2-positive locally advanced breast cancer (T2-3, N2-3, M0 or T4a-c, any N, M0), inflammatory breast cancer (T4d, any N, M0), or operable breast cancer (T2-3, N0-1, M0).

The previous weekly paclitaxel plus cisplatin regimen study conducted by our center is a stage II, single-arm study to explore the effectiveness and safety of the weekly paclitaxel plus cisplatin chemotherapy regimen in locally advanced breast cancer patients. The enrolled patients are breast cancer patients with the primary lesion > 2cm.

4 Study content

4.1 Study Population

Patients with stage IIA-III (according to AJCC Version 8 anatomic staging criteria), HER2-positive, primary invasive breast cancer.

4.2 Sample Size Calculation

The primary endpoint was the rate of total pCR (tpCR), defined as the absence of residual viable tumor (invasive or non-invasive) cells under the microscopic examination of the breast and the axillary lymph nodes at surgery (ypT0 ypN0). Based on the results of clinical trials such as NeoSphere and NeoALTTO as well as the results of the weekly paclitaxel plus cisplatin for locally advanced breast cancer patients in the Phase II clinical trial published by our center, assuming that tpCR increased from 50% of the trastuzumab/paclitaxel/cisplatin regimen to 70% of this study regimen, $\alpha=0.05$ (bilateral) and $\beta=0.2$ were set, and at least 47 subjects needed to be enrolled were calculated according to the degree of certainty of 80%. If a dropout rate of 10% was considered, 52 subjects needed to be enrolled.

4.3 Specific study content

This is a prospective, open-label, single-armed, phase II clinical study to investigate the efficacy and safety of pyrotinib/trastuzumab against HER2 double targets in combination with weekly paclitaxel/cisplatin neoadjuvant chemotherapy regimen in patients with HER2-positive locally advanced breast cancer. At the same time, we compared the efficacy and safety of HER2-positive breast cancer patients who previously received weekly paclitaxel/cisplatin chemotherapy regimen combined with trastuzumab single-target neoadjuvant therapy. To compare the efficacy and safety of HER2-positive breast cancer patients in different cohorts who simultaneously received weekly paclitaxel/cisplatin chemotherapy regimen combined with trastuzumab or trastuzumab/pertuzumab. In addition, the predictive value and prognostic value of molecular biological indicators, cardiac function related indicators are explored using blood and other body fluid samples.

5 Study design

5.1 Eligibility criteria (diagnostic criteria, inclusion criteria, and exclusion criteria)

5.1.1 Inclusion criteria: Patients must meet all of the following criteria

1) Female, aged ≥18 years old, ≤70 years old;
2) Patients with primary invasive breast adenocarcinoma confirmed by pathology and with clinical stages of stage IIA-III (according to the anatomic staging standard of AJCC Version 8) before neoadjuvant chemotherapy; If bilateral breast cancers are found at the same time, patients with invasive breast cancer on one side and carcinoma in situ on the other side can be enrolled;
3) The patient has at least one measurable lesion before neoadjuvant treatment according to RECIST Version 1.1;
4) The pathology in Renji Hospital, School of Medicine, Shanghai Jiaotong University, clearly shows that HER2 is positive in primary breast lesions or axillary metastasis, and HER2 positivity is defined as HER2(3+) by immunohistochemistry or amplification by fluorescence in situ hybridization (FISH), and the specimens from external hospital must be consulted by the Pathology Department of Renji Hospital, School of Medicine, Shanghai Jiaotong University;
5) During the period of neoadjuvant therapy, drug-induced ovarian castration is allowed to be given at the same time;
6) ECOG score 0 to 1 point (see Appendix I for ECOG scores);
7) Hematological and biochemical tests before enrollment should be as follows: WBC ≥ 4.0× 10^9/L, ANC ≥ 1.5× 10^9/L, PLT ≥ 100× 10^9/L; Hemoglobin (Hb) ≥ 90 g/L; Total bilirubin (TBIL)≤1.5× upper limit of normal (ULN), AST (sGOT), ALT (sGPT)≤1.5×ULN, urea nitrogen (BUN)≤1.5×ULN, and creatinine (Cr) ≤ 1.5 × ULN;
8) Creatinine clearance ≥50mL/min (Cockcroft-Gault formula);
9) Echocardiography should meet the requirement of left ventricular ejection fraction (LVEF) ≥ 50%;
10) A 12-lead ECG showed that the Fridericia-corrected QT interval (QTcF) < 470 ms;
11) The subjects voluntarily participated in the study and signed informed consent.

5.1.2 Exclusions criteria: Exclusions from a study were considered to be met if either of the following were met:

1) Pregnant or lactating women, women of childbearing potential who are fertile and have a positive baseline pregnancy test or who are reluctant to use effective contraception throughout the trial;
2) Patients with distant metastasis of breast cancer confirmed by imaging or pathology before enrollment;
3) Patients with bilateral invasive breast cancer are found at the same time;
4) Being unable to swallow, chronic diarrhea and intestinal obstruction, and there are many factors that affect the taking and absorption of drugs;
5) Other targeted therapeutic drugs (such as lapatinib, pertuzumab, and T-DM1);
6) Application of anti-tumor therapy other than the research protocol during the experiment;

7) Allergies to study drugs or auxiliary materials;
8) Patients with sensory or motor nerve disease evidence;
9) A history of immunodeficiency, including HIV-positive tests, or other acquired or congenital immunodeficiency diseases, or a history of organ transplantation;
10) Has suffered from any heart disease, including angina pectoris, arrhythmia requiring medical treatment or with clinical significance, myocardial infarction and heart failure, and any other heart disease judged by the researcher to be unsuitable for participating in the experiment;
11) Has a previous history of other malignancies, except for cured basal cell carcinoma of the skin or squamous cell carcinoma of the skin, and carcinoma in situ of the cervix;
12) In the judgment of the investigator, there are concomitant diseases (including but not limited to severe uncontrollable hypertension, diabetes, active infection, and thyroid disease) that seriously jeopardize the safety of the patient or affect the patient's completion of the study;
13) Has a clear history of neurological or psychiatric disorders, including epilepsy or dementia;
14) Any other circumstances in which the investigator concluded the patient was not eligible for participation in the study.

5.2 Subgroups of Subjects

This clinical trial was a single-arm study with no subgroups.

5.3 Study treatment

5.3.1 Administration plan, drug dosage and administration time.

Dual anti-HER2 blockade of pyrotinib and trastuzumab combined with weekly paclitaxel and cisplatin weekly chemotherapy regimen;

Trastuzumab: the first dose was 4mg/kg, and then 2mg/kg was maintained every week for 16 weeks.

Pyrotinib: 400mg once a day, orally within 30 minutes after breakfast, continuously for 16 weeks;

Paclitaxel: 80mg/m^2, intravenous drip, on the 1^{st}, 8^{th}, 15^{th} and 22^{nd} days, with a cycle every 28 days, for a total of 4 cycles;

Cisplatin: 25mg/m^2, intravenous drip, on the 1^{st}, 8^{th} and 15^{th} day, with a cycle every 28 days, for a total of 4 cycles;

30 minutes before chemotherapy, intravenous dexamethasone 10mg (intravenous dexamethasone 20mg for the first chemotherapy) was given to prevent gastrointestinal adverse reactions and allergic reactions. 15 minutes before chemotherapy, intravenous antiemetics were given according to clinical habits).After cisplatin administration, 500ml of normal saline was given for intravenous hydration.

For patients who have completed 4 consecutive treatment cycles, the investigator will decide whether to operate, the specific operation method and the arrangement of operation time.

According to the scheme, the investigator can decide whether to adjust the drug dose and administration time according to the adverse reactions of the subjects. The subjects continued to take the drug until disease progression, intolerable toxicity, withdrawn of the consent, or researcher judgement that the drug must be stopped, and the researcher decided whether to

advance the operation and the follow-up treatment plan. In case of missed administration of pyrotinib or suspension of administration due to adverse events, the subjects should be recorded in detail the time when the drug should have been taken and the reason why it was not taken, and then continue to take the drug according to the plan, without supplementary administration or cycle adjustment.

5.3.2 Dose adjustment of paclitaxel and cisplatin.

Dose reduction should follow the following principles:

1) For the toxicity that will not cause serious or life-threatening events (such as hair loss, taste change, and non-hemolytic anemia that can be alleviated by blood transfusion, etc.), when the researcher thinks it is appropriate, it is unnecessary to adjust the dose or suspend the medication;
2) In case of grade 1 toxicity (or alopecia of any grade), the patients should be treated with the original dose on schedule;
3) If the researcher determines that the toxicity is caused by only one drug (such as nephrotoxicity after cisplatin treatment), it is unnecessary to adjust the dosage of other drugs;
4) According to the adverse reactions of the subjects, the dose of paclitaxel can be gradually reduced from 80mg/m^2 to 50mg/m^2, and the dose of cisplatin can be gradually reduced from 25mg/m^2 to 15mg/m^2;
5) Once the dose is lowered, it should not be raised in the sequent treatment;
6) If multiple toxicities of different levels occur at the same time, the maximum adjustment range shall be implemented.

5.3.3 Remedial drugs and supportive treatment for paclitaxel/cisplatin treatment (necessary treatment measures in case of SAE related to research).

1) Antiemetic treatment.

Chemotherapy is often accompanied by digestive tract toxic reactions such as nausea and vomiting. Acute vomiting or delayed vomiting are common, and 5-HT3 receptor antagonist can be used to stop vomiting.

2) Use of granulocyte colony stimulating factor (G-CSF).

Preventive G-CSF support therapy is prohibited, and it is recommended that G-CSF should be given when grade 3/4 neutropenia occurs. Treatment guidelines should be followed for febrile neutropenia.

3) Platelet transfusion and/or platelet elevation therapy.

It is recommended to treat with cytokines or interleukin -11 or thrombopoietin when grade 3/4 thrombocytopenia occurs. When necessary, researchers decide whether to give platelet transfusion.

4) Red blood cell infusion and/or erythrocytosis therapy.

It is recommended to give erythropoietin (EPO) treatment when grade 3/4 hemoglobin drops, and if necessary, researchers will decide whether to give red blood cells infusion.

5) Others.

Patients can receive the best supportive treatment. Clinical complications and other adverse events should be actively treated, and symptomatic treatment can be given according to the

judgment of clinicians. All drugs used in combination should be recorded in the case report form in strict accordance with GCP. It is not recommended that patients treated with traditional Chinese medicine at the same time.

5.3.4 Remedial drugs and supportive treatment for the treatment of trastuzumab (necessary treatment measures in case of SAE related to research).

As trastuzumab may cause symptoms of infusion reaction, such as nausea, fever, diarrhea, chills, fatigue and headache, such reactions generally occur during or shortly after infusion. Therefore, the administration of trastuzumab should be carried out in an environment with first aid equipment, and medical personnel should be trained in monitoring and dealing with medical emergency rescue. Monitor the subjects for any adverse reactions during each infusion, and monitor at least 90 minutes after the first infusion of trastuzumab.

In case of transfusion-related symptoms (such as fever, chills, headache, fatigue, itching, nausea, vomiting and diarrhea), the infusion of trastuzumab should be slowed down or stopped. Oxygen inhalation, β-agonists, antihistamines, antipyretics and corticosteroid support therapy are helpful to relieve symptoms. Patients with infusion-related symptoms during or after infusion may be pretreated with analgesics and antihistamines during subsequent infusion. Patients with grade 4 or higher allergic reactions or infusion-related acute respiratory distress syndrome should stop treatment. Because the infusion reaction may cause treatment delay, if there is any problem, the subject should contact the attending doctor.

The dose of trastuzumab was calculated according to the actual weight of the subject, and the baseline and the weight of the subject at each visit should be recorded. If the increase or decrease of the subject's body weight from the baseline is more than 10%, the dosage will be recalculated.

Dose adjustment is not allowed except for the change of body weight. In case of toxicity (including cardiac toxicity), the treatment should be interrupted or suspended. It is allowed to suspend trastuzumab several times during the test. See Table 2 for specific countermeasures.

Table 2 Management for adverse events in the treatment of trastuzumab.

Adverse events	Treatment of therapeutic process
Cardiac toxicity	
Symptomatic LVSD（heart failure）	Stop trastuzumab treatment.
Asymptomatic LVEF decrease<20% and LEVF≥50%	Continue trastuzumab treatment.
Asymptomatic LVEF decrease≥20% and LEVF≥50% OR Asymptomatic LVEF decrease<10% and LEVF<50%	Continue trastuzumab treatment, and reexamine LVEF every 3 weeks.
Asymptomatic LVEF decrease≥10% and LEVF<50%	Suspend trastuzumab treatment, reexamine LVEF every 3 weeks. Stop trastuzumab treatment if LVEF decreases by ≥10% and LVEF is < 50%. If LVEF decreases by < 10% or LVEF≥50%,

	continue trastuzumab treatment.
Grade 4 allergic reaction or acute respiratory distress syndrome	Stop trastuzumab treatment.
Other non-hematological toxicities	
Grade 1	Continue trastuzumab treatment.
Grade 2	According to the tolerance of subjects, researchers may continue to treat with trastuzumab or continue to treat with trastuzumab after the severity of the event resolved to ≤ grade 1.
Grade 3~4	Suspend trastuzumab treatment If the severity of the event resolved to ≤ Grade 1 within 2 weeks, the treatment with trastuzumab was resumed; If the severity of the event does not resolve to ≤ Grade 1 within 2 weeks, treatment with trastuzumab is discontinued

LVSD，Left Ventricular Systolic Dysfunction；LVEF，Left Ventricular Ejection Fraction

5.3.5 Dose adjustment of pyrotinib

Multiple suspensions and multiple dose adjustments of pyrotinib with a gradient of 400mg, 320mg, and 240mg were permitted during the trial (see Table 3).

Table 3 Provisions on dose adjustment of pyrotinib

NCI CTCAE 5.0*	**Treatment of therapeutic process (After active clinical treatment or observation)**	**Dose adjustment for dose resumption**
Cardiac toxicity		
≥ Grade 2 decreased LVEF without symptoms/ LVEF is below the lower limit of normal (LVEF decrease≥10% and LEVE<50% OR heart failure)	Discontinued permanently	-
Diarrhea		
Grade 4	Discontinued permanently	-
Grade 3		First time 400 mg

Grade 1~2 with complications (Including mild to severe abdominal colic, ≥ grade 2 nausea or vomiting, decreased ECOG score, fever, pyemia, neutropenia, hemorrhage or dehydration)	Dose interruption, until recovery to grade 0-I and disappearance of complications	Second time 320 mg
Other adverse events		
≥2 Grade non-hematological adverse event (except alopecia, fatigue, fatigue, etc.)	Dose interruption, until recovery to grade 0-I	First time 400 mg Second time 320 mg
≥Grade 3 hematological adverse event	Dose interruption, until recovery to grade 0-I	First time 400 mg Second time 320 mg

LVEF，Left Ventricular Ejection Fraction.

*Grades of hand-foot syndrome and other adverse events that are not included are seen in Section 9.2.2. Investigators can give clinically active treatment or observation (≤14 days) according to the condition of subject and adverse event, and adjust the dose in accordance with the tablet if it is still present.

After administration adjustment, if the subject still has ≥ Grade 3 diarrhea or Grade 1–2 diarrhea with complications that are clinically uncontrollable (that is, the diarrhea still exists after ≤14 days of clinical treatment or observation and occurs ≥2 times), or other adverse events that are ≥Grade 2, at the discretion of the investigator, it is allowed to reduce the dose by one gradient again when the drug administration resumes after the suspension.

Multiple drug pauses were allowed during treatment, with each pause resuming after the adverse event had returned to Grade 0–1 and the complications had disappeared.

5.3.6 Management, distribution and retrieve of pyrotinib

The management, distribution and retrieve of the test drug pyrotinib shall be the responsibility of specific personnel. The investigator must ensure that all test drugs pyrotinib is only used for subjects participating in this clinical trial, its dose and use should be in accordance with the trial protocol, and the remaining drugs shall be returned to the sponsor. Clinical drugs should not be transferred to any non-clinical trial participants.

The test drug, pyrotinib, was retained as labeled. The drug receipt must be signed by two personnel at the time of drug distribution, in duplicate, one for each of the clinical study unit and the sponsor. The remaining drugs and empty boxes were retrieved after the study. The two parties signed a drug retrieve form. The release and retrieve of each test drug should be recorded in a timely manner on a special record sheet. The auditor is responsible for monitoring the supply, use, storage and handling of the remaining drug product of the test drug, pyrotinib.

5.3.7 Compliance with pyrotinib

In cases where test article pyrotinib has been dispensed to individual subjects, these subjects should be required to return unused test drugs on return for each subsequent visit. Subjects recorded daily dosing information in a diary.

Test drug and adherence to dosing (e.g., the daily dose of the medicine is consistent with that described above) will be evaluated at each clinical visit (except for follow-up visits) while the subject is on medication. At each study visit, study center personnel will compare the returned test drug with the dose information reported by the subject and with the prescribed dose for monitoring compliance. The number of test drugs returned will be recorded. Deviations between the number of test drugs returned and the dosing information reported by the subject should be checked with the subject during the visit. Adherence and unclear deviations will be documented in the source file and on the drug inventory record. Any deviations from compliance should be documented and explained accordingly.

Calculation of dose compliance:

% Compliance = Tablets taken/Tablets expected to be taken ×100. A subject may be considered to be noncompliant with dosing if the compliance percent calculated using the above formula is less than 80% or greater than 120%.

If a subject's adherence exceeded the above range, a protocol deviation was recorded. The online drug electronic case report form (CRF) for test drugs should reflect the cross-checked amount of drug information provided by the subject.

5.3.8 Criteria for concomitant medications

1) Drugs prohibited or used with caution during the study

Adverse reactions of the subjects should be closely observed and proactive treatment should be given if necessary, and the drugs used should be recorded and described on the CRF.

The following medications were prohibited during the study:

- Systemic high-dose corticosteroids, that is dexamethasone > 20mg per day (or an equivalent dose of other corticosteroids) treated continuously for > 7 days;
- Hormonal contraceptives administered by oral administration, injection or implantation.

The following medications were used with caution during the study:

- Drugs that interfere with liver P450 enzymes:
  - ✓ Inducers (catamidate, rifampin, and phenobarbital) and inhibitors (ketoconazole, itraconazole, erythromycin, and clarithromycin) of CYP3A4;
  - ✓ Substrates for CYP3A4 (simvastatin, cyclosporine, and midazolam);
  - ✓ Other drugs metabolized by CYP3A4 (such as benzodiazepines, dihydropyridines, calcium antagonists, and HMG-COA reductase inhibitors);
  - ✓ Substrates for CYP2C9 (diclofenac, phenytoin, piroxicam, S- warfarin, and toluenesulfonylurea) and CYP2C19 (diazepam, promethazine, lansoprazole, and S-mephenytoin).
- Drugs that prolong the QT interval: including antibiotics, antiarrhythmic drugs, antipsychotic drugs, antifungal drugs, antimalarial drugs, antidepressants and other drugs (such as clarithromycin, quinidine, risperidone, fluconazole, mefloquine, amitriptyline, azithromycin, sotalol, flufenadine, ketoconazole, chloroquine, imipramine, erythromycin, amiodarone, droperidol, clomipramine, roxithromycin, propiramine, haloperidol, dulpine, metronidazole, procainamide, thiolizine, doxepin, moxifloxacin, pimozide, olanzapine

2) Medications that may be used in combination during the study

Subjects were allowed to receive drug-induced ovarian castration and bisphosphate therapy. Clinical combined diseases and various adverse events (AEs) should be actively treated.

Concomitant use of each drug should be recorded in the CRF in strict accordance with GCP regulations.

5.3.9 Supportive treatment for pyrotinib (necessary treatment measures in case of SAE related to research).

When adverse events occur in the course of research, researchers should take active treatment, and make detailed records of combined treatment and medication in the course of disease and CRF. According to the data of pre-clinical trials of pyrotinib, the single drug of pyrotinib is well tolerated. The incidence of grade 3/4 adverse events was 25% (2/8) in the 400mg dose group and 11.1% (1/9) in the 320mg dose group. Therefore, it is suggested to adjust the dosage of pyrotinib according to Table 2 in the study.

Researchers should carry out medical treatment according to the actual clinical situation, and the following treatment methods are for reference.

1) Diarrhea: Before subjects start oral administration of pyrotinib, researchers should inform in detail the possibility of diarrhea and the treatment measures for diarrhea. When diarrhea occurs, symptomatic treatment should be given first, followed up or observed closely (≤14 days). Clinically, it is suggested that oral montmorillonite powder, 3g/ bag, three times a day should be given on the day of diarrhea. Patients with severe diarrhea can take electrolyte orally or intravenously. For grade 3 diarrhea that still cannot be alleviated or grade 1~2 diarrhea with complications, it is recommended to suspend the administration. After AE is restored to grade 0~1, resume administration according to relevant regulations in Table 2.
2) Hand-foot syndrome and rash: give symptomatic treatment first and follow up closely. Suggested symptomatic treatment: strengthen skin care, keep skin clean and avoid secondary infection; Avoid pressure or friction; Use moisturizer or lubricant, and locally use emulsion or lubricant containing urea and corticosteroid; If necessary, local use of antifungal or antibiotic treatment. For grade 2 hand-foot syndrome which still cannot be alleviated, it is suggested to suspend pyrotinib. After AE is restored to grade 0~1, resume administration according to relevant regulations in Table 2.
3) Abnormal liver function: The researcher should give symptomatic treatment or observation to protect liver according to the subjects and AE, and the frequency of blood biochemical examination should be increased according to clinical needs. After active treatment or observation (≤14 days), liver function abnormality of grade ≥2 still exists, it is suggested to suspend pyrotinib. After AE is restored to within Grade 1, resume administration according to relevant regulations in Table 2.
4) Vomiting: First give symptomatic treatment, and follow up closely. According to the judgment of the researcher, it is recommended to suspend pyrotinib for ≥grade 2 vomiting which still cannot be alleviated. After AE is restored to within Grade 1, resume administration according to relevant regulations in Table 2. If vomiting is close to the medication time of the day, it is necessary to record the occurrence time of vomiting in detail. However, regardless of whether vomiting affects the absorption of the study drug, the patients will continue to take the drug according to the program cycle, and will not take supplements or adjust the cycle.

5.4 Criteria for Early Withdrawal/Termination

5.4.1 Criteria for Early Withdrawal

1) The subject voluntarily withdrew the informed consent form at any time;
2) Subjects were found to have seriously violated the inclusion criteria.

5.4.2 Criteria for Termination

Subjects discontinued from study treatment but continued to be followed-up as required by study:

1) Medical imaging or clinical progress;
2) Use of anti-tumor drugs other than the research protocol during the test;
3) Medications were not given according to the dose, method and course of treatment specified in this study protocol;
4) Any clinical adverse event, laboratory abnormality, or other medical condition that results in the possibility that the subject may no longer benefit from continuing to take the drug;
5) Gestation events that occurred in the subject during the study;
6) The research on the influence of medical or ethical reasons continues;
7) The subject is lost to follow-up;
8) Any other circumstances that the investigator deems necessary to withdraw from the study.

5.4.3 Treatment discontinue from Subject

Every effort must be made to complete the study end/exit visit as per protocol, and for subjects who discontinue study treatment, safety, efficacy (if required), and survival visits will be conducted as specified in the follow-up period.

According to the actual situation of the patient, the investigator can recommend or provide new or alternative treatments to the patient.

5.5 Evaluation method of efficacy and toxicity

5.5.1 Efficacy evaluation:

1) Evaluate the status of primary lesions and regional lymph nodes according to RECIST 1.1 standard (see Appendix II for details) in combination with the results of physical examination, B-ultrasound and MRI;
2) The curative effect was evaluated according to the results of pathological examination

- Total pathological complete response (tpCR, ypT0 ypN0): the absence of invasive and in situ cancer in the breast and axillary lymph nodes
- Locoregional pathological response rate (ypT0/is ypN0): absence of invasive cancer in the breast and no pathological involvement of axillary lymph nodes

5.5.2 Toxicity evaluation:

1) Physical examination, vital signs and ECOG score

The physical examination is in charge of the research doctors. The examination contents include: general condition, skin mucosa, lymph nodes, head and neck, chest, abdomen, musculoskeletal, nerve reflex, respiratory system, cardiovascular system, urogenital system, mental status, etc. Weight needs to be measured at each physical examination, but height only needs to be measured once during the screening period.

Vital signs include the following: body temperature, blood pressure, respiratory rate and heart rate.

The ECOG score was evaluated by the study doctor according to the contents in Appendix I "physical condition scoring criteria (ECOG)".

2) Laboratory examination

The laboratory indicators are sampled and tested by the research center. See Table 4 for the content requirements. For subject safety reasons, unplanned clinical laboratory tests may be performed at any time.

3) Electrocardiogram

12 lead ECG should be in the charge of a qualified doctor.

All ECG examinations are required to be performed after the subjects rested in a quiet position for at least 10 minutes. The content shall at least include: heart rate, QT, QTc and P-R interval.

To assess the safety of the subjects, the doctor will compare the ECG results with the baseline test. If the QTc interval is more than 30msec higher than the baseline, or the absolute value of QTc interval is ≥ 470msec in any specified ECG measurement, it is necessary to add two ECG inspections at an interval of at least 10 minutes to determine the accuracy of the original measurement and eliminate abnormal ECG caused by incorrect conductor layout. If the QTc value read by the machine is prolonged, according to the above description, if a qualified physician determines that the QTc value is within the acceptable range, repeated measurement may not be carried out.

Table 4 Contents and requirements of laboratory inspection

Routine blood test	**Blood biochemistry**	**Routine stool test**	**Routine urine test[a]**
Hemoglobin Red blood cell white blood cell Neutrophil count Lymphocyte count Platelet count	Total bilirubin Conjugated bilirubin ALT AST Alkaline phosphatase γ- GTT Total protein Albumin Urea nitrogen Creatinine Uric acid Blood sugar Triglyceride Cholesterol Blood lipase Blood amylase Potassium Sodium Chlorine	Stool character Fecal occult blood	Urine protein Urine sugar Urine occult blood

	Calcium Phosphorus		
Coagulation function	**Infectious disease screening**	**Others**	
PT APTT TT Fbg	Hepatitis B five items HIV antibody HCV antibody	Proportion of blood immune cells Urinary microglobulin	

Note: a. If the semi quantitative method shows protein ≥ 2 + (e.g., urine test paper), conduct 24-hour urinary protein quantitative examination.

4) Echocardiogram

Echocardiogram should be in charge of qualified doctors. During the administration of the study protocol, the study doctor will evaluate and monitor left ventricular ejection fractions (LVEF) at the time point specified in the protocol. In the course of administration, if the investigator judges that the subject has symptoms of heart failure or clinically significant decrease of LVEF, it shall be treated and monitored according to standard medical guidelines, and consult a cardiologist if necessary. In case of clinically uncontrollable symptoms of severe heart failure (NYHA class III or IV) or significant decrease of LVEF (Below the lower limit of normal value or below 50%), the administration of test drug shall be suspended according to the provisions in 5.3.5 "dose adjustment of pyrotinib", and treatment and monitoring shall continue in accordance with standard medical guidelines.

5) 6-minute walking test

The 6-minute walking test should be carried out by a qualified doctor. During the administration of the study protocol, the study doctor will evaluate and monitor according to the time point specified in the protocol.

6) Cardiac magnetic resonance enhancement

Cardiac magnetic resonance enhancement should be the responsibility of a qualified doctor. During the administration of the study protocol, the study doctor will evaluate and monitor according to the time point specified in the protocol.

7) Adverse events/reactions

Content and classification refer to NCI CTCAE 5.0 standard. During the test, the symptoms and signs of the subjects after medication should be closely observed. Adverse events/reactions should be handled timely and effectively to ensure the safety and interests of subjects. After timely and effective treatment of adverse drug events/reactions, their types, symptoms, occurrence time, degree (or grade), symptomatic treatment methods and outcomes should be recorded, and then adverse events should be analyzed, evaluated and counted as the basis for the continuation of the trial.

6 Trial procedure

See Appendix III for trial procedures.

6.1 Screening Period

For the inspection before the test, the following items shall be completed within 14 days before the test:

1) Informed consent;
2) Collect medical history and demographic data;
3) Concomitant diseases, combined medication and treatment;
4) Physical examination: height, weight, body surface area, vital signs (heart rate, blood pressure, respiration, body temperature, etc.);
5) ECOG score;
6) Laboratory examination: blood routine, urine routine, stool routine, blood biochemistry, infectious disease screening, proportion of blood immune cells, urinary microglobulin, etc.;
7) 12 lead electrocardiogram;
8) Echocardiogram: all reports within 28 days before the enrollment are acknowledged (including qualified echocardiogram completed before signing the informed consent);
9) 6-minute walking test: all reports within 28 days before the enrollment are acknowledged (including the qualified 6-minute walking test completed before signing the informed consent);
10) Imaging examination: including chest CT, ultrasound (breast, axillary, supraclavicular lymph nodes, abdomen, gynecology, etc.), skull MRI, cardiac magnetic resonance enhancement, RGD examination of primary breast / regional lymph nodes, bone scan, etc. Reports within 28 days before enrollment can be used (including qualified tumor imaging examination completed before signing the informed consent);
11) Tumor evaluation: including physical examination and imaging examination of tumor related symptoms and superficial lesions, such as ultrasound, mammograph and MRI;
12) Collection and preservation of biological specimens such as blood and/or tissue;
13) Confirm the inclusion and exclusion criteria.

According to the above results, the researcher decides whether the patient is enrolled or not, and the qualified patient will obtain the enrollment number.

6.2 Treatment period

After obtaining the enrollment number, the subjects received neoadjuvant therapy. The following inspection items shall be completed within the time window listed in the test process. In case of national legal holidays, they can be advanced or postponed accordingly, and the reasons for exceeding the window should be recorded in CRF. During the treatment, visit should be taken once a week (D7 ± 3).

The investigator can increase the examination items or increase the vist frequency according to the clinical situation of the subjects:

1) ECOG score: check according to the visit frequency;
2) Vital signs: check by visit frequency;

3) Physical examination: pre administration examination at the beginning of each treatment cycle;
4) Blood routine examination: according to the visit rate of visit; check once or twice a week. If bone marrow suppression occurs, increase the number of tests as needed;
5) Urine routine examination: according to the visit frequency; If the urine routine shows urinary protein + + or above, then check the 24h urinary protein quantification;
6) Stool routine: check once every two treatment cycles;
7) Blood biochemistry: check according to the visit rate; If necessary, myocardial zymogram examination can be carried out, which shall be determined by the researcher according to the situation of the subject;
8) Proportion of blood immune cells: check once every two treatment cycles;
9) Urinary microglobulin: check once every treatment cycle;
10) 12 lead ECG: once per treatment cycle; During the test, if the QTc interval is more than 30msec higher than the baseline, or the absolute value of QTc interval is ≥ 470msec in any specified ECG measurement, it is necessary to add two ECG examinations (at least 10min interval);
11) Echocardiogram: if there are symptoms such as chest pain and palpitation during the study, it can be checked as appropriate; check every 12 weeks of targeted therapy after enrollment (± 7 days);
12) 6-minute walking test: if there are symptoms such as chest pain and palpitation during the study, it can be checked as appropriate; check every 12 weeks of targeted therapy after enrollment (± 7 days);
13) Imaging examination: cardiac magnetic resonance enhancement was examined once after targeted treatment for 12 weeks (± 7 days), and ultrasound and breast MRI were evaluated once every two treatment cycles;
14) Concomitant medication/treatment: record the concomitant medication/treatment information during the study at any time;
15) Collection and preservation of biological specimens such as blood and/or tissue;
16) Adverse events: observe and record the adverse events during the study at any time.

6.3 Post Treatment Surgery

For cases that have completed or not completed four consecutive treatment cycles, the clinical investigator could decide whether to operate, the specific operation method and the operation time.

The efficacy was evaluated according to pathology after operation.

1) ECOG score, physical examination, ECG, electrocardiogram, 6-minute walking test, cardiac magnetic resonance enhancement, ultrasound, chest CT, breast MRI and laboratory examination were performed within 7 days before operation, and vital signs were recorded;
2) The treatment effect was evaluated according to pathology after operation;
3) One biological specimen such as blood and/or saliva should be taken within 7 days before operation, and one tumor specimen and one adjacent breast tissue specimen shall be taken during operation.

6.4 End of Study Treatment / Withdrawal from Study

The subject continued to use the drug until disease progression, intolerable toxicity, withdrawal of consent, surgery or the investigator judged that the drug must be terminated. At the end of study treatment or withdrawal from the study, if the patient has not been examined within 7 days before the end of the study (except cardiac color Doppler ultrasound and imaging evaluation), the following examinations should be performed:

1) ECOG score;
2) Vital signs;
3) Physical examination;
4) Blood routine examination: if not conducted within the previous 7 days;
5) Urine routine: if not conducted within the previous 7 days;
6) Stool routine: if not conducted within the previous 7 days;
7) Blood biochemistry: if not conducted within the previous 7 days;
8) Proportion of blood immune cells: if not conducted within the previous 7 days;
9) Urinary microglobulin: if not conducted within the previous 7 days;
10) 12 lead ECG: if not conducted within the previous 7 days;
11) Electrocardiogram : if it has not been performed within the previous 4 weeks;
12) 6-minute walking test: if not conducted within the previous 4 weeks;
13) Imaging examination: if not performed within the previous 8 weeks;
14) Combined medication / treatment: real-time record;
15) Collection and preservation of biological specimens such as blood and/or tissue: for patients who have withdrawn due to disease progression, it is recommended to collect and preserve biological specimens such as blood and/or saliva and subsequent puncture or intraoperative tissue specimens; For patients who have withdrawn due to intolerable toxicity, it is recommended to collect and preserve biological samples such as blood and/or saliva;
16) Adverse events: recorded in real time.

6.5 Follow Up Period

Follow-up period started the next day after subject withdrawal, and the following follow-up was required until the survival follow-up was completed:

1) Safety follow-up: follow up and final evaluation should be made for the adverse events that have not recovered after the discontinuation of the administration scheme in the trial. All subjects received safety follow-up until 28 days after the last administration; Adverse events, concomitant medication / treatment and unplanned examinations during the period were recorded in detail.
2) Efficacy follow-up: all subjects need to receive efficacy follow-up. After entering the follow-up period, ask the subjects themselves, their families or local doctors by telephone or clinical follow-up at least once every 12 weeks (± 7 days), and collect the efficacy (date and content of the first DFS event) and information after the end of the study treatment (including the treatment received), until the end of DFS data collection or the loss of follow-up (whichever comes first). Each efficacy follow-up should be recorded in detail and recorded in CRF.

3) Survival follow-up (OS data collection): all surviving subjects who have completed safety follow-up and efficacy follow-up (whichever is completed later) shall receive survival follow-up. Every 12 weeks (± 7 days) from the date of completing safety follow-up and efficacy follow-up (whichever is completed later) the subjects themselves, their families or local doctors shall be inquired by telephone or followed up clinically at least once, and the survival (date and cause of death) and information after the end of study treatment (including treatment received) should be collected until death, loss of follow-up of subjects or the end of OS data collection (whichever comes first). The situation of each survival follow-up should be recorded in detail and recorded in CRF.

7 Start and End of trial

The beginning of the study was defined as the beginning of the enrollment screening of the first patient.

The end of the study was defined as the completion of surgery in the last patient enrolled.

8 Clinical criteria for early termination of trials

This study may be terminated or suspended early with good reasons. If the study is terminated or suspended in advance, the principal investigator must immediately report to the ethics committee and provide corresponding reasons.

Termination criteria for this study (including but not limited to the following):

1) The study is found to have unexpected, significant or unacceptable risks to subjects;
2) In the study process, the execution of the scheme is found to have a significant error;
3) The study drug/test treatment is invalid, or is meaningless to continue to test;
4) It is extremely difficult to complete the study due to severe delay in enrollment of subjects or frequent protocol deviations.

9 Data security and monitoring plan

9.1 Overview of Data Management

The purpose of data management is to ensure the reliability, integrity and accuracy of data. Its goal is to obtain high-quality real data for statistical analysis.

In this study, the online electronic case report form (CRF) will be used for the collection and management of research data. The online electronic case report form is provided by Xinyu Technology (scientific research treasure) company.

9.1.1 Requirements for investigators in data filling

1) For all patients who have filled in the informed consent form and qualified to enter the trial, any item in the case report form must be recorded carefully and in detail, and no blank item or omission is allowed;
2) All data in the case report form should be checked with the subject's medical record data to ensure that it is correct;
3) As the original data, the case report form can only be crossed when making any correction, and the modified data can be annotated with the signature and date of the researcher;
4) The data that are significantly high or beyond the range of clinical acceptance should be verified and the researcher should make necessary explanations;
5) Fill in the description with reference to the case report form.

9.1.2 Traceability of data

The original record is the study medical record for proper preservation. The case report form is from the study medical record and filled by the researcher. Each selected case must complete the case report form.

9.2 Reporting and Collection of Adverse Events and Serious Adverse Events

9.2.1 Adverse event

Adverse event (AE) refers to any adverse medical event that occurs after the patient or the subject of the clinical trial signs the informed consent, but the event does not necessarily have a causal relationship with the study drug. An adverse event may be any unexpected, adverse symptom, sign, disease or abnormal examination result, whether or not it is related to the study drug. Adverse events include the following situations:

1) Medical conditions/diseases existing before the start of study treatment are recorded as adverse events only when they deteriorate after the start of study drug use;
2) Any new adverse events;
3) Abnormal changes in laboratory results constitute adverse events only if they are considered to be of clinical significance.

9.2.2 Criteria for judging the severity of adverse events

Refer to the classification standard of adverse drug events in NCI CTCAE version 5.0.

In case of adverse events not listed in NCI CTCAE version 5.0 table, the following standards can be referred to:

Grade 1: mild; No clinical symptoms or mild clinical symptoms; Only clinical or diagnostic findings; No treatment is required.

Grade 2: moderate; Minimal, local or non-invasive treatment is required; Age matched instrumental activities of daily living (ADL) were limited. Instrumental activities of daily living refer to cooking, shopping, making phone calls, financial management, etc.

Grade 3: severe or of medical significance, but not immediately life-threatening; Resulting in hospitalization or prolonged hospitalization; Cause disability; Self-care activities of daily living (ADL) are limited. Self-care activities of daily living refer to bathing, dressing, undressing, eating, toilet, taking medicine, etc. they are not bedridden.

Grade 4: with life-threatening consequences; Urgent treatment is needed.

Grade 5: death related to adverse events.

9.2.3 Adverse event reporting period

From signing the informed consent form to the date of the last visit, the adverse events during this period, whether related to the study drug or not, should be filled in the case report form. In addition, any adverse event occurring after the adverse event reporting period, which the investigator estimated may be related to the study drug, should also be reported as an adverse event.

9.2.4 Serious adverse events (SAE)

Serious adverse event (SAE) refers to medical events that require hospitalization or prolonged hospitalization, disability, affect working ability, endanger life or death, cause congenital malformation and so on. Adverse events that meet one or more of the following criteria are SAE:

1) Cause death;
2) Life threatening (defined as the risk of immediate death of the subject at the time of the event);
3) Lead to hospitalization or prolonged hospitalization;
4) Permanent or severe disability/loss of function;
5) Congenital malformation/defect;
6) Important medical events: these adverse events may not cause death, endanger life, or require hospitalization, but according to medical judgment, the events may endanger the subject and require medical or surgical intervention to prevent any of the above results.

9.2.4.1 Potential drug-induced liver damage

If the AST and/or ALT levels are abnormal and the total bilirubin level is abnormally elevated, the following conditions are met and there are no other causes of liver injury, it will be considered as drug-induced liver injury. Such situations should always be regarded as important medical events (see Table 5 for details).

The subject should return to the Research Center for evaluation as soon as possible (preferably within 48 hours) after learning the abnormal results. The evaluation should include laboratory examination, detailed medical history and physical evaluation, and should consider the possibility of liver tumor (primary or secondary).

Table 5 Evaluation criteria of potential drug-induced liver dysfunction

<table>
<tr><td>Baseline Period</td><td colspan="2">Normal (AST / ALT and total bilirubin)</td><td colspan="2">Abnormal (AST / ALT / total bilirubin)</td></tr>
<tr><td rowspan="2">Treatment Period</td><td>ALT≥ 3×ULN</td><td>AST≥ 3×ULN</td><td>ALT or AST ≥ 2 × Baseline level and ≥ 3 × ULN</td><td>AST or ALT ≥ 8 × ULN</td></tr>
<tr><td colspan="2">Meet one of the above two, with total bilirubin ≥ 2 × ULN and alkaline phosphatase ≤ 2 × ULN without hemolysis</td><td colspan="2">Meet one of the above two, with total bilirubin ≥ 3 × ULN or increase ≥ 1 × ULN</td></tr>
</table>

In addition to repeated testing of AST and ALT, laboratory tests should also include albumin, creatine kinase, total bilirubin, direct and indirect bilirubin γ- Glutamyltransferase, prothrombin time (PT) / international normalized ratio (INR), alkaline phosphatase. Further examination may also include the detection of acute hepatitis A, B, C and E and liver imaging (such as biliary tract).

The detailed medical history collection should include: drinking history, acetaminophen, soft drugs, various supplements, family history, occupational exposure, sexual behavior history, travel history, contact history with patients with jaundice, surgery, blood transfusion, liver disease or allergic disease history, etc.

If the repeated test still confirms that it meets the definition of the above laboratory standards, the possibility of potential drug-induced liver damage should be considered without waiting for all the etiological test results of liver function. Such potential cases of drug-induced liver damage should be reported as SAE.

9.2.4.2 Disease progression

Disease progression (including symptoms and signs of progression) should not be reported as a serious adverse event, but death due to disease progression within the test or safety reporting period, i.e. within 28 calendar days (including 28 days) after the last use of the study protocol, should be reported as a serious adverse event. Hospitalization due to symptoms and signs of disease progression should not be reported as a serious adverse event. During the trial or safety reporting period, if the final result of cancer is death, the event leading to death must be reported as a serious adverse event.

9.2.4.3 Hospitalization

Adverse events resulting in hospitalization or prolonged hospitalization in clinical studies should be regarded as serious adverse events. Any initial admission by a medical institution (even less than 24 hours) meets this standard.

The following hospitalizations do not constitute SAE:

- Rehabilitation institutions

- Sanatorium
- Routine emergency room admission
- Intra-day surgery (e.g., outpatient/intra-day/ambulatory surgery) hospitalization or prolonged hospitalization not related to adverse events is not a serious adverse event itself. For example:

✓ There were no new adverse events or aggravation of the original disease (for example, in order to check the laboratory examination abnormalities that continue to exist before the test);

✓ Hospitalization for management reasons (e.g. annual routine physical examination);

✓ Hospitalization specified in the trial protocol during the clinical trial (e.g. operation according to the requirements of the trial protocol);

✓ Elective hospitalization unrelated to adverse events (e.g. elective cosmetic surgery);

✓ Scheduled treatment or surgery shall be recorded in the whole trial protocol and / or baseline data of individual subjects;

✓ Hospitalized only for the use of blood products.

Diagnostic or therapeutic invasive (such as surgery) and non-invasive procedures should not be reported as adverse events. However, when the disease condition leading to this operation meets the definition of adverse event, it should be reported. If the acute appendicitis occurred during the reporting period of adverse event shall be reported as an adverse event, and the appendectomy therefore should be recorded as the treatment method of the adverse event.

9.2.5 Reporting route of serious adverse events

The report of serious adverse events should start from the subject's signing of informed consent to 28 calendar days (including 28 days) after the last use of the study drug. During the trial, in case of serious adverse events, whether the first report or follow-up report, the investigator must immediately fill in the serious adverse event (SAE) report form, sign and indicate the date, and immediately notify the ethics committee of the sponsor and report to the drug safety team of Hengrui tumor business department within 24 hours after the investigator learned the SAE (see Table 6 for details).

Table 6 Serious adverse event reporting contact

Company	Contact Department	Reporting Method	Contact Information
Sponsor	Sponsor ethics committee	Hand in	
Jiangsu Hengrui Pharmaceutical Co., Ltd	Drug safety group of cancer Division	E-mail (signed scanned copy)	Telephone: 021-60453192-818 Mailbox: hengrui_drug_safety@shhrp.com

Serious adverse events occurring 28 days after the last administration are generally not reported unless they are suspected to be related to the study drug. For serious adverse events, the symptoms, severity, occurrence time, treatment time, measures taken, combined medication, follow-up time and method, prognosis, etc. should be recorded in detail. If the

investigator considers that the serious adverse event is not related to the investigational drug, but potentially related to the study conditions (such as termination of the original treatment, or complications during the trial), this relationship should be described in detail in the narrative section of the serious adverse event page in the case report form. If the intensity of the serious adverse event or its relationship with the test drug changes, the serious adverse event follow-up report should be sent to the sponsor immediately. All serious adverse events should be followed up until recovery, remission to level 1 or baseline level, or stability.

9.2.6 Records and reports

The investigator should explain in detail to the patients and ask them to truthfully reflect the changes of their condition after medication. Doctors should avoid inducing questions. While observing the treatment effect, pay close attention to the adverse events, analyze the causes, make judgments, track, observe and record, and count the incidence of adverse reactions.

Adverse events include all unexpected clinical manifestations. As long as these events occur after the signing of informed consent, they should be reported as adverse events regardless of whether they are related to the test drug or whether they are used.

The time, symptoms, degree, duration, treatment measures and outcome of adverse events during the test should be recorded in the case report form to evaluate their correlation with the test drug, which should be recorded in detail, signed and dated by the researcher. The severity of adverse events will be graded according to NCI CTCAE version 5.0. For each symptom, the highest grade of adverse events since the last follow-up should be reported. The relationship between adverse events and investigational drugs is determined according to grade 5: definitely related, possibly related, possibly unrelated, irrelevant and unable to be evaluated (Table 7). The first two were counted as adverse reactions, and the incidence of adverse reactions was counted.

1) Affirmation: the occurrence of the reaction conforms to the reasonable time sequence after medication, and the reaction conforms to the known reaction type of the suspected drug; The reaction was improved after drug withdrawal and reappeared after repeated administration.
2) Possibly relevant: the occurrence of the reaction conforms to the reasonable time sequence after medication, and the reaction conforms to the known reaction type of the suspected drug; The patient's clinical status or other treatment methods may also produce this reaction.
3) Possibly irrelevant: the reaction does not accord with the reasonable time sequence after medication, and the reaction does not accord with the known reaction type of the suspected drug; The patient's clinical status or other treatment methods may produce this reaction.
4) Irrelevant: the reaction does not conform to the reasonable time sequence after medication, and the reaction is in line with the known reaction types of non test drugs; The patient's clinical state or other treatment methods may produce this reaction, the disease state improves or stops other treatment methods, the reaction is eliminated, and the reaction of repeated use of other treatment methods appears.

5) Unable to evaluate: the occurrence of the reaction has no clear relationship with the time after administration. It is similar to the known reaction type of the drug, and other drugs used at the same time may cause the same reaction.

Table 7 Relationship between adverse events and investigational drugs

	Affirmation	Possibly relevant	Possibly irrelevant	Suspicious	Irrelevant
There is a reasonable time sequence with the experimental medication	+	+	+	+	-
Known types of drug reactions	+	+	+	-	-
The reaction alleviated or disappeared after drug withdrawal	+	+	±	±	-
The reaction occurred repeatedly after re administration	+	?	?	?	-
It cannot be explained by the subject's disease	+	+	-	±	-

The trial starts from the day when the subject signs the informed consent form and needs to be evaluated for drug safety within 28 days after the last administration. All adverse events (serious and non-serious) shall be recorded in the adverse event report page of the case report form, and the adverse events shall be reported in accurate medical terms.

Related AES: follow up is required until any of the following conditions occur:

1) Disappeared or improved to baseline level;
2) Reassessment considered that it had nothing to do with the study protocol;
3) Death;
4) Start a new anti-tumor treatment program;
5) The researchers confirmed that no further improvement was expected and the patient's condition was stable;
6) Clinical data or final database will no longer be collected.

Unrelated AES: follow up is required until any of the following conditions occur:

1) Disappeared or improved to baseline level;
2) The severity is improved to within level 1;
3) Death;
4) Start a new anti-tumor treatment program;
5) The researchers confirmed that no further improvement was expected;
6) Clinical data or final database will no longer be collected.

9.3 Medical Safety Measurements

In order to ensure that the trial can be carried out in strict accordance with the clinical research plan, in the whole process of the clinical trial, the clinical researchers and clinical sponsors should operate in strict accordance with the requirements of Good Clinical Practice (GCP), and be sure to achieve standardized test procedures, accurate test data and reliable research conclusions.

Before the clinical trial, clinicians participating in the trial should be trained uniformly. Doctors participating in clinical trials should be relatively fixed. The subjects should return to the clinic in time according to the requirements of the scheme. The researchers should follow up the cases that are not returned to the clinic in time. The compliance of patients should be supervised by nurses or family members or nursing staff. The relevant adverse reactions of patients should be handled by clinicians in time, treated actively, and reported to the Ethics Committee Department in time.

9.4 Communication with Ethics Committee and Superior Drug Administration Department

If there is any change in the research plan, scope and content, it will communicate with the ethics committee and the superior drug administration department in time.

9.5 Internal Data Analysis Plan

Estimated enrollment time of the first subject: March 2019;

It is estimated that the last subject will be enrolled in March 2020;

It is estimated that the last subject will be out of the group in July 2020;

Expected completion time of the study: September 2020.

9.6 Frequency of Submission of Data Security and Monitoring Reports to the Ethics Committee

Data security report shall be conducted once a year.

10 Compliance with Ethical Principles and Related Regulations

10.1 Laws and Regulations

Before the start of this study, the clinical study can be carried out only after obtaining the approval or filing of the corresponding regulatory department. The clinical study also needs to be carried out in accordance with all applicable regulatory requirements.

10.2 Code of Ethics

This research process must be carried out in strict accordance with the requirements of CFDA code for quality management of drug clinical trials and Helsinki declaration.

10.3 Ethics Committee

Before the clinical study, the trial protocol, informed consent and other data provided to the subjects must be reviewed and approved by the ethics committee of the team leader unit, and the relevant approval documents should be provided to the reviewer.

10.4 Informed Consent

Before receiving the treatment of this trial, the subjects must give informed consent to participate in this trial, so as to protect the legitimate rights and interests of the subjects. The researcher is responsible for completely and comprehensively introducing the purpose of this study, the effects of drugs, possible toxicity and side effects and possible risks to the subjects or their designated representative, and should let the subjects know their rights, risks and benefits. Conversation is a very important informed consent process. If the subject and his legal representative are illiterate, the witness shall participate in the informed consent process. After the oral consent of the subject or his legal representative, he shall sign the informed consent form. The signature of the witness shall be on the same day as the signature of the subject. The informed consent form shall indicate the version and formulation date or modification date.

11 Statistical analysis plans

11.1 Sample Size

The primary endpoint of this study was the rato of tpCR (defined as ypT0 ypN0). According to the results of clinical trials such as NeoSphere and NeoALTTO, and the results of the phase II clinical trial of the neoadjuvant therapy of weekly paclitaxel combined with cisplatin published by our center, it is assumed that tpCR increased from 50% of the trastuzumab / paclitaxel / cisplatin regimen to 70% of the study plan,α= 0.05 (bilateral), β= 0.2, at least 47 subjects need to be enrolled according to the 80% power. If the 10% dropout rate is considered, 52 subjects need to be enrolled in each group.

11.2 Statistical Analysis Data Set

1) Full analysis set (FAS): according to the principle of intention to treat analyze (ITT), analyze the efficacy of all cases enrolled and used drugs at least once. For the case data that cannot observe the whole treatment process, the last observation data shall be carried forward to the final result of the trial (last observation carried forward, LOCF).

2) Per protocol (PP) analysis set: refers to the collection of cases that meet the inclusion criteria and complete the treatment scheme, that is, the cases that meet the test scheme, have good compliance, do not take prohibited drugs and complete the contents specified in the case report form. There is no imputation for missing data.

The efficacy analysis of this study protocol was conducted for the full analysis set and the per protocol analysis set.

3) Safety analysis set: all enrolled patients who have used the study protocol at least once and have post medication safety records belong to the safety analysis set.

The safety analysis of this research scheme is based on the safety analysis set.

11.3 Statistical Methods

The statistical description method is used. The measurement data should list the mean, standard deviation, median, maximum value and minimum value, and the counting data and grade data should list the frequency (constituent ratio), rate and confidence interval.

1) Measurement data: t-test, paired t-test, rank sum test, paired rank sum test, etc.;
2) Counting data: Chi square test and Fisher exact probability method were used for non-grade data; Rank sum test was used for grade data.
3) Analysis of efficacy indexes: the counting data were analyzed by logistic regression, Fisher exact probability method, etc.; The measurement data were analyzed by analysis of variance or rank sum test according to the characteristics of the data; The survival data were analyzed by Kaplan Meier method or Cox regression.
4) FAS analysis and PP analysis: FAS analysis and PP analysis were performed simultaneously for the main study endpoint.

11.4 Statistical Expression

1) The report is mainly expressed in tables, which are self-evident, that is, there are table questions, table notes and number of cases.

2) All statistical tests adopt two-sided test. If the p value is less than or equal to 0.05, it will be considered that the tested difference is statistically significant, and the confidence interval adopts 95% confidence.

11.5 Statistical Software

STATA Statistics SE 14 (Stata Corp LP, College Station, TX, USA) and R statistical software (version 4.1.0) were used for analysis.

11.6 Contents of Statistical Analysis

The statistical analysis plan is written by statisticians, including data management, statistical methods and analysis contents. The main analysis contents include:

1) Case distribution: Chi square test will be used to compare the total abscission rate and the abscission rate due to adverse events.
2) Efficacy analysis: FAS and PP analysis are used for main indicators and overall indicators.
3) Safety analysis: firstly, according to the requirements of adverse reaction correlation, list and describe adverse events and adverse reactions (in which adverse reactions are defined as "adverse events with a relationship with the study drug as' definitely related/possibly related/undecidable'). The laboratory test results describe the normal conditions before the test but abnormal conditions after treatment, and the relationship with the test drug in case of abnormal changes. Chi square test was used for statistical analysis of adverse reactions.

Appendix I

Physical Status Score (ECOG)

Activity Status	Description
0	Asymptomatic, fully active, and able to carry on all predisease performance without restrictions.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature, eg, light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours： in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bedridden.
4	Completely disabled. Cannot carry on any self-care. Totally bedridden.
5	Dead

Appendix II

RECIST guideline (version 1.1)

1. Definitions

At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows:

1.1 Measurable

Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

• 10 mm by CT scan (CT scan slice thickness no greater than 5 mm; see Appendix II on imaging guidance).

• 10 mm caliper measurement by clinical exam (lesions
which cannot be accurately measured with calipers should
be recorded as non-measurable).

• 20 mm by chest X-ray.

• Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be P15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed (see Schwartz et al. in this Special Issue15). See also notes below on ‘Baseline documentation of target and non-target lesions’ for information on lymph node measurement.

1.2 Non-measurable

All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with P10 to <15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

1.3. Special considerations regarding lesion measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions:.

• Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

• Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.

• Blastic bone lesions are non-measurable.

Cystic lesions:.

• Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

• 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above.

• However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

2. Response criteria

2.1. Evaluation of target lesions

1) Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
2) Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
3) Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
4) Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

2.2. Evaluation of non-target lesions

While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only qualitatively at the time points specified in the protocol.

1) Complete Response (CR): Disappearance of all non-target lesions and normalisation of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).
2) Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
3) Progressive Disease (PD): Unequivocal progression (see comments below) of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

2.3 Overall response

Target lesions	NON-Target lesions	New lesions	Overall response
CR	CR	no	CR
CR	Non-CR/non-PD	no	PR
CR	Not evaluated	no	PR
PR	Non-PD or not all evaluated	no	PR
SD	Non-PD or not all evaluated	no	SD

Not all evaluated	Non-PD	no	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD
CR= complete response	PR= partial response	SD= stable disease,	PD= progressive disease, NE= unevaluable

3. Results Report

All patients who enter the study, even those who deviate from the main treatment plan and are out of the group according to the conditions, should be evaluated for efficacy. Each patient can be classified into one of the following categories:

- Complete response CR
- Partial response PR
- Disease progression PD
- Disease stable SD
- Early death due to tumor
- Early death due to therapeutic toxicity
- Early death due to other causes
- Unable to classify (unable to evaluate or incomplete information)

Appendix III

Research Flow Chart

	Screening period	**Trial period**			**Follow up period**		
	D-14~D-1	C1~C4	Within 7 days before operation	Exit study	Security	Curative effect[17]	Survival[18]
Sign informed consent	×						
Demographic data[1]	×						
Medical history[2]	×						
ECOG score[3]	×	Check once a week	×	×			
Vital signs[3]	×	Check once a week	×	×			
Physical examination[4]	×	Check once before administration at the beginning of each treatment cycle	×	×			
Routine blood test[5]	×	Check once a week	×	×			
Routine urine test[6]	×	Check once a week	×	×			
Routine stool test[7]	×	Check once every two treatment cycles	×	×			
Proportion of blood immune cells[7]	×	Check once every two treatment cycles	×	×			
Urinary microglobulin[8]	×	Check once per treatment cycle	×	×			
Blood biochemistry[9]	×	Check once a week	×	×			

Infectious disease screening[10]	×						
12 lead ECG[11]	×	Check once per treatment cycle	×	×			
Echocardiogram / 6-minute walking test[12]	×	Targeted therapy: check once every 12 weeks (± 7 days)	×	×			
Imaging examination[13]	×	See instructions for details	×	×			
Pyrotinib[14]		Once a day, continuous administration					
Combined medication/ treatment[15]	×	×					
Adverse event observation record[16]	×	×					
Time of progression / death						×	×
Follow up tumor treatment[19]					×	×	×

Note: the following inspection items shall be completed within the time window listed in the test process. In case of national legal holidays, the reason for exceeding the window can be recorded in CRF in advance. During the treatment, visit once every weekend (D7 ± 3).

1. Demographic data (abbreviation, gender, nationality, marital status, date of birth, height and weight, and body surface area BMI shall be calculated accordingly);

2. Medical history enquiries: including past medical history and treatment history (clinical/pathological diagnosis, diagnosis time, clinical/pathological stage, HER2/ER/PR expression, previous treatment for breast cancer), smoking and drinking history (frequency, quantity, duration), drug allergy history (drug name, allergy symptom), past diseases or accompanying diseases/symptoms (name of disease symptoms, name of combined medication, dose, usage and prognosis), stool habits (times);

3. Vital signs and ECOG score: including body temperature, blood pressure, respiratory rate and heart rate; check once during screening period, check according to interview video rate during neoadjuvant treatment period, check once within 7 days before operation and once at the end / withdrawal of treatment (if not within 7 days before);

4. Physical examination: including general condition, skin mucous membrane, lymph node, head and neck, chest, abdomen, musculoskeletal, neuro-reflex, respiratory system, cardiovascular system, urogenital system, mental condition, etc.; one examination before administration at the beginning of screening period and each treatment cycle, one examination within 7 days before operation, and one examination at the end/withdrawal of treatment (if not conducted within the previous 7 days);

5. Blood routine examination: including absolute counts of WBC, ANC, LC, RBC, Hb and PLT; one examination during screening period, one examination according to the visit rate during neoadjuvant treatment period, one examination within 7 days before operation and one examination at the end/withdrawal of treatment (if it is not carried out within 7 days before); if bone marrow suppression occurs, the examination times can be increased as needed;

6. Urine routine: including urine protein. If the urine routine shows urine protein + + or above, then check the 24h urine protein quantification; check once in the screening period, according to the visit rate in the neoadjuvant treatment period, once in 7 days before operation and once at the end/exit of treatment (if it is not done in the previous 7 days);

7. Stool routine and proportion of blood immune cells: including stool characteristics and stool occult blood; check once during the screening period, once every two treatment cycles, once within 7 days before operation and once at the end / withdrawal of treatment (if not within 7 days before);

8. Urinary microglobulin: including α 2-microglobulin and β 1-microglobulin; check once in the screening period, once in each treatment cycle, once in 7 days before operation, and once at the end / withdrawal of treatment (if not in the previous 7 days);

9. Blood biochemistry: including glucose, TP, A/G, alt, AST, ALP γ- GTT, ALB, TBIL, DBIL, IBIL, TG, CHOL, UA, bun, Cr, K +, Na +, Mg2 +, Cl -, Ca2 +, p; check once during the screening period, according to the follow-up frequency during the neoadjuvant treatment period, once within 7 days before operation and once at the end/withdrawal of treatment (if not within 7 days before); if necessary, check the myocardial zymogram, which is determined by the researcher according to the situation of the subjects;

10. Infectious disease screening: including hepatitis B five items, HIV antibody, HCV antibody test; screening period 1 times;

11.12 lead ECG: observe QT, QTc and P-R time, check once in screening period, once in each treatment cycle, once in 7 days before operation, and once at the end/withdrawal of treatment (if not in the previous 7 days); during the test, if the QTc interval is more than 30msec higher than the baseline, or the absolute value of QTc interval is ≥ 470msec in any specified ECG measurement, it is necessary to add two ECG examinations (at least 10min interval);

12. Echocardiogram/6-minute walking test: it should be conducted within 28 days before enrollment. If there are symptoms such as chest pain and palpitation during the study period, it can be checked as appropriate; it should be checked once 12 weeks (± 7 days) after enrollment; once within 7 days before operation; and once at the end of treatment / withdrawal (if it has not been conducted within the previous 4 weeks);

13. Imaging examination: including chest CT, B-ultrasound (breast, axillary, supraclavicular lymph nodes, abdomen, gynecology, etc.), brain MRI, cardiac magnetic resonance enhancement, bone scan, etc., which can be reported within 28 days before enrollment; during the study, cardiac magnetic resonance enhancement can be checked every 12 weeks after enrollment (± 7 days); ultrasound and breast MRI are evaluated once every two treatment cycles; check once cardiac magnetic resonance enhancement, ultrasound, chest CT and breast MRI within 7 days before operation; check once at the end/withdrawal of treatment (if not carried out within the previous 8 weeks);

14. Pyrotinib: 400mg once a day, oral administration within 30 minutes after breakfast, continuous administration for 16 weeks; the dose can be adjusted according to the adverse reactions of the subjects according to the scheme, and the subjects continue to use the drug until the disease progresses, the toxicity is intolerable, the withdrawal is informed, or the researchers judge that the drug must be terminated;

15. Concomitant medication/treatment: record the concomitant medication within 28 days before enrollment and during the trial; once the subject interrupts the trial treatment, only the concomitant medication and concomitant treatment used for new or unresolved adverse events related to the trial treatment shall be recorded;

16. Observation record of adverse events: monitoring should be started from the day when the informed consent is signed until at least 28 days after the last treatment; adverse events, concomitant medication/treatment and unplanned examination during the period shall be recorded in detail;

17. Efficacy follow-up: all subjects need to receive efficacy follow-up. After entering the follow-up period, the subjects themselves, their families or local doctors should be inquired by telephone or followed up clinically at least once every 12 weeks (± 7 days), so as to collect the efficacy (date and content of the first DFS event) and the information after the end of the study treatment (including the treatment received) Until the end of DFS data collection or the subject's loss of follow-up (whichever comes first). Each efficacy follow-up shall be recorded in detail and recorded in CRF;

18. Survival follow-up (OS data collection): all surviving subjects who have completed safety follow-up and efficacy follow-up (whichever is completed later) shall receive survival follow-up. Every 12 weeks (± 7 days) from the date of completing safety follow-up and efficacy follow-up (whichever is completed later) At least once, the subjects themselves, their families or local doctors should be inquired by telephone or followed up clinically, and the survival (date of death or cause of death) and information after the end of study treatment (including treatment received) should be collected until death, loss of follow-up of subjects or the end of OS data collection (whichever comes first). The situation of each survival follow-up should be recorded in detail and recorded in CRF;

19. Follow up of anti-tumor treatment: the subjects need to record whether they use other anti-tumor treatment during the period from leaving the group to the end of survival follow-up; survival follow-up only records the tumor treatment, and does not need to record the concomitant medication due to other diseases.