CLINICAL STUDY PROTOCOL

Phase II neoadjuvant pyrotinib combined with epirubicin and cyclophosphamide followed by docetaxel in Luminal/HER2-low-expressing early or locally advanced breast cancer (the PILHLE-001 study): a single-arm, non-randomized, single-center, open label trial

Protocol code: PILHLE-001

Coding description: Pyrotinib in Luminal/HER2-low-expressing-001

Version: 2.0, Date: November 07, 2022

Ethic Committee Approved Date: December 14, 2022

Principal Investigators: Pro. Erwei Song and Pro. Chang Gong

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PROTOCOL APPROVAL PAGE

I have carefully read protocol of "Phase II neoadjuvant pyrotinib combined with epirubicin and cyclophosphamide followed by docetaxel in Luminal/HER2-low-expressing early or locally advanced breast cancer (the PILHLE-001 study): a single-arm, non-randomized, single-center, open label trial." I agree to conduct this study as outlined herein and in compliance with Good Clinical Practices (GCP), all applicable regulatory requirements and with the ethical principles laid down in the Declaration of Helsinki.

I agree not to disclose or otherwise make available any of the confidential information to anyone except those employees and agents of institution who need to know. I agree that this protocol is only used for this study. All data pertaining to this study will be stored at Sun Yat-sen Memorial Hospital, Sun Yat-sen University. The policy requires that any presentation or publication of study data by clinical investigators be reviewed by principal investigator and DMC/IDMC, before release, as specified in the protocol.

Principal Investigator's Signature: Date:

Summary of Amendment Implemented by Protocol

This protocol has been amended in efficacy/safety outcomes and the sample size calculation. The following changes were made

Page	Item	Before	After	
cover		Version 1.0, approved date: May 19, 2021	Version 2.0, approved date: Dec 14, 2022	
P14	2.1 Primary Endpoint	Pathological complete response (pCR) rate	Residual cancer burden (RCB) 0/I rate	During that pat (E/LAE signific or RCI assessm popula 0/I rate treatme patients Follo ethics c to RCB
P14	2.2 Secondary Endpoint	- Residual cancer burden (RCB) 0/I rate	- Pathological complete response (pCR) rate	See abo
P14	2.2 Secondary Endpoint	- Frequency of Grade 3 or higher incidence of diarrhea	- AEs	Conside
P30	10.1 Sample Size	We calculate the sample size based on one-stage design. The primary outcome in this study is the pCR rate after neoadjuvant therapy. The null hypothesis of the	We calculate the sample size based on one-stage design. The primary outcome in this study is the RCB 0/I rate after neoadjuvant therapy. The null hypothesis of the RCB 0/I rate with neoadjuvant	The san

		pCR rate with neoadjuvant chemotherapy in Luminal/HER2-low E/LABC was 8%. The combination of pyrotinib and chemotherapy will increase the pCR rate from 8% to 21%. The trial has 80% power to detect true difference at one-sided alpha level of 0.05. Using the PASS software (version 15.0), a total of 43 samples is required for the trial. If more than 6 responders are observed in 43 patients, it has clinical significance.	chemotherapy in Luminal/HER2-low E/LABC was 15%. The combination of pyrotinib and chemotherapy will increase the RCB 0/I rate from 15.0% to 30.0%. The trial has 80% power to detect true difference at one-sided alpha level of 0.05. Using the PASS software (version 15.0), a total of 48 samples is required for the trial. If more than 11 responders are observed in 48 patients, it has clinical significance.	
P30	10.4.2 Analysis of Efficacy Parameters	The primary endpoint of the study is the pCR rate, defined as the proportion of patients with no residual invasive tumor cells in the breast and axillary nodes, regardless of ductal carcinoma in situ as evaluated by two independent pathologists.	The primary endpoint of the study is the RCB 0/I rate after neoadjuvant therapy, which was defined as the proportion of patients who were classified into RCB 0 or RCB I according to the online Residual Cancer Burden Calculator provided by the MD Andersson Cancer Center and were classified into four levels: RCB 0 (score = 0, equivalent to pCR), RCB I (score > 0-1.36), RCB II (score > 1.36-3.28), and RCB III (RCB score > 3.28).	See above.
P30	10.4.2 Analysis of Efficacy Parameters	The RCB 0/I rate is defined as the proportion of patients who were classified into RCB 0 or RCB I according to the online Residual Cancer Burden Calculator provided by the MD Andersson Cancer Center and were classified into four levels: RCB 0 (score = 0, equivalent to pCR), RCB I (score > 0-1.36), RCB II (score > 1.36-3.28), and RCB III (RCB score > 3.28).	The pCR rate is defined as the proportion of patients with no residual invasive tumor cells in the breast and axillary nodes, regardless of ductal carcinoma in situ as evaluated by two independent pathologists.	See above.
P31	10.4.4 Analysis of Safety Parameters	Safety analyses will be performed on the All-treated Population. Frequency of Grade 3 or higher incidence of diarrhea will be recorded and evaluated for severity according to the Medical Dictionary for Regulatory	Safety summaries will be included in the form of tables and listings. The frequency (number and percentage) of treatment emergent AEs will be reported by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class and Preferred Term. Summaries will	See above.

		Activities (MedDRA Version 19.1) and the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. Study drug-related AEs are those assessed by investigator as related.	also be presented by the severity of the AEs (per Common Terminology Criteria for Adverse Events, v5.0) and by relationship to study drug. Laboratory shift tables containing counts and percentages will be prepared by laboratory parameter. Figures of changes in laboratory parameters over time will be generated. Results of vital sign assessments will be tabulated and summarized.	

In addition, the remaining parts related to the above amended regulations had also been amended accordingly, including the study synopsis, background, efficacy assessment, safety assessment, etc.

Study synopsis

Protocol Number:	PILHLE-001
Protocol Title	Phase II neoadjuvant pyrotinib combined with epirubicin and cyclophosphamide followed by docetaxel in Luminal/HER2-low-expressing early or locally advanced breast cancer (the PILHLE-001 study): a single-arm, non-randomized, single-center, open label trial
Version No./Date	2.0/Dec 14, 2022
Phase	Phase II
Study type	Investigator-initiated clinical trials
Study design	Single-arm, prospective, non-randomized, single-center, open label
Study objective	To evaluate the efficacy and safety of neoadjuvant pyrotinib combined with epirubicin and cyclophosphamide followed by docetaxel in Luminal/HER2-low early or locally advanced breast cancer
Criteria for Inclusion/Exclusion	**Inclusion Criteria:** 1) Signed informed consent, compliance with the study protocol, women whose age ≥ 18 years old and ECOG performance status ≤ 1. 2) Centrally confirmed, newly diagnosed, unilateral, primary invasive, hormone receptor positive and HER2-low early or locally advanced breast cancer: -HER2: immunochemistry 2+ with fluorescent in situ hybridization negative, immunochemistry 1+ was not included. -Hormone receptor positive: estrogen receptor and/or progesterone receptor ≥ 1% stained cells. 3) The tumor is greater than 2 cm (cT2-4) or between 1 cm and 2 cm (cT1c) with histopathological involved lymph nodes. Note: for tumors with TNM stage-IIA, histologic grade III or Ki67 ≥ 20% or MammaPrint high-risk are required. 4) At least one evaluable target lesion according to Response Evaluation Criteria in Solid Tumors version 1.1. 5) Consented to contraception both during the trial and within 6 months after the last administration of the test drug. 6) Requisite laboratory values: - Left ventricular ejection fraction at least 55%. - White blood cell count: ≥ 3.0 × 10^9/L, absolute neutrophil count: ≥ 1.5 × 10^9/L, platelet count: ≥ 100 × 10^9/L; hemoglobin: ≥ 90 g/L. - Aspartate aminotransferase and alanine aminotransferase: ≤ 2.5 × upper limit of normal, alkaline phosphatase: ≤ 2.5 × upper limit of normal, blood total bilirubin: ≤ 1.5 × upper limit of normal; serum creatinine: ≤ 1.5 × upper limit of normal. **Exclusion Criteria:** 1) Metastatic or bilateral breast cancer, occult breast cancer, inflammatory breast cancer without assessable focus, or eczema like breast cancer. 2) Known history of hypersensitivity to the test drugs. 3) Severe dysfunction of the heart, lung, liver, or kidney.

	4) Patients who need receive other anti-tumor treatments (except for ovarian function inhibitors) during neoadjuvant therapy. 5) Patients who underwent breast cancer-free surgery within 4 weeks or had not fully recovered after BC-free surgery. 6) Patients who had basic gastrointestinal diseases (especially long-term history of diarrhea or/and constipation), inability to swallow, intestinal obstruction or other factors will affect drugs administration and absorption. 7) Serious or uncontrolled infections that may affect study treatment or evaluation of study results. 8) History of other malignant tumors in the past 5 years. 9) Not suitable for the clinical trial due to other reasons.
Study endpoints	**Primary outcome**: the residual cancer burden 0/I rate after neoadjuvant therapy, will be analyzed in the all-treated population. **Secondary outcomes**: 1) pathological complete response rate; 2) objective response rate; 3) breast conservation surgery rate; 4) 5-year disease-free survival; 5) 5-year overall survival; 6) exploratory biomarkers analysis; 7) adverse events.
Dose Regimen and Route of Administration	Patients in PILHLE-001 study will receive pyrotinib 320mg orally once daily, and epirubicin 90 mg/m² plus cyclophosphamide 600 mg/m² intravenously on day 1 for four 3-week cycles followed by docetaxel 100 mg/m² intravenously on day 1 or four 3-week cycles.
Sample size and Statistical Methods	We calculated the sample size based on one-stage design. To test the null hypothesis that 15% or fewer patients will achieve an RCB 0/I (not considered clinically meaningful), the planned sample size of 48 patients provides an 80% power to test a difference of 15% versus 30% in overall response at a two-sided significance level of 0.05. Efficacy and safety analyses are conducted on all-treated population, defined as all patients receiving at least one dose of pyrotinib. Measurement data are summarized by means, standard deviation, median, inter-quartile range; count data are summarized by frequency and percentage; time-to-event data are summarized by Kaplan-Meier model to estimate survival rate and draw survival curve.

1. Background

1.1 Luminal/HER2-low BC

Breast cancer (BC) is still the most frequently diagnosed malignant tumor in women worldwide, with an estimated 2.3 million new cases and estimated 68 hundred thousand deaths reported in 2020.[1] According to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines, human epidermal growth factor receptor 2 (HER2)-low BCs account for 45%-55% of all breast carcinomas and are defined by immunohistochemistry (IHC) 2+ with fluorescent in situ hybridization (FISH)-negative or IHC 1+,[2,3] with more than 60% of them being hormone receptor (HR)-positive (Luminal).[4,5]

1.2 Therapeutic options for Luminal/HER2-low E/LABC

Luminal/HER2-low early or locally advanced breast cancer (E/LABC, TNM stage II-III) is treated with curative intent, with the goal to reduce the risk of future recurrence. A multimodality approach is used, including surgery, radiation, and systemic therapies. Chemotherapy and endocrine therapy as the mainstay of systemic treatment for patients with Luminal/HER2-low E/LABC, have significantly improved their prognosis.[6] Nevertheless, there is still an about 20% incidence of recurrence, metastases, or death within 5 years,[7] which is further exacerbated among those presenting with high-risk clinical or genomic features such as TNM stage-IIB/III,[8] histologic grade III,[7] Ki67 ≥ 20%,[9] or MammaPrint high-risk.[10] Several studies have explored whether targeted therapies could provide benefits. Although Luminal/HER2-low BC expresses a certain degree of targetable HER2, this patient population do not derive any incremental benefit from traditional anti-HER2 drugs, such as trastuzumab, HER2-derived vaccine nelipepimut-S plus trastuzumab, or trastuzumab combined with pertuzumab in adjuvant setting.[11-13] Additionally, in neoadjuvant setting, CDK4/6 inhibitors or monoclonal anti-VEGF antibody, showed only faint anti-tumor activity.[14-18] Only pembrolizumab (PD-1 inhibitor) demonstrated excellent treatment benefits in tumors with molecular high risk.[19] Novel antibody-drug conjugates (ADCs), trastuzumab deruxtecan or Trastuzumab duocarmazine, demonstrated an excellent response in HER2-low advanced BC,[20,21] their anti-tumor activity in HER2-low EBC is unclear and under evaluation (NCT04553770). Therefore, more effective strategies are needed for this population.

1.3 Preclinical basis and clinical studies of protinib

Pyrotinib, a well-absorbed irreversible pan-HER tyrosine kinase inhibitor (TKI) targeting HER1, HER2, and HER4, has shown excellent response in HER2-positive BC,[22] and has reported more effective results compared to lapatinib, a traditional anti-HER2 drug.[23] It is unclear whether pyrotinib can be effective for Luminal/HER2-low E/LABC. The I-SPY2 trial evaluated an irreversible pan-HER TKI in HER2-negative (IHC 2+/FISH-negative, 1+, or 0) EBC but did not find any additional benefit.[24] This could be attributed to the inclusion of patients with HER2 IHC 0 or 1+ tumors, which may not respond to pan-HER TKI treatment. Supporting this hypothesis, a previous *in vitro* study indicated that irreversible pan-HER TKIs exhibit more pronounced anti-tumor activity against Luminal/HER2-low BC cells with higher levels of HER2 expression.[25] In our own comprehensive *in vitro* study, we investigated the impact of chemotherapy and the specific TKI, pyrotinib, either as independent treatments or in combination, on primary BC cells and BC cell lines. Consistently, we observed that when it came to BC cells classified as Luminal/HER2-low (IHC 2+/FISH-negative), pyrotinib monotherapy exhibited substantial anti-tumor activity and TKI combined with chemotherapy demonstrated a powerful synergistic effect in inhibiting tumor growth. However, in cases with HER2 IHC 1+, neither the substantial anti-tumor activity of pyrotinib monotherapy nor the synergistic effect of pyrotinib plus chemotherapy was observed (**Figure 1A and 1B** below). Furthermore, we extended our research to

establish BC patient-derived xenograft (PDX) models (**Figure 1C** below) and observed similar results. In Luminal/HER2-low (IHC 2+/FISH-negative) PDX models, the combination of TKI and chemotherapy displayed a synergistic anti-tumor effect, whereas TKI monotherapy had limited impact in HER2 IHC 1+ models (**Figure 1D** below). Additionally, no synergistic anti-tumor effect was observed when TKI and chemotherapy were used together in HER2 IHC 1+ models. Importantly, our research showed that the combined therapy did not lead to significant toxicity in the mice, indicating the safety of this approach. Overall, our findings emphasized the differential responses to TKI treatment based on HER2 expression levels and underscore the potential clinical significance of combining TKI and chemotherapy in Luminal/HER2-low (IHC 2+/FISH-negative) BC cases.

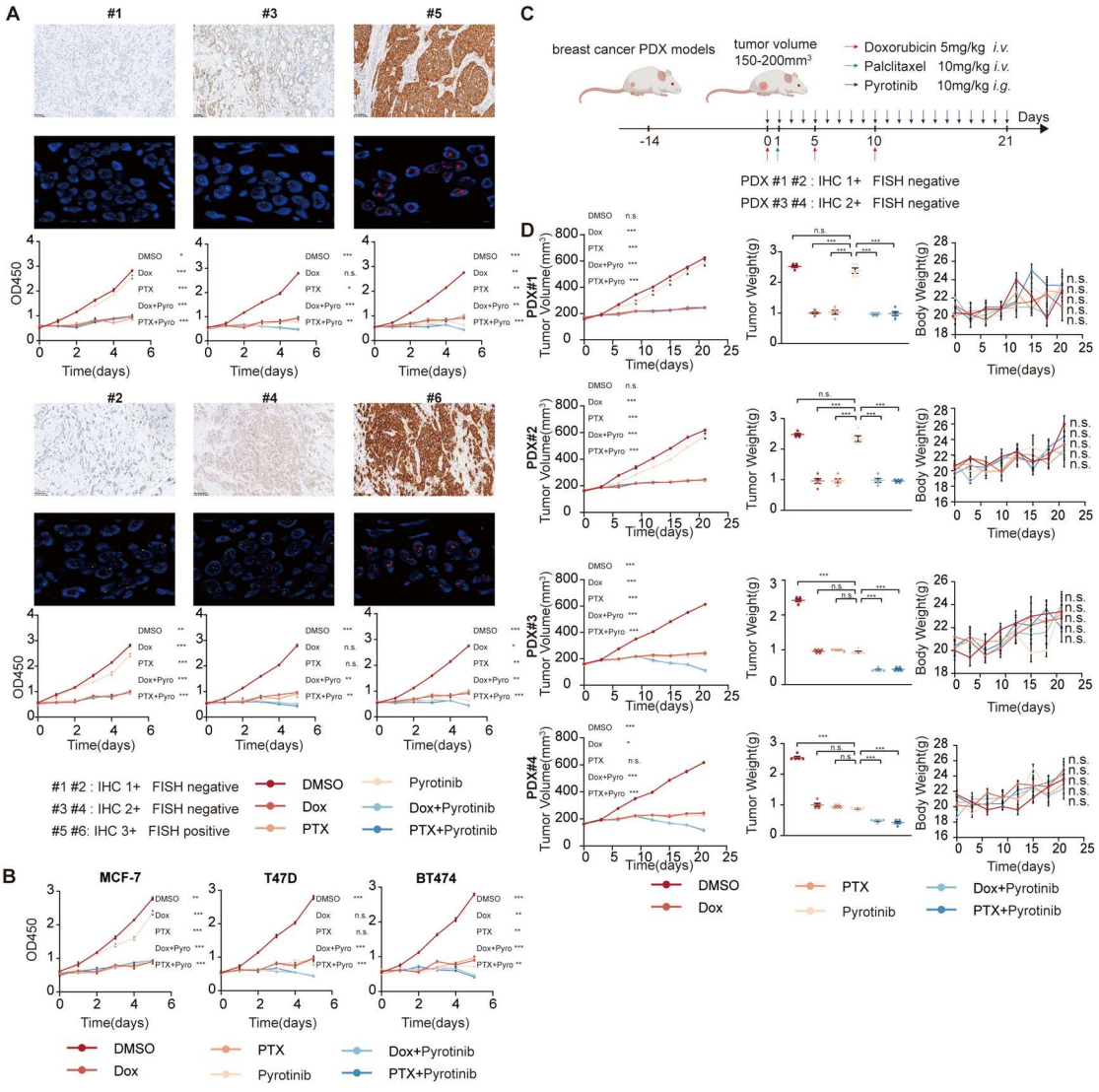

Figure 1

A. CCK8 detection of primary breast cancer cells (#1-#6, both were hormone receptor-positive) after treatment (0.2% DMSO, 50 nmol/L TKI, 100 nmol/L Dox, 20 nmol/L PTX).

B. CCK8 detection of ER+/HER2-low MCF-7 (expression level: 2), ER+/HER2-low (expression level: 4) T47D, and ER+/HER2-positive (expression level: 7) BT474.

C. Profile of patient-derived xenograft (PDX) models experiments.

D. Tumor-bearing (primary breast cancer cells #1-#4, both were hormone receptor-positive) mice were divided into equal cohorts (n = 5) treated with CMC, TKI, Dox, PTX, Dox + TKI, and PTX + TKI. Tumor volumes (mm^3), tumor

weight (g), and body weight (g) were measured at the indicated days.
CMC=carboxymethyl cellulose. TKI=pyrotinib. Dox=doxorubicin. PTX=paclitaxel.

Cell lines and Cell Counting Kit-8 (CCK8)

Primary BC cells (#1-#6) were established by our laboratory. ER+/HER2-low (expression level: 2) MCF-7, ER+/HER2-low (expression level: 4) T47D, and ER+/HER2-positive (expression level: 7) BT474 cells were obtained from American Type Culture Collection (ATCC). All cell lines were grown according to standard protocols. The CCK8 assay was performed by CCK8 assay kit manual (Dojindo, Japan). Primary BC cells (#1-#6, both were hormone receptor-positive), MCF-7, T47D, and BT474 cells were seeded in 96-well plates (3000 cells/well). CCK-8 solution was added to the cells and incubated for 2 hours at 37°C. The absorbance at 450 nm was measured using a spectrophotometer.

Establishment of BC PDX models

To establish the PDX models, tissues were maintained in PRI DMEM with 10% fetal bovine serum and 1% penicillin/streptomycin. Tissues were then cut into 1×1×1 mm^3 pieces and rinsed with fresh PRI DMEM twice. These tissue pieces were subsequently implanted subcutaneously into the fat pad of NOD/SCID mice. When the xenografted tumor tissues reached a size of 1-2 cm^3, they were harvested following the protocols mentioned earlier and transplanted into subsequent generations of NOD/SCID mice.

In vivo animal experiments

Tumor-bearing mice were randomly assigned to different groups, with each group consisting of 5 mice, when the tumors reached an average volume of 150-200 mm^3. The groups included the vehicle group (0.5% carboxymethyl cellulose, CMC), the doxorubicin group at a dose of 5 mg/kg/day, the paclitaxel group at a dose of 10 mg/kg/day, the pyrotinib group at a dose of 10 mg/kg/day, and the combination group receiving doxorubicin or paclitaxel in combination with pyrotinib. Oral gavage administration was conducted for 21 days. Tumor volumes were measured twice a week using calipers (volume = $L \times W^2/2$), and mouse weights were recorded every 3 days. At the end of the treatment period, the mice were euthanized, and the tumors were collected for further analysis.

1.4 Neoadjuvant approach for Luminal/HER2-low E/LABC

The neoadjuvant approach, which is highly effective in assessing response to therapy based on biological heterogeneity, providing prognostic information, and identifying biomarkers for predicting efficacy, is an ideal strategy to test new treatment options in patients with Luminal/HER2-low E/LABC. To our knowledge, anthracycline-taxane chemotherapy is commonly used as a foundation for testing new drugs in this population. Pathological complete response (pCR) was the primary endpoint commonly used in the neoadjuvant setting.[18,26,27] During the course of the study, on December 11, 2021, a multicenter pooled analysis demonstrated that patients with Luminal/HER2-low early or locally advanced breast cancer who achieved residual cancer burden (RCB) 0, equal to pCR, or RCB I had a significantly improved long-term prognosis compared to those with RCB II or RCB III disease.[28] The RCB class adds substantially to the binary assessment of pCR versus non-pCR in predicting long-term survival in this population. Therefore, the RCB 0/I rate can provide a more accurate reflection of the efficacy of neoadjuvant pyrotinib plus chemotherapy in Luminal/HER2-low E/LABC patients compared to the pCR rate.

1.5 Benefits/Risks

In the phase III PHOEBE trial,[23] 267 patients were randomly assigned to 400mg of pyrotinib plus capecitabine arm or lapatinib plus capecitabine arm. The main grade 3 adverse event (AE) was diarrhea, which was higher in pyrotinib group than that in lapatinib group (31% *vs.* 8%). Similar numbers of patients required treatment interruption or dose reduction because of diarrhea in the

pyrotinib and lapatinib groups. Such a high incidence of diarrhea may affect the compliance of patients who treated with 400mg of pyrotinib plus chemotherapy. According to the phase I clinical study,[29] there was no significant difference in pharmacokinetic C_{max} or areas under the curve between 400 mg and 320 mg of pytotinib. These results suggest that it is feasible to use 320 mg of pyrotinib in clinical trial to reduce toxicity effects and maintain the efficacy. Thus, the primary administration-dose of pyrotinib is 320mg in this trial.

1.6 Summary and conclusion

The design and conduct of this study are supported by an understanding of the natural history and current therapies for subjects with Luminal/HER2-low E/LABC, knowledge of the activity and safety of HER TKIs, and the available nonclinical and clinical information regarding pyrotinib.

2. Objectives

The PILHLE-001 study is aimed to assess the efficacy and safety of neoadjuvant pyrotinib combined with epirubicin and cyclophosphamide followed by docetaxel in patients with Luminal/HER2-low E/LABC.

2.1 Primary Endpoint

The primary outcome is the residual cancer burden (RCB) 0/I rate after neoadjuvant therapy. RCB are evaluated according to the online Residual Cancer Burden Calculator provided by the MD Anderson Cancer Center.

2.2 Secondary Endpoints

The secondary outcomes include:

- The pathological complete response (pCR, ypT0/is ypN0, defined as no residual invasive tumor cells in the breast and axillary nodes, regardless of ductal carcinoma in situ) rate.
- Objective response rate [ORR, defined as the percentage of patients who achieved a complete or partial response according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1[30]] at the end of cycle 2 neoadjuvant therapy and the end of all neoadjuvant therapy.
- Breast conservation surgery (BCS, calculated as the percentage of patients who had successful breast conservation surgery after neoadjuvant therapy) rate.
- Disease-free survival (DFS, defined as the time from the first dose of study drug until any relapse, secondary malignancy, or death from any cause).
- Overall survival (OS, defined as the time from the first dose of study drug to death, irrespective of cause).
- Exploratory analysis of biomarkers that predict the efficacy.
- AEs, judged based on Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

3. Study Design

PILHLE-001 study is a single-arm, prospective, non-randomized, single-center, open label phase II clinical trial. As planned, 48 patients in this study will be enrolled at Sun Yat-sen Memorial Hospital and treated with neoadjuvant pyrotinib combined with epirubicin and cyclophosphamide followed by docetaxel until completion of all neoadjuvant therapy cycles, withdrawal of consent, disease progression, death, intolerable toxicity, or protocol violation. After completion of the above treatments, patients received surgery and subsequent therapy as recommended by the National Comprehensive Cancer Network guidelines (version 3.2021). Patients after surgery will enter the follow-up phase during which subsequent treatment conditions and long-term survival information will be collected. The main

treatment schema is exhibited in Figure 2.

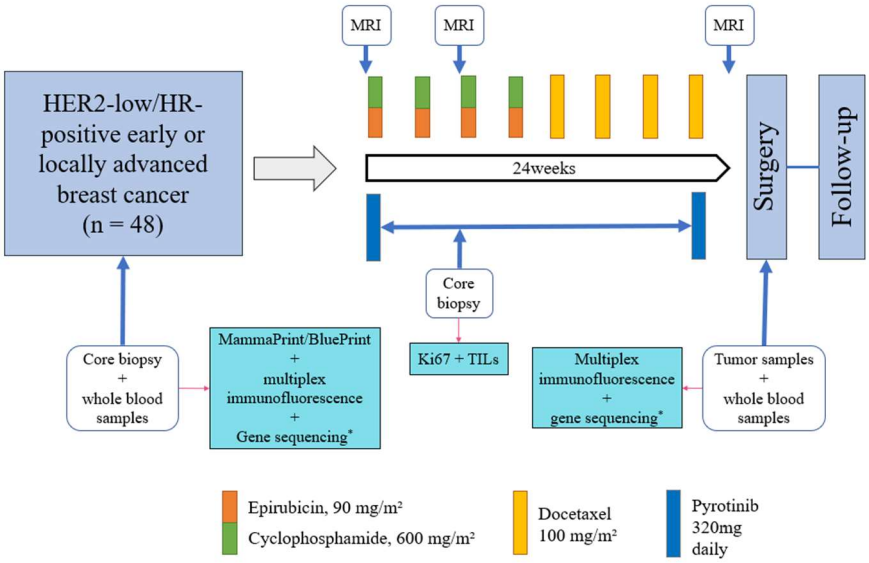

Figure 2. The PILHLE-001 study design. *Including NGS panel of genes variation and ctDNA/HRD status detection. MRI=magnetic resonance imaging. TILs=tumor infiltrating lymphocytes. ctDNA=circulating tumor DNA. HRD=homologous recombination deficiency.

4. Study Subjects

All patients meeting the inclusion criteria and do not meet any exclusion criteria must be provided with detailed information about this study, and written informed consent for participation must be obtained. The patients will then start to receive neoadjuvant pyrotinib combined with chemotherapy.

4.1 Enrollment

Enrollment of a subject into the study will be performed according to the following procedures:

- The study center will notify the clinician when a clinically eligible subject is identified and is ready to screen, to ensure enrollment availability on the study.

- After the subject has signed and dated the Informed Consent Form (ICF), all screening procedures have been completed, and eligibility has been confirmed, the subject can be officially enrolled in the study. The enrollment date will be the date that the investigator confirms enrollment.

4.2 Inclusion Criteria

Patients must fulfill all the following criteria to be eligible for this study.

1) Signed informed consent, compliance with the study protocol, women whose age ≥ 18 years old and ECOG performance status ≤ 1 (Table 1).

2) Centrally confirmed, newly diagnosed, unilateral, primary invasive, hormone receptor (HR) positive and HER2-low E/LABC.

- HER2-low is defined as immunochemistry (IHC) 2+ with fluorescent in situ hybridization (FISH) negative, IHC 1+ was not included.

- HR positive is defined as estrogen receptor (ER) and/or progesterone receptor (PR) > 1% stained cells.

3) The tumor is greater than 2 cm (cT_{2-4}) or between 1 cm and 2 cm (cT_{1c}) with histopathological involved lymph nodes. Note: for tumors with TNM stage-IIA, histologic grade III or Ki67 ≥ 20% or MammaPrint high-risk are required.

4) At least one evaluable target lesion according to Response Evaluation Criteria in Solid Tumors

(RECIST) version 1.1.

5) Consented to contraception both during the trial and within 6 months after the last administration of the test drug

6) Requisite laboratory values:

- Left ventricular ejection fraction at least 55%.
- White blood cell count: ≥ 3.0 × 10^9/L; absolute neutrophil count: ≥ 1.5 × 10^9/L; platelet count: ≥ 100 × 10^9/L; hemoglobin: ≥ 90 g/L.
- Aspartate aminotransferase and alanine aminotransferase: ≤ 2.5 × upper limit of normal (ULN); alkaline phosphatase: ≤ 2.5 × ULN; blood total bilirubin: ≤ 1.5 × ULN; serum creatinine: ≤ 1.5 × ULN.

Table 1. ECOG PERFORMANCE STATUS

0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair

4.3 Exclusion Criteria

Patients meeting any of the following criteria are not eligible for this study.

1) Metastatic or bilateral BC, occult BC, inflammatory BC without assessable focus, or eczema like BC.

2) Known history of hypersensitivity to the test drugs.

3) Severe dysfunction of the heart, liver, or kidney.

4) Patients who need receive other anti-tumor treatments (except for ovarian function inhibitors) during neoadjuvant therapy

5) Patients who underwent BC-free surgery within 4 weeks or had not fully recovered after BC-free surgery

6) Patients who had basic gastrointestinal diseases (especially long-term history of diarrhea or/and constipation), inability to swallow, intestinal obstruction or other factors will affect drugs administration and absorption.

7) Serious or uncontrolled infections that may affect study treatment or evaluation of study results.

8) History of other malignant tumors in the past 5 years.

9) Not suitable for the clinical trial due to other reasons.

4.4 Exit/withdraw criteria

The investigator, in consultation with the medical monitor, may withdraw any subject from study treatment, if in the investigator's opinion, it is not in the subject's best interest to continue. Any subject has the right to withdraw from the study at any time. In addition, subjects may be withdrawn from study treatment for the following reasons:

1) Any event that may cause the patient who continues to take the drug to no longer benefit, such as clinical adverse events, abnormal laboratory tests, pregnancy events or other medical conditions.

2) Subjects cannot participate in further trials (including new clinical indications generated during the trial or problems that are not discovered in time).

3) It is necessary to suspend the experiment from the perspective of medical ethics.

4) Any subject who becomes pregnant should be removed from study treatment.

5) The patient has poor compliance, no longer receiving drugs or tests before completing all trials, or receiving other anti-tumor treatments at the same time before the completion of the trials and unable to persist in completing the trials as planned.

4.5 Termination criteria

If there are sturdy reasons, the study may be terminated or suspended early. The decision-making party will provide a written notice explaining the reason for the early termination or suspension, and submit it to the investigator, sponsor, ethics committee and relevant departments.

Reasons for termination of this study include but are not limited to the following:

- Serious mistakes in the clinical trial protocol are found in the trial, making it difficult to evaluate the drug;
- The investigator requests termination;
- The relevant department or the ethics committee ordered the termination of the trial for some reason.

5. Study Intervention

5.1 Study Drugs

- Pyrotinib (Irene), 80mg per table, po.
- Epirubicin (Pharmorubicin), injectable, iv.
- Cyclophosphamide (Endoxan), injectable, iv.
- Docetaxel (Asu), injectable, iv.

5.2 Drugs Administration

Patients in the PILHLE-001 study received pyrotinib 320mg orally once daily, and epirubicin 90 mg/m² plus cyclophosphamide 600 mg/m² intravenously on day 1 for four 3-week cycles followed by docetaxel 100 mg/m² intravenously on day 1 or four 3-week cycles.

The dosage of the above-mentioned drugs can be adjusted according to the protocol and the adverse reactions of the subjects. The subject continues to take the drug until completion of all cycles, withdrawal of consent, disease progression, death, intolerable toxicity, or other reasons as determined by the investigator. The date of the course of treatment will be determined from the date the subject first used the drug. During the testing process, any suspended, missing, or underused test drugs will continue to be administered according to the plan, without the need for supplementary use or regular adjustments.

5.3 Dose Modification and Discontinuation

When an AE occurs during the study period, the investigator should take active symptomatic treatment, and record the combined treatment and drug treatment in detail during the neoadjuvant treatment according to the following recommendations and dose levels.

- Pynotinib, dose levels are level $_{-1}$ (240 mg/m²) and level $_{-2}$ (stop), listed in Table 2.
- Epirubicin, dose levels are level $_{-1}$ (75 mg/m²) and level $_{-2}$ (60 mg/m²), listed in Table 3.
- Docetaxel, dose levels are level $_{-1}$ (80 mg/m²) and level $_{-2}$ (60 mg/m²), listed in Table 3.
- Cyclophosphamide, dose levels are level $_{-1}$ (500 mg/m²) and level $_{-2}$ (400 mg/m²), listed in Table 4.

Table 2 Dose Adjustment of Pynotinib	
AE	**Dose modification**
Grade 1/2 of diarrhea	Dose modifications are not recommended and can intensify antidiarrheal therapy as appropriate
Grade 3 of diarrhea	- First appearance: Interrupt therapy until resolved to grade 0 or 1 and begin the next cycle at the starting dose (320 mg). - Second appearance: Interrupt therapy until resolved to grade 0 or 1 and begin the next cycle at the level $_{-1}$ dose (240 mg) - Third appearance: Level $_{-2}$ (stop), discontinue therapy permanently.
Grade 4 of diarrhea	First appearance: Level $_{-2}$ (stop), discontinue therapy permanently
If the subject has AEs that are not up to the above level or other AEs not listed above, symptomatic treatment should be given as far as possible, and suspension of therapy or dose adjustment should be administered according to the investigator's judgment.	

Table 3 Dose Adjustment of Epirubicin or Docetaxel	
AEs	**Dose modification**
Grade ≥ 3 of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood bilirubin increased, or serum creatinine increased	- First appearance: Interrupt therapy until resolved to grade 0 or 1 and begin the next cycle at the level $_{-1}$ dose (75 mg/m² for epirubicin and 80 mg/m² for docetaxel). - Second appearance: Interrupt therapy until resolved to grade 0 or 1 and begin the next cycle at the level $_{-2}$ dose (60 mg/m² for epirubicin and 60 mg/m² for docetaxel) - Third appearance: Discontinue therapy permanently.
Grade ≥ 3 of neutrophil count decreased or platelet count decreased	
Febrile neutropenia or severe infections	
Grade ≥ 3 of Hand-Foot syndrome or stomatitis	
Severe skin reactions or symptoms/signs involving central neuropathy	
If the subject has AEs that are not up to the above level or other AEs not listed above, symptomatic treatment should be given as far as possible, and suspension of therapy or dose adjustment should be administered according to the investigator's judgment.	

<table>
<tr><th colspan="2">Table 4 Dose Adjustment of Cyclophosphamide</th></tr>
<tr><th>AEs</th><th>Dose modification</th></tr>
<tr><td>Grade ≥ 3 of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood total bilirubin increased, or serum creatinine increased</td><td rowspan="5">- First appearance: Interrupt therapy until resolved to grade 0 or 1 and begin the next cycle at the level $_{-1}$ dose (500 mg/m²).
- Second appearance: Interrupt therapy until resolved to grade 0 or 1 and begin the next cycle at the level $_{-2}$ dose (400 mg/m²)
- Third appearance: Discontinue therapy permanently.</td></tr>
<tr><td>Grade ≥ 3 of neutrophil count decreased or platelet count decreased</td></tr>
<tr><td>Febrile neutropenia or severe infections</td></tr>
<tr><td>Grade ≥ 3 of stomatitis</td></tr>
<tr><td>Symptoms/signs involving central neuropathy</td></tr>
<tr><td colspan="2">If the subject has AEs that are not up to the above level or other AEs not listed above, symptomatic treatment should be given as far as possible, and suspension of therapy or dose adjustment should be administered according to the investigator's judgment.</td></tr>
</table>

In principle, only the dose of one drug can be adjusted at a time, and it is only allowed to be reduced twice, otherwise the subject will withdraw from the study. Treatment should be discontinued if severe toxicity may be related to the compound. If toxicity recovered within 3 weeks to grade 0 or 1, a restart of treatment could be considered. Regardless of the cause of the delay, patients who discontinue dosage for more than 4 weeks should terminate treatment and withdraw from the trial. Recommendations for the selection of organic system showing the greatest toxicity must be based on the judgment of the Investigators. The doses reduced due to toxicity shall not be allowed to re-escalate.

5.4 Investigational products management, distribution and recycle.

The management, distribution, and recycle of clinical drugs in this trial are handled by dedicated personnel. Investigators must ensure that all investigational products are used only for subjects participating in clinical trials, and their dosage and usage should follow the trial protocol, and the remaining drugs should be returned to sites. Do not transfer clinical drugs to any non-clinical trial participants. The investigational products should be stored according to the instructions on the label that is affixed to the package containing the drug product. The issuance and recovery of medicines are carried out in accordance with GCP requirements. The remaining medicines and empty boxes are recovered after the study. The dispense and recycle of each medicine should be recorded in a timely manner on a special record sheet. The monitor is responsible for supervising the supply, use, storage, and disposal of surplus drugs in clinical trials.

5.5 Patient compliance

If test drugs have been assigned to individual subjects, these subjects should be required to return unused test drugs each time they return during neoadjuvant treatment. The subjects recorded the drug information on the diary card every day. When the subject is taking medication, the sites will evaluate the compliance of the investigational products at each treatment visit (except follow-up). Record the number of returned investigational products, compare the returned investigational products with the dose

information reported by the subject, and compare with the prescribed dose to monitor compliance. Compliance and reasons for deviation will be recorded in source files and drug inventory records. Any deviation from compliance should be explained accordingly.

Calculation of drug dose compliance: compliance (%) = number of tablets taken/ expected number of tablets to be taken×100.

If the compliance percentage calculated according to the above formula is less than 80% or greater than 120%, it can be considered that the subject does not comply with the dose. If the subject's compliance exceeds the above range, it shall be recorded as a protocol deviation. The eCRF of the investigational product should reflect the verified drug dosage information provided by the subject.

6. Concomitant Therapy

6.1 Prohibited Drugs during the study

During the treatment period, other anti-tumor drugs and adjuvant drugs related to tumor treatment other than the study drugs specified in this protocol should be stopped, including anti-tumor Chinese medicines and immune preparations.

6.2 Permitted concomitant therapy requiring caution

If the subjects have adverse reactions, they should be closely observed, and if necessary, actively treat the symptoms, and record and explain the drugs used on the eCRF form. The following drugs should be used with caution during the research:

Pyrotinib:

Medications to be used with caution during pyrotinib in this study are listed below. This list is not comprehensive and is only meant to be used as a guide. These medications should be excluded from patient use if possible. If they must be given, then use with caution and consider a pyrotinib interruption if the concomitant medication is only needed for a short time.

a) CYP3A4 inducers (dexamethasone, phenytoin sodium, carbamazepine, rifampicin, rifabutin, rifapentin) and inhibitors (ketoconazole, itraconazole, erythromycin, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, voriconazole, citrus paradisi macf., etc.);

b) CYP3A4'substrates (Simvastatin, pimozide);

c) Other drugs metabolized by CYP3A4 (benzodiazepines, dihydropyridine, calcium ion antagonist HMG-COA reductase inhibitor);

d) CYP2C9" substrates (diclofenac, phenytoin, piroxicam, S-warfarin and tolbutamide) and CYP2C19"

e) Drugs that prolong the QT interval: Including antibiotics, antiarrhythmic, antipsychotic, antifungal, antimalarial and antidepressant drugs (such as clarithromycin, quinidine, risperidone, fluconazole, mefloquine, amitriptyline, azithromycin, sotalol, fluphenazine, ketoconazole, chloroquine, imipramine, erythromycin, amiodarone, droperidol, clomipramine, roxithromycin, disopyramide, haloperidol, dosulepin, metronidazole, procainamide, thioridazine, doxepin, moxifloxacin, pimozide, olanzapine and clozapine).

Epirubicin:

Epirubicin is extensively metabolized by the liver. Changes in hepatic function induced by concomitant therapies may affect epirubicin metabolism, pharmacokinetics, therapeutic efficacy, and/or toxicity. Toxicities associated with epirubicin, especially hematologic and gastrointestinal events, may be increased when doxorubicin is used in combination with other cytotoxic drugs (progesterone,

verapamil, cyclosporine, dexrazoxane, phenobarbital, phenytoin, streptozocin, saquinavir, live vaccines, etc.).

Cyclophosphamide:

Cyclophosphamide is a pro-drug that is activated by cytochrome P450s.

An increase of the concentration of cytotoxic metabolites may occur with:

Protease inhibitors: concomitant use of protease inhibitors may increase the concentration of cytotoxic metabolites. Use of protease inhibitor-based regimens was found to be associated with a higher Incidence of infections and neutropenia in patients receiving cyclophosphamide than use of a Non-Nucleoside Reverse Transcriptase Inhibitor-based regimen. Combined or sequential use of cyclophosphamide and other agents with similar toxicities can potentiate toxicities.

Increased hematologic toxicity and/or immunosuppression may result from a combined effect of cyclophosphamide and, for example:

a) ACE inhibitors: ACE inhibitors can cause leukopenia;
b) Thiazide diuretics;
c) Zidovudine.

Increased pulmonary toxicity may result from a combined effect of cyclophosphamide and, for example:

a) Amiodarone;
b) G-CSF, GM-CSF (granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor): Reports suggest an increased risk of pulmonary toxicity in patients treated with cytotoxic chemotherapy that includes cyclophosphamide and G-CSF or GM-CSF.

Increased nephrotoxicity may result from a combined effect of cyclophosphamide and, for example:

a) Amphotericin B;
b) Indomethacin: Acute water intoxication has been reported with concomitant use of indomethacin.

Increase in other toxicities:

a) Azathioprine: Increased risk of hepatotoxicity (liver necrosis);
b) Busulfan: Increased incidence of hepatic veno-occlusive disease and mucositis has been reported;
c) Etanercept: In patients with Wegener's granulomatosis, the addition of etanercept to standard treatment, including cyclophosphamide, was associated with a higher incidence of non-cutaneous malignant solid tumors;
d) Metronidazole: Acute encephalopathy has been reported in a patient receiving cyclophosphamide and metronidazole. Causal association is unclear. In an animal study, the combination of cyclophosphamide with metronidazole was associated with increased cyclophosphamide toxicity;
e) Coumarins: Both increased and decreased warfarin effect have been reported in patients receiving warfarin and cyclophosphamide;
f) Cyclosporine: Lower serum concentrations of cyclosporine have been observed in patients receiving a combination of cyclophosphamide and cyclosporine than in patients receiving only cyclosporine. This interaction may result in an increased incidence of graft-versus-host disease;
g) Depolarizing muscle relaxants: Cyclophosphamide treatment causes a marked and persistent inhibition of cholinesterase activity. Prolonged apnea may occur with concurrent depolarizing muscle relaxants (e.g., succinylcholine). If a patient has been treated with cyclophosphamide within 10 days of general anesthesia, alert the anesthesiologist.

Docetaxel:

The metabolism of docetaxel is catalyzed by cytochrome P450 isoenzymes CYP2C8 and CYP3A4. Caution should be exercised when docetaxel is concomitantly administered with known substrates (e.g., midazolam, buspirone, felodipine, lovastatin, eletriptan, sildenafil, simvastatin, and triazolam), inhibitors (e.g., atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, and telithromycin), and inducers (e.g., rifampin and carbamazepine) of CYP3A4. Caution should also be exercised when docetaxel is concomitantly administered with known substrates (e.g., repaglinide and rosiglitazone), inhibitors (e.g., gemfibrozil), and inducers (e.g., rifampin) of CYP2C8.

6.3 Drugs and treatments that can be used in combination during the study

Patients can receive the best supportive treatment, and actively treat the clinical complications and various AEs. Preventive and prophylactic management such as antidiarrheal drugs (loperamide, moisturizing creams), antiemetic drug, and G-CSFs during neoadjuvant therapy is allowed. In. In strict accordance with the GCP guidelines, all drugs used in combination are recorded in the eCRF. From the 4 weeks before the study treatment to the end of the safety follow-up, the combination medication/treatment should be recorded.

7. Study Procedures

Before starting the study, patients must read and sign the current ethics committee approved informed consent form. All study steps must be performed within the time frame specified in the study plan. Tables 5 lists all assessments and indicates with an “x”, the visits on which they are performed.

Table 5. Investigation Summary Table

Visits	Baseline Assessment	Cycle 1	Cycle 2 to 8	End of treatment	Surgery	1 month after surgery	Follow-up (3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 54, 60 months)
Written Informed Consent	X						
Demographic data	X						
Verification of inclusion/exclusion criteria	X						
Medical history	X						
Physical examination	X		At the end of each cycle	X		X	X
Vital Signs	X		At the end of each cycle	X		X	X
Pregnancy test	X						
Menopausal state	X						X
ECOG PS	X		At the end of each	X		X	X

			cycle				
HIV and hepatitis testing	**X**						
Blood E2 and FSH*	**X**			**X**			**X#**
Hematology test	**X**	**Every week**		**X**		**X**	**X**
Blood chemistry test	**X**			**X**		**X**	**X**
Urine routine test	**X**			**X**		**X**	**X**
Breast tumor related indicators (CEA, CA 15.3, etc.)	**X**						**X**
ECG	**X**		**At the end of each cycle**	**X**		**X**	**X**
Echocardiography	**X**			**X**			
Tumor samples histopathology	**X**		**At the end of cycle 2**		**X**		
Combination medication/treatment	**X**	**X**	**At the end of each cycle**	**X**		**X**	
Safety assessment	**X**	**X**	**At the end of each cycle**	**X**		**X**	
Mammogram	**X**			**X**			**Every year**
Breast ultrasound	**X**	**Every second cycle**		**X**		**X**	**X**
Breast magnetic resonance imaging	**X**	**At the end of cycle 2**		**X**			
Chest computerized tomographic scanning	**X**			**X**			**Every 6 months**
Treatment (pyrotinib plus chemotherapy)		**X**	**X**				
Ultrasonography (abdomen, gynecologic, etc.)	**X**						**X**

*Administered in premenopausal patients. #Administered in patients with ovaries with amenorrhea until it's clear the patient is postmenopausal. E2=Estradiol;

FSH=Follicle stimulating hormone

7.1 Screening

After signing the informed consent form, the subjects entered the screening period. The time window for histopathological biopsy was within 4 weeks before the first neoadjuvant therapy cycle, and for other examinations was within 1 week before the first neoadjuvant therapy cycle. Unless otherwise stated, the following screening steps must be completed within 4 weeks before starting study drug treatment.

[Demographics] Initials, gender, race, marital status, date of birth, height, weight, etc.;

[Medical history] Past medical history and treatment history including menopausal status;

[ECOG performance status] Refer to Table 1 for the scoring criteria;

[Vital Signs] Body temperature, blood pressure, respiratory rate, pulse;

[Physical examination] General conditions, skin and mucous membranes, lymph nodes, head and neck, chest, abdomen, musculoskeletal, nerve reflexes, respiratory system, cardiovascular system, genitourinary system, mental status, etc.;

[Tumor samples histopathology] Including histological type, grade, HER2, ER, PR, Ki67, tumor infiltrating lymphocytes (TILs), and collection of tumor sample for other biomarkers.

[Blood E2 and FSH]

[Hematology] WBC, ANC, RBC, Hb, PLT, neutrophils count, lymphocytes count;

[Blood biochemistry] ALT, AST, ALP, AKP, γGT, TBIL, DBIL, GLU, IBIL, BUN, creatinine, blood electrolytes (K +, Na +, Cl-, Ca2 +, Mg2 +). If necessary, the investigator can conduct other tests;

[Urine routine] Urine protein, urine glucose, urine occult blood;

[HIV and hepatitis testing]

[Breast tumor related indicators] Including CEA, CA153, CA125, CA199, etc.

[Pregnancy test] Only applicable to blood or urine tests for women of childbearing age;

[ECG] Must include QT, QTc and P-R interval. If there is an abnormality, the investigator can judge and perform other necessary inspections;

[Echocardiography] Must include LVEF. If symptoms such as chest pain and palpitations occur, other tests can be performed as appropriate.

[Tumor imaging examination] Including mammogram, breast MRI, breast/axillar ultrasound, and chest computerized tomographic scanning.

[Combination medication/treatment] Participate in the combination medication part;

[Safety assessment] From the day the subjects signed the informed consent form, it was recorded until 30 days after the last medication.

Subjects must meet all admission criteria to be included in the study.

7.2 Treatment phase

After enrolment, the subjects received treatment according to the protocol. On the last day of each cycle (± 2 days), a treatment period visit was performed, and the reason for the out of visit window was recorded in the eCRF. Investigators can add inspection items or increase the frequency of video visits based on the subject's clinical conditions, and the inspection results will be recorded in the eCRF "unscheduled visits".

[Vital signs] Heart rate, breathing rate, body temperature, blood pressure.

[Physical examination] General conditions, skin and mucous membranes, lymph nodes, head and neck, chest, abdomen, musculoskeletal, neural reflexes, respiratory system, cardiovascular system, genitourinary system, mental status, etc.;

[ECOG performance status] Refer to Table 1 for the scoring criteria;

[Tumor samples histopathology] Including histological type, grade, Ki67, TILs, and collection of tumor sample for other biomarkers. Additional core biopsies will be conducted at the end of cycle 2 neoadjuvant therapy with consent.

[Hematology] WBC, ANC, RBC, Hb, PLT, neutrophils count, lymphocytes count;

[Blood biochemistry] ALT, AST, ALP, AKP, γGT, TBIL, DBIL, GLU, IBIL, BUN, creatinine, blood electrolytes (K +, Na +, Cl-, Ca2 +, Mg2 +). If necessary, the investigator can conduct other tests;

[Urine routine] Urine protein, urine glucose, urine occult blood;

[ECG] Must include QT, QTc and P-R interval. If there is an abnormality, the investigator can judge and perform other necessary inspections;

[Tumor imaging examination] Including breast MRI and breast/axillar ultrasound. Ultrasound will be administered every second cycle and at the end of neoadjuvant therapy. MRI will be conducted the end of cycles 2 and the end of neoadjuvant therapy.

[Combination medication/treatment] Participate in the combination medication part;

[Safety assessment] From the day the subjects signed the informed consent form, it was recorded until 30 days after the last medication.

7.3 End-of-treatment visit

A pre-surgery visit will be performed at the end of the neoadjuvant treatment (including patients who interrupt treatment prematurely), between last study dose and surgery. The following examinations should be performed:

[Vital signs] [Physical examination] [ECOG performance status] [Blood E2 and FSH] [Hematology] [Blood biochemistry] [Urine routine] [Breast tumor related indicators] [ECG] [Echocardiography] [Tumor imaging examination] [Combination medication/treatment] [Safety assessment]

7.4 Surgery

The surgery should take place take place within 3 weeks (± 3 days) after the last dose of chemotherapy. If feasible by institutional logistics and timing, these windows should be maintained for patients proceeding to curative surgery after early treatment discontinuation. Letrozole should be continued up to surgery.

Breast and axillary surgery will follow Local practice. However, pre-surgical SLNB is not allowed. Information on the type of surgery will be collected and recorded. Surgery samples of the remaining tissue (or tumor bed if pCR/RCB 0 is achieved) will be collected regardless of whether they completed full neoadjuvant treatment. Tumor samples from breast cancer surgery will be collected for primary/secondary trial objectives, biomarkers assay, and translational purposes. Local histological examination will include determination of RCB.

7.5 Follow-up

A post-surgery visit will be performed within 30 days (± 7 days) from surgery. Assessment of adverse events and general safety will be collected at this visit. Patients withdrawn from the study before surgery due to confirmed progressive disease, physician's choice, intolerable toxicity, or reasons will attend to a follow-up visit 30 days after the administration of the last treatment dose. Following assessments will be done:

[Vital signs] [Physical examination] [ECOG performance status] [Hematology] [Blood biochemistry] [Urine routine] [ECG] [Tumor imaging examination] [Combination medication/treatment] [Safety assessment]

Then all patients will attend to survival follow-up and visits will be performed at 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 54, 60 months after surgery. Following assessments will be done:

[Vital signs] [Physical examination] [ECOG performance status] [Blood E2 and FSH] [Hematology] [Blood biochemistry] [Urine routine] [Breast tumor related indicators] [ECG] [Tumor imaging examination] [Ultrasonography (abdomen, gynecologic, etc.)]

8. Efficacy Assessment

8.1 Primary Endpoints

The primary endpoint of the study is the RCB 0/I rate after neoadjuvant therapy, which was defined as the proportion of patients who are classified into RCB 0 or RCB I according to the online Residual Cancer Burden Calculator provided by the MD Anderson Cancer Center and are classified into four levels: RCB 0 (score = 0, equivalent to pCR), RCB I (score > 0-1.36), RCB II (score > 1.36-3.28), and RCB III (RCB score > 3.28).

8.2 Secondary Endpoints

Pathological complete response (pCR) rate, defined as the proportion of patients with no residual invasive tumor cells in the breast and axillary nodes, regardless of ductal carcinoma in situ.

Objective response rate (ORR) at the end of cycle 2 neoadjuvant therapy and the end of all neoadjuvant therapy: the percentage of patients who achieved a complete or partial response according to the RECIST, version 1.1.

Breast conservation surgery (BCS) rate: the proportion of patients who had successful breast conservation surgery after neoadjuvant therapy.

Disease-free survival (DFS): the time from the first dose of study drug until any relapse, secondary malignancy, or death from any cause.

Overall survival (OS): the time from the first dose of study drug to any-cause death.

Exploratory analysis of biomarkers that predict the efficacy.

AEs: judged based on Common Terminology Criteria for Adverse Events Version 5.0.

9. Safety Assessment

9.1 Adverse Events (AEs)

9.1.1 Definition of AEs

An AE is defined as any untoward medical occurrence during the period from registration to the 30th day after the last neoadjuvant therapy cycle or to the most recent follow-up, regardless of causal attribution with the study drug. An AE can be any of the following: a symptom, a sign, abnormal examination results, or a disease, which may occur at any time since the initiation of treatment.

An AE should be accurately recorded during the study, including its time, severity, duration, management, and prognosis.

9.1.2 Severity of AEs

Severity of AEs is graded according to Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. The CTCAE Version 5.0 manual can be found at the following URL: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf. Grades of AEs that are not listed in it are as follows:

Grade I: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade II: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL), which refer to preparing meals, shopping, using the telephone, managing money, etc.

Grade III: Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care ADL, which refer to bathing, dressing, and undressing, feeding self, using the toilet, taking medication, and not bedridden.

Grade IV: Life-threatening consequences; urgent intervention indicated (experiences which cause the subject to be in imminent danger of death)

Grade V: Death related to AE (experiences which result in subject death)

9.1.3 Judgment criteria for the causal association between AEs and Study Treatment

Regardless of whether it is related to the investigational product, or even the investigational product has not been accepted, all unexpected clinical manifestations after signing the informed consent form should be reported as AEs. Any adverse reactions complained by subjects and abnormal laboratory test results during treatment should be truthfully recorded. In addition, indicate the severity, duration, management, outcome, and concomitant medication/treatment of the AE. Investigators should comprehensively evaluate the relationship between AEs and investigational product. The causality can be divided into five categories, which are definitely related, possibly related, possibly not related, definitely not related, and unassessable.

The relationship between AEs and the study drug should be assessed by investigators according to the judgment criteria are as follows:

1) Definitely related: An AE that follows a reasonable temporal sequence from administration of the study intervention, follows a known or expected response pattern to the suspected intervention, and is confirmed by improvement on stopping and reappearance of the event on repeated exposure.

2) Probably related: An AE that follows a reasonable temporal sequence from administration of the study intervention, follows a known or expected response pattern to the suspected intervention, but that could readily have been produced by the patient's clinical conditions or other treatments.

3) Probably unrelated: An AE that does not follow a reasonable temporal sequence from administration of the study intervention, does not follow a known or expected response pattern to the

suspected intervention, and could readily have been produced by the patient's clinical conditions or other treatments.

4) Unrelated: An AE that does not follow a reasonable temporal sequence from administration of the study intervention, but follows a known or expected response pattern to other treatments, and could readily have been produced by the patient's clinical conditions or other treatments. The AE can be relieved by improvement of the clinical conditions or stopping other treatments, and reappears after repeating other treatments.

5) Unable to determine: An AE that does not follow a reasonable temporal sequence from administration of the study intervention, but follows a known or expected response pattern to the study intervention, and could readily have been produced by other treatments.

9.1.4 AEs recording and reporting

The investigator should record in detail any AEs experienced by the subject, including: AEs description and all related symptoms, occurrence time, severity, duration, management, and final outcomes (recovery/cure, sequelae, response, death, unknown).

After signing the informed consent form, the safety assessment starts and ends within 30 days after the last administration. Any AEs (serious and non-serious) should be recorded on the AE report page of the case report form. Ensure that accurate medical terminology is used to report AEs.

Regardless of whether within 30 days after the last medication, treatment-related AEs (investigator's judgment) continue to be followed up until any of the following occurs:

1) Disappeared or resolved to baseline;

2) Re-assessment confirmed that there is no causality between AEs and the test drug (pyrotinib and/or chemotherapy);

3) Death;

4) Start a new anti-tumor treatment;

5) AEs are not expected to be further improved (investigator's assessment), and the patient's condition is stable;

6) No longer collect clinical data or final database lock.

Regardless of whether within 30 days after the last medication, non-treatment-related AEs (investigator's judgment) continue to be followed up until any of the following occurs:

1) Disappeared or resolved to baseline;

2) Resolved to ≤ grade 1;

3) Death;

4) Start a new anti-tumor treatment;

5) AEs are not expected to be further improved (investigator's assessment);

6) No longer collect clinical data or final database lock.

9.2 Serious Adverse Events (SAEs)

9.2.1 Definition of SAEs

Serious AE (SAE) refers to a medical event that requires hospitalisation or prolonged hospitalisation, causes disability, affects workability, is life-threatening, causes death, and causes congenital anomaly. AEs that meet one or more of the following criteria are SAE:

1) Results in death.

2) Is life-threatening.

3) Requires or prolongs hospitalization.

4) Causes persistent or significant disability or incapacity.

5) Results in congenital anomalies or birth defects.

Note that hospitalizations for the following reasons should not be reported as serious adverse events:

1) Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (i.e., to perform study related assessments).
2) Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent.
3) Social reasons and respite care in the absence of any deterioration in the patient's general condition.

Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event.

9.2.2 SAEs Reporting

The report of SAEs should start from signing the informed consent form until 30 calendar days (including 30 days) after the last medication. If a SAE occurs during the trial, whether it is the first report or follow-up report, the PI must immediately fill in the "Serious Adverse Event (SAE) Report Form", sign and date it. This includes the investigator's assessment of the causal relationship. The PI is responsible of reporting SAEs to ethics committee and the State Food and Drug ministration (SFDA) (also to the drug manufacturer within 24 hours if the SAE considered to be related to the study drug).

SAEs that occur 30 days after the last administration are only reported when they are suspected to be related to the study drug.

The symptoms, severity, occurrence time, treatment time, management, concomitant medication, follow-up time, follow-up method, and outcome of SAEs should be recorded in detail. For SAEs that are independent to the investigational product and potentially related to the research conditions (such as termination of the original treatment, or comorbidities during the trial), the causal relationship is detailed in the narrative section of the SAE page of the case report form. If the severity of a SAE or its causality with the test drug changes, the follow-up report should be sent to the sponsor immediately. Follow-up for SAEs should continue until it is recovered, resolved grade 1, baseline, or stable.

Table 6: Contact for serious adverse events

Affiliated Institution	Contact Person	Contact Details
China Food and Drug Administration	Drug Research and Supervision Office of Department under the Drug and Cosmetics Registration	Tel: 010-68313344-1003 Fax: 010-88363228 Address: Budling 2, No 26 Xidajie, Xuanwumen, Xicheng district, Beijing (100053)
Jiangsu Hengrui Pharmaceuticals Co., Ltd.	Pharmacovigilance Department	Tel: 021-60453192-818 Email: hengrui_drug_safety@shhrp.com
Sun Yat-sen Memorial Hospital	Ethics Committee	Tel: 020-81332587
Sun Yat-sen Memorial Hospital	Breast tumor center	Tel: 020-34070870 or 13925089353

9.2.3 Pregnancy and Pregnancy Outcome

Pregnant subjects meet the exclusion criteria. The investigator must report to Hengrui within 24 hours of knowing the pregnancy and fill in the "Hengrui Clinical Trial Pregnancy Report/Follow-up Form" at the same time. The mother and the fetus must be followed up at least until the birth of the infant and one month after the birth of the infant. And report the results to Hengrui. If a pregnancy results in an abnormal

outcome (stillbirth, spontaneous abortion, defect/congenital anomaly), this must be reported as an SAE. At the same time, fill in the "Hengrui Clinical Trial Pregnancy Report/Follow-up Form" and the NMPA SAE report form, and report by the SAE time limit.

Email address for sending pregnancy report: hengrui_drug_safety@shhrp.com

For subjects with SAE, investigators need to fill in the SAE report form and report in accordance with SAE requirements.

10. Statistics

10.1 Sample Size

We calculate the sample size based on one-stage design. The primary outcome in this study is the RCB 0/I rate after neoadjuvant therapy. The null hypothesis of the RCB 0/I rate with neoadjuvant chemotherapy in Luminal/HER2-low E/LABC was 15%. The combination of pyrotinib and chemotherapy will increase the RCB 0/I rate from 15.0% to 30.0%. The trial has 80% power to detect true difference from initial RCB 0/I rate of 15.0%, to an expected RCB 0/I rate of 30.0% at one-sided alpha level of 0.05. Using the PASS software (version 15.0), a total of 48 samples is required for the trial.

10.2 Analysis population

The efficacy for all efficacy endpoints and safety analyses will be performed on the all-treated population, defined as all patients who received at least one dose of pyrotinib.

10.3 Missing data handling

General Considerations: Subjects who dropped out during neoadjuvant therapy will be included in statistical analyses up to the point of their last evaluation and seem as RCB II/III.

Disease-free Survival: Data for subjects without disease progression or death will be censored at the date of the last tumor assessment and before the initiation of alternative anticancer therapy. The censoring rules details will be provided in the Statistical Analysis Plan (SAP).

Overall Survival: Data for subjects who have not died will be censored at the date of the last date known to be alive.

Safety: Missing or partial start and end dates for AEs and concomitant medications will be imputed according to prespecified, conservative imputation rules. No other imputation of values for missing data will be performed.

10.4 Statistical Methods

10.4.1 Demographics and Baseline Characteristics

Additional analyses will include summaries of subject demographics, baseline characteristics, compliance, and concurrent treatments.

10.4.2 Analysis of Efficacy Parameters

Primary Efficacy Endpoint

The primary outcome is the RCB 0/I rate after neoadjuvant therapy, defined as the proportion of patients who are classified into RCB 0 (score = 0, equivalent to pCR) or RCB I (score > 0-1.36) according to the online Residual Cancer Burden Calculator provided by the MD Anderson Cancer Center as evaluated by two independent pathologists who are blind to this study. The corresponding 95% two-sided confidence interval using of exact methods based on binomial, Clopper-Pearson method.

Secondary Efficacy Endpoint

pCR rate

The pCR rate is defined as the proportion of patients with no residual invasive tumor cells in the breast and axillary nodes, regardless of ductal carcinoma in situ. pCR and the corresponding 95% two-sided

confidence interval using of exact methods based on binomial, Clopper-Pearson method.

Objective response rate

ORR is defined as the percentage of patients who achieved a complete or partial response according to the RECIST, version 1.1, based on MRI. ORR and the corresponding 95% two-sided confidence interval using of exact methods based on binomial, Clopper-Pearson method.

Breast conservation surgery rate

BCS rate is defined as the proportion of patients who had successful breast conservation surgery after neoadjuvant therapy. BCS rate and the corresponding 95% two-sided confidence interval using of exact methods based on binomial, Clopper-Pearson method.

Disease-free survival

DFS is referred as the time from the first dose of study drug until any relapse, secondary malignancy, or death from any cause. Kaplan-Meier methods will be used to estimate the disease-free curves and the corresponding 95% two-sided confidence interval (including the median). A log-rank test is done to determine the significance of survival differences between groups, and the Cox proportional hazards regression model was used to estimate the hazard ratios (HRs) and 95% CIs.

Overall Survival

OS is referred as the time from the first dose of study drug until the date of death. Kaplan-Meier methods will be used to estimate the OS curves and the corresponding 95% two-sided confidence interval (including the median). A log-rank test is done to determine the significance of survival differences between groups, and the Cox proportional hazards regression model is used to estimate the hazard ratios (HRs) and 95% CIs.

10.4.3 Analysis of Safety Parameters

Safety summaries will be included in the form of tables and listings. The frequency (number and percentage) of treatment emergent AEs will be reported by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class and Preferred Term. Summaries will also be presented by the severity of the TRAEs (per Common Terminology Criteria for Adverse Events, v5.0) and by relationship to study drug. Laboratory shift tables containing counts and percentages will be prepared by laboratory parameter. Figures of changes in laboratory parameters over time will be generated. Results of vital sign assessments will be tabulated and summarized.

10.4.4 Exploratory analyses

Exploratory analyses including following assessments:

• The association between baseline MammaPrint/BluePrint signature and RCB.

• The association between intratumoral/stromal density of immune cell populations by multiplex immunofluorescence and RCB.

• The association between baseline MRI parameters and RCB.

• The association between early on-treatment tumor response at the end of cycle 2 neoadjuvant therapy and RCB, including objective response as evaluated by MRI or changes in MRI parameters, Ki67, and TILs.

• The association between gene sequencing and RCB.

Student's t-test or Wilcoxon test and ANOVA or Kruskal-Wallis test are used to compare continuous variables between different patient groups. The χ^2 test or Fisher's exact test is used to assess associations between two categorical variables. The above background variables will be compared using statistical test (at a two-sided significance level of 0.05).

11. Study administration and investigator obligations

11.1 Regulatory and ethical compliance

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" as well as "Guideline for Good Clinical Practice (GCP)" and relevant laws and regulations of the SFDA, whichever affords the greater protection to the individual.

11.2 Institutional review board and independent ethics committee

The investigator will submit this protocol, the ICF, IB, and any other relevant supporting information (eg, all advertising materials) to the appropriate Institutional review board (IRB)/Independent Ethics Committee (IEC) for review and approval before study initiation. A signed protocol approval page, a letter confirming IRB/IEC approval of the protocol and informed consent, and a statement that the IRB/IEC is organized and operates according to GCP and the applicable laws. The study will be initiated only after the protocol is approved by the ethics committee of the Sun Yat-sen Memorial Hospital, Sun Yat-sen University. Any changes to the protocol during the study should be reported and approved by IRB/IEC.

11.3 Informed Consent

Before enrollment, study physicians are responsible for a complete and comprehensive presentation to patients of the study purpose, the properties of the drug, its possible side effects, and potential risks. Patients should be informed of their rights, risk, and benefit. It should be emphasized that they can withdraw from the trial at any stage of the trial without affecting their subsequent treatment. Subjects should be promptly informed of any updates of the study, and a renewed informed consent to continue in the study should be obtained. Patients should sign the informed consent in duplicate with their name and date. The two copies are given to the patient and kept in study archives, respectively.

11.4 Study monitoring and quality assurance

Monitoring will be conducted through visits with the investigator and site staff as well as any appropriate communications by email, wechat, or telephone. The purpose of monitoring is to ensure compliance with the protocol and the quality and integrity of the data.

To ensure accordance with study protocols, physicians are asked to strictly follow the requirements of GCP throughout the trial, to achieve standard procedures, accurate data, and reliable conclusions. Specific requirements are as follows:

1) Obtain informed consent that is signed by each subject or their agents.

2) Complete the case report form (CRF) as required.

3) Follow-up on schedule.

4) Keep complete records of laboratory examinations, clinical records, and the original medical documents of the subjects.

11.5 Case Report Form (CRF)

The CRF will be completed by investigators in a timely manner to ensure the accuracy and timeliness of the content. Generally, the CRF should not be altered. If there are any errors to be corrected, the original record should be crossed out with a horizontal line, and the modified text should be signed and dated. The completed CRFs are reviewed by the quality control officer for data input. No further modification of CRFs is allowed once the database is locked.

11.6 Record Retention and Database Establishment

The investigator and other appropriate study staff are responsible for maintaining all documentation relevant to the study. Mandatory documentation includes copies of study protocols and amendments, IRB/IEC approval letters, signed ICFs, SAE forms, subject files (source documentation) that substantiate

CRF entries, and all relevant correspondence and other documents pertaining to the conduct of the study.

Statisticians will have questions in the CRFs checked with investigators, who should reply and return the CRFs promptly. Statisticians should establish the database in a timely manner, and the data will be locked by investigators, statisticians, and research assistants after the database has been reviewed. To ensure data security, a non-permitted person cannot modify the data, and the data must be backed up. According to the principle of GCP in China, research data should be stored for at least five years.

11.7 Independent Data Monitoring Committee

In this study, an Independent Data Monitoring Committee (IDMC) will be established to review the efficacy and safety in necessity. Any changes in the protocol will be discussed with IDMC. IDMC will be composed of at least three clinicians and statisticians who are not employed by Jiangsu Hengrui Medicine Co., Ltd. And participate in this study with no conflict of interests. IDMC review meetings will be held according to IDMC charter, during which, the study will continue. After the results review, IDMC will propose to terminate, continue the study, or modify the protocol. The principal investigators will ultimately decide whether to adopt the IDMC recommendations.

11.8 Protocol Modifications

All protocol modifications must be discussed with IDMC and submitted to the IRB/IEC. Approval must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to the trial patients, or when the change involve only logistical or administrative aspects of the trial. Additionally, under this circumstance, information on the increased risk and/or change in scope must be provided to subjects already actively participating in the study, and they must read, understand, and sign any revised IRB/ICF confirming willingness to remain in the trial.

11.9 Publication of study results

Authorship, in general, will follow the recommendations of the International Committee of Medical Journal Editors (International Committee of Medical Journal Editors 2016).

11.10 General investigator responsibility

The principal investigator must ensure that:

1) She will personally conduct or supervise the study.

2) Her staff and all persons who assist in the conduct of the study clearly understand their responsibilities and have their names included in the Study Personnel Responsibility/Signature Log.

3) The study is conducted according to the protocol and all applicable regulations.

4) The protection of each subject's rights and welfare is maintained.

5) Signed and dated informed consent and, when applicable, permission to use protected health information are obtained from each subject before conducting nonstandard of care study procedures. If a subject or subject's legal guardian withdraws permission to use protected health information, the investigator will obtain a written request from the subject or subject's legal guardian and will ensure that no further data be collected from the subject.

6) The consent process is conducted in compliance with all applicable regulations and privacy acts.

7) The IRB/IEC complies with applicable regulations and conducts initial and ongoing reviews and approvals of the study.

8) Any amendment to the protocol is decided by discussion with IDMC.

9) Any amendment to the protocol is submitted promptly to and get permission with the IRB/IEC.

10) Any significant protocol deviations are reported to Acerta Pharma and the IRB/IEC according to the guidelines at each study site.

11) Electronic CRF pages are completed promptly.

12) All IND Safety Reports and SUSAR Reports are submitted promptly to the IRB/IEC.

13) All SAEs are reported to the AstraZeneca Representative within 24 hours of knowledge and to the IRB/IEC per their requirements.

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