Neoadjuvant Pyrotinib plus Trastuzumab and Chemotherapy for Stage I–III HER2-Positive Breast Cancer: A Phase II Clinical Trial

JUNCHENG XUHONG,† XIAOWEI QI,† PENG TANG,† LINJUN FAN, LI CHEN, FAN ZHANG, XUANNI TAN, WENTING YAN, LING ZHONG, CHENG HE, YAN LIANG, LIN REN, MINGHAO WANG, YI ZHANG, JUN JIANG

Department of Breast and Thyroid Surgery, Southwest Hospital, Army Medical University, Chongqing, People’s Republic of China

†Contributed equally.

Key Words. Pyrotinib • Trastuzumab • HER2-positive breast cancer • Phase II trial • Neoadjuvant therapy • Tyrosine kinase inhibitor

Trial Information

• ClinicalTrials.gov Identifier: ChiCTR1900022293
• Sponsor: Southwest Hospital
• Principal Investigators: Jun Jiang, Yi Zhang
• IRB Approved: Yes

Lessons Learned

• This is the first trial to explore the neoadjuvant therapy of pyrotinib in HER2-positive operable and locally advanced breast cancer, in combination with epirubicin plus cyclophosphamide followed by docetaxel plus trastuzumab.
• Results primarily showed that pyrotinib in combination with epirubicin plus cyclophosphamide followed by docetaxel plus trastuzumab was effective and safe in HER2-positive operable and locally advanced breast cancer.
• A subsequent randomized controlled trial is still warranted to confirm these results.

Abstract

Background. The efficacy and safety of neoadjuvant therapy of pyrotinib, a new irreversible tyrosine kinase inhibitor (TKI), was first estimated in patients with HER2-positive breast cancer in this phase II study, in combination with trastuzumab and chemotherapy.

Methods. Between February 19, 2019, and November 20, 2019, 20 female Chinese patients with stage I–III HER2-positive breast cancer were assigned to receive eight cycles of neoadjuvant pyrotinib (P) in combination with four cycles of epirubicin (E) and cyclophosphamide (C) followed by four cycles of docetaxel (T) and trastuzumab (H), once every 3 weeks, referred to as P+E C - T H.

Results. A total of 19 patients completed the therapy and final surgery. The total pathological complete response (tpCR) rate was 73.7% (95% confidence interval [CI], 48.8–90.9), and no recurrence or metastasis occurred during the short-term follow-up period. The objective response rate (ORR) was 100% (95% CI, 82.4–100). The most common adverse events (AEs) were diarrhea and leukopenia in 18 of 20 patients (90%), but no grade 5 AEs were reported.

Conclusion. This study showed that in HER2-positive operable or locally advanced breast cancer, the tpCR rate of P + EC-TH neoadjuvant therapy was about twice as high as that of EC-TH neoadjuvant therapy reported in other trials, with tolerable side effects.

Discussion

Pyrotinib is a novel oral, irreversible TKI drug targeting HER1, HER2, and HER4 [1]. Combination therapy consisting of pyrotinib plus capcitabine exhibited promising antitumor effect and good tolerability in HER2-positive relapsed or metastatic breast cancer [2–5]; however, its activity in neoadjuvant treatment remains unknown.

In this study, we first explored the safety and efficacy of P + EC-TH neoadjuvant therapy in HER2-positive breast cancer. A total of 20 eligible patients were finally enrolled, and 19 patients completed eight cycles of P + EC-TH neoadjuvant therapy and underwent definitive surgery (Fig. 1).

Fourteen of 19 patients achieved tpCR (73.7%; 95% CI, 48.8–90.9). In terms of the subgroup analysis, patients with...
later clinical TNM stage and higher levels of tumor-infiltrating lymphocytes (TILs) in the previous treatment biopsy samples were more likely to have pathological complete response (pCR).

After completing the first four cycles of P + EC treatment, a single targeted treatment of pyrotinib plus chemotherapy illustrated an early relative antitumor effect, with a 75% ORR. After completing eight cycles of P + EC-TH neoadjuvant therapy, 5 participants had clinical complete response (CR) and 14 participants had clinical partial response (PR), exhibiting a 100% ORR. It is worth noting that two patients with HER2-positive stage IIIC breast cancer also achieved pCR. These two patients were histologically diagnosed with ipsilateral subclavian metastasis and ipsilateral supraclavicular metastasis, respectively, by core needle biopsy.

Diarrhea and leukopenia were the most common AEs associated with P + EC-TH neoadjuvant therapy, followed by decreased hemoglobin, alopecia, vomiting, reduced appetite, and oral ulceration. The most serious adverse reactions consisted of grade 4 leukopenia and neutropenia. However, no grade 5 AEs were reported. During the course of treatment, all of the patients were found to be tolerant to the therapeutic dosage, and no one interrupted therapy because of AEs. With the exception of one patient who still eventually achieved pCR, the dosage of pyrotinib was reduced in the seventh cycle because of grade 3 diarrhea.

In conclusion, a high pCR rate and tolerable AEs were reported in this study, which primarily illustrated that a neoadjuvant combination therapy consisting of pyrotinib plus trastuzumab and chemotherapy is effective and safe in HER2-positive breast cancer. However, the limitations of this study include the small sample size and the lack of a control group. A subsequent open-label, randomized, and controlled phase III trial is ongoing to confirm these results.

---

**TRIAL INFORMATION**

| Disease          | Breast cancer                                      |
|------------------|---------------------------------------------------|
| Stage of Disease/Treatment | Neoadjuvant                                      |
| Prior Therapy    | None                                              |
| Type of Study    | Phase II, single arm                              |
| Primary Endpoint | Total pathological complete response               |
| Secondary Endpoints | Overall response rate, safety, tumor-infiltrating lymphocytes, residual cancer burden, Neo-Bioscore |

**Additional Details of Endpoints or Study Design**

**Patient eligibility:** Eligible Chinese women were those aged at least 18 years who had newly diagnosed clinical stage I–III breast cancer and histologically confirmed invasive HER2-positive breast cancer (immunohistochemistry 3+, or immunohistochemistry 2+ along with HER2 gene amplification confirmed by fluorescent in situ hybridization). Before neoadjuvant chemotherapy, distant metastases were excluded by abdominal ultrasound, chest x-ray, and a whole-body bone scan. All participants displayed a performance status of 0 to 1 on the Eastern Cooperative Oncology Group score, normal organ function, and ability to follow the prescribed treatment schedule. Patients were not eligible if (a) they were pregnant or lactating or unwilling to take effective contraceptive measures throughout the trial; (b) they had serious heart, liver, kidney, or endocrine system disease, associated with an expected survival time of the above diseases of less than 2 years; (c) they had other factors (e.g., inability to swallow, intestinal obstruction, influence on drug administration, and absorption or allergic history of the drug); (d) they had a Karnofsky scale lower than 60; (e) they had bilateral breast cancer, inflammatory breast cancer, or distant metastases; and (f) they were not suitable for the clinical trial for other reasons.

**Study design and treatment plan:** Patients were assigned to receive four cycles of epirubicin (100 mg/m²) and cyclophosphamide (600 mg/m²) intravenously, once every 3 weeks, followed by four cycles of docetaxel (100 mg/m²) and trastuzumab...
Investigator’s Analysis: The primary outcome measure was the rate of tpCR. This was defined as the absence of any residual invasive cancer observed in the H&E staining of the excised breast specimen and all ipsilateral lymph nodes sampled after the completion of systemic neoadjuvant therapy.

Endpoints: The primary outcome measure was the rate of tpCR. This was defined as the absence of invasive cancer components in the breast and involvement of the axillary lymph nodes. However, intraductal carcinoma in situ (DCIS) was permitted, known as ypT0/is ypNO. The secondary outcome measures included ORR, which was defined as the proportion of patients who achieved a complete or partial response during the eight neoadjuvant treatment cycles according to RECIST, version 1.1. This was evaluated by the researchers: breast conserving surgery rate; TILs of the previous treatment biopsy samples of all patients and surgical specimens of patients who did not achieve pCR in accordance with the recommendations of the international TILs working group in 2014 [7], categorized as low (0%–49%), intermediate (10%–49%), and high (≥50%), respectively; residual cancer burden [8]; and Neo-Bioscore, a new staging system for breast cancer treated with neoadjuvant chemotherapy, are assigned by presenting clinical stage, final pathologic stage, and the biologic markers [9].

Efficacy and Safety Measures: Efficacy measures have been previously described. The safety assessment was conducted on days 7, 14, and 21 of every cycle, including the vital signs, laboratory examination, breast ultrasound, and dosage adjustment. Compiled adverse event profiles included the type, incidence, and severity grade according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0.

Statistical Analysis: A total of 20 patients were desired for enrollment to detect any adverse events, clinical response, and pCR rate. Safety and primary efficacy were analyzed in patients who received at least one cycle (3 weeks) of treatment. The proportion of patients with pCR was assessed and reported with 95% CIs calculated using the Clopper-Pearson method. Subgroup analyses were prespecified for the stratification factors of lymph node status, clinical tumor stage, hormone receptor status, Ki-67, and pretreatment TILs. All analyses were conducted using SAS, version 9.4. The study protocol was approved by the ethics committee of Southwest Hospital, Army Medical University, and all subjects participated in the study voluntarily and signed an informed consent form. The subjects were informed of the right to withdraw from the study at any time without any discrimination and punishment. This study is registered with the Chinese Clinical Trial Registry (ChiCTR1900022293).

Drug 1

| Generic/Working Name | Epirubicin |
|----------------------|------------|
| Trade Name           | Ellence    |
| Company Name         | Hanhui Medicine |
| Drug Type            | Other      |
| Drug Class           | Other      |
| Dose                 | 100 milligrams (mg) per squared meter (m²) |
| Route                | IV         |
| Schedule of Administration | Once every 3 weeks for four cycles: cycles 1–4 |

Drug 2

| Generic/Working Name | Cyclophosphamide |
|----------------------|------------------|
| Trade Name           | Endoxan          |
| Company Name         | Baxter Oncology GmbH |
| Drug Type            | Other            |
| Drug Class           | Other            |
| Dose                 | 600 milligrams (mg) per squared meter (m²) |
### Drug 3

| Route                      | IV |
|----------------------------|----|
| **Schedule of Administration** | Once every 3 weeks for four cycles: cycles 1–4 |

| **Generic/Working Name**          | Docetaxel          |
| **Trade Name**                   | Docetaxel          |
| **Company Name**                 | Jiangsu Hengrui Medicine |
| **Drug Type**                    | Other              |
| **Drug Class**                   | Other              |
| **Dose**                         | 100 milligrams (mg) per squared meter (m²) |
| **Route**                        | IV                 |
| **Schedule of Administration**   | Once every 3 weeks for four cycles: cycles 5–8 |

### Drug 4

| Route                      | IV |
|----------------------------|----|
| **Schedule of Administration** | Once every 3 weeks for four cycles: cycles 5–8 |

| **Generic/Working Name**          | Trastuzumab          |
| **Trade Name**                   | Herceptin            |
| **Company Name**                 | Roche                |
| **Drug Type**                    | Antibody             |
| **Drug Class**                   | Her2/Neu             |
| **Dose**                         | 8 mg/kg first load followed by 6 mg/kg milligrams (mg) per kilogram (kg) |
| **Route**                        | IV                   |

### Drug 5

| Route                      | Oral (po) |
|----------------------------|-----------|
| **Schedule of Administration** | Once every day |

| **Generic/Working Name**          | Pyrotinib          |
| **Trade Name**                   | Pyrotinib Maleate Tablets |
| **Company Name**                 | Jiangsu Hengrui Medicine |
| **Drug Type**                    | Small molecule     |
| **Drug Class**                   | Her2/Neu           |
| **Dose**                         | 400 milligrams (mg) per flat dose |
| **Route**                        | Oral (po)          |

### Patient Characteristics

| **Number of Patients, Male**   | 0 |
| **Number of Patients, Female** | 20 |
| **Stage**                     | I — 3 (15%) |
|                               | II — 15 (75%) |
|                               | III — 2 (10%) |
| **Age**                       | Median (range): 47.5 (30–66) years |
| **Number of Prior Systemic Therapies** | None |
| **Performance Status: ECOG**  | 0 — 16 |
|                               | 1 — 4 |
|                               | 2 — 0 |
|                               | 3 — 0 |
|                               | Unknown — 0 |

**Detailed Characteristics, n (%)**

| Menstrual status | 10 (50) |
Menopausal 10 (50)
Lymph node status
  Positive 13 (65)
  Negative 7 (35)
Tumor size
  1 4 (20)
  2 16 (80)
  3 0 (0)
cTNM
  I 3 (15)
  II 15 (75)
  III 2 (10)
HR status
  Positive 5 (25)
  Negative 15 (75)
Ki-67
  ≤20% 5 (25)
  >20% 15 (75)
Pre-TILs
  Low 5 (25)
  Intermediate 12 (60)
  High 3 (15)

| Cancer Types or Histologic Subtypes | HER2-positive breast cancer: 20 |
|------------------------------------|---------------------------------|

**PRIMARY ASSESSMENT METHOD: TOTAL PATHOLOGICAL COMPLETE RESPONSE RATE**

| Number of Patients Screened | 20 |
|-----------------------------|----|
| Number of Patients Enrolled | 20 |
| Number of Patients Evaluable for Toxicity | 20 |
| Number of Patients Evaluated for Efficacy | 19 |
| Evaluation Method | Miller-Payne grading system |
| Outcome Notes |

From February 2019 to April 2019, a total of 20 eligible newly diagnosed patients with stage I–III HER2-positive breast cancers were assigned to be administered P + EC-TH neoadjuvant treatment (Fig. 2). Informed consent was obtained from all patients. Eventually, a total of 19 patients completed the study treatment and surgery, whereas one patient discontinued therapy because of consent withdrawal after completing the fourth cycle of treatment (Fig. 3).

A pathological complete response was achieved in 14 of 19 patients, and the pCR rate was 73.7% (95% CI, 48.8–90.9). There were 12 patients (63.2%) who had neither invasive residuals nor DCIS in the removed breast and axillary specimens (ypT0 ypN0). In the other two patients, DCIS was observed in the breast tissue, and immunohistochemistry revealed that one was luminal B and another was a triple-negative breast cancer subtype. We also performed a subgroup analysis based on the lymph node status, tumor size, clinical TNM stage, hormone receptor status, Ki-67 percentage, and pretreatment TIL status, as shown in Figure 4.

**SECONDARY ASSESSMENT METHOD**

| Number of Patients Screened | 20 |
|-----------------------------|----|
| Number of Patients Enrolled | 20 |
| Number of Patients Evaluable for Toxicity | 20 |
| Number of Patients Evaluated for Efficacy | 19 |
| Evaluation Method | RECIST 1.1 |
| Response Assessment CR | n = 5 (26.3%) |
| Response Assessment PR | n = 14 (73.7%) |
| Response Assessment SD | n = 0 (0%) |
One patient who withdrew consent after completing the first four cycles of treatment was also included in the analysis of the clinical response at the end of fourth cycle. At the end of the fourth cycle, 1 (5%) patient achieved a CR, 14 (70%) patients achieved a PR, and 5 (25%) patients achieved clinical stable disease. The ORR (CR + PR) after four treatment cycles was 75% (15 of 20; 95% CI, 54.4–94.0). After eight cycles of neoadjuvant treatment were completed, 5 (26.3%) patients had CR, and 14 (73.7%) patients had PR. The ORR was 100% (95% CI, 82.4–100). As shown in the swimmer plot (Fig. 1), six patients had PR during the first month, nine patients had PR in the first 2 months, and the first patient achieved CR in the third month. In terms of surgery, 1 out of 19 patients (5.3%) underwent breast conserving surgery, and others had mastectomy. Furthermore, the distribution of patients in the other different response scoring systems was also assessed (Fig. 5).

| Name                                      | All Cycles | Grade 1, % | Grade 2, % | Grade 3, % | Grade 4, % | Grade 5, % | All grades, % |
|-------------------------------------------|------------|------------|------------|------------|------------|------------|---------------|
| Diarrhea                                  | 10         | 20         | 25         | 45         | 0          | 0          | 90            |
| White blood cell decreased                | 10         | 30         | 30         | 20         | 10         | 0          | 90            |
| Alopecia                                  | 35         | 30         | 35         | 0          | 0          | 0          | 65            |
| Vomiting                                  | 40         | 50         | 10         | 0          | 0          | 0          | 60            |
| Skin ulceration                           | 45         | 55         | 0          | 0          | 0          | 0          | 55            |
| Nausea                                    | 50         | 40         | 10         | 0          | 0          | 0          | 50            |
| Skin hyperpigmentation                    | 50         | 50         | 0          | 0          | 0          | 0          | 50            |
| Neutrophil count decreased                | 50         | 15         | 20         | 10         | 5          | 0          | 50            |
| Alanine aminotransferase increased        | 60         | 20         | 5          | 15         | 0          | 0          | 40            |
| Fatigue                                   | 55         | 40         | 5          | 0          | 0          | 0          | 45            |
| Headache                                  | 55         | 40         | 5          | 0          | 0          | 0          | 45            |
| Weight loss                               | 70         | 30         | 0          | 0          | 0          | 0          | 30            |
| Aspartate aminotransferase increased      | 75         | 15         | 5          | 5          | 0          | 0          | 25            |
| Platelet count decreased                  | 75         | 25         | 0          | 0          | 0          | 0          | 25            |
| Rash acniform                             | 85         | 15         | 0          | 0          | 0          | 0          | 15            |
| Dizziness                                 | 90         | 10         | 0          | 0          | 0          | 0          | 10            |
| Blood bilirubin increased                 | 80         | 20         | 0          | 0          | 0          | 0          | 20            |
| Cough                                     | 90         | 10         | 0          | 0          | 0          | 0          | 10            |
| Hypokalemia                               | 90         | 10         | 0          | 0          | 0          | 0          | 10            |
| Upper respiratory infection               | 90         | 10         | 0          | 0          | 0          | 0          | 10            |
| Stomach pain                              | 90         | 5          | 5          | 0          | 0          | 0          | 10            |
| Palpitations                              | 90         | 10         | 0          | 0          | 0          | 0          | 10            |

Adverse Events Legend

In 19 patients who completed eight cycles of neoadjuvant therapy and underwent the final surgery, the median duration of the treatment was 5.7 months (range: 5.3–6.1 months). The safety of P + EC-TH neoadjuvant sequential therapy was surveyed in 20 patients, including one patient who withdrew consent after completing four cycles of neoadjuvant therapy. Diarrhea and leukopenia were the two most common AEs (90%, 18 of 20) of P + EC-TH. Diarrhea was also associated with the highest proportion of grade 3 AEs (45%, 9 of 20), followed by leukopenia (20%, 4 of 20), neutropenia (1%, 2 of 20), increased ALT (10%, 2 of 20), and increased AST (5%, 1 of 20). Grade 4 leukopenia and neutropenia were the most severe AEs, which were observed in two and one patients, respectively, whereas no grade 5 AEs were reported. Grade 3 diarrhea was managed by appropriate drugs, including montmorillonite powder (brand name, simida, produced by Ipsen) and probiotics. The injection with recombinant human granulocyte stimulating factor was applied for situations of leukopenia. At the last visit, grade 1 increased ALT levels occurred in two cases (10%) and grade 1 increased AST levels occurred in one case (5%). No patients discontinued treatment because of toxic effects. One patient adjusted the dosage of pyrotinib from 400 mg to 240 mg because of grade 3 diarrhea during the seventh cycle, but still achieved pCR.

Abbreviation: NC/NA, no change from baseline/no adverse event.
Breast cancer remains a predominant cause of death in women with malignant tumors [10]. Human epidermal receptor 2 (HER2) overexpression or gene amplification accounts for approximately 15%–20% of breast cancer cases [11] and is associated with early metastasis and poor prognosis [12–14].

Currently, specific monoclonal antibodies and tyrosine kinase inhibitors (TKIs) are two targeted strategies for HER2-positive breast cancer, including trastuzumab, pertuzumab, trastuzumab emtansine, lapatinib, neratinib, and pyrotinib [3, 15–18]. Trastuzumab is the first targeted drug specifically binding to the extracellular segment of HER2 [19], which prolongs the survival time of patients to more than 10 years.

Doxorubicin (A) plus cyclophosphamide (C) followed by paclitaxel (pac) and trastuzumab (H), known as A+C-pac+H, was first brought forward in adjuvant therapy and developed from an A+C-pac scheme. The BCIRG-006, NCTGT 9831, NSABP B-31, and HERA trials demonstrated better outcomes of A+C-pac+H adjuvant therapy compared with AC-T adjuvant therapy and also established the cornerstone position of standard therapy involving 1 year of trastuzumab [20–26]. Recently, epirubicin (E), a new anthracycline-based drug, has been successfully used to ameliorate cardiac toxicity in chemotherapy recipients for cancers. As for neoadjuvant therapy, treatment with trastuzumab in addition to docetaxel followed by epirubicin plus cyclophosphamide was shown to confer a higher pathological complete response (pCR) rate compared with the EC-T chemotherapy regimen (25.8% vs. 19.0%) (Table 1) [27]. In the TECHNO study, 217 participants with stage I–III HER2-positive breast cancer were assigned to receive E+C-pac+H neoadjuvant therapy, and the results indicated that the 3-year disease-free survival (88% vs. 73%) and overall survival (OS) (96% vs. 86%) of the patients with pCR were higher than in patients without pCR [29]. In NeoSphere, patients treated with dual-targeted neoadjuvant therapy consisting of pertuzumab and trastuzumab plus docetaxel had a higher pCR rate compared with patients treated with trastuzumab plus docetaxel (45.8% vs. 29.0%) [34]. However, anthracycline has cardiotoxicity, and it can lead to congestive heart failure when combined with trastuzumab. Moreover, the NOAH study showed that about 42% patients treated with trastuzumab relapsed within 5 years [35]. Therefore, finding a more effective therapeutic regimen with fewer adverse reactions is urgently needed.

Over the past decades, TKIs have been gaining increased attention because of the advantages of restricting multiple targets, oral administration, and decreased cardiac toxicity in contrast to that of monoclonal antibodies [36]. In HER2-positive breast cancer, some studies had demonstrated that more patients could attain pCR from dual-targeted treatment of TKI plus trastuzumab. In the NeoALTTO study (NCT00553358), patients with HER2-positive breast cancer were randomly administered lapatinib, trastuzumab, or lapatinib plus trastuzumab, with 12-week paclitaxel sequential treatment [30]. Subsequently, a significantly higher pCR rate was reported for the lapatinib and trastuzumab combination therapy (51.3%), superior to trastuzumab monotherapy (29.5%), as well as lapatinib monotherapy (24.7%). Moreover, the CHERLOB trial (NCT00429299), in which 121 patients with HER2-positive stage I–IIIA breast cancer were randomized to receive neoadjuvant treatment with lapatinib, trastuzumab, or lapatinib plus trastuzumab, combined with chemotherapy, also found a similar pCR rate of 26.3%, 25%, and 46.7%, respectively [32]. However, after a 3-year follow-up of the NeoALTTO study, the benefit of the pCR rate in the combination treatment group did not translate into long-term event-free survival and OS benefits [31]. NSABP-FB7 (NCT01008150) investigated neoadjuvant therapy with neratinib or trastuzumab, or neratinib plus trastuzumab in 141 patients with HER2-positive breast cancer. Both groups were combined with weekly paclitaxel and followed by doxorubicin and cyclophosphamide. The pCR rate was 38.1%, 33.3%, and 50.0%, respectively, with no observed significant differences [33].

In our study, the pCR (ypT0/TiS ypNO) rate of this regimen was 73.7%, and the clinical objective response rates for four and eight cycles reached 75% and 100%, respectively. However, in previous neoadjuvant studies combined pertuzumab with trastuzumab for six or eight cycles, the KRISTINE and BERENICE trials resulted in a pCR rate of 55.7% and 60.7%, respectively [37, 38].

As expected, diarrhea was the most common adverse event (AE) observed in our study, and the reports of grade 3 diarrhea were higher than that with the addition of pertuzumab in neoadjuvant setting, but no grade 4 diarrhea occurred, and most was controllable. Other AEs were similar to the known toxicity profiles of the regimen of cyclophosphamide with pertuzumab plus trastuzumab in the neoadjuvant setting. Concurrent administration of pyrotinib and an anthracycline did not increase risk of cardiac dysfunction.

In China, we are currently conducting a single-arm, multicenter study with an expanded sample size and hope to further confirm the results of this study.

Our present study reported a higher pCR rate compared with studies involving neoadjuvant therapy of TKIs in combination with trastuzumab and chemotherapy. However, several unsolved questions remain regarding the optimal time, combination, dosage, and cycle of neoadjuvant treatment involving pyrotinib treatment for patients. It is unknown whether pyrotinib would continue to work after the dosage reduction because of AEs, the characteristics of the subgroup of patients with a better response, and whether dual-targeted neoadjuvant therapy with pyrotinib plus trastuzumab is more effective than trastuzumab.

**Acknowledgments**

We thank the patients who participated in this study and their family members, the study investigators, and the research
Neoadjuvant Pyrotinib in Breast Cancer

1. Zhu Y, Li L, Zhang G et al. Metabolic characterization of pyrotinib in humans by ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. J Chromatogr B Analys Technol Biomed Life Sci 2016;1033–1034: 117–127.

2. Ma F, Li Q, Chen S et al. Phase I study and biomarker analysis of pyrotinib, a novel irreversible pan-ERbB receptor tyrosine kinase inhibitor, in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer. J Clin Oncol 2017;35:3105–3112.

3. Fei Ma, Quchang Ouyang, Wei Li et al. Pyrotinib or lapatinib combined with capcitabine in HER2-positive metastatic breast cancer with prior taxanes, anthracyclines, and/or trastuzumab: A randomized, phase II study. J Clin Oncol 2019;37:2610–2619.

4. Meng J, Liu XY, Ma S et al. Metabolism and disposition of pyrotinib in healthy male volunteers: Covalent binding with human plasma protein. Acta Pharmacol Sin 2018;40:980–988.

5. Jiang Z, Yan M, Hu X et al. Pyrotinib combined with capcitabine in women with HER2-metastatic breast cancer previously treated with trastuzumab and taxanes: A randomized phase III study. J Clin Oncol 2019;37(suppl 15):1001a.

6. Wolff AC, Hammond MEH, Allison KH et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/Collaboration of American Pathologists clinical practice guideline focused update. J Clin Oncol 2018;36:2105–2122.

7. Salgado R, Denkert C, Demaria S et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: Recommendations by an International TILs Working Group 2014. Ann Oncol 2015;26:259–271.

8. Symmans WF, Peintinger F, Hatzis C et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007;25:4414–4422.

9. Mittendorf EA, Vila J, Tucker SL et al. The Neo-Bioscore update for staging breast cancer treated with neoadjuvant chemotherapy incorporation of prognostic biomarker factors into staging after treatment. JAMA Oncol 2016;2:929–936.

10. Bray F, Ferlay J, Soerjomataram I et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.

11. Wolff AC, Hammond ME, Hicks DG et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/Collaboration of American Pathologists clinical practice guideline update. J Clin Oncol 2013;31:3997–4013.

12. Piccart-Gebhart MJ, Procter M, Leyland-Jones B et al.; Herceptin Adjuvant (HERA) Trial Study Team. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005;353:1659–1672.

13. Slamon DJ, Leyland-Jones B, Shak S FH et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–792.

14. Ménard S, Tagliabue E, Campiglio M et al. Role of HER2 gene overexpression in breast carcinoma. J Cell Physiol 2000;182:150–162.

15. Geyer CE, Forster J, Lindquist D et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006;355:2733–2743.

16. Verma S, Miles D, Gianni L et al.; EMASTudy Group. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012;367:1783–1791.

17. Swain SM, Baselga J, Kim SB et al.; CLEOPATRA Study Group. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N Engl J Med 2015;372:724–734.

18. Chan A, Delarole S, Holmes FA et al.; ExeNET Study Group. Neratinib after trastuzumab-based adjuvant therapy in patients with HER2-positive breast cancer (ExeNET): A multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2016;17:367–377.

19. Hudis CA. Trastuzumab — mechanism of action and use in clinical practice. N Engl J Med 2007;357:39–51.

20. Slamon D, Eiermann W, Robert N et al.; Breast Cancer International Research Group. Adjunct trastuzumab in HER2-positive breast cancer. N Engl J Med 2011;365:1273–1383.

21. Slamon DJ, Eiermann W, Robert NJ et al.; BCIRG-006 Investigators. Ten year follow-up of BCIRG-006 comparing doxorubicin plus cyclophosphamide followed by docetaxel (AC — T) with doxorubicin plus cyclophosphamide followed by docetaxel and trastuzumab (AC — TH) with docetaxel, carboplatin and trastuzumab (TCH) in HER2 + early breast cancer. Cancer Res 2016;76(suppl 4):5504a.

22. Romond EH, Perez EA, Bryant J et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005;353:1673-1684.

23. Perez EA, Suman VJ, Davidson NE et al. Sequential versus concurrent trastuzumab in adjuvant chemotherapy for breast cancer. J Clin Oncol 2011;29:4491–4497.

24. Perez EA, Romond EH, Suman VJ et al. Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2-positive breast cancer: Planned joint analysis of overall survival from NSABP B-31 and NCTG N9831. J Clin Oncol 2014;32:3744–3752.

25. Cameron D, Piccart-Gebhart MJ, Gelber RD et al.; Herceptin Adjuvant (HERA) Trial Study Team. 11 years’ follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: Final analysis of the HERceptin Adjuvant (HERA) trial. Lancet 2017;389:1195–1205.

26. Goldhirsch A, Gelber RD, Piccart-Gebhart MJ et al.; Herceptin Adjuvant (HERA) Trial Study Team. 2 years versus 1 year of adjuvant trastuzumab for HER2-positive breast cancer (HERA): An open-label, randomised controlled trial. Lancet 2013;382:1021–1028.

27. Pierga JY, Delaloge S, Espie M et al. A multicenter randomized phase II study of sequential epirubicin/cyclophosphamide followed by docetaxel with or without celecoxib or trastuzumab according to HER2 status, as primary chemotherapy for localized invasive breast cancer patients. Breast Cancer Res Treat 2010;122:439–437.

28. Untch M, Rezai M, Loibl S et al. Neoadjuvant treatment with trastuzumab for HER2-positive breast cancer: Results from the geoparquattro study. J Clin Oncol 2010;28:2024–2031.

29. Untch M, Fasching PA, Konecny GE et al. Pathologic complete response after neoadjuvant chemotherapy plus trastuzumab predicts favorable survival in human epidermal growth factor receptor 2-overexpressing breast cancer: Results from the TECNO trial of the AGO and GBG study groups. J Clin Oncol 2011;29:3351–3357.

30. Baselga J, Bradbury I, Eidtmann H et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoadJITO): A randomised, open-label, multicentre, phase 3 trial. Lancet 2012;379:633–640.

31. de Azambuja E, Holmes AP, Piccart-Gebhart MJ et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoAdJITO): Survival outcomes of a randomised, open-label, multicentre, phase 3 trial and their association with pathological complete response. Lancet Oncol 2014;15:1137–1146.

32. Guanieri V, Frassoldati A, Bottini A et al. Preoperative chemotherapy plus trastuzumab, lapatinib, or both in human epidermal growth factor receptor 2-positive operable breast cancer: Results of the randomized phase II CHER-LOB study. J Clin Oncol 2012;30:1989–1995.

33. Jacobs SA, Robidoux A, Abraham J et al. NSABP FB-7: A phase II randomized neoadjuvant trial with paclitaxel + trastuzumab and/or neratinib receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Physicians. JAMA Oncol 2016;2:929–936.

The Oncologist published by Wiley Periodicals LLC on behalf of AlphaMed Press.
Figure 2. P + EC-TH neoadjuvant treatment procedure.
Abbreviations: C, cyclophosphamide; E, epirubicin; H, trastuzumab; ORR, objective response rate; P, pyrotinib; pCR, pathological complete response; T, docetaxel.
Figure 3. Trial profile. Nineteen patients who completed eight cycles of P + EC-TH neoadjuvant treatment and final surgery were included in ultimate efficacy analysis. Twenty patients were contained in safety and primary efficacy analysis, including one patient who withdrew consent after completing the first four cycles of therapy.

Abbreviation: P + EC-TH, pyrotinib in combination with epirubicin plus cyclophosphamide followed by docetaxel plus trastuzumab.

Figure 4. Subgroup analysis. Subgroup analysis of tpCR rate in terms of lymph nodes status, tumor size cTNM, HR status, Ki-67, and pre-TILs.

Abbreviations: cTNM, clinical TNM stage; HR, hormone receptor; pre-TILs, previous treatment tumor-infiltrating lymphocytes; tpCR, total pathological complete response.
Table 1. Summary of clinical trials of TKIs plus trastuzumab neoadjuvant therapy in HER2-positive breast cancer

| Trial         | Arm                          | Number | tpCR (%) | bpCR (%) | Safety                                                                 | Principal findings                                                                 |
|--------------|------------------------------|--------|----------|----------|----------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| 2010 [27]    | E + C → T                   | 37     | 19.0     | 19.0     | There was no obvious cardiac toxicity or treatment-related death.    | Trastuzumab added to ECT led to increased pCR rates in patients with HER2-positive breast cancer. |
|              | E + C → T + H               | 34     | 25.8     | 32.1     |                                                                      | Patients with HER2-positive tumors who were treated with trastuzumab and chemotherapy had a higher pCR than others with HER2-negative tumors treated in the same study with the same chemotherapy but without trastuzumab. |
| GeparQuattro | 2010 [28]                   |        |          |          |                                                                      | Neutropenia (48.4%) and leucopenia were the most common AEs, and five patients had LVEF decreased less than 45%. |
|              | E + C → T + H               | 146    | 32.9     |          |                                                                      | Three-year DFS of patients with pCR was higher than patients without pCR (88% vs. 73%), as well as 3-year OS (96% vs. 86%). But pCR was the only significant prognostic feature for DFS. |
|              | E + C → T + X + H           | 144    | 31.3     |          |                                                                      | Three-year follow-up showed no significant difference in EFS or OS between treatment groups. However, patients who achieved pCR had longer EFS and OS than those who did not. |
|              | E + C → X + H               | 136    | 34.6     |          |                                                                      |                                                                                            |
| TECHNO 2011  | E + C → pac + H             | 217    | 38.7     | 42.3     | Neutropenia (48.4%) and leucopenia were the most common grade 3–4 hematologic toxicities. Cardiac toxicities were reported in 3.7% of patients. |                                                                                           |
| Neoadj TO    | 2012 [30, 31]               | L → L + pac | 154      | 24.7     | 33.78 16.25 More than one third of the patients who received lapatinib did not complete the treatment as planned because of diarrhea, hepatotoxicity, and other AEs. |                                                                                           |
|              | H → H + pac                 | 149    | 29.5     | 36.49 22.76 |                                                                                            |
|              | H + T → L + H + pac         | 152    | 46.8     | 61.33 41.56 |                                                                                            |
| CHERLOB 2012 | H + pac → F + E + C + H     | 36     | 25.0     |          | Diarrhea, dermatologic toxicities, and hepatic toxicities were the most common AEs, and approximately half of patients interrupted lapatinib. | Chemotherapy plus trastuzumab and lapatinib significantly increased pCR rate compared with chemotherapy plus either trastuzumab or lapatinib. |
|              | L + pac → F + E + C + L     | 39     | 26.3     |          |                                                                      |                                                                                            |
|              | H + L + pac → F + E + C + H + L | 46  | 46.7     |          |                                                                      |                                                                                            |
| NSABP-FB7 2019 | H+ pac → A + C            | 41     | 39.0     | 51.7     | Diarrhea was the most frequent AE. Neratinib dose reduction was reported in 38% of patients in arm 2 and 52% of patients in arm 3. | Chemotherapy plus trastuzumab and neratinib did not prominently improve pCR rate in contrast with chemotherapy plus either trastuzumab or neratinib. But HR+ tumors had a higher pCR rate than HR- tumors in all three treatment arms, with the highest pCR rate in the combination arm. |
|              | N+ pac → A + C              | 42     | 33.3     | 46.2     |                                                                      |                                                                                            |
|              | H + N+ pac → A + C          | 42     | 50.0     | 73.7     |                                                                      |                                                                                            |
| NeoSphere 2012 | T + H                     | 29.0  36.8 | 20.0  | 24.0  30.0 | The most common AEs were neutropenia, febrile neutropenia, and leucopenia. There were no substantial differences in tolerability among the four groups. | Patients treated with pertuzumab and trastuzumab plus docetaxel had a significantly higher pCR rate compared with those treated with trastuzumab plus docetaxel. |
|              | T + H + P                   | 45.8  63.2 | 26.0  | 24.0  30.0 |                                                                      |                                                                                            |
|              | H + P                       | 16.8  27.3 | 5.9   | 17.4   |                                                                      |                                                                                            |
|              | T + P                       | 20.0  |          |          |                                                                      |                                                                                            |
| P + EC-TH    | E + C + pro → T + H + pro   | 19     | 73.7     | 87.5     | 63.6                                                                 |                                                                                            |

Abbreviations: A, doxorubicin; AE, adverse event; bpCR, breast pathological complete response; C, cyclophosphamide; DFS, disease-free survival; E, epirubicin; EFS, event-free survival; F, fluorouracil; H, trastuzumab; HER2, human epidermal growth factor receptor 2; HR-, hormone receptor negative; HR+, hormone receptor positive; ITT, intention-to-treat; L, lapatinib; LVEF, left ventricular ejection fraction; N, neratinib; OS, overall survival; P, pertuzumab; pac, paclitaxel; pro, pyrotinib; T, docetaxel; tpCR, total pathological complete response (breast and axillary); X, capecitabine.
Figure 5. Distribution of patients in the different response scoring systems. (A): Distribution of posttreatment tumor-infiltrating lymphocyte levels in patients with residual disease. (B): Distribution of the RCB scores. (C): Distribution of the Neo-Bioscore scores. Dark gray indicates pCR; light gray indicates residual disease. Abbreviations: pCR, pathological complete response; post-TILs, posttreatment tumor-infiltrating lymphocytes; RCB, residual cancer burden.