The Oncologist®

Clinical Trial Results

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 + EC-TH.

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. ***The Oncologist*** 2020;25:e1909–e1920

Discussion

Pyrotinib is a novel oral, irreversible TKI drug targeting HER1, HER2, and HER4 [1]. Combination therapy consisting of pyrotinib plus capecitabine 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

Correspondence: Jun Jiang, M.D., Department of Breast and Thyroid Surgery, Southwest Hospital, Army Medical University, Chongqing 400038, People's Republic of China. Telephone: +8613508319495; e-mail: jcbd@medmail.com.cn; or Yi Zhang, M.D., Department of Breast and Thyroid Surgery, Southwest Hospital, Army Medical University, Chongqing 400038, People's Republic of China. Telephone: +8613983701489; e-mail: zy53810@163.com Received September 28, 2020; accepted for publication September 7, 2020; published Online First on October 20, 2020. http://dx.doi.org/10.1002/onco.13546

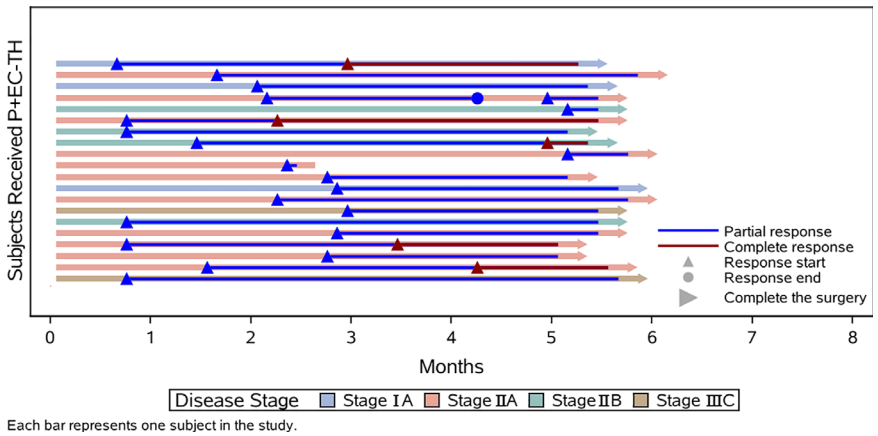

Figure 1. Swimmer plot of clinical response. Triangle indicates the start time of clinical response. Circle indicates the end time of the former clinical response. Arrows indicate patients completed the neoadjuvant therapy and received final surgery.
Abbreviation: P + EC-TH, pyrotinib in combination with epirubicin plus cyclophosphamide followed by docetaxel plus trastuzumab.

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 (immunochemistry 3+, or immunochemistry 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/m^2) and cyclophosphamide (600 mg/m^2) intravenously, once every 3 weeks, followed by four cycles of docetaxel (100 mg/m^2) and trastuzumab

(8 mg/kg first load followed by 6 mg/kg) intravenously, once every 3 weeks. The patients also received 400 mg pyrotinib orally once per day. Dosage reduction of pyrotinib was permitted from 400 mg to 320 mg or 240 mg if pyrotinib-related AEs were experienced (e.g., grade 3 or 4 diarrhea, leukopenia, increased alanine aminotransferase [ALT], or increased aspartate aminotransferase [AST]). Dose reescalation of pyrotinib was permitted for patients in whom the related AEs were relieved. After the completion of the above treatment cycle, the operation was performed. After the operation, trastuzumab was continuously used for 1 year. At baseline, a primary tumor of the breast was histologically confirmed by core needle biopsy, including estrogen receptor, progesterone receptor, and HER2 status evaluation by immunohistochemistry. HER2 status was defined as positive if the immunohistochemistry result was 3+ or immunohistochemistry was 2+ in conjunction with *HER2* gene amplification confirmed by fluorescent in situ hybridization following the HER2 testing guidelines of American Society of Clinical Oncology/College of American Pathologists in breast cancer [6]. Breast and axillary lymph node ultrasound and magnetic resonance imaging (MRI) were performed, and an ultrasound-guided fine-needle aspiration or core biopsy was required in cases of suspicious axillary lymph nodes. Breast ultrasound was performed during the third week of each cycle, and MRI was conducted every four cycles (12 weeks) to assess the tumor response. Before each chemotherapy cycle, routine blood, liver, and kidney function were assessed, and the blood function was rechecked on the third, sixth, and ninth days after chemotherapy. Tumor samples were collected during surgery after the completion of neoadjuvant treatment and evaluated with a local pathology review. The primary endpoint of the pathological complete response (ypT0/is ypN0) 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 by 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 ypN0. 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%–9%), 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 less 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).

Investigator's Analysis	Active and should be pursued further

Drug Information

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 (m^2)
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 (m^2)

Route	IV
Schedule of Administration	Once every 3 weeks for four cycles: cycles 1–4
Drug 3	
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 (m^2)
Route	IV
Schedule of Administration	Once every 3 weeks for four cycles: cycles 5–8
Drug 4	
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
Schedule of Administration	Once every 3 weeks for four cycles: cycles 5–8
Drug 5	
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)
Schedule of Administration	Once every day

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	
Premenopausal	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%)

Response Assessment PD	*n* = 0 (0%)
Response Assessment OTHER	*n* = 0 (0%)

Outcome Notes

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).

Adverse Events

	All Cycles						
Name	**NC/NA, %**	**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 acneiform	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.

Assessment, Analysis, and Discussion	
Completion	Study completed
Investigator's Assessment	Active and should be pursued further

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, NCCTG 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 II–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 ypN0) 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

staff. Jiangsu Hengrui Medicine provided pyrotinib for the study. This study was supported by grants from the Talents Training Program (2018XLC2004) and the Military Medical Staff Innovation Plan of Southwest Hospital (SWH2018BJLC-04 and SWH2016ZDCX1008), as well as from the Army Medical University (XZ-2019-505-042) and Chongqing Science and Technology Commission (CSTC2015shms-ztzx10005).

Data Sharing Statement: Individual participant data that underlie the results reported in this article will be available after deidentification, as will the study protocol, statistical analysis plan, informed consent form, clinical study report, and analytic code. Data will be available immediately after publication, with no end date. Researchers should provide a request containing the research objectives, data requirements, publication plan, aims in the approved proposal, and qualifications of the researchers.

Disclosures

The authors indicated no financial relationships.

References

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 Analyt 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 capecitabine 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 capecitabine 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/College 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 NeoBioscore update for staging breast cancer treated with neoadjuvant chemotherapy incorporation of prognostic biologic 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/College 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.; EMILIA Study 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, Delaloge S, Holmes FA et al.; ExteNET Study Group. Neratinib after trastuzumab-based adjuvant therapy in patients with HER2-positive breast cancer (ExteNET): 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. Adjuvant 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):S5-04a.

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 NCCTG 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;122:429–437.

28. Untch M, Rezai M, Loibl S, et al. Neoadjuvant treatment with trastuzumab in her2-positive breast cancer: Results from the geparquattro 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 TECHNO 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 (NeoALTTO): A randomised, open-label, multicentre, phase 3 trial. Lancet 2012;379: 633–640.

31. de Azambuja E, Holmes AP, Piccart-Gebhart M et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): 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. Guarneri 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 CHERLOB 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 followed by chemotherapy and post-operative trastuzumab in HER2(+) breast cancer. Breast Cancer Res 2019;21:133.

34. Gianni L, Pienkowski T, Im YH et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): A randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13:25–32.

35. Gianni L, Eiermann W, Semiglazov V et al. Neoadjuvant and adjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer (NOAH): Follow-up of a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet Oncol 2014;15:640–647.

36. Xuhong JC, Qi XW, Zhang Y et al. Mechanism, safety and efficacy of three tyrosine kinase inhibitors lapatinib, neratinib and pyrotinib in HER2-positive breast cancer. Am J Cancer Res 2019;9:2103–2119.

37. Hurvitz SA, Martin M, Symmans WF et al. Neoadjuvant trastuzumab, pertuzumab, and chemotherapy versus trastuzumab emtansine plus pertuzumab in patients with HER2-positive breast cancer (KRISTINE): A randomised, open-label, multicentre, phase 3 trial. Lancet Oncol 2018;19:115–126.

38. Swain SM, Ewer MS, Viale G et al. Pertuzumab, trastuzumab, and standard anthracycline- and taxane-based chemotherapy for the neoadjuvant treatment of patients with HER2-positive localized breast cancer (BERENICE): A phase II, open-label, multicenter, multinational cardiac safety study. Ann Oncol 2018;29:646–653.

Figures and Table

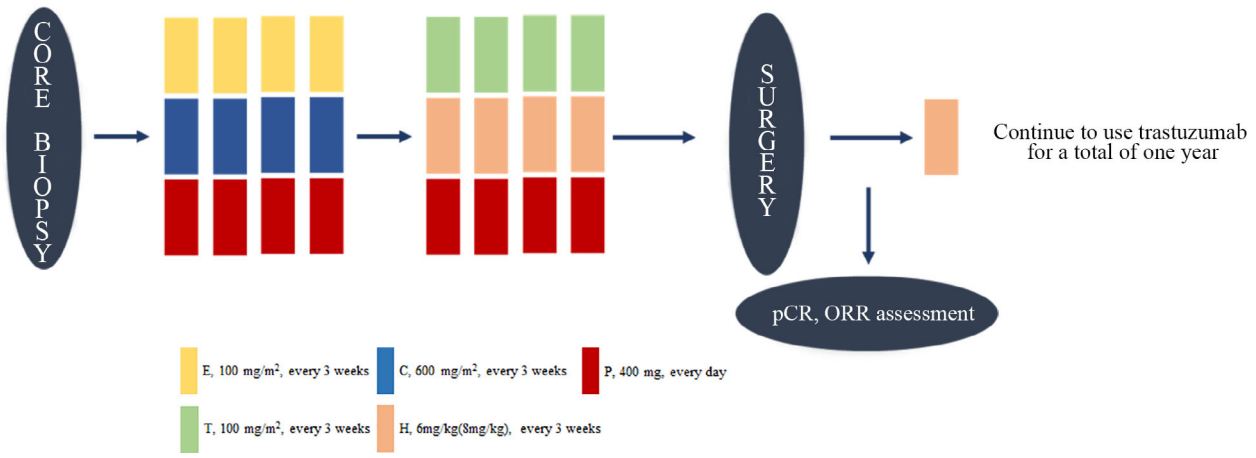

Figure 2. P + EC-TH neoadjuvant treatment procedure.
Abbreviations: C, cyclophosphamide; E, epirubicin; H, trastuzumab; ORR, objective response rate; P, pyrotinib; pCR, pathological complete response; T, docetaxel.

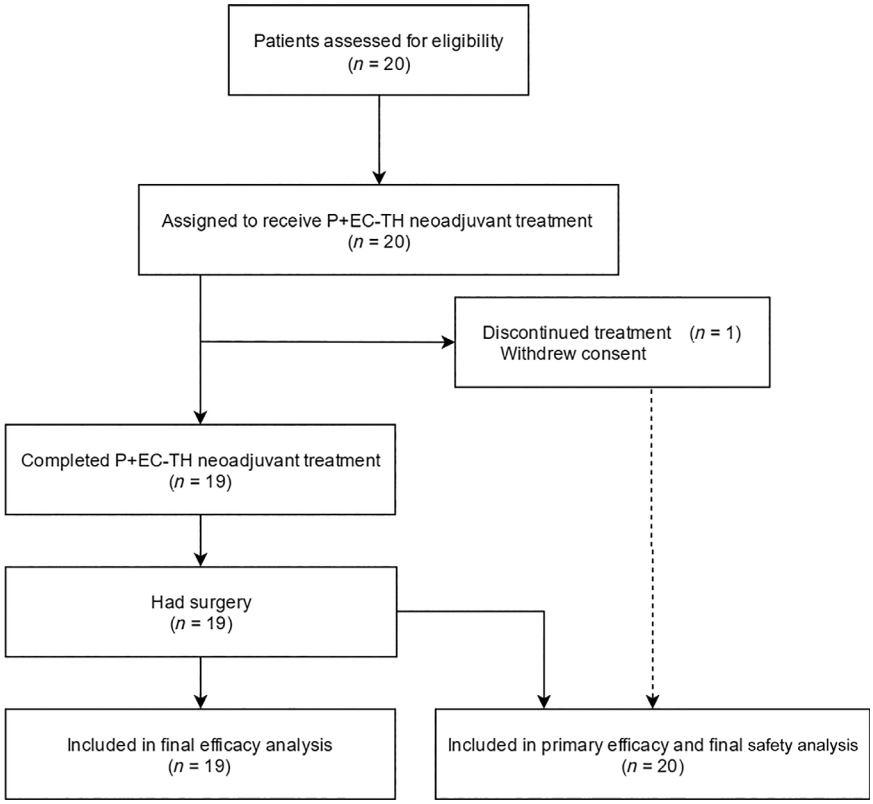

Figure 3. Trial profile. Nineteen patients who completed eight cycles of P + EC-TH neoadjuvant treatment and final surgery were included in ultimate efficacy analysis. Twenty patients were contained in safety and primary efficacy analysis, including one patient who withdrew consent after completing the first four cycles of therapy.
Abbreviation: P + EC-TH, pyrotinib in combination with epirubicin plus cyclophosphamide followed by docetaxel plus trastuzumab.

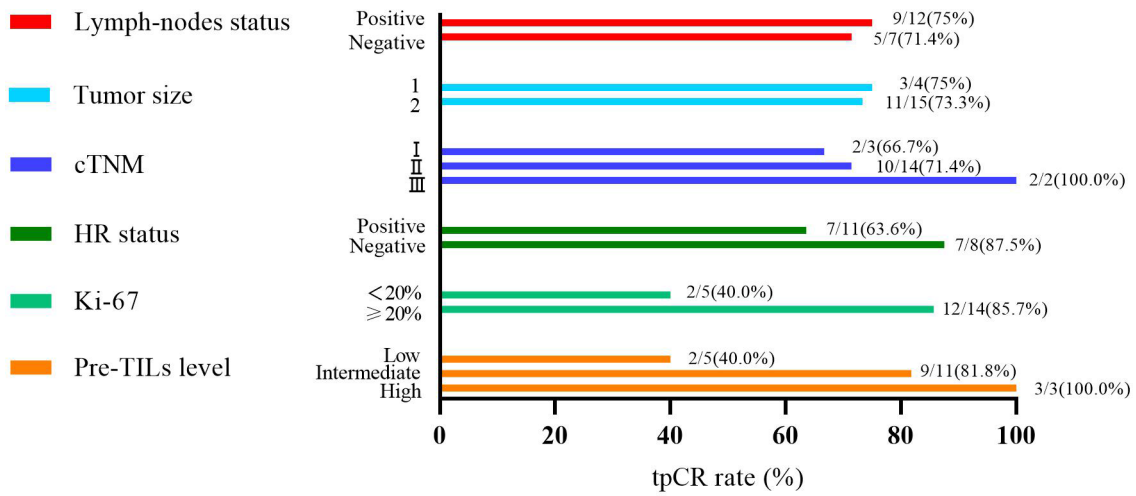

Figure 4. Subgroup analysis. Subgroup analysis of tpCR rate in terms of lymph nodes status, tumor size cTNM, HR status, Ki-67, and pre-TILs.
Abbreviations: cTNM, clinical TNM stage; HR, hormone receptor; pre-TILs, previous treatment tumor-infiltrating lymphocytes; tpCR, total pathological complete response.

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
			ITT	HR−	HR+	ITT	HR−	HR+		
2010 [27]	E + C → T	37	19.0	19.0	18.9				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	20.6					
GeparQuattro 2010 [28]	E + C → T + H	146	32.9						Leucopenia and neutropenia were the most common AEs, and five patients had LVEF decreased less than 45%.	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.
	E + C → T + X + H	144	31.3							
	E + C → X + H	136	34.6							
TECHNO 2011 [29]	E + C → pac + H	217	38.7	42.3	35.4				Neutropenia (48.4%) and leucopenia were the most common grade 3–4 hematologic toxicities. Cardiac toxicities were reported in 3.7% of patients.	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.
NeoALLTO 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.	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.
	H → H + pac	149	29.5			36.49	22.76			
	H + T → L + H + pac	152	46.8			61.33	41.56			
CHERLOB 2012 [32]	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 [33]	H+ pac → A + C	41	39.0	51.7	29.6				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	27.6					
	H + N + pac → A + C	42	50.0	73.7	30.4					
NeoSphere 2012 [34]	T + H					29.0	36.8	20.0	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		
	H + P					16.8	27.3	5.9		
	T + P					24.0	30.0	17.4		
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.

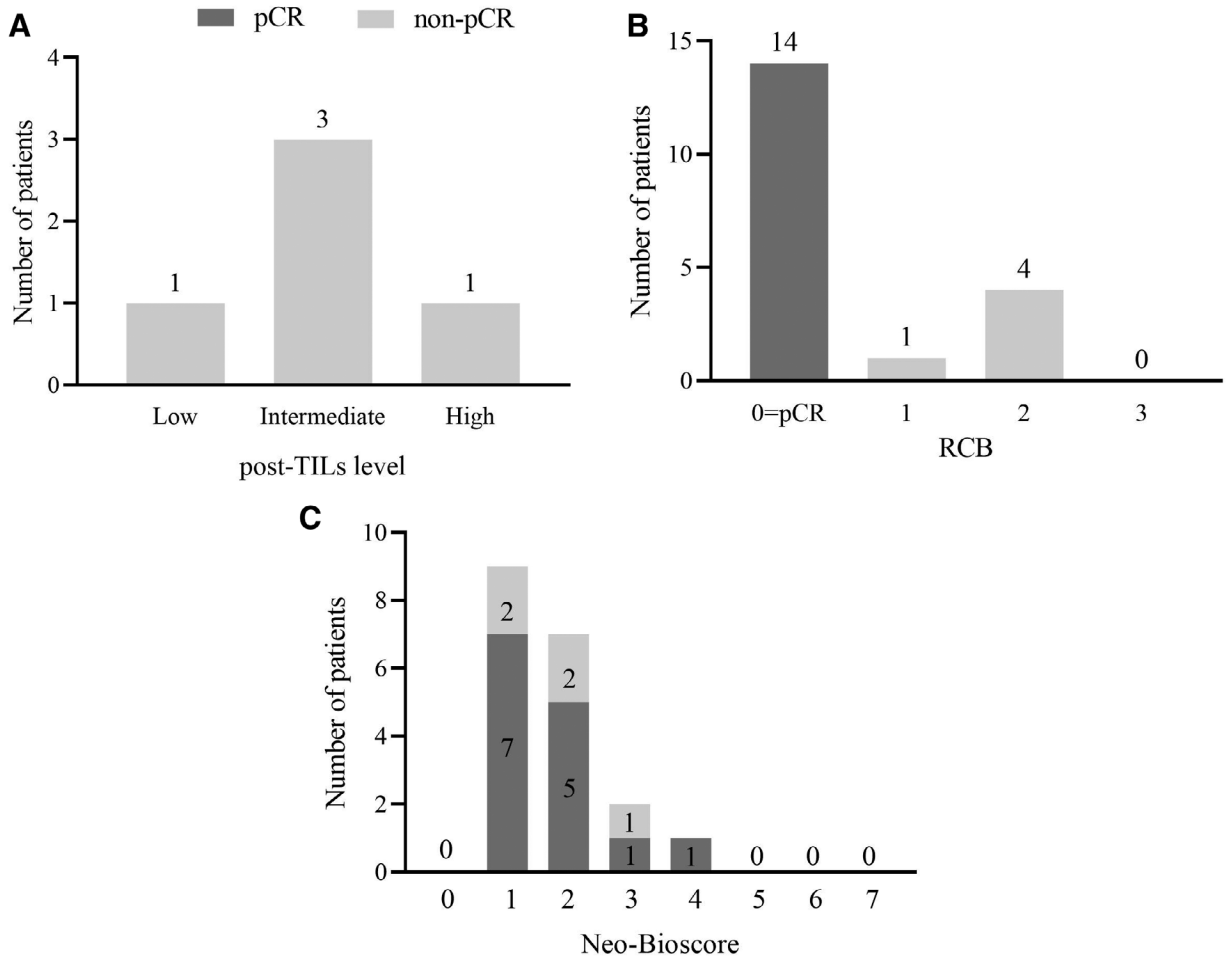

Figure 5. Distribution of patients in the different response scoring systems. **(A):** Distribution of posttreatment tumor-infiltrating lymphocyte levels in patients with residual disease. **(B):** Distribution of the RCB scores. **(C):** Distribution of the Neo-Bioscore scores. Dark gray indicates pCR; light gray indicates residual disease.
Abbreviations: pCR, pathological complete response; post-TILs, posttreatment tumor-infiltrating lymphocytes; RCB, residual cancer burden.