

Review Article

Mechanism, safety and efficacy of three tyrosine kinase inhibitors lapatinib, neratinib and pyrotinib in HER2-positive breast cancer

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Abstract: The incidence of breast cancer ranks first among female malignant tumors that affect women's health. Epidermal growth factor receptor (EGFR) family overexpression, especially human epidermal receptor2 (HER2), features prominently in breast cancer with a significant relation to poor prognosis. Currently, specific monoclonal antibodies and tyrosine kinase inhibitors (TKIs) are the two HER2 targeting strategies that have successfully improved the prognosis of patients with HER2-positive breast cancer. This paper focuses on three officially approved TKIs for HER2 breast cancer, namely, lapatinib, neratinib and pyrotinib, and systematically reviews the mechanism, safety, efficacy and resistance of these TKIs.

Keywords: HER2-positive breast cancer, lapatinib, neratinib, pyrotinib, tyrosine kinase inhibitors

Introduction

Breast cancer is the most common malignant tumor for women [1]. It has four molecular subtypes based on immunochemistry, including Luminal A, Luminal B, HER2-enriched and Triple negative. Luminal A refers to estrogen receptor (ER) and/or progesterone receptor (PR) positive with Ki-67 less than 14%; Luminal B (HER2+) refers to ER and/or PR with HER2 positive, while Luminal B (HER2-) refers to ER and/or PR, with HER2 negative and Ki-67 more than 14%; HER2-enriched refers to both ER and PR negative and HER2 positive; Triple negative, also called basal-like, refers to ER, PR and HER2 negative [2]. HER2 overexpression or gene amplification represents 15-20% of all breast cancer cases, which is closely related to aggressive phenotypes and poor outcomes [3, 4]. In 2018, American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) updated the guideline recommendations for HER2 testing in breast cancer and advocated the improvement of the accuracy of HER2 testing by immunohistochemistry (IHC) or in situ hybridization (ISH). HER2 positive criteria

were defined as HER2 protein overexpression (IHC, microscopic field of vision > 10% of adjacent homogeneous tumor tissue cell region with complete and intense circumferential membrane staining) or gene amplification (ISH, average HER2 copy number ≥ 6.0 signals/cell or average HER2 copy number ≥ 4.0 signals/cell and HER2/chromosome enumeration probe 17 (CEP17) ratio ≥ 2.0). If indeterminate results appear, a reflex test using an alternative assay (IHC or ISH) is required. If the test results do not conform to other histological tests, they should be repeated. The test results from laboratories should be highly consistent with the validated HER2 test, and the test should be carried out in the laboratories certified by CAP or other authorized institutions [5].

EGFR family

Human epidermal growth factor receptor 2 (HER2/ErbB2/NEU) is a transmembrane protein in human cells encoded by oncogene ERBB2 [6], which is a member of human epidermal growth factor receptor (EGFR/ERB) family of tyrosine kinase receptors, along with epider-

mal growth factor receptors (EGFR)/HER1/ErbB1, HER3/ErbB3, and HER4/ErbB4.

The molecular structure of EGFR family consists of a large extracellular region, a single spanning transmembrane (TM) domain, an intracellular juxtamembrane (JM) region, a tyrosine kinase domain and a C-terminal regulatory region [7], while HER3 is the only tyrosine kinase-defective receptor [8]. The extracellular domain of EGFR family can bind to 11 ligands, but the ligand of HER2 is still unidentified [9]. In most cases, the combination of extracellular regions and ligands result in receptor-mediated dimerization of EGFRs, but it is widely believed that HER2 undergoes ligand-independent heterodimerization with other 3 EGFR family members because of its constitutively active conformation [10], or homodimerization in the cases of high concentration [11]. Furthermore, because of the lack of tyrosine kinase, HER3 homodimer shows no signaling transition. Besides inactive HER3 homodimer, the signal of HER1, HER2 and HER4 homodimer is weak compared with HER2 heterodimer, and HER2 dimer is formed prior when HER2 is over-expressed [12].

Homodimer and heterodimer formation brings the intracellular domains closer to each other, leading to the asymmetric interaction of intracellular kinase domain between the amino-terminal lobe of one tyrosine kinase and the carboxy-terminal lobe of the other, and promoting the autophosphorylation of the tyrosine kinase domains [7]. Then, several pathways such as PI3K/Akt, MAPK, PLC γ , ERK1/2, JAK/STAT are activated, regulating differentiation, apoptosis, migration, growth and adhesion of normal cells [12]. MAPK and PI3K/Akt are the two main pathways activated by EGFR family, especially HER2 heterodimer, which feature prominently in breast cancer [13]. Activated MAPK pathway promotes relative gene transcription, subsequently improving proliferation, migration, differentiation, angiogenesis and drug resistance of cancer cells [14, 15]. And in PI3K/Akt pathway, phosphorylated Akt acts on a series of transcription factors including MDM2, mTOR, p27, GSK3 β , BAD, NF- κ B, FKHR, enhancing proliferation, survival, and suppressing apoptosis [16, 17]. The mechanism of HER2 targeted drugs and EGFR family in breast cancer is summarized in **Figure 1**.

Tyrosine kinase inhibitors (TKIs) targeting HER2

At present, there are two HER2 targeting strategies, namely, specific monoclonal antibodies and TKIs. And five drugs were officially approved by the U.S. Food and Drug Administration (FDA) for the treatment of HER2-positive breast cancer, known as trastuzumab, pertuzumab, trastuzumab emtansine (TDM-1), lapatinib and neratinib. Additionally, the Chinese State Drug Administration has recently authorized a new TKI, pyrotinib, for the treatment of patients with HER2-positive recurrence and metastasis breast cancer. TKI refers to a series of oral small molecular drugs active in promoting apoptosis and inhibiting proliferation of cancer cells. It competitively binds intracellular adenosine triphosphate (ATP) binding domains of EGFR family due to the homological structure of the ATP, resulting in inhibiting tyrosine kinase phosphorylation, subsequently blocking downstream signals [18]. TKI has the advantage of oral administration, multiple targets, and less cardiotoxicity compared with intravenous monoclonal antibodies. In terms of brain metastasis cancer treatment, the efficacy of monoclonal antibodies might be limited in crossing blood-brain-barrier (BBB), while small molecular TKIs, such as lapatinib, are thought to have the permeability through the BBB [19]. This paper reviews the mechanism, safety, efficacy and resistance of these three TKIs, namely, lapatinib, neratinib and pyrotinib. All the HER2 targeted drugs are summarized in **Tables 1 and 2**.

Lapatinib

Lapatinib is a TKI with the capacity of reversibly blocking HER1 and HER2. On March 13, 2007, lapatinib in combination with capecitabine obtained the approval of the FDA for HER2-overexpressed/amplified breast cancer patients who previously received therapies including anthracycline, taxane, and trastuzumab [20]. And in February 2010, therapeutic regimen of lapatinib plus letrozole attained the approval of the FDA as a first-line therapeutic option for the post-menopausal women with co-expressing hormone receptors and HER2 metastatic breast cancer.

Mechanism: Lapatinib restricts phosphorylation of HER1 and HER2 by reversibly and competitively inhibiting ATP-binding sites of the in-

Tyrosine kinase inhibitors in HER2-positive breast cancer

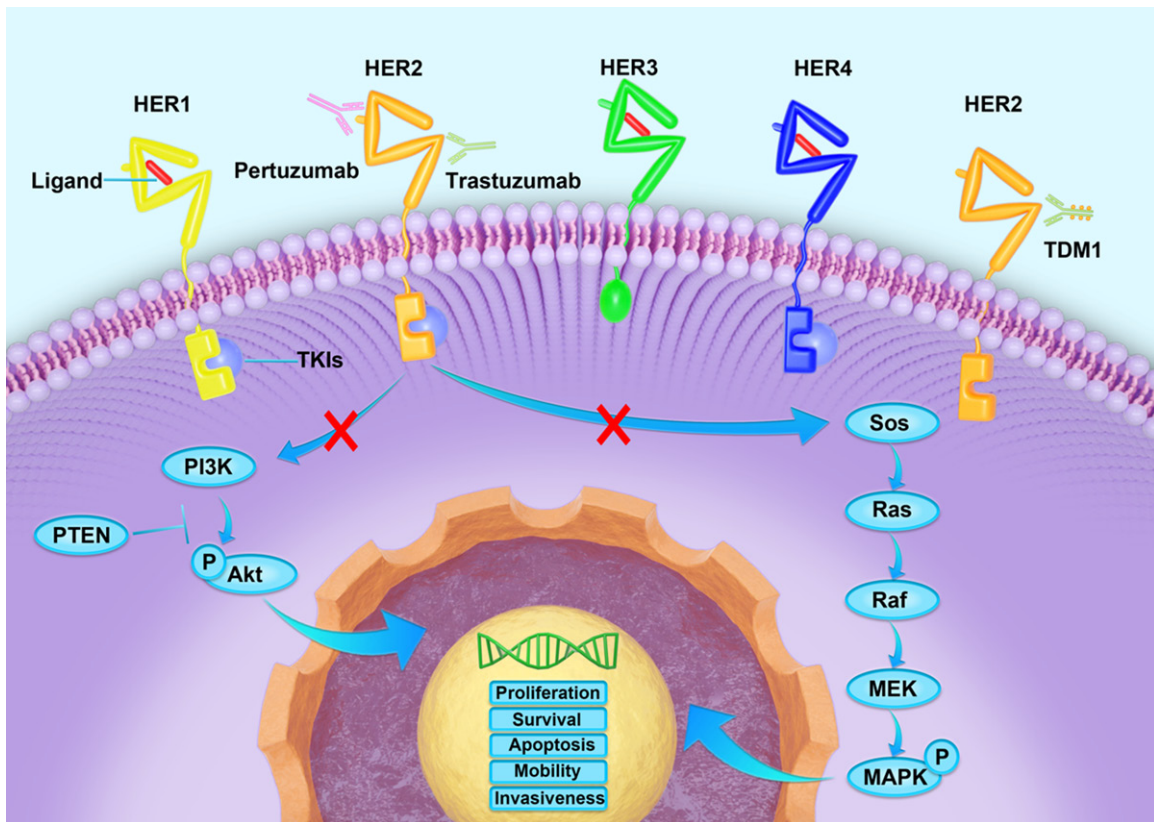


Figure 1. Mechanism of HER2 targeted drugs and EGFR family in breast cancer. Trastuzumab, pertuzumab and TDM-1 bind to the juxtamembrane domain of HER2. Lapatinib is reversible TKI of HER1 and HER2, while neratinib and pyrotinib are irreversible HER 1, 2 and 4 inhibitors. These drugs inhibit downstream signals of EGFR family, especially PI3K/Akt and MAPK pathway, improving proliferation, survival, migration, angiogenesis, drug resistance and suppressing apoptosis of cancer cells.

tracellular kinase region and subsequently interrupting the downstream signals, namely, Raf, AKT, ERK and PLC γ , resulting in a significant efficacy in inducing the apoptosis and restricting the development and migration of HER2-overexpressing cancer cells [21, 22]. But the HER1 expression level is irrelevant to the antineoplastic effect of lapatinib in HER2-overexpressing breast cancer cells [23]. Moreover, drug interaction of lapatinib and trastuzumab combination was discovered in vitro, and lapatinib still plays an antitumor role in cell lines with trastuzumab resistance [22].

Safety: The safety of lapatinib was estimated by several clinical researches in a single dose or in the combination therapy [24-29]. 1250 mg once daily in combination with capecitabine, and 1500 mg once daily in combination with letrozole, were considered as safe and effective dosages of lapatinib. The most frequent adverse events (AEs) are grade I/II, including

diarrhea, nausea, fatigue, vomiting, anorexia, rash, and musculoskeletal pain. And grade 3/4 diarrhea is the most severe AE. Furthermore, left ventricular ejection fraction (LVEF) reduction, as well as the elevation of ALT and AST, was observed in patients [24, 27], which means a potential hepatic and cardiac toxicity of lapatinib.

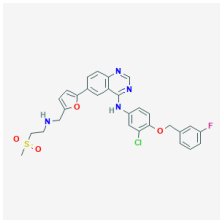
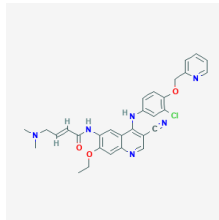
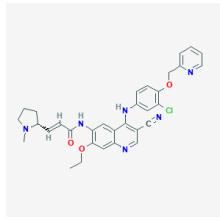
Salvage treatment: Lapatinib monotherapy was primarily assessed in some phase II studies, revealing marginal antitumor activity with tolerated toxic effects [25, 27-30]. However, stronger efficacy of lapatinib in combination with other antineoplastic drugs was witnessed in some phase III trials. One phase III research (NCT00078572) evaluated lapatinib and capecitabine combination therapy versus capecitabine monotherapy in women with HER2-positive metastatic breast cancer who previously received treatment with anthracycline, a taxane, and trastuzumab [31]. 324 participants

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Table 1. Summary of HER2 targeted drugs

Drug	Brand Name	Monoclonal Antibodies	Route of Administration	TKIs	Antibody-drug Conjugate	Target			
						HER1	HER2	HER3	HER4
Trastuzumab	Herceptin	√	Injection				+		
Pertuzumab	Perjeta	√	Injection				+		
Trastuzumab emtansine	Kadcyla		Injection		√		+		
Lapatinib	Tykerb		Oral	√		+	+		
Neratinib	Nerlynx		Oral	√		+	+		+
Pyrotinib	Irene		Oral	√		+	+		+

Table 2. Summary of HER2 Targeted TKIs

Drug	Lapatinib	Neratinib	Pyrotinib
Molecular Formula	$C_{29}H_{26}ClFN_4O_4S$	$C_{30}H_{29}ClN_6O_3$	$C_{32}H_{31}ClN_6O_3$
Chemical Structure			
Mechanism	Reversible	Irreversible	Irreversible
Target	HER1, HER2	HER1, HER2, HER4	HER1, HER2, HER4
In vivo efficiency	Moderate	Strong	Strong
Safety Window	-	-	High
MTD	1250 mg/1500 mg	240 mg	400 mg

were randomized to be administrated with combination therapy (n=163) or monotherapy (n=161). The results illustrated that lapatinib and capecitabine combination therapy extended the time to progression (TTP) versus capecitabine monotherapy (8.4 vs 4.4 months), accompanied with a prolonged progression-free survival (PFS) (8.4 vs 4.1 months) and a favored overall response rate (22% vs 14%). After an additional 75 patients participated in this study by the time enrollment was suspended, the latest analysis of 399 women was carried out [32]. Updated TTP was 6.2 months for combination therapy and 4.3 months for capecitabine alone, and the overall response rate was 24% versus 14%, respectively.

The same therapeutic combination was also evaluated in another phase III trial in patients with progression on trastuzumab treatment, compared with capecitabine alone [33, 34]. It was found that there was no dominant difference of median overall survival (OS) between 75 weeks and 64.7 weeks. But the median TTP

of lapatinib plus capecitabine (31.3 weeks) was longer than capecitabine monotherapy (18.6 weeks) in patients who received only one previous trastuzumab-based treatment. However, another phase III trial (NCT00820222), CERE-BEL, demonstrated there was no differences between lapatinib and trastuzumab both in combination with capecitabine, in terms of occurrence of central nervous system (CNS) metastases [35]. Moreover, EMILIA trial illustrated lapatinib plus capecitabine failed in challenging trastuzumab emtansine with less efficacy and tolerability [36]. Trastuzumab emtansine delayed the time to symptom worsening compared with lapatinib and capecitabine combination arm (7.1 vs 4.6 months), and more patients in trastuzumab emtansine group showed clinically significant improvement than those in combination groups (55.3% vs 49.4%).

In a phase III study (NCT00281658), lapatinib plus paclitaxel dramatically improved median OS (27.8 vs 20.5 months) of patients with HER2 metastasis breast cancer, as well as median

PFS (9.7 vs 6.5 months), compared with paclitaxel and placebo [37]. Another phase III trial also showed an improved objective response rate (ORR) (63.3% vs 37.8%), clinical benefit rate (CBR) (69.4% vs 40.5%) and TTP (36.4 vs 25.1 weeks) in patients of lapatinib and paclitaxel combination group versus lapatinib plus placebo [38]. In another phase III trial, improved PFS was observed in lapatinib plus trastuzumab combination treatment as 12.0 weeks versus 8.1 weeks, as well as favored CBR (24.7% vs 12.4%) and OS (51.6 vs 39.0 weeks) compared with lapatinib monotherapy, in HER2-positive metastatic breast cancer patients who had previously progressed on trastuzumab-based therapy [24].

Moreover, lapatinib and letrozole combination therapy was found to have dramatically improved PFS of patients with hormone receptors and HER2 co-expressed metastatic breast cancer, compared with placebo plus letrozole (8.2 vs 3.0 months), as well as CBR (48% vs 29%) [39]. In an ALTERNATIVE trial, 355 patients with HER2 and ER positive breast cancer were randomly administered with lapatinib, trastuzumab or trastuzumab plus lapatinib, both combined with aromatase inhibitor (AI) [40]. PFS of participants who received trastuzumab plus lapatinib and AI had a longer PFS in contrast with trastuzumab plus AI (11 vs 5.7 months). And PFS of lapatinib plus AI group was also higher than trastuzumab plus AI group (8.3 vs 5.7 months).

Adjuvant treatment: Lapatinib monotherapy as adjuvant treatment versus placebo was estimated in HER2-positive early-stage breast cancer female patients by TEACH research (NCT00374322), a randomized, controlled and multicenter phase III trial [41]. 3147 participants who received prior adjuvant chemotherapy without trastuzumab were administered with lapatinib (n=1571) or placebo (n=1576). Eventually, only 2490 patients were confirmed HER2-positive after central review, and DSF event is 13% (157 of 1230) in lapatinib group and 17% (208 of 1260) in placebo group (HR 0.82, 95% 0.67-1.00; P=0.04), showing a modest antitumor efficacy. And in ALTO phase III research (NCT00490139), 8381 HER2-positive early stage breast cancer patients were enrolled and randomly assigned to receive one-year adjuvant treatment of trastuzumab (n=2097), lapa-

tinib (n=2100), trastuzumab plus lapatinib (n=2093), or their sequence (n=2091). Eventually, noninferiority of improving DFS and OS and increased toxicity were found in the combination treatment and the sequence treatment compared with trastuzumab monotherapy [42].

Neoadjuvant treatment: In a non-comparative, randomized, phase II trial, CHERLOB study, neoadjuvant therapy of preoperative chemotherapy plus trastuzumab and lapatinib relatively increased pCR rate (46.7% vs 25% vs 26.3%), in contrast with chemotherapy combined with trastuzumab or lapatinib [43]. Another phase II randomized neoadjuvant study (NCT00429299) estimated the safety and efficacy of letrozole plus lapatinib in contrast with letrozole plus placebo in 92 postmenopausal women with stage II to IIIA hormone receptor-positive/HER2-negative breast cancer. Eventually, neither of the two groups achieved a pathological complete response (pCR), but a higher clinical response rate was concluded out in the letrozole and lapatinib combination group as 70% compared with letrozole plus placebo (63%) [44]. In a randomized, five-arm phase II research, 215 HER2-positive breast cancer patients were enrolled and randomly assigned therapy consisting of trastuzumab, lapatinib, and paclitaxel neoadjuvant treatment, with or without prolongation of anti-HER2 therapy, and with or without endocrine treatment in ER-positive patients. Though there was no divergence in pCR rate between the prolonged exposure groups and the standard groups, ER-negative patients had a superior comprehensive pathological complete response (CpCR) rate than ER-positive patients (63.0 vs 36.1%) [45]. However, in a randomized phase III trial (NCT00567554), GeparQuinto, lapatinib as a neoadjuvant treatment in contrast with trastuzumab both in combination with epirubicin and cyclophosphamide followed by docetaxel, were valued in untreated HER2-positive operable or locally advanced breast cancer patients. pCR rate of lapatinib group was dominantly lower than trastuzumab group as 22.7% to 30.3% respectively [46].

NeoALTO (NCT00553358), a randomized and multicenter phase III trial, assessed neoadjuvant combination therapy of lapatinib plus trastuzumab in HER2-positive early breast cancer participants [47, 48]. 455 eligible women from

23 countries were randomized to receive lapatinib (n=154), trastuzumab (n=149), or lapatinib plus trastuzumab (n=152) at the first 6 weeks and weekly paclitaxel for a further 12 weeks. Then after definitive surgery, lapatinib and trastuzumab dominantly ascended patients' pCR rate compared with trastuzumab monotherapy and lapatinib monotherapy (51.3% vs 29.5% vs 24.7%), but increased toxicity of grade 3 diarrhea and liver-enzyme alterations. After 3.77 years' follow-up, 3-year EFS of lapatinib, trastuzumab and combination group was 78%, 76% and 84%, respectively, and 3-year OS was 93%, 90% and 95%, respectively. Though EFS and OS made no difference between treatment groups, longer EFS and OS were observed in participants who came through pCR compared with those who did not.

Neratinib (HKI-272)

Neratinib (HKI-272), whose brand name is Nerlynx, is an irreversible TKI of HER1, HER2, and HER4. On July 17, 2017, neratinib was approved by the FDA as an extended adjuvant treatment for patients with early-stage HER2-overexpressed/amplified breast cancer after surgery and trastuzumab-based adjuvant therapy.

Mechanism: Neratinib inhibits phosphorylation of ErbB family as well as downstream pathways including ERK and Akt, by covalently combining with cysteine residues Cys-773 and Cys-805 of ATP-binding domain of HER1, HER2, and HER4 [49]. The inhibition of downstream signal transduction after neratinib treatment causes reduced phosphorylated retinoblastoma protein and cyclin D1 expression and ascending p27 level, and then arrests the G1-S phase transition, eventually resulting in the down-regulation of cell proliferation [50]. In addition, neratinib can also down-regulate HER2 expression via leading to HSP90 dissociation and subsequently inducing ubiquitylation and endocytic degradation [51]. Moreover, a study found that neratinib can inhibit ATP-binding cassette transporter and thereafter reverse multidrug resistance of cancer cells [52].

Safety: The safety of neratinib was estimated by several trials [53-60] as a single drug or plus other antitumor agents. Maximum tolerated dose (MTD) of neratinib was assessed by dose escalation in these trials, and 240 mg

orally once a day was designated as the recommended dosage. Common adverse reactions include diarrhea, nausea, vomiting, fatigue, stomachache, headache, rash, decreased appetite, muscle spasms and dizziness at grade 1 or 2. And diarrhea is the most frequent AE from grade 1 to 3. There was almost no grade 4 AE. Furthermore, elevated AST and ALT level was also observed in a small part of the participants.

Salvage treatment: For patients who received prior trastuzumab and had stage IIIB, IIIC, or IV HER2-positive breast cancer, a phase II trial (NCT00777101) recruited 233 participants and explored the safety and efficacy of neratinib monotherapy versus lapatinib plus capecitabine. In consequence, neratinib monotherapy illustrated no non-inferiority in prolonging PFS and OS compared with lapatinib plus capecitabine [61]. And another phase II study (NCT01494662) investigated the efficacy of neratinib in the treatment of HER2-positive breast cancer patients with brain metastasis and progression on one or more lines of CNS targeted therapy [62]. However, low CNS ORR (8%) and median PFS (1.9 months) were excluded from in neratinib monotherapy. And in NEFERT-T trial (NCT00915018), 479 women patients were randomized to receive neratinib plus paclitaxel or trastuzumab plus paclitaxel. It turned out that neratinib plus paclitaxel did not lengthen the PFS of patients in contrast with trastuzumab plus paclitaxel, but might delay CNS metastasis and reduce the CNS recurrence [63].

Adjuvant treatment: An inspiring result was found in a multicenter, randomized phase III study of extended neratinib adjuvant therapy, ExteNET trial (NCT00878709) [60, 64]. 2840 eligible female patients who had early-stage breast cancer and had completed over 2-year neoadjuvant and adjuvant trastuzumab treatment were finally enrolled and randomly assigned to receive neratinib (n=1420) or placebo (n=1420). Patients received 240 mg neratinib orally once daily for one year, versus placebo. Finally, 2816 patients (1408 in neratinib group, 1408 in placebo group) completed the trial and were included in the safety analysis. After two-year and five-year follow up, neratinib adjuvant therapy revealed an arresting diminution in the recurrence rate of breast cancer

compared with placebo group (93.9% vs 91.6% and 90.2% vs 87.7% iDFS, respectively).

Neoadjuvant treatment: An adaptive randomized phase II study, I-SPY-2, explored neoadjuvant of neratinib in high-risk breast cancer [65]. I-SPY-2 is an experimental platform for evaluating the efficacy of new drugs for different breast cancer subtypes in a relatively short period of time, by using data from a small number of patients receiving neoadjuvant chemotherapy. Thus, appropriate drugs would be administered prior to the subgroup with specific molecular signature. Participants with HER2 overexpressed and hormone receptor-negative breast cancer received 12-week paclitaxel plus 4 cycles of doxorubicin and cyclophosphamide every 2 to 3 weeks, and randomly received neratinib (n=115) or trastuzumab (n=78) in the first 12 weeks. In consequence, pCR rate of neratinib group was 56% (37% to 73%) compared with control group, 33% (11% to 54%). Neratinib and/or trastuzumab plus chemotherapy as neoadjuvant therapy in locally advanced HER2-positive breast cancer was investigated by a phase II study (NCT01008150), NSABP-FB7. 141 patients were enrolled and randomized to be administered with neratinib, or trastuzumab, or neratinib plus trastuzumab both combined with weekly paclitaxel and followed by doxorubicin and cyclophosphamide. The result was announced at the San Antonio breast cancer symposium in December 2015 [66]. The overall pCR rate was 38.1%, 33.3% and 50.0%, respectively. And pCR rate of HER2-positive and hormone receptor-negative patients were higher at 57.1%, 46.2% and 73.7%, respectively.

Pyrotinib (SHR1258)

Pyrotinib (SHR1258), whose brand name is Irene, is a new generation of anti-HER2 therapeutic target drug developed by Jiangsu Hengrui Pharma. In August 2018, Chinese State Drug Administration first conditionally approved pyrotinib for the combination treatment of capecitabine for patients with HER2-positive advanced or metastatic breast cancer and those who had received anthracycline or taxane chemotherapy previously.

Mechanism: It is a small-molecular irreversible dual pan-ErbB TKI with activity against HER1 (IC₅₀, 5.6 ± 3.9 nM), HER2 (IC₅₀, 8.1 ± 2.3

nM), and HER4 [67]. By covalently binding with ATP binding sites of intracellular kinase regions, the drug inhibits the formation of homologous/heterodimer and auto-phosphorylation of HER family, thus blocking the activation of RAS/RAF/MEK/MAPK, PI3K/AKT signaling pathways and tumor cell cycle in G1 phase and restricting tumor development.

Kai Zhang et al. also found the combination of palbociclib, a CDK4/6 inhibitor, and pyrotinib showed a synergistic effect of proliferation and colony formation suppression both in trastuzumab-sensitive and trastuzumab-resistant cell lines of human HER2-positive breast cancer. The combined treatment promotes RB P70-S6K phosphorylation and suppresses AKT and HER3 phosphorylation, inducing G0-G1 cell cycle arrest and apoptosis. Furthermore, in the xenograft model experiment, the combination of palbociclib and pyrotinib corroborate inferior antitumor activity compared with either agent alone without manifest toxicity increase [68].

Preclinical study: The pharmacokinetics of pyrotinib was investigated in two researches [69, 70]. After one single oral administration, pyrotinib is absorbed into blood in 1 hour, and reaches peak blood concentration in 4 hours, while after multiple oral administrations, the plasma concentration gets to a plateau on day 8. Pyrotinib is transported in blood plasma by covalently binding with the amino acid residue Lys190 of human serum albumin. Between the 4th and 12th hour, pyrotinib is practically metabolized into 24 products, but the parent drug still accounts for the largest part in circulation. Among these metabolites, SHR150980 (M1), SHR151468 (M2), and SHR151136 (M5) are the three major substances detected in plasma. The enzyme CYP3A4 features prominently in biotransformation of pyrotinib. The elimination of pyrotinib is quickly processed within 36 hours, and no metabolites can be detected after that. Eventually, pyrotinib and its metabolites are excreted in feces (90.9%) and urine (1.7%) as the intact parent drug.

Clinical study: Hitherto, one phase I and one phase I/II clinical trial about pyrotinib in patients with HER2-positive metastatic breast carcinoma have been completed. The safety, efficacy, pharmacokinetics, and biomarkers of pyrotinib were investigated by the first-in-patients phase I study (NCT01937689) [67]. A total of 38

HER2-positive metastatic breast cancer patients without prior exposure to TKIs were selected to receive pyrotinib orally once daily, in the dosage of 80 mg (n=3), 160 mg (n=8), 240 mg (n=8), 320 mg (n=9), 400 mg (n=8) or 480 mg (n=2). Eventually, the MTD was designated as 400 mg according to the study. The most common AE was diarrhea, followed by nausea, oral ulceration, asthenia, and leukopenia, with the percentage of 44.7%, 13.2%, 13.2%, 10.5% and 10.5%, respectively. And the most severe AE was grade 3 diarrhea, observed in 5 patients, which is dosage limiting. And only 2 patients administered with 480 mg of pyrotinib discontinued the treatment due to diarrhea. Pharmacokinetics analysis illustrated median Tmax (3.00-5.00 hours) and mean t_{1/2} (11.4-15.9 hours) of pyrotinib after the first dosage intake from 80 mg to 400 mg. The peak of multiple-dose plasma concentration stabilized on day 8, and the exposure was 1.22-1.57 times that of a single dose. Thus, dose dependence rather than major accumulation of pyrotinib exposure was confirmed by these pharmacokinetic results. Meanwhile, the antitumor activity of pyrotinib was primarily assessed in this trial. Two patients in 480 mg who discontinued treatment because of diarrhea were excluded, and the rest 36 patients were enrolled in further analysis of efficacy. The overall response rate was 50.0%, and the median PFS was 35.4 weeks. Interestingly, trastuzumab-naïve patients had a considerably higher overall response rate compared with trastuzumab-pretreated patients as 83.3% (10 of 12) versus 33.3% (8 of 24).

In a randomized, open, controlled I/II clinical study, the efficacy and safety of pyrotinib plus capecitabine in contrast with lapatinib plus capecitabine were evaluated in the treatment of HER2-positive recurrent or metastatic breast cancer [71]. 128 participants previously administered with anthracycline or taxanes (including adjuvant therapy or relapse and metastasis therapy) and no more than 2 lines of chemotherapy after relapse/metastasis were recruited in this trial. Participants were randomized to receive pyrotinib (400 mg once daily) plus capecitabine (1000 mg/m² twice daily) (n=65) or lapatinib (1250 mg once daily) plus capecitabine (1000 mg/m² twice daily) (n=63). Gastrointestinal reactions, skin reaction, metabolic and nutritional diseases, hepatobiliary diseases,

systemic reactions, blood system diseases were common AEs of combination treatment of pyrotinib and capecitabine, and diarrhea accounts for 96.9%, the largest proportion, followed by palmoplantar erythrodysesthesia (78.5%), vomiting (46.2%), nausea (38.5%), anorexia (32.3%), oral mucositis (29.2%). Grade 3 AE was only diarrhea, whose incidence rate was 13.2%. And no over grade 4 AEs were found. ORR was 78.5% (n=51) of the combination therapy of pyrotinib and capecitabine and 57.1% (n=36) of the combination therapy of lapatinib and capecitabine. PFS of investigators assessment was 18.1 months versus 7.0 months, while the Independent Review Committee (IRC) assessment was 12.6 months and 5.6 months, respectively. Moreover, efficacy outcomes were further analyzed in subgroup based on whether or not trastuzumab had been used in the past. The combination of pyrotinib plus capecitabine revealed a dramatically anti-tumor efficacy of HER2-positive recurrence or metastasis breast carcinoma.

The result of phase III trial assessing pyrotinib versus placebo both in combination with capecitabine in women with HER2-positive metastatic breast cancer who received prior taxanes and trastuzumab therapy was reported at ASCO in June 2019. Patients were randomly assigned to be administered with pyrotinib plus capecitabine (n=185) or placebo plus capecitabine (n=94). The median PFS for the combination group was 11.1 months, and that for placebo group was 4.1 months. Furthermore, 71 patients in placebo group whose disease progressed received pyrotinib monotherapy afterwards, revealing single drug response rate of 38.0% and the median PFS of 5.5 months [72].

Additionally, there are many clinical trials underway to further confirm the efficacy and safety of pyrotinib (<https://clinicaltrials.gov>, <http://www.chictr.org.cn>) (**Table 3**).

Mechanism of resistance

At present, intrinsic and acquired resistance of lapatinib is already found in patients. Multiple genes and pathways function in lapatinib resistance, including EGFR family, PI3K/Akt/mTOR, Ras/Raf/MEK/MAPK, FOXM1/FOXO3a, eEF2/PP2A, autophagy, tumor metabolism and other members of receptor tyrosine kinase family

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Table 3. Clinical trials of pyrotinib in HER2 breast cancer treatment

NCT Number	Study Type	Trial Arm	Subjects	Patients	Status	Location
NCT01937689	I	Pyrotinib	40	HER2-positive advanced breast cancer	Completed	China
NCT02361112	I	Pyrotinib + capecitabine	38	HER2-positive metastatic breast cancer	Completed	China
NCT02500199	I	Pyrotinib	50	HER2-positive advanced solid tumors, including breast cancer, non-small cell lung cancer	Recruiting	USA
NCT03772353	I/II	Pyrotinib + letrozole + SHR6390	32	HER2-positive and HR-positive relapsed or metastatic breast cancer	Recruiting	China
NCT02422199	I/II	Pyrotinib + capecitabine vs lapatinib + capecitabine	128	HER2-positive metastatic breast cancer	Active, not recruiting	China
NCT03805399	I/II	Pyrotinib + capecitabine	140	Luminal androgen receptor subtype triple-negative and HER2 mutation breast cancer	Recruiting	China
NCT04001621	II	Pyrotinib + capecitabine	100	HER2-positive advanced breast cancer with Trastuzumab-resistant	Recruiting	China
NCT03923179	II	Pyrotinib + etoposide	32	HER2-positive advanced breast cancer	Recruiting	China
NCT03993964	II	Pyrotinib + CDK4/6 Inhibitor	20	HER2-positive metastatic breast cancer	Not yet recruiting	China
NCT03933982	II	Pyrotinib + vinorelbine	30	HER2-positive CNS metastatic breast cancer	Recruiting	China
NCT03919253	II	Pyrotinib + nab-paclitaxel	80	HER2-positive advanced breast cancer	Not yet recruiting	China
NCT03735966	II	Pyrotinib + trastuzumab + docetaxel	51	HER2-positive early stage or locally advanced breast cancer	Not yet recruiting	China
NCT03923166	II	Pyrotinib+ capecitabine	35	HER2-positive advanced breast cancer	Recruiting	China
NCT03691051	II	Pyrotinib + capecitabine	102	HER2-positive metastatic breast cancer	Not yet recruiting	China
NCT03997539	II	Pyrotinib + vinorelbine vs Treatment of Physician's Choice	256	HER2-positive locally advanced or metastatic breast cancer	Not yet recruiting	China
NCT03412383	II	Pyrotinib	14	HER2 non-amplified but HER2 mutant metastatic breast cancer	Recruiting	China
NCT03876587	II	Pyrotinib + docetaxel	79	HER2-positive metastatic breast cancer	Not yet recruiting	China
NCT03910712	II	Pyrotinib+ trastuzumab + aromatase inhibitor vs trastuzumab + aromatase inhibitor	250	HER2-positive and HR-positive metastatic or inoperable locally advanced breast cancer	Not yet recruiting	China
NCT03980054	III	Pyrotinib vs placebo	1192	HER2-positive early stage or locally advanced breast cancer	Not yet recruiting	China
NCT03080805	III	Pyrotinib + capecitabine vs Lapatinib + capecitabine	240	HER2-positive metastatic breast cancer	Recruiting	China
NCT02973737	III	Pyrotinib + capecitabine vs placebo + capecitabine	350	HER2-positive early stage or locally advanced breast cancer	Active, not recruiting	China
NCT03588091	III	Pyrotinib + trastuzumab + docetaxel vs placebo + trastuzumab + docetaxel	294	HER2-positive early stage or locally advanced breast cancer	Recruiting	China
NCT03863223	III	Pyrotinib + trastuzumab + docetaxel vs placebo + trastuzumab + docetaxel	590	HER2-positive metastatic breast cancer	Not yet recruiting	China
NCT03908749	-	Pyrotinib	300	HER2-positive advanced breast cancer	Not yet recruiting	China
NCT03947242	Not Applicable	Pyrotinib + trastuzumab + vinorelbine	48	HER2-positive early stage or locally advanced breast cancer	Not yet recruiting	China
NCT03847818	Not Applicable	Pyrotinib + trastuzumab + docetaxel + carboplatin	268	HER2-positive early stage or locally advanced breast cancer	Not yet recruiting	China
NCT03756064	Not Applicable	Pyrotinib + trastuzumab + docetaxel + carboplatin vs placebo + trastuzumab + docetaxel + carboplatin	100	HER2-positive early stage or locally advanced breast cancer	Not yet recruiting	China
ChiCTR1900022844	I	Pyrotinib + different chemotherapy regimens	60	HER2 positive advanced breast cancer	Not yet recruiting	China
ChiCTR1900022557	I	Pyrotinib + chemotherapy	40	HER 2-positive and HR low-positive/negative advanced recurrent/metastatic breast cancer	Not yet recruiting	China
ChiCTR1800020217	II	Epirubicin + cyclophosphamide + pyrotinib + sequential docetaxel + trastuzumab + pyrotinib	60	HER2-positive early stage or locally advanced breast cancer	Recruiting	China

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ChiCTR1900023653	II	Pyrotinib + paclitaxel(albumin-binding)	55	HER2 positive advanced breast cancer	Not yet recruiting	China
ChiCTR1900022293	III	Epirubicin + cyclophosphamide + pyrotinib + trastuzumab vs epirubicin + cyclophosphamide + trastuzumab	210	Stage I to III HER2-positive breast cancer	Not yet recruiting	China
ChiCTR1800020226	III	Pyrotinib + trastuzumab + carboplatin + docetaxel vs trastuzumab + carboplatin + docetaxel	336	HER2-positive early stage or locally advanced breast cancer	Not yet recruiting	China
ChiCTR1900021819	IV	Pyrotinib	1000	HER2-positive locally advanced breast cancer	Not yet recruiting	China
ChiCTR1900020670	IV	Pyrotinib + standard treatment	48	HER2-positive brain metastatic breast cancer	Not yet recruiting	China
ChiCTR1800020449	IV	Pyrotinib + trastuzumab + paclitaxel + cisplatin	40	HER2-positive early stage or locally advanced breast cancer	Not yet recruiting	China
ChiCTR1900023152	IV	Pyrotinib	60	HER2 positive advanced breast cancer	Not yet recruiting	China

[73]. In a nutshell, activation of compensatory pathways, HER2 tyrosine kinase domain mutation and gene amplification of NIBP (TRAPPC9, trafficking protein particle complex 9) account for the three main mechanisms of lapatinib resistance [74]. Furthermore, phosphorylation-mediated reprogramming of glycolytic activity also features prominently in lapatinib resistance of breast cancer cell lines, and glycolysis inhibitors employment increases the sensitivity of resistant cells [75]. High PTEN or wild-type PIK3CA expression was found in most patients with HER2-positive breast cancers who were administrated with neoadjuvant lapatinib plus trastuzumab and finally achieved pCR [76]. On the contrary, low PTEN and PIK3CA mutations were associated with the resistance of neoadjuvant lapatinib and trastuzumab [77].

In terms of neratinib, Susan Breslin et al. found out that enhanced activity of metabolism enzyme cytochrome P4503A4 leads to neratinib resistance and cross-resistance to trastuzumab, lapatinib and afatinib [78]. One case report found that a HER2 mutant breast cancer patient, who attained HER2 gatekeeper mutation, had induced a neratinib resistance, after neratinib treatment [79]. And Seyhan et al. had identified a collection of genes related to neratinib resistance by using a genome-wide RNAi screen combined with a lethal dose of neratinib, including oncogenesis, transcription factors, cellular ion transport, protein ubiquitination, cell cycle, and genes known to interact with breast cancer-related genes [80].

The expression of RB1CC1, ERBB3, FOXO3a and NR3C1 was also upregulated in HER2 TKI-sensitive breast cancer cell lines after treatment of lapatinib, afatinib and neratinib [81]. And in phase I study of pyrotinib, after analyzing ctDNA and T-primary tumor tissues of 18 patients' blood samples, the authors suggested that PIK3CA or TP53 mutations in ctDNA were more related to the efficacy of pyrotinib [67, 82].

Side effects

Diarrhea is the most common side effect of these three TKI drugs, mostly grade 1 and 2. The mechanism of TKI-induced diarrhea is still unclear, which is different from chemotherapy induced intestinal epithelium injury. One of the hypothesis suggests that TKIs inhibit EGFR downstream signals of intestinal epithelium le-

ading to decreased growth and regeneration [83]. While another hypothesis involved in TKI reversing EGFR negative regulatory in chloride secretion and activates basolateral membrane potassium (K⁺) channels, resulting in chloride secretory diarrhea [84, 85]. At present, management of TKI-induced diarrhea resembles that of chemotherapy induced diarrhea, including patient education, dietary control and pharmacologic management, within valid assessment [86, 87]. Rash, nausea, vomiting, anorexia, fatigue and oral ulceration are also common AEs. Moreover, these drugs still have potential hepatic toxicity. Put slightly differently, neratinib can also cause headache and dizziness, and lapatinib might decrease LVEF of heart, and pyrotinib might cause leukopenia.

Conclusion and suggestions for future research

Currently, specific therapies for HER2-overexpression breast cancer include monoclonal antibodies and small molecular TKIs. Trastuzumab is the first monoclonal antibody used in HER2 specific therapy, but increased drug resistance and cardiotoxicity are its shortcomings. TKI has attracted public attention by the advantages of oral administration, multiple targets and low cardiotoxicity. More importantly, it might cross BBB as a potential therapy of HER2-positive breast cancer with CNS metastasis. The FDA has so far approved lapatinib and neratinib, and Chinese State Drug Administration has approved a new TKI, pyrotinib, in HER2 targeted treatment of breast cancer. A series of clinical trials have demonstrated that the TKIs are promising anti-HER2 drugs, especially in the terms of metastatic breast cancer. In the results of the current clinical studies, pyrotinib initially showed decent tolerance, safety and efficacy, though its efficacy needs to be confirmed by further research. Exploring new combination therapy of currently available HER2 targeted drugs, as well as in combination with PI3K inhibitor, AKT inhibitor, mTOR inhibitor, BET inhibitor, CDK4/6 inhibitor, or PD1/PDL1 antibodies, may also discover effective therapy for HER2 positive breast cancer.

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Disclosure of conflict of interest

None.

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