Tyrosine Kinase Inhibitors in the Combination Therapy of HER2 Positive Breast Cancer

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Abstract

Human epidermal growth factor receptor 2 (HER2)-positive breast cancer (BC) accounts for about 20% to 30% of all BC subtypes and is characterized by invasive disease and poor prognosis. With the emergence of anti-HER2 target drugs, HER2-positive BC patient outcomes have changed dramatically. However, treatment failure is mostly due to drug resistance and the special treatment needs of different subgroups. Small molecule tyrosine kinase inhibitors can inhibit multiple targets of the human epidermal growth factor receptor family and activate PI3K/AKT, MAPK, PLC γ, ERK1/2, JAK/STAT, and other pathways affecting the expression of MDM2, mTOR, p27, and other transcription factors. This can help regulate the differentiation, apoptosis, migration, growth, and adhesion of normal cells and reverse drug resistance to a certain extent. These inhibitors can cross the blood-brain barrier and be administered orally. They have a good synergistic effect with effective drugs such as trastuzumab, pertuzumab, t-dm1, and cyclin-dependent kinase 4 and 6 inhibitors. These advantages have resulted in small-molecule tyrosine kinase inhibitors attracting attention. The new small-molecule tyrosine kinase inhibitor was investigated in multi-target anti-HER2 therapy, showed a good effect in preclinical and clinical trials, and to some extent, improved the prognosis of HER2-positive BC patients. Its use could lead to a de-escalation of treatment in some patients, possibly preventing unnecessary procedures along with the associated side effects and costs.

Introduction

Human epidermal growth factor receptor (HER2)-positive breast cancer (BC) accounts for 20% to 30% of all BC[1] and is characterized by invasive disease and poor prognosis. HER2-positive criteria were defined as HER2 protein overexpression (immunohistochemistry (IHC): microscopic field >10% of the

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cell area of surrounding homogeneous tumor tissue, with intact and strong staining of peripheral membrane) or gene amplification (in situ hybridization (ISH), average HER2 copy number ≥ 6.0 signal/cell or average HER2 copy number ≥ 4.0 signal/cell and HER2/chromosome counting probe 17 (CEP17) ratio ≥ 2.0).[2,3] At present, targeted nanotechnology and new PET-CT technology are also integrated into the diagnosis of HER2-positive BC.

The introduction of trastuzumab (approved in 1998) humanized monoclonal antibody binding to extracellular domain IV of HER2 and has led to dramatic improvements in the prognosis of patients with HER2-positive BC. Currently, the FDA has approved trastuzumab, pertuzumab, ado-trastuzumab emtansine (T-DM1), lapatinib, and neratinib for the treatment of HER2-positive BC, and the Chinese Drug Administration has also approved pyrotinib for the therapy of HER2-positive BC.[4] According to the data of the neosphere trial, the efficacy of the treatment of patients with HER2-positive BC has led to a 60% pathological complete response (PCR) with the introduction of the THP (trastuzumab and pertuzumab plus docetaxel) regimen in the neoadjuvant setting. In addition, according to the data of the Cleopatra study, the overall survival (OS) of patients with HER2-positive metastatic BC can be up to about 5 years.[5] However, there is still a chance for recurrence and death, and drug toxicity and primary or acquired resistance may limit the application of these treatments. The annual risk is around 10% of patients with metastatic HER2-positive BC exhibiting central nervous system metastases, constituting the most common site of first-time recurrence.[6] Many patients who cannot tolerate systemic chemotherapy, like elderly people, need a better combination of de-escalation therapy, but the total number of elderly people who have been studied is low. Hence, further studies should be continued, especially for those who are not feeling well and have heart disease. Information on the safety and tolerability of new therapies targeting HER2 is needed in elderly patients. This is reflected in recently approved drugs such as T-DM1, pertuzumab and neratinib.

The tyrosine kinase inhibitors (TKIs) not only target multiple HER family members but also play a role in heterodimerization, compensatory crosstalk, and redundancy existing in the ErbB network.[7-10] HER2 and its homologous dimerization or heterodimerization with HER1, HER3, or HER4 play a significant role in cancer cell survival and proliferation. Because of the lack of tyrosine kinase, HER3 homodimer is less crucial. The signals of the HER1, HER2, and HER4 homodimers are weak compared with that of the HER2 heterodimer. The HER2 dimer is formed when HER2 is overexpressed.[11,12] Gene family mutations may have the potential to alter cellular behavior and efficacy of HER inhibitors and PI3 K inhibitors or other signaling panel inhibition. Somatic mutations in the genes promote oncogenesis and TKI resistance in HER2-positive BC.[13] Then, several pathways such as PI3K/AKT and MAPK are the main pathways activated by the ErbB family. In Figure 1, we show the current network mechanism of anti-HER2 therapy and the HER family.

At present, there are many TKIs for HER2-positive BC, and 4 of them have been approved for marketing. In 2007, lapatinib was approved by the FDA, in combination with the oral pyrimidine analogue capecitabine, for the treatment of patients with HER2-positive metastatic BC who were pretreated with anthracycline, trastuzumab, and a taxane.[14] In 2017, neratinib was approved by the FDA because of the resulting enhanced disease-free survival (DFS) in the ExteNET trial for extended therapy after 1-year adjuvant therapy with trastuzumab.[8,15] In 2018, pyrotinib was approved by the China Drug Administration for treatment of HER2-positive metastatic BC in patients who had received prior anthracyclines, taxanes, and/or trastuzumab.[9] In 2020, tucatinib was approved in the USA for the treatment of HER2-positive metastatic BC.[16] There are 2 TKIs (poziotinib, and afatinib), which although not approved, have shown good performance in many clinical trials. In this article, we will focus on these 6 drugs for the therapy of HER2-positive BC and their mechanisms, metabolism, trials, side-effects, biomarkers, resistance mechanisms, and future studies. These TKIs are in clinical development (Table 1).

Pertuzumab and trastuzumab act on the second and fourth extracellular domain of HER2 protein, T-DM1 targets binding HER2 protein, then DM1 enters into the cells through endocytosis, leading to DNA damage. Targeted therapeutic TKIs include pyrotinib, poziotinib, tucatinib, afatinib, neratinib, lapatinib, which have extensive inhibitory effects on her family. Through binding with RTK binding sites in the intracellular segment of cells, the downstream channels are mainly PI3 K / Akt / mTOR pathway, RAS / Raf / MEK / MAPK pathway, and PLC-γ pathway.TKIs first binds to RTK and then phosphorylates some tyrosine residues. The phosphorylated receptor binds to grb2-sos complex or PI3 K and then activates Ras protein, PLC—γ protein, PIP3, etc, finally, the downstream pathways would be activated. At the same time, there is cross-talk with the ER pathway.PI3 K / Akt / mTOR is mainly related to cell growth and proliferation. Ras / Raf / MEK / MAPK pathway has close relations to transcription and translation.

Lapatinib

Lapatinib, an oral, reversible TKI, inhibits HER1 and HER2. It inhibits the growth of tumor cells mainly through PI3K/Akt and MAPK pathways and connects HER1 and HER2 kinase domains, especially with adenosine triphosphate (ATP) binding sites. Lapatinib can inhibit the activation of tyrosine kinase and lead to new signal transduction through the HER receptor dimer and reduce RAF, ERK, Akt, and PLC γ 1 protein phosphorylation. Moreover, because of the accumulation of HER2 on the cell surface, it can promote trastuzumab-dependent ADCC.[37] It can inhibit the phosphorylation of p95her2, Akt, and MAPK and the growth of cells expressing truncated receptors. Lapatinib, in combination with capecitabine, was approved in 2007 for the treatment of patients with HER2-positive metastatic BC after the treatment with anthracyclines, taxanes, and trastuzumab.[14] In 2013, lapatinib, in combination
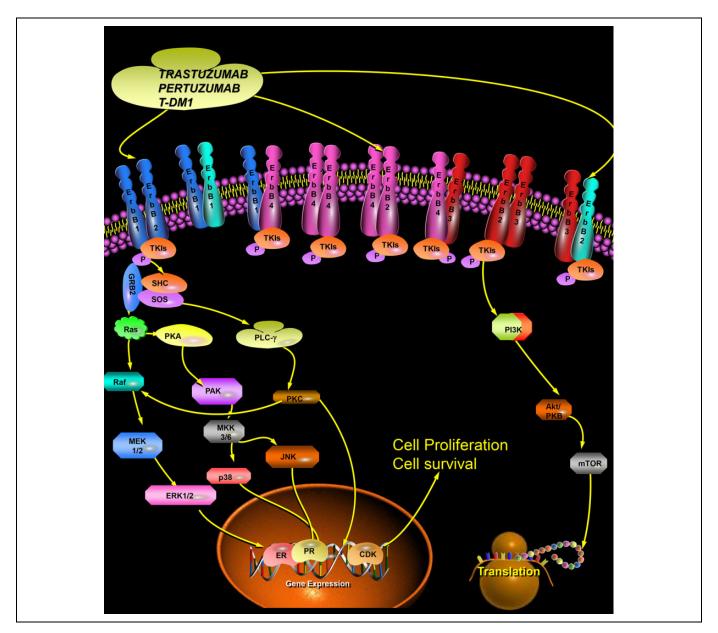
Figure 1. Anti HER2 therapy and HER family.

with trastuzumab, was approved as a chemotherapy-free combination for patients with hormone receptor (HR)-negative and HER2-positive advanced BC after standard treatment with trastuzumab and chemotherapy.[19] As for patients with triple-positive (HER2 and hormone receptor-positive) metastatic BC, for postmenopausal women, the approval was extended to a multi-blockade combination with letrozole.[38] Lapatinib has tolerated toxicity, and the most common adverse effect (AE) is diarrhea. The mechanisms of diarrhea are unclear, and diarrhea could be controlled well with a prophylactic. Lapatinib also potentially causes cardiotoxicity,[39] although early clinical studies demonstrated their cardiac safety profile. The use of preventive interventions against trastuzumab-induced cardiac dysfunction, including beta-blockers, remains controversial.

Lapatinib plays an important role in metastatic BC. The combination of lapatinib and chemotherapy is efficient. In a phase III, double-blind, randomized study, designers assessed the effect of paclitaxel and/or lapatinib on patients with HER2-positive metastatic BC (mBC). The combination treatment significantly improved OS compared with paclitaxel; the median OS from the combination therapy was 27.8 months and median OS from paclitaxel alone was 20.5 months. The median progression free survival (mPFS) was prolonged by 3.2 months. Compared to paclitaxel alone, the overall response rate (ORR) was higher in patients receiving the combination therapy (69% vs. 50%, respectively; P < .001).[40] In many phase III trials, the datas also showed that paclitaxel-lapatinib treatment could significantly improve time to progression(TTP), event-free survival (EFS), ORR, and clinical benefit rate (CBR). However,

Table 1. Summary of Her2 Targeted Drugs.

Drug	Target HER family	Route of administration	MTD	Grade 3/4 adverse events	Reported results of efficacy in HER2-positive advanced disease	CNS ORR/mPFS in the combination therapy	Phase of development
lapatinib[17-20]	reversible HER1,2	oral	1250mg/1500mg	nausea; vomiting;diarrhea	single-agent ORR 20%; In combination with everolimus, capecitabine mPFS 6.2 m and OS 24.2 m; In combination with trastuzumab ORR 21.7%, mPFS 3.9 m, OS 21.6M	ORR 21.4%(+capecitabine) ORR 28%(+everolimus, capecitabine)(phase 1b/II)	US FDA approved in the salvage setting with capecitabine in 2007; with trastuzumab(patients HR negative) in 2013; In HR-positive breast cancer with letrozole in 2010;
neratinib[21-23]	irreversible HER1,2,4	oral	240mg	diarrhea;dyspnea; abnormalities in liver enzyme levels	Single-agent ORR 56%(phase II);	ORR 49%(+ capecitabine phaseII)	US FDA approved only in the adjuvant setting
pyrotinib[9,24,25]	irreversible HER1,2,4	oral	400mg	anemia;diarrhea	Single-agent ORR 50%, CBR 61%, PFS 35.4wphase(I); In combination with capecitabine ORR 78.5% PFS 18m(phaseII)	NA	China NMPA approved in combination with capecitabine for the treatment of HER2-positive, advanced or metastatic breast cancer
tucatinib[26-31]	reversible HER2	oral	300mg(with chemotherapy)/ 750mg(with trastuzumab and without chemotherapy)	diarrhea	In combination with capecitabine and trastuzumab: ORR 61% PFS of 7.8 m; OS 18.1months; In combination with T-DM1: ORR 48% PFS 8.2m(phase1b)	ORR 42%(+trastuzumab and capecitabine) mPFS 4.1 months (+ trastuzumab) mPFS 9.9 months(+ trastuzumab+ capecitabine)	III
poziotinib[32-34]	irreversible HER1, HER2, HER4	oral	12mg	diarrhea;stomatitis; rashes	Single-agent DCR75% PFS 4.04m(phaseII);	NA	III
afatinib[35,36]	irreversible HER1, HER2	oral	20mg	diarrhea	Single-agent ORR 27%; In the combination with vinorelbine mPFS 5.5 m; In the combination with trastuzumab ORR 11% DCR 39% mPFS 111days	ORR 10.7%(+vinorelbine)	III(not approved yet)

Notes:
HER, human epithelial receptor; MTD, maximum tolerance dose; CBR, clinical benefit rate; CNS, central nervous system; PFS, progression-free survival; US FDA, United States Food and Drug Administration; OS, overall survival; ORR, overall response rate; m, months; HR, hormone receptor; DCR, disease control rate; NMPA, National Medical Products Administration; NA, not applicable.

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in a randomized, open-Labe, phase III study, CEREBEL (EGF111438), patients with HER2-positive mBC were treated with lapatinib/trastuzumab plus capecitabine. There was no difference between the lapatinib arm and trastuzumab arm regarding the incidence of central nervous system metastasis. Patients treated with trastuzumab-capecitabine had a better outcome. Moreover, in a phase III study, EMILIA (TDM4370g/BO21977), patients with HER2-positive locally advanced or mBC were treated with capecitabine and lapatinib or T-DM1. The time to symptom worsening was 7.1 months in the T-DM1 arm and 4.6 months in the capecitabine-plus-lapatinib arm. Patients treated with T-DM1 exhibited a more clinically dramatic improvement in symptoms from baseline versus in the capecitabine and lapatinib arm.

As for the triple-positive BC, the combination treatment with lapatinib and an aromatase inhibitor (AI) can be a treatment option. In a phase III trial, patients with hormone receptor (HR)-positive metastatic BC were treated with lapatinib-plus-letrozole and letrozole-plus-placebo as the salvage therapy for mBC. The PFS in patients with HER2-positive BC was dramatically longer in the lapatinib + letrozole group (8.2 months) than in the letrozole group (3 months). The quality of life in the HER-2 positive patients was dramatically better in the lapatinib-plus-letrozole arm. Letrozole seems to be the best partner aromatase inhibitor with lapatinib.[41] Then, in a phase III study, ALTERNATIVE, postmenopausal women with triple-positive mBC were recruited. They were randomly assigned to receive lapatinib (LAP) + trastuzumab (TRAS) + aromatase (AI), TRAS + AI, or LAP + AI. The ORR, CBR, and OS also favored LAP + TRAS + AI. The mPFS with LAP + TRAS + AI versus LAP + AI versus TRAS + AI was 11 versus 8.3 versus 5.7 months. The incidence of AEs was similar between the 3 groups.[10]

As for the chemotherapy-free option for patients, the combination treatment with lapatinib can be safe and potent. In a phase III study, EGF104900, the data demonstrated that lapatinib plus trastuzumab continued to show superiority to lapatinib monotherapy in PFS (HR, 0.74; P = .011) and resulted in better OS (HR, 0.74; P = .026).[42] And in a real world study, Trastyvere, the data also showed that lapatinib-plus-trastuzumab was more efficient than monotherapy.[43]

To improve outcomes, numerous trials designed novel combinations with lapatinib or new administration regimens for this disease. In a phase Ib/II trial, TRIO- US B-09, after treatment with the combination of lapatinib, everolimus, and capecitabine, the best CNS ORR of recruited patients was 28%. The mPFS was 6.2 months, and the mOS was 24.2 months.[44] Morikawa et al. had reported a regimen with an escalated dose of lapatinib to treat patients with CNS metastasis.[45]

In the adjuvant treatment, lapatinib was not effective. In a phase 3 trial (TEACH), 8381 women with early-stage HER2-positive BC were treated with adjuvant trastuzumab (T) and/or lapatinib (L). The median follow-up was 4.5 years, and the HR of DFS was 0.84 when using L+T compared to T. The lapatinib and trastuzumab arm did not dramatically improve DFS compared with monotherapy with trastuzumab and added toxicity.

In a phase 3 study (ALTTO), the data also showed that the DFS was similar between 3 groups.[46] And according to the subanalysis of the ALTTO trial, dual HER2 blockade with trastuzumab + lapatinib is a safe regimen from a cardiac perspective, but cardiac risk factors like baseline LVEF < 55% should also be taken into crucial consideration.[47]

Lapatinib has been explored in the field of neoadjuvant treatment. In a phase 2 trial, TBCRC 006, patients with early HER2-positive BC were recruited and treated with lapatinib-plus-trastuzumab with hormonal therapy. For all the population, the PCR was 27% (the ER-positive arm, 21%; the ER-negative arm, 36%). Another study also explored that treatment with lapatinib for 24 weeks resulted, which resulted in a statistically significant increase in pCR rate compared with 12 weeks.[48] However, in a phase 2 trial, patients were treated with lapatinib, paclitaxel, and trastuzumab, and there was no difference in the PCR rate by prolonging exposure as well as adding endocrine treatment.[49] In the GeparQuinto randomized phase 3 trial, data showed that patients treated with chemotherapy-plus-trastuzumab had a better PCR rate than when treated with chemotherapy-plus-lapatinib (30.3% and 22.7%, respectively).[50] In a phase 2 trial, EORTC 10054, a modest pCR rate increase with trastuzumab, chemotherapy, and lapatinib was reported.[51] Then, in a phase II study, treatment-naive patients with inflammatory breast cancer (IBC) were treated with lapatinib and weekly paclitaxel. The combined clinical response rate was 78.1%.[52] In a randomized phase 3 trial (CALGB 40601), evaluating the efficacy of neoadjuvant treatment, paclitaxel (P) plus trastuzumab (H) with or without lapatinib (L) in early-stage HER2-positive BC, the PCR rate was 56% with THL and 46% with TH.[53] However, in the randomized phase III study, NeoALTTO, the data demonstrated that although lapatinib, in combination with trastuzumab, statistically improved PCR rates compared with either drug alone, the EFS or OS were similar between all groups. Moreover, in a phase 2 trial, NCT 02073487 (Teal study), patients treated with lapatinib, T-DM1 as well as nab-paclitaxel had better outcomes compared to patients who received the standard treatment, particularly in the triple-positive group.[17]

Biomarkers and the Mechanisms of Resistance

Further exploration of the biomarkers and the mechanisms of resistance of treatment with lapatinib can improve the efficacy of therapies with lapatinib. The HER2E subtype may be a favorable predictor for the clinical benefit of HER2-targeting therapy, particularly with trastuzumab-based treatment.[54-56] Quantitative assessment of the immune infiltrate also seems to play a role in predicting HER2-targeting benefits.[57-60] The mechanism of drug resistance of lapatinib is reticular, mainly through PI3K/AKT/mTOR and MEK/MAPK pathways, and other pathways also have effects, such as on metabolism, autophagy, and bypass activation.[61-65] The mTOR/PI3 K inhibitor NVP-BEZ235 can reverse PI3 K hyperactivation, which results in lapatinib resistance.[63] BAY 80-6946 is an intravenous pan-class I PI3 K inhibitor with predominant activity

against the alpha/delta isoform.[64,65] About 18% of HER2-positive BCs directly inhibited the HER2 inhibitors via down-regulation of MEK/MAPK but not AKT signaling.[66] The combination of MEK inhibitor refametinib and lapatinib could also improve the prognosis of HER2-positive BC. The ATP-binding cassette (ABC) family of transporters[67] includes P-glycoprotein (P-GP; ABCB1). The inhibition of ABCB1 and ABCG2 transporters by elacridar substantially enhanced the penetration of lapatinib into the central nervous system. Furthermore, treatment with EXEL-7647 inhibits the heregulin-EGFR-HER3 autocrine signaling axis and is reported to be an additional therapeutic avenue that blocks the activation of genes engaged by multiple HER2 resistance kinases. And the recent data demonstrated that targeting the EphB4 receptor tyrosine kinase can improve the effect of lapatinib.[68]

Neratinib

Neratinib is an oral, irreversible, next-generation, multi-target TKI that inhibits HER1, HER2, and HER4 in the intracellular tyrosine kinase receptor sites and has shown encouraging anti-tumor outcomes in patients previously heavily treated with trastuzumab-based therapy. It is covalently bound to the ATP binding site of receptor kinase and has a cell cycle arrest and anti-proliferative effect.[69,70] The regulation of downstream signal transduction by neuratinib leads to the stagnation of G1-S phase transition, the increase of the p27 level, and the decrease of phosphorylated retinoblastoma protein (PRB) and cyclin D1. Neratinib has been approved by the FDA and EDQM as the extended adjuvant treatment of adult patients with early-stage BC based on outstanding 5-year data from ExteNET.[8,15] Neratinib dramatically improved five-year invasive disease-free survival (iDFS) in patients who were previously treated with trastuzumab-based adjuvant treatment for early BC. In combination therapy, neratinib showed primary anti-tumor activity in patients with BC and central nervous metastases. The most common all-grade adverse events of neratinib were diarrhea and nausea. It can also have rare related cutaneous side effects, which is similar to the EGFR inhibitors.[71]

In the salvage treatment, the combination therapy with neratinib proved to be more efficient than lapatinib and well-tolerated.[21,22,72] In a phase II trial, designers evaluated the efficacy of neratinib plus capecitabine in 68 eligible HER2-positive mBC patients pretreated with trastuzumab as well as lapatinib. The ORR was 57% and the mPFS was 35.9 weeks.[69] In the NEfERT-T trial, 479 women with HER2-positive mBC were randomized to the neratinib-paclitaxel arm [n = 242] or trastuzumab-paclitaxel arm [n = 237]. The mPFS was similar in 2 arms (12.9 months).[73] Xu et al. reported data on Asian populations; the ORR was 66.4%, and the mPFS was 55.6 weeks.[74] The NSABP Foundation Trial also explored novel combination treatment[75] (FB-10, phase Ib), 27 patients were recruited and treated with neratinib and T-DM1. The ORR was 63% of 19 evaluable patients. The dose-limiting toxicities (DLTs) were diarrhea and nausea. And the recent data from the NALA trial also demonstrated that neratinib+capecitabine significantly improved PFS and time to intervention for CNS disease versus lapatinib+ capecitabine.[29]

For patients with measurable progressive HER2-positive brain metastases, the combination treatment with neratinib is efficient. In the NEfERT-T trial, with the treatment of neratinib-paclitaxel, the incidence of CNS recurrences was lower, and time to CNS metastases was delayed.[73] In a phase II study, TBCRC 022[21], 59 patients with measurable progressive HER2-positive brain metastases were recruited and divided into cohort 3A (lapatinib-naive) and cohort 3B (lapatinib-treated). Patients were treated with neratinib, in combination with trastuzumab. The composite CNS ORR was 49% in cohort 3A and 33% in cohort 3B. The mPFS and mOS were higher in cohort 3A, which were 5.5 months and 15.1 months, respectively. The data from the NALA trial also showed that fewer interventions for CNS disease occurred with neratinib+capecitabine versus lapatinib+ capecitabine (P = 0 .043).[29]

In the adjuvant treatment, ExteNET is the most famous trial wherein 2840 eligible women in 40 countries who completed trastuzumab-based adjuvant therapy for early-stage BC were recruited and randomly assigned (1:1) to receive neratinib or placebo. The data showed that the 2-year iDFS rate and 5-year iDFS were higher in the neratinib group, which were 93.9% and 90.2%, respectively.[8] However, based on 5-year data from ExteNET, patients who received neratinib following adjuvant trastuzumab did not experience this benefit.[76] For triple-positive BC, hormone therapy can be recommended to further improve the efficacy. The ERBB RTK pathway has compensatory crosstalk with the ER pathway. Treatment with neratinib or the combination of fulvestrant and neratinib strongly inhibited growth, and the levels of ER reporter activity, P-AKT, P-ERK, and cyclin D1 were also be downregulated. Inactivation of cyclin D1 enhanced fulvestrant action.[77]

In the neoadjuvant therapy, a phase II study, NSABP FB-7 (NCT01008150) explored neoadjuvant therapy for early-stage HER2-positive BC patients. They were treated with trastuzumab and/or neratinib and weekly paclitaxel, then they received standard doxorubicin plus cyclophosphamide. The PCR rate in the combination arm was 50% greater than that for the trastuzumab arm (38% or neratinib 33%).[78] In the I-SPY 2 trial, they also reported the efficacy for neratinib and efficiently identified responding tumor subtypes. With 115 patients and 78 concurrently randomized controls, neratinib graduated in the HER2+/HR−signature, with a mean PCR rate of 56% vs. 33% for controls. The combination therapy with neratinib is highly likely to improve PCR rates in HER2+/HR2212 BC. Furthermore, confirmation in I-SPY 3, a phase III neoadjuvant trial, is ongoing.[79]

Drug Resistance Mechanism

In view of the clinical development of neratinib resistance, many new combinations are being explored. A collection of genes reported resulted in neratinib resistance including genes involved in transcription factors, oncogenesis, protein

ubiquitination, cellular ion transport, and genes known to interact with BC-associated genes as well as the cell cycle.[80] The resistance of neratinib could lead to cross-resistance to afatinib, lapatinib, and trastuzumab. Neratinib-resistant cells were more aggressive and associated with increased CYP3A4 activity.[81] Furthermore, the corresponding targeted inhibitors like IGF1 R inhibitor figitumumab and neratinib combination have shown good, synergistic effects.[82,83] Hyperactivation of TORC1 also can drive resistance.[84] Hence, it is likely that only combination therapy will kill all clonal variants of the tumor. These findings may guide the design of future experiments.

Pyrotinib

Pyrotinib is a novel, oral TKI that acts against multiple members of the HER family (HER1, HER2, and HER4) and suppresses cell proliferation via PI3K/AKT/mTOR and MEK/MAPK pathways. It can block the tumor cell cycle in the G1 phase as well as inhibit tumor proliferation.[85] According to the positive results in a phase II trial, pyrotinib was recently approved in China, in combination with capecitabine, for patients with HER2-positive, advanced, or mBC after treatment of anthracycline or taxane chemotherapy.[86] The metabolic process of pyrotinib in humans primarily showed its safety and rational clinical application as well as the related toxicities of the key metabolites. Meng et al. and Zhu et al. discovered that pyrotinib was abstracted into the blood by 1 hour and at 4 hours reached its peak level. CYP3A4 is the most active enzyme leading to the transformation of pyrotinib, which could be responsible for potential CYP3A4-mediated drug-drug interactions in human. Finally, the excretion route of pyrotinib was mainly in the feces (about 90%), with some in the urine.[87,88]

In the salvage treatment, pyrotinib showed good efficacy, and previous trastuzumab treatment has a great impact on the efficacy of pyrotinib.[24,9,86,89,90] In a phase I study, Ma et al. reported that pyrotinib exposure was dose-dependent. The dose-limiting toxicity (DLT) was grade 3 diarrhea, and the maximum tolerated dose (MTD) was 400 mg. The ORR was 50.0%, and the CBR was 61.1%. The median PFS was 35.4 weeks. In trastuzumab-naive patients and in trastuzumab-pretreated patients, the ORR was 83.3% and 33.3%, respectively.[25] Li et al. also reported the results of pyrotinib in combination with capecitabine in patients with HER2-positive metastatic BC. The ORR was 78.6%, and the CBR was 85.7%. The mPFS was 22.1 months.[91] In a Phase II Study, patients were treated with pyrotinib/lapatinib and capecitabine. The ORR was 78.5% with pyrotinib and 57.1% with lapatinib. The mPFS was 18.1 months with pyrotinib and 7.0 months with lapatinib. The most frequent grade 3 to 4 AEs were hand-foot syndrome, diarrhea, and decreased neutrophil count.[9] In June 2019, the American Society of Clinical Oncology (ASCO) reported the results of phase III trials to evaluate the efficacy of pyrotinib/placebo combined with capecitabine in the therapy of HER2-positive mBC patients who had previously received taxane and trastuzumab. The mPFS was 11.1 months in the combination arm and 4.1 months in the placebo arm. The response rate was 38.0%, and the mPFS was 5.5 months.[92] The real world data also support the efficacy of the combination therapy with pyrotinib, For all the patients, the mPFS was 8.07 months. And in second-line therapy and third-or-higher-line therapy, the mPFS were 8.10 months and 7.60 months, respectively.[93]

Since the use of pyrotinib has shown different clinical benefit rates in different subgroups of the population, it is important to find more effective biomarkers and novel combination therapies. The mTBI was suggested to detect disease progression.[91] Ma et al. also discovered that mutations in baseline ctDNA had a close relationship with PFS outcomes.[94] The combination of pyrotinib and CDK4/6 inhibitor (palbociclib) showed synergistic antitumor activity in inhibiting tumor proliferation and colony formation. Combined therapy can also significantly reduce the activation of pAKT and pHER3, induce cell cycle arrest of G0-G1, and increase the apoptosis rate. In the xenograft model, the combination therapy showed stronger antitumor activity than the single use of any drug, with no significant increase in toxicity.[95]

Afatinib

Afatinib is a targeted therapy that irreversibly inhibits HER1, HER2, HER4,[96] and tumor cell proliferation *in vivo*.[97] Afatinib is under investigation as a monotherapy or in combination with HER2-targeting therapy. However, it does not have FDA approval.[98] While phase I and phase II trials have primarily shown anti-tumor results in HER2-positive BC, the phase III randomized trials of afatinib in BC, which had progressed under trastuzumab treatment, were halted early because of the higher toxicities and similar CBR to trastuzumab arms. A future direction for afatinib in HER2-positive BC may be developing better management of its related toxicities and joining in the novel combination therapy of HER2-positive BC.

In the salvage treatment, compared with the combination of trastuzumab and chemotherapy, the combination of afatinib and chemotherapy drugs did not show a better clinical benefit rate, on the contrary, it was more toxic, and the de-escalation treatment of afatinib and trastuzumab also did not show better results. Therefore, the best benefit population subgroups needed to be further analyzed to find the potential application of afatinib. In an open-label, randomized, phase 3 trial (LUX-Breast 1, NCT01125566),[35] 508 eligible patients were recruited. The independent data monitoring committee assessed the benefit-risk, which was unfavorable for the afatinib arm. Median PFS in the afatinib group and the trastuzumab group was 5.5 months and 5.6 months, respectively (HR 1.10). The most common grade 3/4 AEs were higher in the afatinib group. Because of the results of the LUX-Breast 1 trial, the other phase II Trial (NCT01325428) of afatinib with or without vinorelbine was terminated early.[99] As for the special type of BC, HER2-positive inflammatory breast cancer (IBC) has a higher frequency of TP53 gain-of-function mutations and a high mutational burden. IBC patients usually are treatment-

resistant and have worse ORR, PFS, and OS prognosis. In this trial, the CBR was 35% in afatinib monotherapy. All patients had drug-related adverse events. Afatinib showed no better anti-tumor activity in IBC patients. Afatinib, in combination with chemotherapy, did not achieve better clinical efficacy. In a phase I study, patients with locally advanced or metastatic HER2-positive BC were recruited. In the afatinib combined with trastuzumab arm, the ORR and DCR were 11% and 39%, respectively, with an mPFS 111.0 days. Then, in a multicenter, open-label, phase 2 trial (LUX-Breast3, NCT01441596),[36] there was no difference in CBR between patients treated with afatinib-containing treatment and trastuzumab alone. However, the combination treatment with afatinib was less tolerated. Since the most important toxic side-effect of afatinib is diarrhea, whether the dosage of afatinib can be increased and its clinical efficacy can be further improved by inhibiting diarrhea has been explored. However, the results of current experimental investigations are negative. In 1 phase I trial, patients with HER2-positive mBC received afatinib daily in combination with 3-weekly trastuzumab with optimal anti-diarrhea management. The results showed the MTD of afatinib was 20 mg daily despite optimal management of diarrhea, which limits the possibility of increasing the dose of afatinib.[100]

In the neoadjuvant phase, the combination therapy showed no advantage in clinical safety and efficacy. In a phase II trial, DAFNE, NCT015591477, 65 patients with early-stage HER2-positive BC were treated with afatinib, trastuzumab, and chemotherapy. Patients with HR-negative and LPBC showed higher pCR rates. Patients with or without PIK3CA tumor mutations had similar PCR rates. The clinical responses were 96.3%, and the rate of breast-conserving surgery was 59.4%. The PCR rate was 49.2%.[101]

Tucatinib

Tucatinib is highly selective for HER2, has no effect on EGFR, and has less off-target effects like rashes and diarrhea.[26,28] It potently inhibits HER2 and HER3 through the MAPK and PI3K/AKT pathways.[102] It was approved for the treatment of HER2-positive metastatic BC. The combination therapy with tucatinib is generally well-tolerated and has shown encouraging clinical activity in patients with advanced HER2-positive BC, including brain metastases, who have received severe treatment.[6]

Tucatinib has shown excellent efficacy and safety in the treatment of advanced disease, especially in brain metastasis, but this requires further confirmation in clinical trials. In 1 phase I trial, NCT 01983501, 57 T-DM1 naive patients who had undergone 1–3 earlier HER2 therapies were treated with tucatinib and T-DM1. Most of the AEs of tucatinib treatment were grade 1 or 2. The grade 3 tucatinib-related toxic reactions were thrombocytopenia (14%) and hepatic transaminitis (12%). The MTD of tucatinib was 300 mg twice daily with dose-limiting toxic reactions seen at a higher dose. According to pharmacokinetic analysis, T-DM1 had no drug-drug interaction with tucatinib. In another phase Ib trial, (NCT 02025192), patients were heavily treated. The MTD was determined to be 300 mg orally twice per day. The tucatinib + trastuzumab + capecitabine tri-drug regimen resulted in a median PFS of 7.8 months, an ORR of 61%, and an mOS of 21.9 months. Grade 3 and worse treatment-related toxicities included fatigue, diarrhea, and palmar-plantar erythrodysesthesia. There was no treatment-related death. A 3-drug regimen containing tucatinib is also effective in BC patients with brain metastases. The CNS response rate of patients with measurable brain metastases was 36%. The brain disease stabilized for more than 6 months in 63% of patients with unmeasured metastatic lesions. And the exciting data of the HER2CLIMB Trial further demonstrated that median CNS-PFS was 9.9 months in the tucatinib + trastuzumab + capecitabine arm versus 4.2 months in the control arm. The tri-drug regimen educed risk of intracranial progression or death by two thirds.[30]

As for the chemotherapy-free option for patients, the combination treatment with tucatinib can be safe and potent. In a phase I study, NCT01921335, the data demonstrated that tucatinib(750 mg once daily) plus trastuzumab resulted in good CNS PFS, 4.1 months.[31]

A meta-analysis of studies showed that PFS was prolonged when tucatinib was used to treat patients with or without brain metastases, with a median PFS (mPFS) of 8.2 months and 7.8 months, respectively. Nearly 20% of patients treated with tucatinib had prolonged PFS.[103] The ongoing phase II clinical trial HER2CLIMB (NCT02614794), reported in the ES, MO breast cancer meeting, showed that in patients with brain metastases, PFS was 24.9% in the tucatinib group and 0%[104] in the placebo group at 1 year.[104]

Poziotinib

Poziotinib (HM781-36b) (NOV120101) is a pan TKI developed by Korea and the United States. It is a new oral broad-spectrum HER inhibitor, which can irreversibly block the signaling of HER1, HER2, and HER3 tyrosine kinase receptors.[34]

Poziotinib has a half-life of 6.6 hours, an average distribution volume of 164 liters, an average apparent clearance rate of 34.5 liters per hour, and a peak serum concentration of 1.75 hours after oral administration. The maximum tolerated dose of poziotinib is 24 mg once a day.

In preclinical models, poziotinib upregulated the cell-surface expression of HER2 and potentiated the activity of T-DM1, which can result in complete tumor regression. In the salvage therapy, poziotinib has good efficacy and is well-tolerated. The NOV120101-203 phase II trial recruited 106 heavily pretreated patients. The median PFS was 4.04 months. Meanwhile, the median OS has not been reached. Diarrhea, stomatitis, and rashes were the most common AEs. HER2 CN amplification, single nucleotide variants(SNVs), and the alteration of PI3KCA pathway were recommended to be the potential predictive biomarkers of the evaluation of the poziotinib treatment response.[32]

Table 2. Part of Ongoing Trials of Pyrotinib, Tucatinib, Poziotinib.

NCT number	Phase	Trial Arm	Status	Patients	Subjects	Location
Pyrotinib						
NCT03772353	I/II	Pyrotinib + letrozole + SHR6390	32	HER2-positive and HR-positive relapsed or metastatic breast cancer	Recruiting	China
NCT03993964	II	Pyrotinib + CDK4/6 Inhibitor	20	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
NCT03588091	III	Pyrotinib VS. Placebo Oral Tablet VS. Trastuzumab VS. Docetaxel	Recruiting	HER2-positive Breast Cancer	294	China
ChiCTR1900021819	IV	Pyrotinib	Not yet recruiting	HER2-positive locally advanced breast cancer	1000	China
ChiCTR1900020670	IV	Pyrotinib + standard treatment	Not yet recruiting	HER2-positive brain metastatic breast cancer	48	China
ChiCTR1800020449	IV	Pyrotinib + trastuzumab + paclitaxel + cisplatin	Not yet recruiting	HER2-positive early stage or locally advanced breast cancer	40	China
Tucatinib						
NCT03054363	Phase 1 Phase 2	Tucatinib in Combination with Palbociclib and Letrozole	Recruiting	Hormone Receptor-Positive and HER2-positive Metastatic Breast Cancer	25	USA
NCT02025192	Phase 1	tucatinib+capecitabine vs. tucatinib+trastuzumab vs. tucatinib+capecitabine+ trastuzumab	Active, not recruiting	HER2 Positive Metastatic Breast Cancers	60	USA
NCT03846583	Phase Ib	Tucatinib + Abemaciclib + Herceptin	Not yet recruiting	Hormone Receptor-Positive and HER2-positive Metastatic Breast Cancer	53	USA and ENGLAND
NCT02614794	Phase 2	Tucatinib + capecitabine + trastuzumab	Active, not recruiting	Advanced HER2+ Breast Cancer	612	USA
NCT03975647	Phase 3	Tucatinib + T-DM1	Recruiting	Advanced or Metastatic HER2+ Breast Cancer	460	USA
Poziotinib						
NCT02659514	Phase 2	poziotinib	Active, not recruiting	HER2-Positive Metastatic Breast Cancer	75	USA
NCT03429101	Phase 2	poziotinib +T-DM1	Active, not recruiting	HER2 Positive Breast Cancer	6	USA

Additionally, we have listed a number of trials of new TKIs, pyrotinib, tucatinib, and poziotinib, which are currently underway, including phase 3 and 4 clinical trials, as well as the latest treatment combinations of these new drugs, in the hope of providing some basis for subsequent preclinical trials and clinical trials. They are shown in Table 2.

TKIs Reversing Resistance to the Treatment of Her2 Positive BC

The addition of small molecule TKIs into antibody/chemotherapy-based therapies may reverse the resistance to HER2-positive BC therapy by blocking compensatory signaling pathways.[37] The use of TKIs can reduce the level of ROS,[105] to some extent, which can lead to the resistance of chemotherapy. ROS is produced by all aerobic cells to regulate cell development, growth, survival, and death.[106-108] ROS plays an important role in the therapeutic principles of many chemotherapies, such as cisplatin and bleomycin. Lapatinib showed anti-tumor activity in p95HER2 mutant tumor xenografts resistant to trastuzumab therapy. Lapatinib inhibited insulin-like growth factor I (IGF-I) signal transduction and blocked the cross-talk between HER2 and IGF-1 R in trastuzumab-resistant HER2 positive BC.[109] Lapatinib increased the fragment of PARP and down regulated the expression of survivin in trastuzumab-sensitive and resistant

HER2 cells. In addition, lapatinib inhibited the activation of nuclear factor kappa B (NF-κb) and the MEK/ERK signaling pathway in BC cells overexpressing HER2, and enhanced radiosensitization.[110] It can also reduce the expression and activity of topoisomerase II α and reduce the resistance of cells to adriamycin, etoposide, and other cytotoxic drugs. Neratinib inhibits multidrug resistance through ATP transporters and improves response to chemotherapeutic drugs used in HER2-positive BC.[111] Similarly, neratinib enhances therapeutic response and counteracts trastuzumab resistance by reducing the HER4 nuclear translocation induced by trastuzumab in HER2-positive BC.

Targeted Nanotechnology

Nanotherapeutic drugs are one of the most promising therapies for BC and other cancers. The current treatment of BC is mainly focused on the development of targeted therapy and target-specific nanotherapy (TSMT).[112] Hence, we look forward to the combination of nanotherapy and anti HER2-targeted therapy, such as in new TKI drugs. Nanotherapeutic drugs involve the use of biomaterials/biodegradable materials (i.e., proteins, polymers, peptides, RNA/DNA aptamers, lipids, inorganic materials) to deliver therapeutic or diagnostic agents to cancer cells in a minimal and precise manner. The use of nanotherapy can improve targeting as well as several pharmacokinetic and pharmacodynamic parameters, such as increasing the cycle time of rapidly degradable therapeutic agents, pH/temperature sensitivity or drug sustained release in the target site and circulation, higher tissue permeability and maximum efficacy, and lower dose.[113,114] Nanotherapeutic drugs approved for the treatment of BC include liposome nanodrugs, polymer nanomicelles, protein drug conjugates, and polydextran-based nanodrugs, such as abraxane, doxil, and myocet.[115] At present, there are still many problems and challenges related to nanocarriers, including permeability and retention in tumor sites, stability of blood circulation, and emergence of new toxicity.

Summary

With the introduction of anti-HER2 drugs, the prognosis of HER2-positive BC patients was significantly improved. However, the PCR rate of neoadjuvant therapy is only about 60%. Because of primary and acquired drug resistance, almost all patients will have tumor recurrence and metastasis, in which central nervous system metastasis is common. Many special patients who cannot tolerate chemotherapy need better de-escalation treatment. The new generation of TKIs has attracted attention for its good synergistic effect with endocrine therapy drugs and monoclonal antibodies. To some extent, it can reverse the drug resistance of multi-drug chemotherapy and anti-HER2 targeted therapy, has low cardiotoxicity, and has better ability to cross the blood-brain barrier, which has attracted attention. It can be used by HER2-positive BC and brain metastasis patients, and can also be used as an important part of the combination therapy for elderly patients and patients who cannot tolerate chemotherapy. Because of their good targeting ability, TKIs can be used as a coupling agent with nanomaterials for the accurate diagnosis and treatment of HER2-positive BC. It can be used as a potential treatment for patients with HER2-positive BC and brain metastasis. The key tasks in the future are to explore novel and effective biomarkers, to predict the specific patient groups that can benefit the most from the combined treatment, to explore the mechanism of drug resistance, to find more targeted inhibitors that have a synergistic effect on TKIs, to explore effective drugs to deal with the grade 3 or 4 adverse events with limited dose, so as to improve the efficacy of anti-HER2 drugs, and to strive to include a larger proportion of elderly patients, expand the eligibility criteria, and include measures for the elderly in research design.

Authors' Note

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Statement

Our study did not require an ethical board approval because it did not contain human or animal trials.

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