Novel treatment strategies for patients with HER2-positive breast cancer who do not benefit from current targeted therapy drugs (Review)

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Abstract. Human epidermal growth factor receptor-2 positive breast cancer (HER2$^+$ BC) is characterized by a high rate of metastasis and drug resistance. The advent of targeted therapy drugs greatly improves the prognosis of HER2$^+$ BC patients. However, drug resistance or severe side effects have limited the application of targeted therapy drugs. To achieve more effective treatment, considerable research has concentrated on strategies to overcome drug resistance. Abemaciclib (CDK4/6 inhibitor), a new antibody-drug conjugate (ADC), src homology 2 (SH2) containing tyrosine phosphatase-1 (SHP-1) and fatty acid synthase (FASN) have been demonstrated to improve drug resistance. In addition, using an effective vector to accurately deliver drugs to tumors has shown good application prospects. Many studies have also found that natural anti-cancer substances produced effective results during *in vitro* and *in vivo* anti-HER2$^+$ BC research. This review aimed to summarize the current status of potential clinical drugs that may benefit HER2$^+$ BC patients in the future.

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Abbreviations: ATA, acetyl tanshinone IIA; AE, aloe-emodin; SHP-1, src homology 2 (SH2) containing tyrosine phosphatase-1; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; rVP1, recombinant DNA-derived viral capsid protein-1; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; TK, tyrosine kinase; HP, hesperetin; NG, naringenin; PI3K, phosphatidylinositol 3-kinase; Ras, ras kinase family; Akt, protein kinase B; RAF, receptor activation factor; TSC2, tuberous sclerosis complex 2; MEK, mitogen/extracellular signal-regulated kinase; mTOR, mechanistic target of rapamycin; MAPK, mitogen-activated protein kinase; CCND1, cyclin D1; P70-S6K, ribosomal protein S6 kinase beta-1; S6RP, S6-ribosomal protein; HIF-1α, hypoxia inducible factor 1α; YB-1, Y-box binding protein 1; RB, retinoblastoma protein; PD-L1, Programmed death ligand 1; PD-1, programmed death 1; TNBC, triple-negative subtypes of breast cancer

Key words: HER2 target therapy, trastuzumab, neratinib, drug resistance, targeted delivery drugs, natural anti-cancer substances

Contents

1. Introduction

Breast cancer (BC) is the most diagnosed disease among women and has the second highest incidence among all types of cancer worldwide (1.7 million cases, 11.9%) (1). Excessive expression of human epidermal growth factor receptor-2 (HER2) has been investigated in 20-30% of BC patients and generally recognized as a marker for invasive disease, which is likely to be highly metastatic, to be drug resistant and to spread rapidly (2-4). HER2 is a member of the HER/EGFR/ERBB group of protein kinase superfamily, which receives signals to regulate cell survival, adhesion, motility, proliferation and resistance to apoptosis (5,6). A tyrosine kinase (TK) domain located in the HER2 receptor plays an important role in the occurrence of BC, as it is induced by phosphorylation after

HER2 homo/hetero dimerization and then activates multiple signal transduction pathways (5,7). The phosphatidylinositol 3-kinase (PI3K) pathway and the Ras/Raf mitogen-activated protein kinase (MAPK) pathway are the most fully investigated of these pathways, both of which may contribute to the acquired resistance against targeted therapy drugs (8-10). Related research has identified that inhibition of the PI3K-Akt pathway with PI3K inhibitors is effective to reverse tumor growth (11-13). When the HER2 proto-oncogene undergoes mutation and conversions into the HER2 oncogene (14), HER2 receptor protein is highly expressed at the cell membrane, and multiple downstream signaling pathways are excessively activated, resulting in uncontrollable cell growth, repeated division of cells, and altered adhesion properties (7,14,15). Based on its unique role in the development of HER2$^+$ BC, HER2 and its downstream signaling pathways have become extremely important targets for therapy. HER2$^+$ BC that is induced by HER2 amplification was significantly reversed by treatments that targeted HER2 and HER2 TK (15,16). Targeted therapy that depends on the expression levels of HER2 has made great progress, although a considerable portion of the population are resistant.

2. Current targeted therapy drugs

Among all of the drugs that target HER2 and HER2 TK, trastuzumab, pertuzumab, trastuzumab emtansine (T-DM1) and lapatinib have been proven to be effective in treating HER2$^+$ BC in several clinical trials compared with chemotherapy drugs alone (17-20). Trastuzumab, the first-generation targeted therapy drug, is a humanized monoclonal antibody targeting the extracellular domain of HER-2 (21,22) and has the ability to downregulate the signaling pathways involving PI3K/Akt and MAPK (23,24), which in turn inhibits the proliferation of BC cells that overexpress HER2. Trastuzumab, both administered as a single agent or injected in combination with a series of chemotherapy agents (such as docetaxel or vinorelbine plus trastuzumab (25-27), showed anti-tumor effects and remarkably improved time to progression, response and survival rate (22,28,29). Furthermore, several randomized control trials have revealed that trastuzumab plus chemotherapy drugs significantly reduce the risk of recurrence and death and promote survival incidence compared to chemotherapy drugs alone (26,27), making trastuzumab the cornerstone of adjuvant treatments for HER2$^+$ BC.

Pertuzumab belongs to the second-generation targeted drug family, which elicits similar effects to trastuzumab and can significantly promote survival outcomes (25,30). Pertuzumab binds to HER2 and thus blocks the signaling pathways by blocking a binding pocket necessary for receptor dimerization (31). Pertuzumab was approved by FDA as a neoadjuvant therapy in combination with trastuzumab and cytotoxic chemotherapy (32). Related research demonstrated that pertuzumab in combination with trastuzumab was more effective in the blockade of HER2 signaling pathways *in vitro* and *in vivo* than either antibody alone (33).

T-DM1, a second-generation antibody, has attracted great interest from researchers for its effective role as an adjuvant and neoadjuvant (34). The phase III EMILIA study and RESA study proved that T-DM1 was efficacious as afunctional treatment and facilitated better prognosis and improvements in health-related quality of life (35-37). T-DM1 has been investigated to promote progression-free survival (PFS) and overall survival (OS) in patients who were HER2$^+$ and previously treated with trastuzumab and taxane (17).

Lapatinib is a reversible HER2 TK inhibitor that reacts with the ATP binding site, which in turn improves PFS and clinical benefit rate (CBR) by inhibiting the autophosphorylation of ErbB1 and ErbB2 and downstream proliferative signaling pathways (25,38,39). Similarly, in some neoadjuvant clinical trials, lapatinib has been used in dual blockade with trastuzumab to treat patients with HER2$^+$ BC (18,40-42).

3. Does every HER2$^+$ BC patient benefit from targeted therapy?

With the development of targeted therapy, drugs targeting HER2$^+$ BC, such as trastuzumab, pertuzumab and lapatinib, have been proven to significantly improve the prognosis of patients. Though the incidence of PFS, OS and overall response rate (ORr) are significantly promoted among HER2$^+$ BC patients by treatment with HER2-targeting drugs (17-19), drug resistance and progression of metastatic breast cancer (MBC) still develop gradually and are detrimental to the prognosis of patients. It has been estimated that about half of HER2$^+$ MBC does not respond to anti-HER2 drugs (28). Abnormal activation of the PI3K-Akt signaling pathway caused by PI3KCA mutations could promote trastuzumab resistance (43). Similarly, phosphatase and tensin homologue (PTEN) loss also correlated with trastuzumab resistance (44,45). HER2$^+$ BC with PTEN drops and/or PI3K mutations had a worse prognosis (45,46), making this drug resistance a rigorous and persistent clinical challenge. So, patients who present both HER2+ tumors and PI3KCA mutations may benefit from the application of HER2-targeted drugs plus PI3K inhibitors. Rexer *et al* found that dual HER2 and PI3K blockade *in vitro* and in xenograft models showed the most effective role in inducing tumor regression, even with a PI3KCA mutation (47). Interestingly, there were also preclinical studies showing that trastuzumab-induced reprogramming of the HER axis resulted in the increase of EGFR and HER3 expression after long-term trastuzumab treatment in cell lines, which may correlate with primary resistance to trastuzumab (48). As mentioned above, trastuzumab resistance in HER2$^+$ BC might be caused by excessive EGFR expression (49,50), making EGFR an effective target to evaluate in response to trastuzumab treatment (51).

In addition to the gradual increase in resistance, another important issue that hampers the application of trastuzumab in clinical patients is side effects, especially cardiomyopathy, which was reported to affect 2.8-3.3% of patient (52,53). More concerning, when trastuzumab was administered in combination with anthracyclines, cardiac dysfunction, such as left ventricular ejection fraction (LVEF) and congestive heart failure (CHF), affected about a quarter of patients as reported by some studies (28,54).

It has been confirmed that pertuzumab could strengthen the effect of trastuzumab (33). Similarly, a clinical study found that trastuzumab plus pertuzumab received an ORr of 24.2% and a CBR of 50% (55). In another clinical trial, a response rate of 18% was demonstrated after treatment with trastuzumab

and pertuzumab combination (56); however, over half of the patients did not benefit from treatment.

It is reported that the objective response rate of T-DM1 was approximately 44% (17), which indicated that more than 50% of eligible patients did not benefit from T-DM1. In addition, preclinical studies showed that T-DM1 resistance could be caused by downregulation of HER2 expression (57,58), consistent with decreased activity of T-DM1 in tumors that express low levels of HER2, suggesting that patients who initially responded to T-DM1 might develop acquired resistance as HER2 levels decline. Though a 43.6% objective response rate was achieved by T-DM1, more than half of patients receive little benefit from treatment (40). At the same time, many clinical trials reported that T-DM1 could bring about side effects, such as fatigue, nausea, thrombocytopenia and headache (59,60).

Lapatinib yielded response rates from 24-53.2% in clinical activity in advanced HER2-overexpression BC (17,19,40). Although lapatinib was speculated to play a vital role in treating trastuzumab-resistant, HER2-overexpressed tumors, the response rates ranged from 1.4-8.8% after lapatinib monotherapy (19,61). The addition of lapatinib and capecitabine received a 51% reduction in the risk of disease progression and a 2-fold time to progression compared to capecitabine alone (16). However, at least half of the patients showed no benefit from treatment.

Furthermore, with the presence of two kinds of drug resistance, 'a primary or inherent resistance and a secondary or acquired resistance' (24,46,62), we are facing two serious challenges: i) Overcoming the drug resistance; and ii) searching for new effective drugs.

4. How should we treat HER2$^+$ BC patients who do not benefit from trastuzumab, pertuzumab, trastuzumab emtansine (T-DM1) and lapatinib?

The inherent or acquired resistance to targeted therapy drugs, as well as the relatively serious side effects, limit the application of targeted therapy drugs. These two kinds of unfavorable factors are the driving forces to continue to develop new drugs. To overcome the development of drug resistance, some studies explored the possible treatment methods and identified some substances that had potential value. Specifically, the discovery and application of Taxol greatly improved the prognosis of patients. Interestingly, several studies investigated natural anti-cancer substances that had shown significant anti-cancer effects, which might prevent the tumorigenesis of BC. Furthermore, the new ADC and targeted delivery of drugs were found to be effective in treating HER2$^+$ BC patients. In this review, we concentrate on some research studies for putative application in clinical treatment. And we summarize the signaling pathways of corresponding effective potential clinical drugs (Fig. 1).

HER2 TK inhibitors. Neratinib is an irreversible kinase inhibitor and a derivative of EKB-569 (EGFR inhibitor) (63-65). Neratinib significantly inhibited EGFR/HER2 kinase after binding to the ATP pocket and blocking downstream signaling pathways (59) and showed anti-cancer bioactivities in HER2-overexpression cell lines and in patients with or without prior trastuzumab treatment (66-68). Neratinib inhibited proliferation and promoted G1-S phase arrest by regulating HER2 and its downstream signaling pathways, specifically through downregulation of pEGFR, pHER2, pAKT, pMEK and pRb levels and cyclin D1 (CCND1) expression and increase of p27 levels in a HER2-dependent manner (69). Some studies also discovered that neratinib improved trastuzumab resistance and restored sensitivity to trastuzumab in HER2$^+$ BC (69,70). Many clinical trials investigated the functions of neratinib in treating HER2$^+$ BC alone or in combination with trastuzumab, which exhibited great clinical application prospects. A more important role of neratinib was observed when combined with paclitaxel in treating patients who received prior taxane, trastuzumab and lapatinib therapies (71). Neratinib was effective as a single agent or in combination with different chemotherapy drugs in the treatment of HER2+ MBC patients and early BC patients (68,72-74). In a multicenter, randomized, double-blind and placebo-controlled phase III trial (ExteNET), which involved 2,840 women, a total of invasive disease-free survival events of 70 patients in the neratinib group and 109 patients in the placebo group occurred, corresponding to 93.9 and 91.6% 2-year invasive disease-free survival rates, respectively (75). All of these results above show that neratinib is quite effective and is close to clinical application.

Lapatinib showed significant activities in treating HER2$^+$ BC either alone or combined with trastuzumab. A considerable portion of HER2$^+$ patients were insensitive to lapatinib, which prompted development of a modified drug form. KU004, a derivative of lapatinib, remarkably inhibited pEGFR, pHER2, pAkt and pMAPK in BT474 and NCI-N87 cells, which were treated with lapatinib or not. Treatment with KU004 achieved a G0/G1 phase arrest and corresponding reduction of cells in S and G2/M phases in BT474 cells. KU004 could induce caspase-dependent apoptosis, as caspase-8 and caspase-3 were significantly activated by KU004. *In vivo* study found that KU004 reduced growth of NCI-N87 xenograft dramatically in a dose-dependent manner by inducing apoptosis. These *in vivo* and *in vitro* results indicate that KU004 has the potential to treat HER2$^+$ BC in the future (76).

Improvements of drug resistance. Trastuzumab, as the first-generation targeted therapy drug, helped many HER2$^+$ BC patients to improve their prognosis and quality of life in the future. As we mentioned above, the resistance to trastuzumab limited its application to a large extent. Thus, research and development of new drugs to overcome trastuzumab resistance may contribute significantly to the treatment of HER2+ BC patients.

It has been proven that CCND1 and CDK4 played a vital role in BC (77,78). CDK4/6 inhibitors have shown great potential in preclinical studies and clinical trials, such as palbocilcib and ribociclib which has been applied in clinical (79,80). Though CDK4/6 inhibitors are mainly used to treat estrogen receptor (ER)$^+$/HER2$^-$ patients, some studies found that they also have potential therapeutic effect on ER$^+$/HER2$^+$ patients (81). In an exploratory, open-label, phase 2 study (NA-PHER2), the combination of palbociclib, fulvestrant, trastuzumab and pertuzumab was found reduced the expression of Ki67 significantly (82). Palbocilcib could also efficiently inhibit the proliferation of residual HER2$^+$ tumor cells that survive T-DM1 in preclinical BC models (83). In

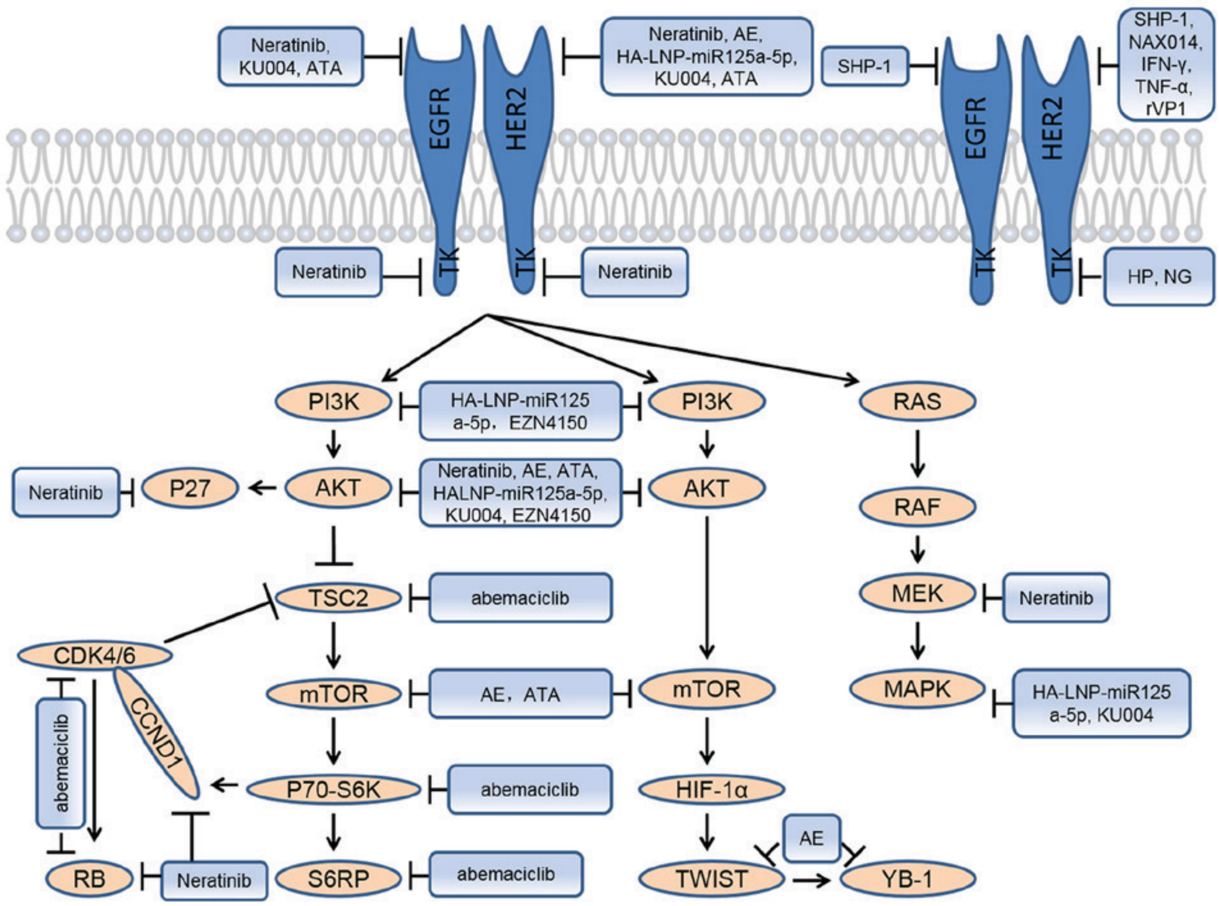

Figure 1. Effectors of EGFR/HER2 downstream signaling pathways and corresponding effective potential clinical drugs. ATA, acetyltanshinone IIA; AE, aloe-emodin; SHP-1, src homology 2 (SH2) containing tyrosine phosphatase-1; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; rVP1, recombinant DNA-derived viral capsid protein-1; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; TK, tyrosine kinase; HP, hesperetin; NG, naringenin; PI3K, phosphatidylinositol 3-kinase; Ras, ras kinase family; Akt, protein kinase B; RAF, receptor activation factor; TSC2, tuberous sclerosis complex 2; MEK, mitogen/extracellular signal-regulated kinase; mTOR, mechanistic target of rapamycin; MAPK, mitogen-activated protein kinase; CCND1, cyclin D1; P70-S6K, ribosomal protein S6 kinase beta-1; S6RP, S6- ribosomal protein; HIF-1α, hypoxia inducible factor 1α; YB-1, Y-box binding protein 1; RB, retinoblastoma protein.

a preclinical study, they found that both residual tumor cells and recurrent tumor cells that were present after treatment presented high levels of CCND1and CDK4, which promoted cell proliferation. In addition, abemaciclib, a CDK4/6 inhibitor, inhibited Rb phosphorylation and significantly postponed the process of recurrent tumors, which showed a CDK4/6-dependent increase in expression of CCND1. Strikingly, reduced sensitivity to both lapatinib and trastuzumab was found when CCND1 was over-expressed in trastuzumab/lapatinib-sensitive BC cell lines. Knockdown of CCND1 in resistant cells partially restored the sensitivity to trastuzumab. Phosphorylation of TSC2 and its downstream effectors, P70-S6K and S6RP, was reduced after abemaciclib treatment, which could be enhanced by combined CDK4/6-HER2 inhibition. Furthermore, *in vivo* study showed that trastuzumab combined with abemaciclib inhibited tumor growth more than either single agent (84). In another preclinical study, abemaciclib with trastuzumab significantly improved tumor growth inhibition and tumor regressions in xenografts progressing on trastuzumab alone (81). In a phase I study, one patient with hormone receptor-negative and $HER2^+$ BC experienced an antitumor effect with a 30% decrease in tumor size from baseline. At the same time, some clinical trials are ongoing to prove whether CDK4/6 inhibitors could be applied to ER^+/$HER2^+$ patients (NCT02947685; NCT02774681; NCT02907918; NCT02675231) (81).

Antibody-drug conjugates (ADCs) are constructed by covalently attaching small-molecule toxins to antibodies (85-87). T-DM1 is a ADCs that has shown significant activity in treating $HER2^+$ BC (86,87). A new biparatopic ADC was constructed, consisting of the trastuzumab scFv unit, the 39S Fv unit, and AZ13599185, which inhibits microtubule polymerization during mitosis. The new biparatopic ADC retained HER2 binding specificity and could effectively deliver cytotoxic agents into the targeted tumors. Compared to T-DM1, the new biparatopic ADC demonstrated at least 10-fold cell killing activity in HER2-expressing cancer cell lines in a HER2-dependent manner and showed activity in cancer cells that were intrinsically resistant to T-DM1. *In vivo* study suggested the possibility of clinical application of the new biparatopic ADC, as it induced full tumor degradation and inhibited tumor growth in a primary BC xenograft model and an intrinsically or acquired T-DM1-resistant xenograft model, which was not observed after treatment with T- DM1 alone or in combination with pertuzumab. These valuable results show that development of the new biparatopic ADC was necessary

to explore a more effective drug for the treatment of HER2+ BC (88).

Due to the putative role of EGFR in trastuzumab resistance, blocking EGFR and its corresponding downstream signaling pathways may produce better prognosis. The src homology 2 (SH2) containing tyrosine phosphatase-1 (SHP-1) regulated the intracellular phosphotyrosine level and was reduced or absent in many kinds of cancer cell lines and tissues (89-91). Overexpression of wild-type SHP-1 sensitized trastuzumab-resistant cells to trastuzumab treatment. Furthermore, overexpression of wild-type SHP-1 also significantly upregulated apoptosis and decreased EGFR and HER2, as well as their phosphorylation levels, in trastuzumab-resistant cells. Wild-type SHP-1 could bind to pEGFR and pHER2, and thus reduced both pEGFR and pHER2 protein levels. *In vivo* study demonstrated that SHP-1-overexpression mice showed delayed tumor progression and growth and achieved a better OS after treatment with trastuzumab compared with the control (92).

Fatty acid synthase (FASN) showed activity in promoting tumorigenesis by activating HER2/PI3K/AKT/mTOR and MAPK signaling pathways (93-95). EGFR and pEGFR increased in trastuzumab resistant cells noticeably, which was consistent with the study of Wu *et al* (92). Pertuzumab combined with EGCG, a classical FASN inhibitor, was more effective than pertuzumab alone against resistant HER2+ BC cells and increased the apoptosis of cancer cells in a trastuzumab plus lapatinib-resistant HER2+ PDX model compared with pertuzumab alone. *In vivo* study showed that dual FASN and HER2 blockade achieved more effective tumor reduction compared with EGCG or pertuzumab as single agent (50).

Targeted delivery drugs. The occurrence of all tumors is due to genetic mutations, so gene therapy is the most effective treatment approach for all cancers. Using an effective vector to accurately deliver drugs to the tumors is a very valid treatment method to stop the tumor progression.

A new delivery platform, which was able to escape from lysosomal degradation and distribute symmetrical in the cytoplasm, was established to send mature tumor suppressor microRNA125a-5p to treat HER2+ MBC. miR125a-5p significantly decreased cellular proliferation and migration in comparison with control cells, which might have been largely caused by knockdown of the HER2 mRNA levels. HA-LNP-miR125a-5p reduced the levels of HER2, PI3K, pAKT, Ki67 and pERK1/2 by over 30, 35, 40, ~40 and 20%, respectively, which indicated that HA-LNP-miR125a-5p could treat BC through inhibiting HER2 and its downstream signaling pathways (96).

Vaccines that target HER2+ BC may become the best way to prevent tumorigenesis. Campbell *et al* (97) had previously shown that the immunostimulatory peptide Hp91 had potent immune effects, and subsequently, Hp91 was encapsulated inside poly (D,L-lactic-co-glycolic) acid nanoparticles (PLGA-NPs) to construct a possible BC vaccine. The new vaccine significantly increased DCs when compared to free Hp91 *in vitro*. Furthermore, *in vivo* study showed that injection of the new vaccine noticeably increased HER2-specific IFN-γ spot-forming cells compared to the control group and the free Hp91 group (whether administered at the same concentration or higher). Hp91 encapsulated in PLGA-NPs delayed the tumor development by approximately three months compared to mice that were injected with free Hp91 and delayed the tumor development by approximately 5 months compared to control mice, and it prolonged the OS. These results proved that Hp91 encapsulated inside PLGA-NPs could be developed into an effective BC vaccine (97).

Natural anti-cancer substances. Natural anti-cancer substances played an important role in the treatment of BC and some new natural anti-cancer substances will play a role in the treatment of HER2+ BC. Paclitaxel is the most successful natural anti-cancer drug, which was extracted first from the bark and wood of Pacific Yew tree (98). Hesperetin (HP) and naringenin (NG) belong to flavonoids, which have shown anti-cancer and pro-apoptotic activity (99). An anthraquinone compound, aloe-emodin (AE), effectively suppressed HER2 expression and cell proliferation in a dose-dependent manner consistent with promotion of apoptosis and G1 cell cycle arrest in HER2+ BC cells. Twist and Y-box binding protein 1 (YB-1) have been investigated for promoting cell proliferation, tumor metastasis, invasion, angiogenesis and anticancer-drug resistance (100,101). *In vitro* study showed that AE observably inhibited cancer cell migration and invasion rates through promoting E-cadherin levels, which restored the epithelial cell adhesion, and inhibiting factors, which activated cancer cell metastasis. These results had important clinical significance and were confirmed by *in vivo* study in a SkBr3 cell xenograft model (102). Irisin is newly discovered, secreted from muscle tissue and adipose tissue, and recognized as an adipokine, which has been proven to participate in breast carcinogenesis (103,104). Irisin or its structural analogues may become new therapeutic drugs (105). Several studies have investigated the use of apigenin decreasing the risk of a variety of cancers, including BC (106-108). NAX014, a derivative of berberine (BBR), was effective in inhibiting a variety of cancer cells (109,110) tended to reduce HER2 expression in tumor tissues (111). Acetyl tanshinone IIA (ATA), a tanshinone IIA derivative (112), had shown activity against BC (113). *In vivo* study showed that injection of ATA reduced tumor volume and tumor weight remarkably (112).

Immune factors. IFN-γ and TNF-α had shown antitumor effects (114). This study emphasized the exciting roles of TNF-α and IFN-γ in treating HER2+ BC. No HER2+ BC cell increase due to induction of cell apoptosis was observed in the dual cytokine-treated group in comparison with the untreated or single cytokine-treated group. To investigate the mechanism of whether TNF-α and IFN-γ induced cell apoptosis, they treated TUBO and 4T1 cells with either dual Th1 cytokines, actinomycin D (positive control), or no treatment (negative control). They found that dual TNF-α and IFN-γ treatment decreased pro-caspase-3 levels significantly, corresponding with marked increase in activated caspase-3 levels. Treatment of Th1 cytokines and selective caspase-3 agonist (PAC-1) induced HER2 loss observably and showed a strong correlation with cancer cell apoptosis. Interestingly, downregulated HER2 expression could be eliminated by caspase-3/7 inhibitor in TUBO cells (115).

Anti-ErbB2 mAb therapy failed to reduce tumor growth in IL21R-/- mice; however, anti-ErbB2 mAb therapy succeeded in WT mice, which indicated that the IL21 signaling pathway had a strong correlation with trastuzumab resistance. Furthermore, IL21R expressed by $CD8^+$ T-cells was required for mice that transferred with WT $CD8^+$ T-cells to achieve anti-ErbB2 mAb therapy success, compared with the mice treated with $IL21R^{-/-}$ $CD8^+$ T-cells. Recombinant IL21 combined with anti-ErbB2 mAb therapy significantly inhibited tumor growth and showed activity in lowering metastatic tumors (116).

Anticancer active substances. Recombinant DNA-derived viral capsid protein-1 (rVP1) has been proven to induce apoptosis and suppress invasion in several cancers. They investigated that both *in vivo* and *in vitro* rVP1 treatment significantly inhibited cancer cell metastasis and invasion, consistent with increased E-cadherin and decreased a-vimentin levels *in vitro*. Furthermore, the mRNA and protein expression of HER2 were inhibited by rVP1 in a dose-dependent manner (117).

Valproic acid (VPA) showed activity in cell apoptosis (118) and controversial effects in inhibiting BC cells (119,120). During HER2-overexpression in SKBR3 BC cells, cell growth was inhibited by VPA in a dose- and time-dependent manner consistent with observably increased p21 WAF1 expression, which could inhibit tumor cell differentiation and growth (121,122). Cleaved caspase-3 levels were upregulated by ~2-fold after the treatment with VPA, corresponding with a higher number of TUNEL positive cells in the SKBR3 cell line compared with the control group (123).

EZN4150, an antisense oligonucleotide, is an inhibitor of the PIK3CA gene that encodes the p110α type I PI3K catalytic subunit. EZN4150 combined with lapatinib or BKM120 (a type I PI3K inhibitor) decreased pAkt and increased cleaved caspase-3 levels, achieving a greater effect in combination than either compound alone. Autophagy is a process that could inhibit tumor initiation but support tumor progression (124,125). The role of autophagy in promoting cell survival was blocked by EZN4150 instead of BKM120 in a p110α-independent manner, which might be mediated by Vps34 ablation, as EZN4150 downregulated both p110α and Vps34 expression. The combined knockdown of p110α and Vps34 significantly decreased cell numbers, increased the level of cleaved caspase-3 and increased lapatinib-mediated growth inhibition in BT474 and SKBR3 cell lines. Although both lapatinib and BYL719 (a p110α-specific PI3K inhibitor (126) treatment robustly induced caspase 3/7 activity, SAR405, an inhibitor of Vps34, more significantly increased caspase 3/7 activity than lapatinib, BYL719 or their combination. These results established Vps34 as a new therapeutic target, and EZN4150 was able to improve clinical prognosis by increasing tumor cell killing (127).

PD-1 and PD-L1 agents. Programmed death ligand 1 (PD-L1) is the ligand of programmed death 1 (PD-1). Anti-PD-1/PD-L1 therapy is a novel immune-checkpoint inhibition therapy and anti-PD-1/PD-L1 agents, such as nivolumab, pembrolizumab, atezolizumab, durvalumab and avelumab have been widely applied to treat various types of cancer (128-130). Anti-PD-1/PD-L1 agents have shown antitumor activity in BC, especially in the triple-negative subtypes of breast cancer (TNBC) (131,132). But PD-L1 expression is associated with $HER2^+$ status and there is an independent poor prognostic impact of PD-L1 in $HER2^+$ BCs (132-134). In a phase 1b trial which 168 patients with MBC received avelumab, avelumab showed an acceptable clinical activity (135). Currently, many clinical trials in $HER2^+$ cohort are ongoing, such as NCT02648477 and NCT02129556 for pembrolizumab, NCT02605915 for atezolizumab and NCT02649686 for durvalumab (131). Anti-PD-1/PD-L1 agents will benefit the patients with $HER2^+$ BC in the future.

5. Conclusions

The advent of targeted therapy drugs is a revolutionary breakthrough in the history of BC treatment. Trastuzumab is the first-generation targeted therapy drug and has been used widely to treat $HER2^+$ BC. Due to the resistance to trastuzumab and severe side effects, second-generation targeted therapy drugs have been successfully developed and applied for clinical treatment, such as pertuzumab, T-DM1 and lapatinib. Although all of them achieved efficacy in the treatment of $HER2^+$ BC, quite a number of patients did not benefit on account of inherent insensitivity, acquired resistance, or potential side effects. How should we treat this subgroup of $HER2^+$ BC patients? Elucidating the mechanism of drug resistance and seeking effective drugs to overcome this hurdle may benefit these patients. In addition, new drugs should be developed for clinical application, and many researchers are actively working in this direction, with particular focus on natural anti-cancer substances and accurate delivery drugs that were identified for putative ability to treat $HER2^+$ BC. Moreover, these new drugs may also strengthen the effect of targeted therapy drugs in treating $HER2^+$ BC patients who are inherently sensitive to targeted therapy drugs. Based on this review, further investigations are needed to strengthen these findings and identify drugs that can overcome targeted therapy drug-resistance or natural anti-cancer substances that are effective in treating $HER2^+$ BC.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YC contributed to the conception of the study. NJ contributed to manuscript preparation and wrote the manuscript. JJL, JW, BNZ, AL, ZYC, SG, BBL, YZD, RYY, HFY, XYF, JLZ and HMY contributed in the writing of the manuscript and assisted in the literature research.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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