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REVIEW

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Triple-positive breast cancer: navigating heterogeneity and advancing multimodal therapies for improving patient outcomes

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Abstract

Triple-positive breast cancer (TPBC), a unique subtype of luminal breast cancer, is characterized by concurrent positivity for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Owing to the crosstalk between the ER and HER2 signaling pathways, the standard of care and drug resistance of this particular subtype are difficult challenges. Recent research and clinical trials have indicated a shift in the treatment paradigm for TPBC from single-target therapies to multi-pathway, multitarget strategies aiming to comprehensively modulate intricate signaling networks, thereby overcoming resistance and enhancing therapeutic outcomes. Among multiple strategies, triple-drug therapy has emerged as a promising treatment modality, demonstrating potential efficacy in patients with TPBC. Moving forward, there is a critical need to perform in-depth analyses of specific mechanisms of cancer pathogenesis and metastasis, decipher the complex interactions between different genes or proteins, and identify concrete molecular targets, thus paving the way for the development of tailored therapeutic strategies to combat TPBC effectively.

Keywords Triple-positive breast cancer, Signaling pathway crosstalk, Drug resistance, Triple therapy

Introduction

The most recent cancer 2024 statistics indicate that breast cancer remains the most prevalent malignant tumor among women, constituting approximately 32% of all female cancer diagnoses. Notably, the incidence of breast cancer continues to rise annually, accompanied by a concerning shift toward affecting younger people [1].

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Breast cancer is distinguished by its profound heterogeneity, which significantly influences the variability in treatment approaches and patient prognoses. Clinically, breast cancer entities are stratified into three principal groups according to estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression: ER-positive, HER2-positive, and triple-negative breast cancer (TNBC). The intrinsic molecular landscape of breast cancer can be further differentiated into five distinct subtypes, each with its own set of biological, clinical, and prognostic traits. These subtypes are luminal A, luminal B, HER2-enriched, basal-like, and claudin-low, offering a nuanced understanding of the disease and guiding tailored therapeutic strategies for patients [2].

Recent research has revealed a novel subtype of breast cancer that has garnered significant interest:

triple-positive breast cancer (TPBC). TPBC is a relatively rare subtype within the luminal breast cancer type, accounting for 5–10% of all pathological types of breast cancer, and is characterized by the concurrent positive expression of ER, PR, and HER2. Therefore, chemotherapy, HER2-targeted therapy, and endocrine therapy have become important treatment modalities for this subtype [3]. Nonetheless, in the therapeutic algorithm, TPBC is uniquely influenced by the activation and intersecting roles of the HER2 and ER signaling pathways, imparting a distinct set of clinical and pathological traits that differ from those observed in other breast cancer subtypes. Confronted with the intricate and heterogeneous nature of TPBC, the selection of pharmacological agents and the strategic sequencing of their administration pose formidable challenges in advancing clinical therapeutics.

Previously, the clinical standard of care primarily involved the combination of targeted therapy with chemotherapy; however, owing to relatively low response rates, unfavorable prognosis, and challenges such as susceptibility to metastasis and the development of drug resistance, there is an urgent need to explore new therapeutic approaches and optimize treatment strategies [4, 5]. This review focuses on the pathological characteristics of TPBC, elucidating the intricate signaling pathways, gene mutation profiles, and molecular underpinnings of drug resistance. A tailored, precision medicine approach that involves multidrug combinations is imperative. Synthesizing and juxtaposing diverse therapeutic modalities and combinatorial strategies could achieve a "triple therapy" regimen integrating endocrine therapy, targeted therapy, and CDK4/6 inhibitors; this approach could maximize patient outcomes and potentially set a new benchmark for TPBC management.

Characteristics of triple-positive breast cancer

Pathologic features of TPBC

Most patients with triple-positive breast cancer are diagnosed between the ages of 45 and 75 years [6]. Clinical studies illuminate a more aggressive biological profile for TPBC, with pathological hallmarks that include large, often calcified, tumor masses, typically graded as III. The tumors frequently exhibit irregular margins, granular calcifications, and evidence of vascular or neural invasion, predisposing them to metastasize to axillary lymph nodes [7]. In a comprehensive analysis of tumor specimens from 2,284 female patients with primary breast cancer, Kast and colleagues reported that TPBC is associated with a significantly greater recurrence rate than that of luminal A breast cancer. Interestingly, despite TPBC's aggressiveness, patients tend to have a more favorable prognosis than those with HER2-overexpressing breast cancers. The predominant pathological subtype is invasive ductal carcinoma, with a propensity for distant metastasis to visceral organs and bones [8]. Moreover, TPBC is characterized by a positive correlation between Ki-67 and HER2 expression, with elevated levels of both proteins indicating a worse prognosis [9]. At the proteomic level, a negative correlation exists between the expression of ER and PR and that of HER2. The interplay between the ER and HER2 pathways has also been identified as a significant contributor to endocrine therapy resistance in TPBC, underscoring the complexity of treatment strategies for this subtype [10].

Major signaling pathways of TPBC

Research has revealed an intricate interplay between multiple segments of the ER- and HER2-mediated signaling cascades in TPBC. The ER signaling pathways can be categorized into two principal types. The initial type, recognized as the genomic or classical pathway, is instigated by the activation of the ER upon encountering a specific stimulus. The quintessential trigger for ER activation is the binding of estrogen (E2) to its cognate receptor (ER). The E2–ER complex subsequently migrates to the nucleus, where it docks onto estrogen response elements (EREs). These EREs are situated within the DNA sequence and are pivotal in modulating the transcriptional activity of genes under ER regulation [11]. The second category of ER signaling is characterized by a nongenomic mechanism. The alternative pathway involves ER activation at the plasma membrane or in proximity to receptors, facilitated by a suite of protein kinase cascades. This activation elicits a rapid cellular response, encompassing the release of nitric oxide (NO), the modulation of transmembrane ion fluxes, and the initiation of the receptor tyrosine kinase (RTK) and proteolipid kinase pathways [12].

In TPBC, amplification of the *ERBB2* gene, which encodes HER2, is typically observed at relatively low levels [13]. Nevertheless, at the cellular level, HER2 can interact with other ERBB family members, including EGFR, HER3, and ERBB3, forming homo- or heterodimers that amplify the activation of downstream signaling pathways via kinase activity. For example, the EGFR/HER2 heterodimer is known to activate the RAS–RAF–MEK–ERK pathway, commonly referred to as the MAPK/ERK pathway, whereas the HER2/HER4 heterodimer triggers the PI3K–AKT–mTOR pathway [14]. These signaling cascades are instrumental in various cellular processes, including cell cycle progression, apoptosis, cell polarity, and cell motility, all of which contribute to breast cancer's growth and metastatic spread [15].

The intricate crosstalk between the ER and HER2 signaling pathways, predominantly via the PI3K–AKT–mTOR and RAS–RAF–MAPK axes, is now recognized as a key regulatory mechanism for ERα activity [16]. The ER interfaces with the HER signaling network at the

cellular membrane and within the nucleus, engaging with and activating various components of the HER signaling cascade, including PI3K, AKT, MAPK, and RAS. Upon estrogen binding, nongenomic ERs interact directly or indirectly (via G proteins) with HER2/HER1-4 dimers, thereby activating downstream Ras-MAPK and PI3K-AKT signaling pathways, which subsequently phosphorylate the ER, a host of transcription factors (TFs), and coregulators such as coactivators (CoAs) and corepressors (Rs). Phosphorylation modulates gene expression patterns within the cell [17]. Moreover, the ER has the capacity to ignite signaling cascades that involve the tyrosine kinase c-Src and other downstream elements of the HER signaling network [18]. Estrogen signaling increases the expression of growth factors such as transforming growth factor-α (TGF-α) and insulin-like growth factor-1 (IGF1) [19, 20] (Fig. 1).

Crosstalk between two signaling pathways is a crucial determinant of the metastatic progression and therapeutic resistance of TPBC. Nonetheless, the intricate mechanisms underlying the interplay of potential molecular and druggable target interactions within these pathways are yet to be fully elucidated. There is a pressing need for researchers to thoroughly investigate novel therapeutic strategies and devise more efficacious approaches to prevent recurrence and metastasis.

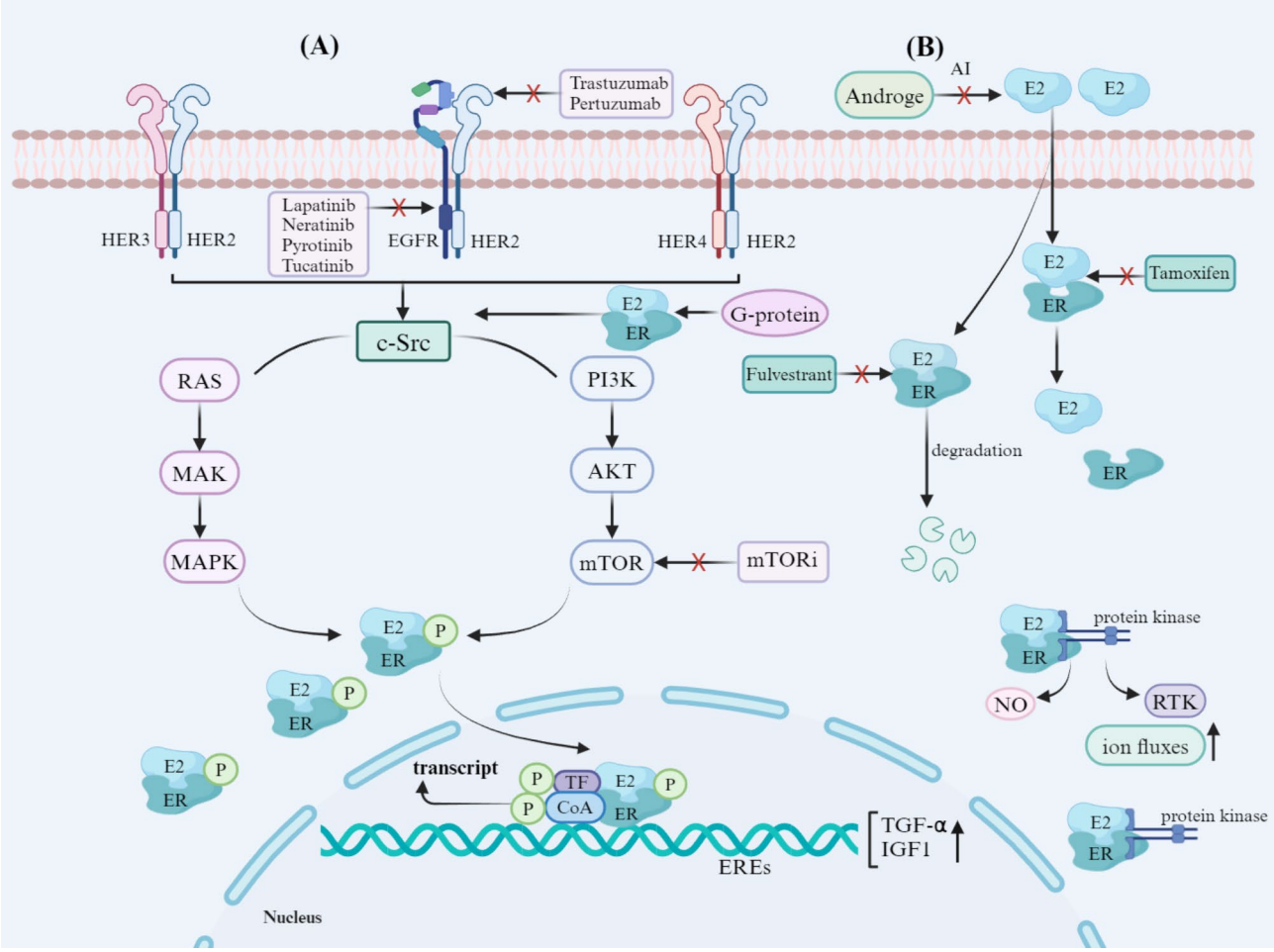

Fig. 1 Schematic diagram of the crosstalk between key targets in different signaling pathways in triple-positive breast cancer and therapeutic drugs. HER2 binds to its family members (such as EGFR/HER3/HER4) and forms homo- or heterodimers. After the ligand binds to HER2, the tyrosine kinase of HER2 is activated. Moreover, after binding to the estrogen receptor (ER), estrogen (E2) can interact with HER2/HER1-4 dimers directly or indirectly (via G proteins), stimulate the tyrosine kinase c-Src, activate the downstream Ras-MAPK and PI3K-AKT signaling pathways, and then phosphorylate the ER and other transcription factors (TFs) and coactivators/coinhibitors (CoAs/R). The E2–ER complex translocates to the nucleus and binds to estrogen response elements (EREs) in DNA, regulating the transcription of ER genes. Furthermore, estrogen signaling affects the expression of growth factors, such as the upregulation of transforming growth factor-α (TGF-α) and insulin-like growth factor 1 (IGF1). Estrogen receptor activation at the plasma membrane or in proximity to receptors is mediated by various protein kinase cascades, resulting in responses such as nitric oxide (NO) release, increased transmembrane ion flux, and activation of RTK and proteolipid kinase pathways. (**A**). Targets of HER2-targeted drugs. (**B**). Mechanism of action of endocrine drugs: AIs block the conversion of androgens into estrogens; tamoxifen competitively binds to the ER and blocks the binding of the ER to E2; and fulvestrant promotes ER degradation

Mutation profiles in TPBC

Research into the genomic lineage heterogeneity of HER2-positive breast cancer indicates that genes such as *TP53*, *CDK12*, *PIK3CA*, and *RARA*, demonstrate high mutation frequencies, in addition to the *ERBB2* gene, pivotal for comprehending the underlying molecular mechanisms of this disease. Comparative analyses between HR+/HER2 + and HR-/HER2 + patients revealed significant differences in mutation types between the two groups (767 vs. 352). Notably, in the HR+/HER2 + cohort, there was a marked amplification of genes such as *SPOP*, *CCND1*, *FGF19*, *FGF4*, *FGF3*, *RNF43*, *RAD51C*, *ADGRA2*, and *MDM4*, alongside frequent mutations in the *GATA3* gene [21]. Mutation of *GATA3* is particularly noteworthy, as this gene directly upregulates ERα and other oncogenes related to the estrogen signaling pathway, such as *DACH1* and *GREB1*, and genes involved in growth factor signaling pathways, such as *KIF16B* and *ERBB4*, which promote the development of luminal-type breast cancer [22]. Moreover, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis revealed significant enrichment of mutated genes in the HR+/HER2 + patient group within pathways such as homologous recombination repair, TGF-β, and WNT [21]. PARP inhibitors are potent targeted therapeutic agents against the DNA damage response (DDR) pathway, suggesting the potential application of PARP inhibitors in the treatment of HR+/HER2 + diseases.

Current status of treatment for TPBC

Neoadjuvant chemotherapy is favored by clinicians for treating TPBC and is chosen by more patients because it can achieve similar efficacy as receiving standard postoperative chemotherapy. Docetaxel (DTX) and albumin paclitaxel are commonly used first-line drugs in chemotherapy and have obvious inhibitory effects on the growth and reproduction of breast cancer cells. However, it is noteworthy that clinical data indicate TPBC patients still exhibit comparatively lower long-term survival rates than certain other breast cancer subtypes even after receiving chemotherapy, likely attributable to the heterogeneous nature of these tumors and variations in treatment response profiles. Since the pathogenesis of TPBC is a direct result of extensive crosstalk between the ER and HER2 signaling pathways, therapeutic strategies that target only one of these pathways usually lead to the upregulation of the other pathway and, ultimately, resistance to treatment. Therefore, modulating and disrupting the crosstalk between these two signaling pathways is essential for more effective TPBC treatment. An increasing number of targeted drugs, endocrine therapeutics, and novel drugs are becoming available on the market; however, it is highly important to explore how to more effectively select optimal treatment strategies and combinatorial approaches among HER2-targeted therapy, endocrine therapy, and chemotherapy.

Monoclonal antibody

With advancements in breast cancer molecular biology research, HER2-targeted therapeutic agents have been developed, and trastuzumab has become the therapeutic agent of choice for patients with HER2-overexpressing breast cancer. Trastuzumab is a recombinant DNA-derived humanized monoclonal antibody that prevents the binding of epidermal growth factor to HER2 by binding to the HER2 extracellular structural domain (ECD) and inhibits the intracellular HER2 signaling pathway, blocking the growth of cancer cells; trastuzumab also inhibits cell cycle blockade, mediates antibody-dependent cell-mediated cellular cytotoxicity (ADCC) effects, and directly kills tumor cells [23]. The treatment of HER2-positive breast cancer is now a focus of "targeted therapy" approache, substantially altering patient outcomes. The addition of trastuzumab to postoperative adjuvant therapy for patients with HER2-positive early-stage breast cancer has long been the standard of care, and the efficacy of trastuzumab in patients with HER2-positive early-stage breast cancer has been confirmed in several important clinical studies, such as the HERA study, which demonstrated that the prognosis of patients with HER2-positive early-stage breast cancer could be significantly improved by treatment with trastuzumab [24]. The B-31 and N9831 studies compared chemotherapy regimens alone with chemotherapy combined with trastuzumab, establishing that the addition of a trastuzumab monoclonal antibody unequivocally improved disease-free survival (DFS) and overall survival (OS) outcomes through 10 years of follow-up [25]. The results of the NOAH study confirmed that chemotherapy in combination with trastuzumab resulted in a higher pathologic complete response (pCR) rate than did chemotherapy alone in HER2-positive patients receiving neoadjuvant therapy; the patients' event-free survival (EFS) was also increased. Patients who achieved pCR had longer DFS and OS durations than those who did not [26]. These findings established trastuzumab as the standard of care in the neoadjuvant treatment of HER2-positive breast cancer. The results of the M77001 study confirmed that paclitaxel combined with trastuzumab significantly improved progression-free survival (PFS) and OS outcomes in patients with HER2-positive advanced recurrent metastatic breast cancer [27]. Thus, anti-HER2 therapy based on trastuzumab is necessary and effective for HER2-positive breast cancer patients, regardless of whether it is applied as an early adjuvant therapy, neoadjuvant therapy, or advanced recurrent breast cancer treatment.

Furthermore, early studies revealed that multiple domains within HER2 can exert synergistic antitumor effects, which led to the development of pertuzumab, the first monoclonal antibody referred to as a "HER dimerization inhibitor." Pertuzumab slows tumor growth by blocking the formation of homo- or heterodimers of HER2 with other HER receptors. Thus, the "dual-target era" has begun, and the APHINITY study revealed that adding pertuzumab to adjuvant trastuzumab and chemotherapy in HER2-positive patients with early-stage breast cancer significantly improved DFS outcomes [28]. The NeoSphere study confirmed that adding pertuzumab to the combination of paclitaxel and trastuzumab can further improve pCR rates in HER2-positive patients [29]. The CLEOPATRA double-blind randomized trial comparing the first-line efficacy of pertuzumab, trastuzumab, and docetaxel with that of trastuzumab and docetaxel in the treatment of HER2-positive metastatic breast cancer revealed that the combination of pertuzumab, trastuzumab, and docetaxel significantly improved the treatment outcome for patients with HER2-positive metastatic breast cancer [30]. Improvements in OS outcomes were found to be maintained after a median follow-up of more than 8 years, and the long-term safety and cardiac safety profiles of the combination were also maintained in the overall safety population and crossover patients [31]. These results constitute a far-reaching milestone in clinical research. The TRAIN-2 study revealed that, in patients younger than 60 years of age with a high tumor load and platinum resistance, compared with anthracycline-containing regimens, paclitaxel and carboplatin in combination with trastuzumab versus pertuzumab resulted in the same rate of pCR and significantly lower toxicity responses, such as neutropenia [32]. With the confirmation of the above clinical findings, dual-targeted therapy is now recommended for patients who are suitable for single-targeted therapy in the neoadjuvant phase.

Small-molecule tyrosine kinase inhibitors (TKIs)

Approximately 30–50% of patients with advanced HER2-positive breast cancer will develop central nervous system (CNS) metastases, with a risk of approximately 10% per year, and half of these patients will die from disease progression in the brain [33]. The combination of trastuzumab, pertuzumab, and docetaxel essentially delays the onset of brain metastases in advanced breast cancer patients [34]. However, the effective utilization of these drugs is greatly reduced because of their inability to penetrate the blood–brain and blood–tumor barriers [35]. TKIs, which are small-molecule targeted drugs, primarily inhibit cancer cell proliferation and promote apoptosis by competitively binding to the binding domain of the epidermal growth factor receptor (EGFR) family with the intracellular homologous structure of adenosine triphosphate (ATP), thereby blocking the signaling pathway downstream of tyrosine phosphorylation and ligand binding [36]. TKIs have a significantly lower molecular weight than monoclonal antibodies, and this allows TKIs to cross the blood–brain barrier more effectively and exert drug effects. Furthermore, TKIs are administered orally and demonstrate less cardiotoxicity, indicating potential advantages in clinical applications. Currently, TKIs are mainly used for anti-HER2 treatment after failure of trastuzumab treatment for HER2-positive advanced breast cancer and intensive treatment after partial completion of 1 year of adjuvant therapy with a trastuzumab monoclonal antibody.

Lapatinib is an orally active quinazoline characterized by its ability to reversibly block the phosphorylation of EGFR1, HER2, HER4, extracellular signal-regulated kinase 1,2 (ERK-1,2), and protein kinase B (PKB/AKT). Additionally, lapatinib inhibits cell cycle protein D expression levels in human tumor cell lines and xenografts [37]. It is used clinically, mainly in combination with capecitabine, in metastatic or advanced breast cancer patients with HER2 overexpression previously treated with anthracycline, paclitaxel, or trastuzumab. The EGF100151 study demonstrated thatlapatinib in combination with capecitabine versus capecitabine alone prolonged patients' time to progression (TTP) and PFS duration [38]. In addition, lapatinib in combination with paclitaxel significantly improved the median OS, median PFS, and objective response rate (ORR) outcomes compared with those of the paclitaxel and placebo groups [39]. However, in the ALTTO study, although the combination therapy arms had higher DFS rates, these differences were not significant, and patients treated with lapatinib experienced higher rates of severe side effects, such as diarrhea, rash, and hepatic toxicity [40]. Therefore, considering the increased toxicity and limited therapeutic improvement associated with lapatinib, this medication is currently not recommended for routine use in the neoadjuvant or adjuvant treatment setting.

Neratinib is an irreversible pan-HER family (HER1, HER2, and HER4) inhibitor that blocks the downstream PI3K/Akt and Ras/Raf/MEK/ERK signaling pathways [41]. Clinical and preclinical studies have shown that the combination of trastuzumab and neratinib for the treatment of breast cancer cells is an effective regimen to counteract both innate and acquired resistance to trastuzumab. Regarding the adjuvant treatment of early-stage breast cancer, the ExteNET study demonstrated that patients with stage II-III HER2-positive breast cancer who completed 1 year of standard trastuzumab therapy followed by 1 year of intensive oral neratinib therapy had significantly improved iDFS outcomes [42]. The results of the NALA study revealed that, in the treatment of advanced breast cancer, neratinib in combination with

capecitabine significantly prolonged PFS durations while delaying the onset of symptomatic brain metastases compared with lapatinib in combination with capecitabine in patients with advanced or metastatic HER2-positive breast cancer who had received ≥ 2 anti-HER2 therapies [43], suggesting that neratinib is a new option after the failure of advanced multiline therapy.

Pyrotinib is a novel orally administered and irreversible TKI with a mechanism similar to that of neratinib. In the PHOEBE phase III study, the combination of pyrotinib with capecitabine significantly improved PFS outcomes in patients who had previously received trastuzumab and paclitaxel analogs [44]. Pyrotinib provides better survival benefits to patients and provides more effective treatment options to combat resistance to trastuzumab, which inevitably occurs in the treatment of metastatic HER2-positive breast cancer.

Different from the three TKIs mentioned above, tucatinib blocks HER2 and not EGFR. Tucatinib is currently used in combination with trastuzumab and capecitabine for the treatment of patients with locally advanced unresectable or metastatic HER2-positive breast cancer who have received at least three lines of anti-HER2 therapies. The HER2CLIMB study revealed that treatment with tucatinib in combination with trastuzumab and capecitabine significantly improved OS and PFS outcomes and significantly reduced the risk of death in the tucatinib arm compared with a placebo in combination with trastuzumab and capecitabine. Moreover, in the subgroup with brain metastases, the median CNS PFS and median OS durations were significantly prolonged in the tucatinib group, and the intracranial ORR was significantly greater in the tucatinib group than in the control group, significantly reducing the risk of progression of the CNS and the risk of death [45]. In summary, the tucatinib combination is expected to become the new standard of care for HER2-positive metastatic breast cancer patients with brain metastases.

Antibody-drug conjugates (ADCs)

ADCs are humanized or human-derived monoclonal antibodies that combine the high specificity of monoclonal antibodies with the potent cytotoxicity of small-molecule drugs. The mechanism involves binding the antibody component to tumor antigens, where the ADC-antigen complex undergoes receptor-mediated endocytosis to enter the cell. Once inside the cell, the active cytotoxic agent is released, leading to the targeted destruction of tumor cells [46]. ADCs have better pharmacokinetic characteristics and cytotoxic effects than traditional targeted drugs and can effectively kill cancer cells, reduce toxic side effects, and have broader application prospects.

Trastuzumab emtansine (T-DM1) is the first ADC approved for anti-HER2 therapy in breast cancer. This pioneering compound results from strategic conjugation between HER2-targeting trastuzumab and the microtubule-inhibiting chemotherapeutic agent emtansine (DM1), facilitated by an innovative nonreducible thioether linker. The EMILIA study confirmed a greater PFS and OS benefit from T-DM1 than capecitabine in combination with lapatinib in patients with HER2-positive locally advanced or metastatic breast cancer who previously received trastuzumab in combination with paclitaxel, independent of the ER/PR status. These benefits were also observed in patients with metastatic disease, particularly those with a disease-free interval of less than six months after completing adjuvant or neoadjuvant treatment with trastuzumab [47]. The KATHERINE study revealed that in patients who did not achieve pCR after neoadjuvant therapy with trastuzumab in combination with paclitaxel, postoperative adjuvant therapy with T-DM1 significantly improved iDFS outcomes compared with trastuzumab [48].

Trastuzumab deruxtecan (DS-8201) is a novel antibody–drug coupling agent comprising trastuzumab and a type I topoisomerase inhibitor linked by an enzymatically cleavable peptide junction. DS-8201 crosses cell membranes more readily and has a more potent cytotoxic effect on tumor cells than T-DM1. In the DESTINY-Breast01 and DESTINY-Breast02 studies, DS-8201 demonstrated favorable therapeutic efficacy in patients with HER-2-positive advanced metastatic breast cancer who had received T-DM1 treatment [49, 50]. Similarly, in the DESTINY-Breast03 study, compared with T-DM1, DS-8201 significantly improved patients' PFS outcomes and reduced the risk ratio for disease progression or death by 72%, establishing the status of DS-8201 after trastuzumab failure [51]. Notably, unlike T-DM1, DS-8201 also kills tumor cells with low HER-2 expression, potentially due to the "bystander effect" of DS-8201, killing adjacent tumor cells with low HER2 expression through effective transport or release of the drug [52]. The DESTINY-Breast04 study compared the efficacy of DS-8201 with that of physician-selected chemotherapy regimens in patients with hormone receptor-positive HER-2 overexpression, suggesting that DS-8201 was significantly more efficacious than chemotherapy in patients who had received 1–2 lines of prior therapy, with the benefit being especially pronounced in the prespecified subgroup of HR-positive patients [53].

PI3K/AKT/mTOR inhibitors

The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway (PAM signaling pathway), which plays an important role in the development of breast cancer, is closely related to resistance to endocrine therapy in advanced breast cancer patients, and anticancer therapies

targeting key molecules in the PAM signaling pathway have become a research focus in recent years. Targeting PAM signaling pathway inhibitors has obvious clinical benefits for advanced breast cancer patients, especially those with hormone receptor-positive, HER2-negative disease.

The emergence of trastuzumab resistance is usually thought to be mediated by the PI3K/AKT/mTOR pathway, and activation of the PI3K pathway is caused mainly by *PIK3CA* mutation or amplification and *PTEN* deletion [54]. *PTEN* activation is a novel mechanism for the antitumor activity of trastuzumab. Conversely, *PTEN* deletion induces trastuzumab resistance in HER2-overexpressing breast cancers [55]. In preclinical models, *PTEN* loss results in a cascade reaction with PI3K, leading to mTOR activation [56]. mTOR, in turn, regulates transcription and translation by phosphorylating downstream proteins, including 40 S ribosomal protein S6 [57].

Everolimus is a mTOR inhibitor, and in an in vivo study, the combination of trastuzumab and everolimus significantly reduced the tumor volume in mice compared with either drug alone ($p < 0.05$). In addition, compared with everolimus alone, the combination of trastuzumab and everolimus reduced the expression levels of Ki67, AKT1, and phosphorylated AKT ($p < 0.05$) [58]. In a phase I study, the coadministration of trastuzumab, vincristine, and everolimus was associated with overall remission in 44% of patients with trastuzumab-resistant breast cancer and 55% of patients with paclitaxel-resistant and trastuzumab-resistant tumors [59, 60]. Similarly, in a phase I pilot study in patients with trastuzumab-resistant, HER2-overexpressing metastatic breast cancer, the addition of everolimus to the combination of paclitaxel and trastuzumab was generally well tolerated and associated with an initially high tumor response rate [59]. These studies enhanced the potential activity of everolimus in the treatment of HER2-overexpressing metastatic breast cancer and the role of mTOR inhibitors in delaying or reversing trastuzumab resistance mediated by the PI3K/AKT/mTOR pathway. In addition, the results of the RAD001 study revealed that the combination of everolimus and trastuzumab resulted in a clinical benefit in 34% of patients with trastuzumab-resistant tumors [61].

A series of studies have confirmed that the therapeutic strategy of combining anti-HER2 targeted drugs with chemotherapy can alter the progression of disease in patients with TPBC, and this combination is often considered the first-line treatment for metastatic breast cancer (Table 1). Some TPBC patients initially exhibit a pronounced response; however, a proportion of patients are nonresponders, leading to rapid tumor progression and recurrence. Therefore, it is imperative to concurrently suppress the expression of both HER2 and HR to enhance the treatment efficacy for these patients.

Endocrine therapy combined with targeted therapy

Endocrine therapy, also referred to as antihormone therapy, mainly regulates hormone secretion and prevents cancer cells from accessing and utilizing the natural hormones (mainly estrogen and progesterone) needed for growth. The mechanism involves changing the breast cancer cells' growth-related endocrine microenvironment, halting proliferation at the G0/G1 stage to control the tumor and promote remission. The different mechanisms of action can be classified into five major categories: selective estrogen receptor modulators (SERMs), ovarian function suppressors (OFSs), aromatase inhibitors (AIs), selective estrogen receptor downregulators (SERDs), and sex hormone analogs. Endocrine therapy not only reduces the risk of recurrence in early-stage breast cancer patients but also prolongs PFS durations in patients with metastatic breast cancer. Endocrine therapy has mild adverse effects on patients' quality of life and is critical for the treatment of HR-positive breast cancer.

Endocrine therapy combined with single-targeted therapy

For patients with advanced TPBC, anti-HER2 targeted therapy combined with endocrine therapy can significantly improve outcomes compared with endocrine therapy alone. Preclinical evidence suggests that crosstalk between HER2 and ER signaling pathways in breast cancer also contributes to resistance to hormone therapy. Trastuzumab in combination with tamoxifen or fulvestrant restores tumor sensitivity to these hormonal agents and may inhibit tumor growth [62]. Thus, it is evident that concurrent inhibition of the HER2 and ER signaling pathways is more efficacious than inhibition of the ER pathway alone.

Patients with TPBC receiving first-line treatment with either an aromatase inhibitor (AI) in combination with lapatinib or trastuzumab monotargeted therapy had better PFS outcomes than did those receiving treatment with an aromatase inhibitor alone. The TAnDEM study demonstrated that trastuzumab, in combination with anastrozole, improved the median PFS compared with anastrozole alone in patients with confirmed hormone receptor positivity [63]. A pilot study revealed that the combination of letrozole and lapatinib significantly reduces the risk of disease progression compared with letrozole–placebo and that the clinical benefit rate (≥ remission or stabilization at 6 months) is significantly improved with lapatinib–letrozole [64]. Notably, the results of the SYSUCC-002 study revealed that in patients with advanced metastatic TPBC, trastuzumab combined with endocrine therapy was no less efficacious than trastuzumab combined with chemotherapy, with the

Table 1 Clinical experimental study of targeted therapy combined with chemotherapy

Trial information	Study design	Sample size	Population	Outcomes
NSABP B-31 and NCCTG N9831 [25]	doxorubicin + cyclophosphamide + paclitaxel (DCP) vs. doxorubicin + cyclophosphamide + paclitaxel + trastuzumab (DCPT)	4046	HER2-positive operable breast cancer, (ER or PR-positive: $n = 2115/4046$, 52%)	DCPT led to a 37% relative improvement in OS ($p < 0.001$) and an increase in 10-year OS rate from 75.2–84%.; in 10-year DFS rate from 62.2–73.7%.
NOAH [26]	Chemotherapy (C) vs. chemotherapy + trastuzumab (TC)	235	HER2-positive locally advanced or inflammatory breast cancer, (HR-positive: $n = 84/235$, 36%)	C vs. TC in the 3-year event-free survival was 56% vs. 71%
M77001 [27]	trastuzumab + docetaxel (TD) vs. docetaxel (D)	186	HER2–positive metastatic breast cancer, (HR-positive: $n = 91/186$, 49%)	TD vs. D in terms of overall response rate was 61% v 34%; median OS was 31.2 v 22.7 months; median time to disease progression was 11.7 v 6.1 months; median time to treatment failure was 9.8 v 5.3 months.
APHINITY [28]	pertuzumab + chemotherapy + trastuzumab vs. placebo + chemotherapy + trastuzumab	4805	node-positive HER2-positive early BC, (HR-positive: $n = 3082/4805$, 64%)	pertuzumab vs. placebo groups:6-year iDFS being 88% vs. 83%.
NeoSphere [29]	pertuzumab + trastuzumab + docetaxel (PTD) vs. trastuzumab + docetaxel (TD)	417	locally advanced, inflammatory, or early-stage HER2-positive breast cancer, (HR-positive: $n = 197/417$, 47%)	pCR 85% in PTD vs. 76% in TD.
CLEOPATRA [30]	pertuzumab + trastuzumab + docetaxel vs. placebo + trastuzumab + docetaxel	808	HER2–positive metastatic breast cancer, (HR-positive: $n = 388/808$, 48%)	placebo versus pertuzumab arm in terms of median PFS of 12·4 vs. 18·7 months; the risk of death in the pertuzumab group was reduced by 34%.
EGF100151 [38]	lapatinib + capecitabine (LC) vs. capecitabine(C)	408	HER2-positive MBC female patients who have received treatment, (ER or PR-positive: $n = 192/408$, 47%)	LC vs. C in terms of median OS times were 75.0 v 64.7 weeks.
NCT00281658 [39]	lapatinib + paclitaxel (LP) vs. placebo + paclitaxel (P)	444	HER2-overexpressing metastatic breast cancer, (ER or PR-positive: $n = 224/444$, 50%)	LP vs. P in terms of median OS was 27.8 v 20.5 months; median PFS was 9.7 v 6.5 months; ORR was 69% v 50%.
ExteNET [42]	Neratinib vs. Placebo	2840	After trastuzumab-based adjuvant therapy in women with HER2-positive breast cancer, (HR-positive: $n = 1631/2840$, 57%)	Neratinib vs. Placebo of the 5-year invasive disease-free survival was 90·2% vs. 87·7%.
NALA [43]	neratinib + capecitabine (NC) vs. lapatinib + capecitabine (LC)	621	HER2-positive, metastatic breast cancer with ≥ 2 previous HER2-directed MBC regimens, (HR-positive: $n = 367/621$, 59%)	NC vs. LC in terms of ORRs was 32.8% vs. 26.7%; median DoR was 8.5 vs. 5.6 months; diarrhea 83% v 66%; and nausea 53% v 42%.
PHOEBE [44]	pyrotinib + capecitabine (PC) vs. lapatinib + capecitabine (LC)	267	HER2-positive metastatic breast cancer, (ER or PR-positive: $n = 120/267$, 45%)	PC vs. LC in terms of median PFS was12·5 months vs. 6·8 months.
HER2CLIMB [45]	tucatinib + trastuzumab + capecitabine (TTC) vs. placebo + trastuzumab + capecitabine (PTC)	291	HER2–positive breast cancer with brain metastases, (HR-positive: $n = 166/291$, 57%)	In the TTC, the risk of intracranial progression or death was reduced by 68%; the risk of death was lowered by 42%. TTC vs. PTC in terms of median PFS was 9.9 versus 4.2 months; median OS was 18.1 vs. 12.0 months; ORR was 47.3% vs. 20.0%.
EMILIA [47]	T-DM1 vs. lapatinib + capecitabine (LC)	991	HER2-positive advanced breast cancer, (HR-positive: $n = 545/991$, 55%)	T vs. LC in terms of median PFS was 9.6 vs. 6.4 months; median OS was 30.9 vs. 25.1 months; ORR was 43.6% vs. 30.8%; Rates of adverse events of grade 3 or above was LC vs. T (57% vs. 41%).
KATHERINE [48]	T-DM1 vs. trastuzumab	1486	HER2-positive early breast cancer after receiving neoadjuvant therapy, (HR-positive: $n = 1074/1486$, 72%)	The estimated percentage of patients who were free of invasive disease at 3 years was 88.3% in the T-DM1 vs. 77.0% in the trastuzumab.
DESTINY-Breast01 [49]	Trastuzumab deruxtecan (T-DXd)	184	HER2-positive metastatic breast cancer who had received previous treatment with T-DM1, (HR-positive: $n = 97/184$, 53%)	The median duration of follow-up was 11.1 months; the median response duration was 14.8 months; and the median PFS was 16.4 months.

Table 1 (continued)

Trial information	Study design	Sample size	Population	Outcomes
DESTINY-Breast02 [50]	T-DXd vs. capecitabine + trastuzumab or capecitabine + lapatinib (TC/LC)	608	HER2-positive metastatic breast cancer who were refractory or resistant to T-DM1, (HR-positive: *n* = 356/608, 59%)	T-DXd vs. TC/LC in the median follow-up was 21·5 months vs. 18·6 months; median PFS was 17·8 months versus 6·9 months; median OS 39 vs. 26.5 months; ORR was 69.7% vs. 29.2%.
DESTINY-Breast03 [51]	T-DXd vs. T-DM1	524	HER2-positive metastatic breast cancer previously treated with trastuzumab and a taxane, (HR-positive: *n* = 265/524, 51%)	T-DXd vs. T-DM1 of those who were alive without disease progression at 12 months was 75.8% vs34.1%; patients who were of alive at 12 months was 94.1% vs. 85.9%; an overall response was 79.7% vs. 34.2%.
DESTINY-Breast04 [53]	T-DXd vs. chemotherapy	557	HR positive and negative HER2 low expression in patients with advanced breast cancer, (HR-positive: *n* = 490/557, 88%)	In the HR–positive cohort, T-DXd vs. C in terms of median PFS was 10.1 vs. 5.4 months; OS was 23.9 vs. 17.5 months. Among all patients, T-DXd vs. C in terms of median PFS was 9.9 vs. 5.1 months; OS was 23.4 vs. 16.8 months.
RAD001 [61]	trastuzumab + everolimus	47	HER2-overexpressing metastatic breast cancer, (ER or PR-positive: *n* = 28/47, 60%)	everolimus + trastuzumab resulting in a clinical benefit rate of 34%, the median PFS was 4.1 month.

former associated with better median PFS and median OS outcomes and fewer adverse effects. For patients with a disease-free interval (DFI) of > 24 months, trastuzumab in combination with endocrine therapy may be of greater benefit [65]. Therefore, HER2-targeted therapy combined with endocrine therapy can replace standard HER2-targeted therapy combined with a chemotherapy approach, and the principle of endocrine therapy preference also applies to patients with advanced metastatic TPBC.

Endocrine therapy combined with dual-targeted therapy

With the widespread use of pertuzumab in the clinic, dual-targeted combination chemotherapy with trastuzumab and pertuzumab has effectively improved the survival benefit of patients with breast cancer. Moreover, dual-targeted combined endocrine therapy has also become mainstream, and the combination of different drugs in combination therapy deserves in-depth clinical exploration to further improve the survival benefit of TPBC patients.

The CLEOPATRA clinical trial evaluated the combination of pertuzumab plus trastuzumab plus docetaxel versus trastuzumab plus docetaxel plus placebo for the first-line treatment of HER2-positive metastatic breast cancer and revealed that the former significantly prolonged PFS durations without a significant increase in cardiotoxic effects [66]. In the most recent PERTAIN study, a randomized trial was conducted in 258 patients with TPBC assigned to either dual-target therapy incorporating trastuzumab and pertuzumab alongside an aromatase inhibitor or a single-target regimen combining trastuzumab with an aromatase inhibitor. The findings indicated that the group receiving dual-target therapy in conjunction with endocrine therapy exhibited superior median PFS and OS benefits. Furthermore, among patients who have not undergone neoadjuvant chemotherapy, the combination of endocrine therapy with dual-targeted treatment has demonstrated enhanced survival benefits [67]. The ALTERNATIVE trial enrolled a total of 355 patients with advanced first- or second-line and above HR+/HER2 + breast cancer who were treated with endocrine medications in combination with anti-HER2 monoclonal antibodies and tyrosine kinase inhibitors. The results indicate that the combination therapy of lapatinib plus trastuzumab and aromatase inhibitors (AI) demonstrates superior PFS benefits compared to trastuzumab plus AI treatment in patients with TPBC, suggesting that the tolerability of the trastuzumab, lapatinib, and AI regimen is favorable, holding promise as a recommended treatment protocol for TPBC [3]. This finding also suggests that for highly selected TPBC patients, chemotherapy may be circumvented, and dual-targeted therapy in conjunction with endocrine treatment could provide an effective and safe therapeutic option.

The HeredERA study is a randomized, open-label, multicenter, phase III study evaluating the efficacy and safety of Giredestrant (an oral SERD drug) in combination with Phesgo after a dual-target subcutaneous agent (Phesgo) + induction chemotherapy (taxanes) compared with Phesgo (+/- endocrine therapy) in patients with previously untreated HER2+/ER + locally advanced or metastatic breast cancer [68]. The oral SERD drug giredestrant inhibits tumor cell proliferation by completely antagonizing the ER, blocking the ER signaling pathway, and enabling ER degradation, potentially providing additional patient benefits.

The results of the above studies demonstrate the benefits of simultaneously inhibiting the HER2 and estrogen receptor signaling pathways. Nonetheless, future investigations are warranted to explore the therapeutic efficacy

of combining diverse endocrine treatments, thereby informing the development of clinical strategies and guiding the selection of endocrine therapeutics for the management of TPBC.

Endocrine therapy and targeted therapy combined with CDK4/6 inhibitors

Given that cell cycle protein-dependent kinase (CDK) 4/6 inhibition combined with endocrine therapy results in favorable PFS and OS benefits for patients with advanced hormone receptor-positive, HER2-nonamplified (HR + HER2-) breast cancer, this study also provides new ideas for the treatment of patients with advanced TPBC.

Cell division activity is orchestrated by the collaborative action of cell cycle proteins (cyclins) and cell cycle protein-dependent kinases (CDKs). In many cancers, the cell cycle protein D-CDK4/6 pathway is overactivated and is no longer dependent on the stimulation of mitotic behavior, thus contributing to the infinite proliferation of tumor cells. CDK4/6 inhibitors can regulate the cell cycle and selectively inhibit CDK4/6 kinase activity, thus blocking the progression of the cell cycle from the G1 to the S phase in cancer cell transformation, inhibiting the unlimited proliferation of tumor cells. Moreover, CDK4/6 inhibitors have synergistic effects with endocrine therapy, inhibiting the expression of the estrogen receptor signaling pathway and delaying and reversing resistance to endocrine therapy [69]. There are currently four approved CDK4/6 inhibitors on the market: palbociclib, ribociclib, abemaciclib, and dalpiciclib. CDK4/6 is overactivated in HR + breast cancer, and blockade of the dual pathways of CDK4/6 and ER can effectively inhibit breast cancer cell proliferation and tumor progression [70].

The NA-PHER2 study explored the administration of trastuzumab, pertuzumab, palbociclib, and fulvestrant during the neoadjuvant phase of therapy up to the time of surgery, with a decrease in Ki67 from 31.9 to 12.1% at baseline and a pCR of 27%, with preliminary data analysis showing an acceptable safety profile of the treatment while simultaneously demonstrating a good antitumor effect [71]. The PATRICIA phase II clinical study demonstrated the efficacy and safety of the CDK4/6 inhibitor palbociclib in combination with trastuzumab in the treatment of advanced HER2-positive breast cancer. The results of the study revealed that patients with advanced TPBC had a better prognosis when treated with palbociclib and trastuzumab and when the combination was followed by letrozole as a "triple therapy" [72]. In the phase II MonarcHER study, 237 patients with advanced TPBC who have received at least second-line anti-HER2 therapy were enrolled and randomized to three treatment groups (Group A: CDK4/6 inhibitor abemaciclib + trastuzumab + fulvestrant, Group B: CDK4/6 inhibitor abemaciclib + trastuzumab and Group C: trastuzumab + chemotherapy). The outcome showed that Group A demonstrated significantly improved PFS and had a significantly higher ORR than those treated with the other two regimens. The regimen administered to Group A also demonstrated a tolerable safety profile, confirming that triple therapy can achieve certain clinical remission rates in the later-line treatment of TPBC patients. Therefore, chemotherapy-free regimens may be an alternative treatment option for patients with hormone receptor-positive, HER2-positive advanced breast cancer [73]. The LORDSHIPS first-/second-line phase Ib clinical study, which enrolled 15 patients with advanced TPBC, revealed that the three-agent combination of dalpiciclib + pyrotinib + letrozole may be the most beneficial in the first-line treatment of patients with advanced TPBC [74].

Therefore, combining anti-HER2 targeted therapy and endocrine therapy with CDK4/6 inhibitors is expected to achieve greater benefits for patients with advanced TPBC. With the increase in clinical research data and the development of precision therapy, the efficacy of salvage therapy for advanced TPBC patients is becoming clearer, and "triple combination" therapy is expected to become one of the best options for advanced TPBC patients (Table 2).

Mechanisms of TPBC drug resistance

Mechanisms of resistance to endocrine therapeutic strategies

ESR1 gene mutations are infrequently observed in primary breast cancer but are found in approximately 20% of patients with metastatic breast cancer who have received endocrine therapy, particularly after treatment with AIs [75]. Analyses of circulating tumor DNA from patients treated with AIs have shown that tumors with Y537S and D538G mutations exhibit increased invasiveness and reduced sensitivity to tamoxifen and fulvestrant [76].

The overexpression of HER2 diminishes sensitivity to antiestrogen therapy, with one of the mechanisms being the activation of the PI3K–AKT–mTOR and mitogen-activated protein kinase (MAPK) pathways [77]. The constituents of the MAPK pathway include *NF1*, *KRAS/NRAS/HRAS*, *BRAF*, and *MAP2K1*; the neurofibromin (*NF1*) gene is frequently associated with metastatic breast cancer [78]. *NF1* encodes neurofibromin, a negative regulator of Ras-GTP activation; loss of *NF1* leads to the activation of *RAS*, thereby triggering downstream activation of the MAPK pathway [79]. The absence of NF1 also promotes estrogen resistance in ER-positive breast cancer cells by inducing the expression of cyclin D1 in a cell cycle-independent manner [80].

DNA methylation contributes to endocrine therapy resistance in ERα-positive breast cancer by

Table 2 Clinical experimental study of targeted combined with endocrine therapy

Trial information	Study design	Sample size	Population	Outcomes
TAnDEM [63]	trastuzumab + anastrozole (TA) vs. anastrozole (A)	212	Postmenopausal women with HER2/HR–copositive MBC	TA vs. A in the median PFS was 4.8 vs. 2.4 months; CBR was 47% vs. 22%; ORR was 21% vs. 9%.
NCT00073528 [64]	letrozole + placebo (LP) vs. lapatinib + letrozole (LL)	1286	Postmenopausal women with HER2/HR–copositive MBC	LL vs. LP in the median PFS was 8.2 v 3.0 months; clinical benefit (responsive or stable disease ≥ 6 months) was 48% v 29%.
SYSUCC-002 [65]	trastuzumab + endocrine therapy (ET) vs. trastuzumab + chemotherapy (CT)	392	HR and HER2-positive metastatic breast cancer	ET vs. CT in the median PFS was 19.2 months vs. 14.8 months; OS was 33.9 vs. 32.5 months.
CLEOPATRA [66]	pertuzumab + trastuzumab + docetaxel (PTD) vs. placebo + trastuzumab + docetaxel (TD)	808	HER2-positive metastatic breast cancer	TD vs. PTD in the median PFS was 12.4 vs. 18.5 months.
PERTAIN [67]	pertuzumab + trastuzumab + AI (PTA) vs. trastuzumab + AI (TA)	129	HER2 and HR–positive metastatic/ locally advanced breast cancer (MBC/LABC)	PTA vs. TA in the median PFS was 18.89 months vs. 15.80 months; rates of grade ≥ 3 AEs were 64 (50.4%) of 127 and 48 (38.7%) of 124.
ALTERNATIVE [3]	lapatinib + trastuzumab + AI (LTA) vs. trastuzumab + AI (TA) vs. lapatinib + AI (LA)	355	Postmenopausal women with HER2 and HR-positive metastatic breast cancer	LTA vs. TA in the median PFS was 11 v 5.6 months.
PATRICIA [72]	palbociclib + trastuzumab (A) vs. palbociclib + trastuzumab + letrozole(B)	71	HER2-positive advanced breast cancer	Primary endpoint was progression-free survival rate at 6 months of A vs. B was 42.8% (12/28) vs. 46.4% (13/28); median PFS was 10.6 vs. 4.2 months.
monarcHER [73]	abemaciclib + trastuzumab + fulvestrant (group A) vs. abemaciclib + trastuzumab (group B) vs. chemotherapy + trastuzumab (group C)	237	HR and HER2-positive advanced breast cancer	In median OS was group A (31.1 months) vs. group B (29.2 months) vs. group C (20.7 months)
LORDSHIPS [74]	dalpiciclib + pyrotinib + letrozole	15	Postmenopausal women with HER2/HR–copositive MBC	The confirmed ORR of study treatment as first line (1 L) and second line (2 L) HER2-targeted therapy was 85.7% (6/7) and 50.0% (4/8); median PFS was 11.3 months (95% CI: 5.3 months to not reached).

downregulating ER expression and modulating ER target genes. Studies have shown that decreased expression of the transcription factor SALL2 leads to the downregulation of ER and PTEN expression, thereby activating the AKT/mTOR signaling pathway and resulting in tamoxifen resistance in ERα-positive breast cancer patients [81]. In HR-positive breast cancer, somatic mutations in histone methyltransferases, such as KMT2B, KMT2D, and KMT2E, and histone demethylases, including KDM4A, KDM5B, KDM5C, and KDM6A, are particularly pronounced [82].

Breast cancer stem cells (BCSCs) are frequently associated with endocrine therapy resistance in breast cancer and are key drivers of metastasis and endocrine resistance in this disease. The receptor Notch 4 is an important regulator of cancer stem cells and is overexpressed in BCSCs [83]. Gene expression profiles derived from breast cancer cells and xenografts treated with tamoxifen or fulvestrant revealed elevated expression of Notch target genes [84]. Death domain-associated protein 6 (DAXX) has been identified as a novel target of the Notch signaling pathway, with its RNA expression inversely correlated with Notch signaling in ER-positive breast tumor samples. High DAXX expression can inhibit tumor stem cells [85].

Mechanisms of resistance to targeted therapeutic strategies

Structural changes, including HER2 truncation mutants (p95HER2) and Δ16HER2, lead to the emergence of drug resistance. More importantly, p95HER2 can form heterodimers with HER3 and activate downstream pathways. Patients with high p95HER2 expression have shorter PFS and OS durations [86]. The overexpression of Δ16HER2 can downregulate the expression of miR-7, thereby activating and upregulating the Src signaling pathway [87].

Both HER2 phosphorylation and PI3K activation are associated with high expression of insulin-like growth factor 1 receptor (IGF-1R), which is a member of the tyrosine kinase receptor family and shares common downstream signaling pathways with HER2 and other EGFRs [88]. Upon ligand binding and subsequent activation, IGF-1R can bypass the activation of the PI3K signaling pathway, thereby contributing to trastuzumab resistance to a certain extent. Other tyrosine kinases, including AXL and EphA2, have also been implicated in trastuzumab resistance [89, 90].

The epithelial–mesenchymal transition (EMT) plays a significant role in the initiation, progression, and metastasis of breast cancer [91]. EMT induction results from

the synergistic action of various signaling pathways and molecules, with the primary inducer being the transforming growth factor TGF-β family, among which TGF-β2 and TGF-β3 are highly expressed in trastuzumab-resistant cells [92]. A multitude of transcription factors also play a role, such as the activation and overexpression of Snail, Twist, ZEB, Goosecoid, and Foxc2, conferring a mesenchymal phenotype upon tumor cells, leading to a significant decline in prognosis [93]. Notably, the overexpression of Snail, Twist, and other proteins during EMT strongly promotes trastuzumab resistance [94].

Mechanisms of drug resistance regulated by the tumor microenvironment

The tumor microenvironment is the tumor's complex internal environment, including tumor cells, various nonmalignant cells (such as fibroblasts and immune cells), and extracellular components (such as growth factors and cytokines) [95]. Complex bidirectional signal transmission among these components jointly regulates the growth and proliferation of tumor cells, participates in angiogenesis, provides nutrients, and promotes the immune escape of tumor cells [96]. Studies have shown that tumor-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), tumor-infiltrating lymphocytes (TILs), and their secreted factors in the tumor microenvironment play key roles in cancer drug resistance [97, 98].

After breast epithelial cells become malignant, the microenvironment components change significantly, leading to uncontrolled proliferation and tumor cell invasion [99]. Studies have reported that CAFs increase the number of cancer stem cells by secreting cytokines such as interleukin 6 (IL-6) and monocyte chemoattractant protein 1 (MCP-1/CCL2), thereby activating multiple pathways (such as the NF-κB, JAK/STAT3, PI3K/AKT/mTOR and MAPK/ERK pathways) and inducing resistance to breast cancer treatment [100, 101]. Interestingly, the fibroblast growth factor receptor (FGFR) on the surface of CAFs is also involved in breast cancer resistance to treatment. For example, in HER2-positive breast cancer trastuzumab-resistant cells, FGFR4 expression is upregulated, and the extracellular structural domain of FGFR4 interacts with fibroblast growth factor to activate β-catenin/TCF-4 signaling through the phosphorylation of GSK-3β, which drives drug resistance in HER2-positive breast cancer [102]. In addition, CAFs can reconstruct the extracellular matrix (ECM), forming a barrier between tumor cells and immune cells, hindering the approach of immune cells and thus achieving immune escape [103]. Notably, the reactive oxygen species (ROS) produced by CAFs during energy metabolism can induce EMT and acquire stem cell characteristics in tumor cells through the COX-2/NF-κB/HIF-1 signaling pathway, thereby leading to tamoxifen resistance in breast cancer [104, 105].

TAMs are derived mainly from circulating monocytes at tumor sites and promote tumor angiogenesis and cancer therapy resistance through metabolic reprogramming [106]. TAMs and breast cancer cells secrete inflammatory cytokines and chemokines, such as TNF-α, IL-6, and CCL18, which cause crosstalk in multiple signaling pathways and promote drug resistance [107]. Studies have shown that TAMs secreting CCL2 can activate the PI3K/AKT/mTOR signaling pathway in breast cancer cells, increasing resistance to trastuzumab and TAM recruitment [108]. In addition, TAMs activate EGFR signaling via the Src/STAT3/ERK1/2 signaling pathway in breast cancer cells by secreting IL-8, promoting resistance to lapatinib [109]. TNF-α and IL-6 secreted by TAMs can activate the NF-κB/STAT3/ERK signaling pathway and ERα hyperphosphorylation in breast cancer cells, leading to the overexpression of cyclin D1, c-Myc, and IL-6 and promoting drug resistance [110].

Mechanisms of resistance regulated by epigenetic modifications

Epigenetics studies reversible and heritable changes in gene function, including DNA methylation, histone modification, chromosome remodeling, and noncoding RNA regulation, without changing the nuclear DNA sequence [111]. Abnormal epigenetic modifications cause cells to exhibit diverse gene expression patterns that affect embryonic development, stem cell differentiation, senescence, tumorigenesis, progression, and drug resistance in complex biological processes.

DNA methylation is an important form of epigenetic modification that mainly occurs on CpG islands in gene promoter regions. In breast cancer, the promoter regions of tumor suppressor genes such as *BRCA1* and *BRCA2* are hypermethylated, causing these genes to be silenced and thereby losing their inhibitory effect on tumor cell growth [112]. In addition, hypomethylation of the promoter region of the drug resistance-related gene *MDR1* leads to upregulation of its expression, promoting drug efflux and increasing tumor cells' resistance to chemotherapeutic drugs [113].

Numerous studies have suggested that dysfunctional epigenetic regulation may be responsible for the development of cellular transformation and endocrine therapy resistance in breast cancer [114]. One study revealed that the histone methylase SET8 directly interacts with the key EMT regulator TWIST, jointly promoting the EMT and metastasis of breast cancer cells. This study revealed that SET8 directly regulates the transcription of the TWIST target genes E-cadherin and N-cadherin in a dual mode through its catalytic product, monomethylated histone H4K20 [115]. KDM6B induces drug resistance

in cells by modifying histones through demethylation and altering the epigenetic landscape and chromatin structure [116]. Interestingly, histone acetyltransferases (HATs) catalyze the acetylation of histones, transcription factors (TFs), and heat shock proteins, which can lead to resistance to tamoxifen treatment [117]. In breast cancer, *ARID1A* determines breast ductal luminal profile fidelity and sensitivity to endocrine therapy, and mechanistically, *ARID1A* deletion reduces chromatin accessibility and the binding of transcription factors (TFs) and decreases the binding of ERα and FOXA1 to chromatin [118]. Notably, under hypoxic conditions, HIF-1α and HIF-2α induce ALKBH5 expression, leading to increased mRNA stability of pluripotency factor genes such as *NANOG* and promoting the self-renewal and proliferation of breast cancer stem cells by increasing the stability of *NANOG* mRNA [119].

Challenge and perspective

A recent study demonstrated that ACTL6A can be an important biomarker for tumor proliferation and drug resistance in TPBC [120]. High GATA3 expression may be associated with poor prognosis in breast cancer patients and may reduce the occurrence of immune infiltration in TPBC [121]. For the precision treatment of TPBC, multiomics analysis, such as genome and transcriptome analysis of single tumor cells, can more accurately reveal the heterogeneity between cells, providing new options for tumor heterogeneity research. Tracing the origin of tumor cells and locking in tumor-initiating and metastasis-initiating cells helps analyze the mechanisms underlying tumor metastasis.

The treatment of TPBC is unique in that it typically begins with targeted combination chemotherapy, is maintained with endocrine cotargeting, is augmented by targeted intensification with TKIs, and relies on ADCs, CDK4/6 inhibitors, and PIK3CA inhibitors for further guarantees, with anti-HER2 therapy as the mainstay. However, it is hoped that patients will obtain substantial benefit regardless of the choice of surgical, neoadjuvant, or adjuvant therapy. The innovative trajectory for the future treatment of TPBC necessitates a holistic approach that balances the considerations of both the HR and HER2 pathways. It is imperative to surmount the crosstalk between these pathways to discern the most efficacious therapeutic strategies and combination modalities among chemotherapy, HER2-targeted therapies, and endocrine treatments, thereby optimizing their pharmacological potential. Harnessing the synergistic effects of a diverse array of pharmaceuticals can significantly enhance therapeutic outcomes for patients with TPBC, mitigate the risks of recurrence and metastasis, and establish a robust foundation for improved clinical outcomes and patient prognoses.

However, tumorigenesis is a complex, multigene, multistep, and long-term process involving numerous connected factors. Although significant advancements have been made in our understanding of the mechanisms underlying cancer development, these represent the tip of the iceberg, and there remains a pressing need for continued diligent exploration. Compared with other types of breast cancer, research on TPBC is relatively rare both domestically and internationally. Moreover, improving early screening and diagnostic methods for TPBC is a future research priority. Although combination therapy can theoretically lead to increased benefit for TPBC patients, the issue of drug resistance remains to be addressed, and research into resistance mechanisms will help clinicians better develop individualized and precise treatment regimens to maximize patient outcomes.

Supplementary Information

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Supplementary Material 1

Author contributions

Jie Xie: Writing - original draft. Zhihui Yang: Writing - original draft. Zhuolin Li: Writing - original draft. Tianyu Zhang: Writing - original draft. Huan Chen: Writing - original draft. Xueru Chen: Writing - original draft. Zehua Dai: Writing - original draft. Tao Chen: Writing - original draft. Jing Hou: Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

CRediT authorship contribution statement

Jie Xie: Writing - original draft. Zhihui Yang: Writing - original draft. Zhuolin Li: Writing - original draft. Tianyu Zhang: Writing - original draft. Huan Chen: Writing - original draft. Xueru Chen: Writing - original draft. Zehua Dai: Writing - original draft. Tao Chen: Writing - original draft. Jing Hou: Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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