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Review Article

Therapeutic challenges in HER2-targeted antibody therapies: trastuzumab and its ADC derivatives in breast cancer

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Abstract: HER2 overexpression is associated with aggressive and poor patient outcome. HER2 has become a crucial target in cancer treatment, and the discovery of effective HER2-targeted therapies marked a significant milestone in treating HER2-positive cancers. This led to the approval of trastuzumab, the first HER2-targeted monoclonal antibody. Later, trastuzumab was used to develop antibody-drug conjugates (ADCs) for breast cancer, which have shown promising results. ADCs are combined trastuzumab with a cytotoxic drug to improve effectiveness while reducing side effects. Two ADCs, trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd), have been approved by the FDA for treating HER2-positive breast cancer. However, drug resistance has become a serious issue, reducing the long-term success of these treatments. This review explores key mechanisms of ADCs resistance including alteration in HER2 expression, antibody and payload-related resistance, altered cell signaling, impaired lysosomal and intracellular activity, and tumor microenvironment. By analyzing recent studies in ADCs resistance, this review provides an insight into ADC resistance mechanisms and potential strategies for improving therapeutic outcomes HER2 positive breast cancer.

Keyword: Antibody-drug conjugates (ADCs), drug resistance, breast cancer, T-DM1, T-DXd

Introduction

Breast cancer (BC) is the most common cancer diagnosed in women in the United States, accounting for about 30% of all new cancer cases. It remains a major cause of cancer-related illness and death in women aged 20 to 59 [1]. BC is classified into several subtypes based on key markers: estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 expression [2]. HER2 belongs to the HER receptor family, which includes EGFR (HER1), HER2, HER3, and HER4. These tyrosine kinases regulate cell growth and differentiation, and their dysregulation can lead to uncontrolled cell proliferation. Approximately 20-30% of BC shows overexpression of HER2, which is classified as HER2-positive BC, a feature associated with more aggressive disease and poor clinical outcomes compared to ER-positive BC [3, 4]. However, advances in pathology, molecular biology, and drug development have significantly improved outcomes of this subtype. This progress began in the 1980s with the discovery of the HER2 gene (also known as ERBB2), which led to the development of HER2-targeted therapies such as trastuzumab [5, 6]. Trastuzumab is the first humanized monoclonal antibody (mAb) for HER2 and was approved nearly 25 years ago. It binds to the extracellular domain of HER2, inhibiting downstream signaling pathways, and activating immune responses such as antibody-dependent cell-mediated cytotoxicity (ADCC) to eliminate tumor cells [7, 8]. The clinical success of trastuzumab was a major breakthrough and laid the foundation for subsequent HER2-targeted treatments, significantly improving out-

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comes in HER2-positive BC [9]. Subsequently, trastuzumab was used to develop antibody-drug conjugates (ADCs) for BC which have shown promising results. ADC is typically composed of mAbs attached to a cytotoxic drug via a chemical linker. It combines both the advantages of highly specific targeting ability and highly potent killing effect to achieve accurate and efficient elimination of cancer cells. This targeted approach increases efficacy, thus it is referred to as the "biological missile" [10]. That increases the therapeutic index by enabling chemotherapy to be directed to cells that express a particular target antigen [11, 12]. To date, the U.S. Food and Drug Administration (FDA) has approved 15 ADCs (**Table 1**), with more than 100 others in clinical development. These trials showed ADCs have good progress and promising. In the BC treatment, two ADCs, trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd), have been used for treating HER2-positive BC while sacituzumab govitecan is used to treat ER-positive and triple-negative BC (TNBC) [13].

T-DM1 is an ADC approved in 2013 that combines trastuzumab with the maytansine-derived microtubule inhibitor DM1 via a stable linker. With a drug-to-antibody ratio (DAR) of approximately 3.5, T-DM1 disrupts mitosis and induces apoptosis in HER2-overexpressing tumor cells, even in cells resistant to trastuzumab or lapatinib. The EMILIA Phase III clinical trial demonstrated that T-DM1 significantly improved progression-free survival (PFS) and overall survival (OS) compared to the combination of capecitabine and lapatinib, leading to its FDA approval for HER2-positive metastatic BC (MBC) [14, 15]. Later, T-DXd was approved in 2019 for patients with HER2-positive metastatic or unresectable BC who had received two or more prior HER2-targeted therapies [16, 17]. T-DXd consists of trastuzumab and the potent topoisomerase I inhibitor DXd, via a cleavable tetrapeptide-based linker. This ADC features a high DAR of 8. Upon intracellular uptake, the cytotoxic payload is efficiently released within tumor cells, reducing off-target toxicity. Moreover, the high membrane permeability of DXd also enables a bystander effect, allowing it to kill neighboring tumor cells with low or no HER2 expression. T-DXd has also been evaluated for various tumors with low HER2 expression [18, 19].

Although T-DM1 and T-DXd have been shown to be highly effective in BC treatment, drug resistance has been observed in many patients who have received the treatment, highlighting the need for a better understanding of the mechanisms that drive drug resistance. Cancer cells can evade the effect of ADC through various mechanisms, such as reduced antigen expression, altered intracellular transport pathways, or acquired payload resistance. The Intrinsic and acquired resistance to ADCs has become a clinical issue. Therefore, this review summarized the mechanisms of drug resistance to T-DXd and T-DM1 and aims to provide insights to overcome these challenges.

Mechanisms of HER2-targeted ADCs resistance

The development of resistance to ADCs remains a significant challenge in oncology, despite their high specificity and potent antitumor effects compared to traditional chemotherapy. While many cancer patients initially respond well to ADC therapy, most of them eventually develop resistance. ADCs work through multiple steps from binding to target antigens on cancer cells to cellular uptake, lysosomal sorting, lysosomal degradation, and payload release, leading to cell death (**Figure 1**). Each of these steps can be a cause of resistance. For example, alterations in the antigen-antibody binding, impaired cellular uptake and drug transport, impaired lysosomal function, increased drug efflux, and changes in the tumor microenvironment (TME) can all contribute to resistance. In addition, resistance to payload itself and enhanced cancer cell survival also contribute to therapeutic failure. The mechanisms underlying resistance to HER2-targeted ADCs in breast cancer are summarized in **Figure 2**. Understanding these diverse resistance mechanisms is crucial for improving the efficacy of ADC therapies and overcoming their limitations in clinical settings.

HER2 related resistance: reduced HER2 expression and inhibition of antibody binding

ADCs first specifically bind to tumor antigens and, after internalization, release their cytotoxic payload into the cytoplasm, leading to targeted cancer cell death. Therefore, one of the mechanisms of resistance is related to the first

Table 1. FDA Approved ADCs

ADCs	Trademark	Target	Disease	Linker	Payload	DAR	Approved
Telisotuzumab vedotin-tllv	Emrelis	c-Met	mNSCLC	valine-citrulline peptide linker	MMAE	~3.5	2025
Datopotamab Deruxtecan	Datroway	Trop2	Metastatic triple-negative breast cancer	Tetrapeptide	DXd	~4	2025
Mirvetuximab soravtansine	ELAHERE	FRα	Platinum-resistant epithelial ovarian	Sulfo-SPDB	DM4	~3.5	2022
Tisotumab vedotin-tftv	Tivdak	Tissue factor	Recurrent or metastatic cervical cancer	MC-Val-Cit-PABC	MMAE	~4	2021
Loncastuximab tesirine-lpyl	Zynlonta	CD19	Diffuse large B-cell lymphoma	Val-Ala dipeptide	PDB dimer	~2.3	2021
Belantamab mafodotin-blmf	Blenrep	BCMA	Relapsed or refractory multiple myeloma	MC	MMAF	4	2020; withdrawn 2022
Sacituzumab govitecan	Trodelvy	Trop-2	Metastatic triple-negative breast cancer	Carbonate	SN38	~7:6	2020
Trastuzumab deruxtecan	Enhertu	HER2	Unresectable or metastatic HER2-positive breast cancer	Tetrapeptide	DXd	~8	2019
Enfortumab vedotin	Padcev	Nectin-4	Advanced or metastatic urothelial carcinoma	MC-Val-Cit-PABC	MMAE	~4	2019
Polatuzumab vedotin-piiq	Polivy	CD79	Relapsed or refractory diffuse large B-cell lymphoma	MC-Val-Cit-PABC	MMAE	~3.5	2019
Moxetumomab pasudotox	Lumoxiti	CD22	Relapsed or refractory hairy cell leukemia	MC-Val-Cit-PABC	PE38		2018
Inotuzumab ozogamicin	Besponsa	CD22	B-cell acute lymphocytic leukemia	Hydrazone	N-acetyl-γ calicheamicin	~6	2017
Trastuzumab emtansine	Kadcyla	HER2	HER2-positive breast cancer	SMCC	DM1	~3.5	2013
Brentuximab vedotin	Adcetris	CD30	Anaplastic large-cell lymphoma, Hodgkin lymphoma	MC-Val-Cit-PABC	MMAE	~4	2011
Gemtuzumab ozogamicin	Mylotarg	CD33	Acute myeloid leukemia	Hydrazone	N-acetyl-γ calicheamicin	~2.5	2000; reapproved 2017

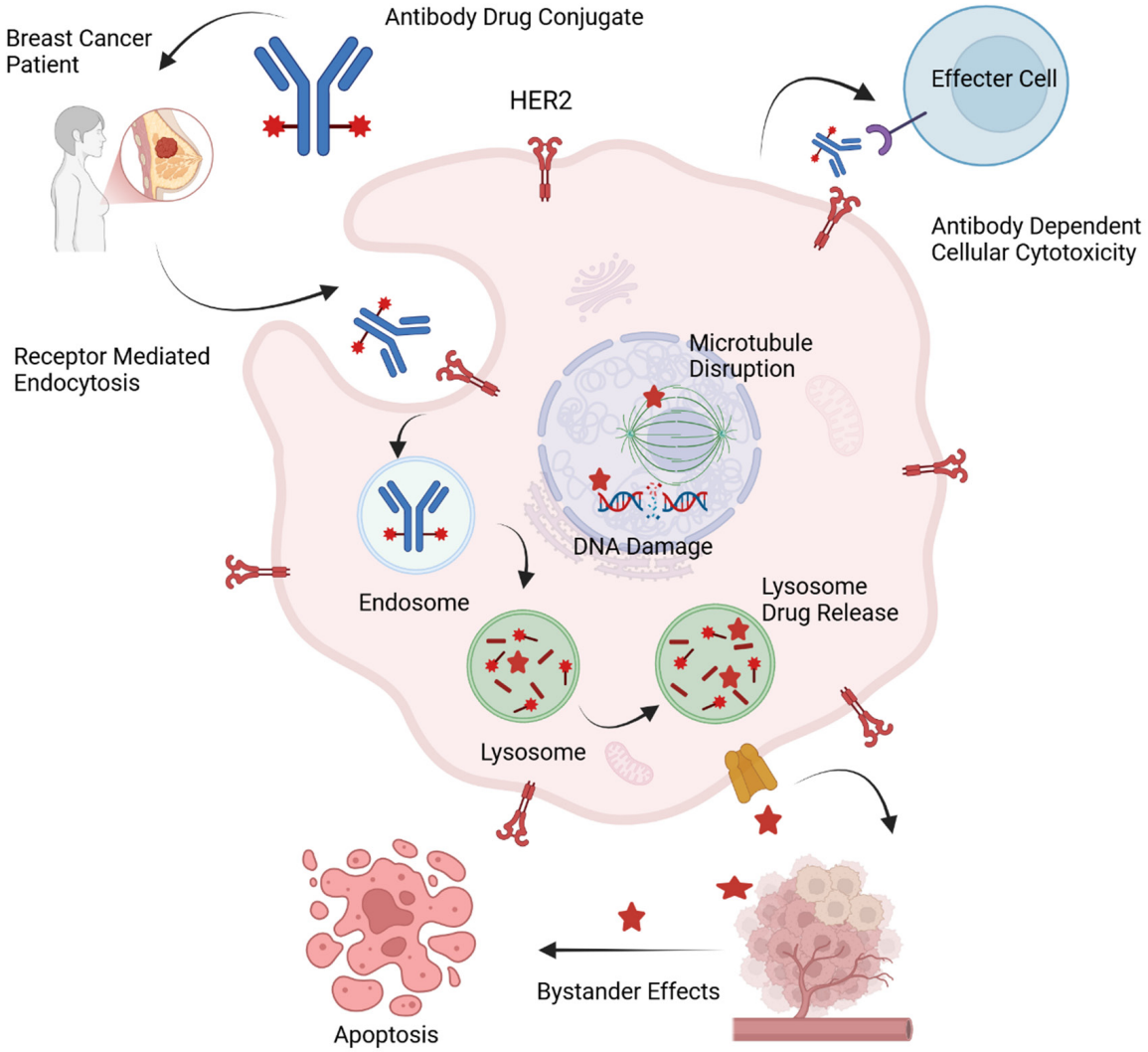

Figure 1. Schematic representation of the mechanism of action of HER2-targeted antibody - drug conjugate. HER2-targeted ADCs function by specifically binding to HER2 antigens expressed on the surface of cancer cells. Upon binding, the ADCs-antigen complex is internalized and trafficked through the endosomal-lysosomal pathway. Within the lysosome, cleavable linkers are degraded by proteolytic enzymes or acidic conditions, resulting in the release of cytotoxic payloads into the cytoplasm. These payloads can disrupt cellular function, leading to apoptosis or cell death due to bystander effects.

step, the recognition of these antigens by mAbs.

Both preclinical and clinical studies have shown that HER2 depletion frequently occurs after trastuzumab treatment [20]. This is a key resistance mechanism common to T-DM1 and T-DXd. For example, a phase II clinical trial revealed a strong association between higher HER2 levels and increased efficacy of T-DM1. Conversely, in vitro studies using T-DM1-resistant cell lines demonstrated diminished HER2 expression and binding, implicating HER2 loss as a primary contributor to resistance [21-23]. HER2 downregulation reduces not only the binding and internalization of ADCs but also the release of cytotoxic payloads, ultimately impairing therapeutic efficacy. Several mechanisms can drive HER2 depletion, including mutations and deletions in the ERBB2 gene that cause reduced HER2 protein production and binding affinity of ADCs.

Moreover, specific HER2 variants further contribute to resistance. One notable example is the HER2Δ16 splice variant (p95HER2), which

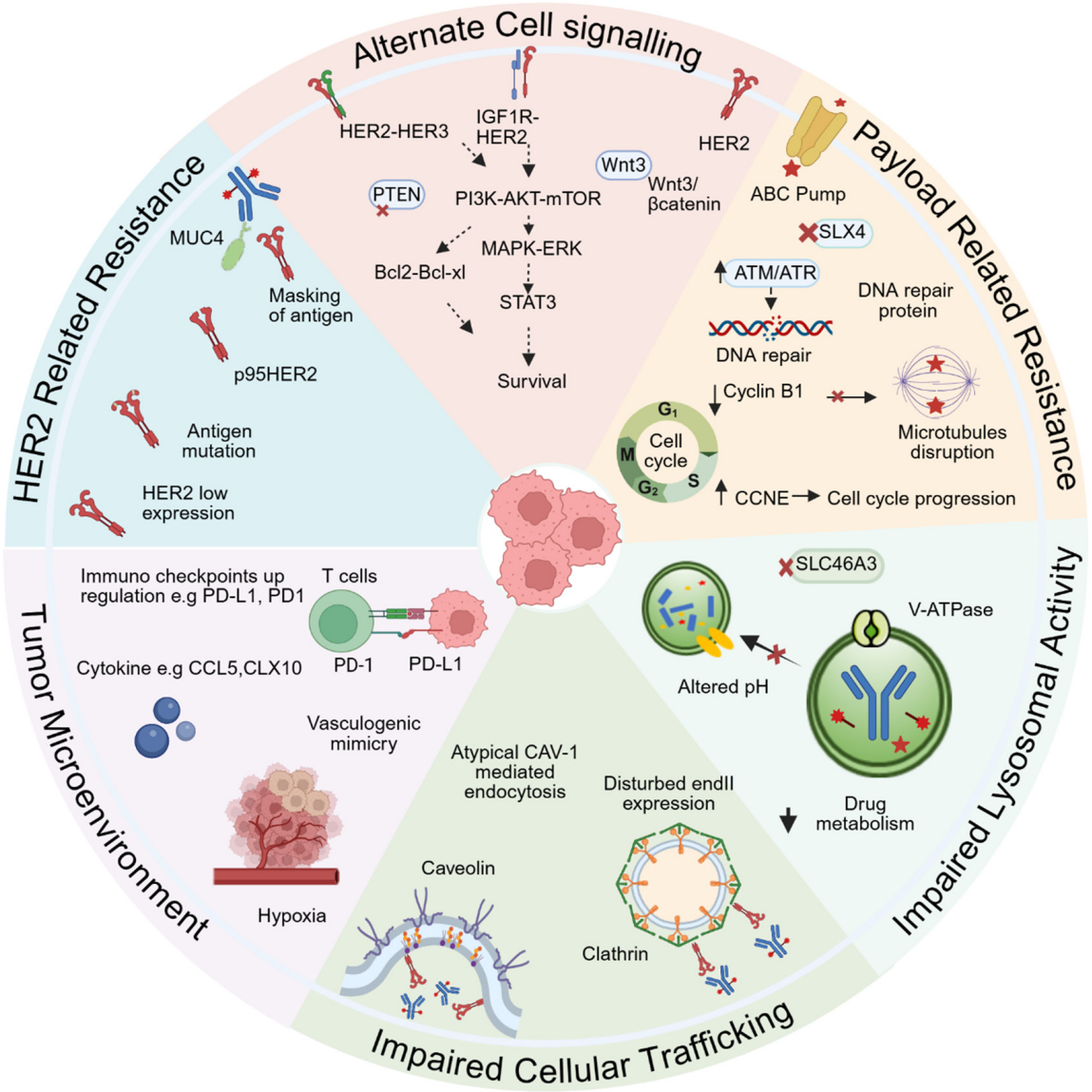

Figure 2. Mechanisms of resistance to HER2-targeted ADCs in breast cancer. Schematic overview of mechanisms of resistance to HER2-Targeted ADCs. Multiple resistance mechanisms limit the efficacy of HER2-targeted ADCs, including reduced HER2 expression, activation of alternative signaling pathways, impaired intracellular trafficking and lysosomal processing, drug efflux, resistance to apoptosis, and modulation by the tumor immune microenvironment.

lacks part of the extracellular domain essential for trastuzumab binding. p95HER2 is expressed in approximately 2-9% of HER2-positive cancers and is associated with reduced trastuzumab efficacy. p95HER2 retains potent oncogenic activity ten times than full-length HER2, enhancing the activation of signaling pathways such as PI3K/AKT to promote tumor growth and survival [24].

It has also been observed that T-DM1 and T-DXd resistance in HER2-positive BC cell lines is associated with genomic alterations affecting the HER2 locus. Specifically, resistant cells exhibited decreased HER2 mRNA levels, accompanied by elevated alternative splicing indices in genes located within the ERBB2 amplicon, including ERBB2, MIEN1, MIR4728, and PGAP3. These splicing events likely contribute to gene downregulation and loss of HER2 protein expression [25]. In the DAISY trial, which aimed to identify T-DXd resistance pathways [26]. HER2 IHC 1+ tumors exhibited lower intratumoral distribution of T-DXd compared with

HER2 IHC 2+ tumors, indicating the significance of the HER2 protein level in controlling drug uptake. In addition, resistance to T-DXd has been linked to the ERBB2 D582N mutation [26, 27].

Beyond HER2 itself, other molecules can interfere the binding of trastuzumab to HER2 on cell surface. For instance, the glycoprotein MUC4, can sterically hinder trastuzumab binding by associating with HER2, HER3, and HER4. Studies have shown that MUC4 knockdown can restore trastuzumab sensitivity in resistant cell lines such as JIMT-1 [28, 29]. Similarly, MUC1 has also been reported to interact with HER2 and be involved in trastuzumab resistance [30].

In summary, reduced expression, structural changes, or blockade of antibody binding of HER2 impairs target recognition by ADCs, resulting in reduced therapeutic efficacy. These mechanisms limit ADC therapy, especially in tumors with high HER2 heterogeneity or unstable HER2 expression [31, 32]. Moreover, prolonged treatment may lead to reduced HER2 expression or structural alterations, resulting in resistance [33].

Alternative cell signaling

HER2 antibodies such as trastuzumab function at least in part by suppressing HER2 signaling. HER2-ADCs also have this effect, but the primary effect of ADCs is due to the cytotoxicity of their payloads, and the impact of HER2 signaling suppression on ADC resistance is likely to be minimal. In fact, it has been shown that HER2-ADCs are effective against trastuzumab-resistant cells. However, activation of AKT and other survival kinases is involved in resistance to various stresses, including chemotherapy and DNA damage, and may therefore also contribute to ADC resistance.

It has been reported that HER2 mediates its oncogenic activity through the formation of homodimers and heterodimers with other receptors, including HER1, HER3, HER4, and insulin-like growth factor 1 receptor (IGF-1R) [34, 35]. While HER2 heterodimer activity can be influenced by ligand binding, homodimers tend to be constitutively active. A key downstream pathway activated by HER2 overexpression is the PI3K/AKT/mTOR signaling cascade, which plays a central role in promoting cell proliferation and survival while suppressing apoptosis. Consequently, HER2-positive breast cancers often show hyperactivation of PI3K signaling, driving tumor progression. Trastuzumab targeting HER2 can inhibit tumor growth by blocking HER2-mediated signaling. However, this inhibition is frequently incomplete, particularly when cancer cells adapt or activate compensatory pathways. One notable mechanism of resistance is the loss or decreased expression of PTEN, a tumor suppressor that negatively regulates PI3K/AKT/mTOR signaling. PTEN dephosphorylates PIP3, thereby inhibiting AKT activation. When PTEN is absent or functionally impaired, AKT remains active despite HER2 inhibition, allowing cancer cells to bypass trastuzumab-induced blockade and continue proliferating [29, 36]. Studies show that in HER2-positive cancers, resistance is often driven by upregulation of EGFR, IGF-1R, and PI3K signaling, even in the presence of trastuzumab. In a study of 155 metastatic breast cancer patients treated with trastuzumab, approximately 25% developed resistance due to such mechanisms [37]. Numerous investigations have demonstrated that, in contrast to the MAPK and PI3K/Akt/mTOR pathways, Trastuzumab can't totally stop signaling because, in addition to HER2, other HER family receptors, including EGFR/HER1, HER3, and HER4, may develop dimers and mediate signaling [38]. Trastuzumab resistance is linked to the upregulation of the PI3K-mediated signaling pathway, EGFR, and IGF-1R, according to a study of 155 individuals who developed metastases and had Trastuzumab treatment [39]. Overexpression of IGF-1R also contributes to resistance. IGF-1R activates pathways similar to HER2 and, when co-expressed at high levels with its ligand IGF-I, can promote tumor cell proliferation and metastasis independently of HER2. Even if trastuzumab inhibits HER2, IGF-1R can continue to support tumor progression through alternative signaling. Similarly, high levels of TGF-α (a ligand for the EGFR family) can bypass HER2 signaling, reducing the efficacy of trastuzumab [40].

Another mechanism of resistance involves DARPP-32 and its truncated isoform, t-DARPP. These proteins are highly expressed in HER2-positive breast tumors and are associated with poor prognosis. t-DARPP enhances trastuzum-

ab resistance by persistently activating the AKT pathway, promoting HER2/IGF-1R dimerization, and supporting glycolytic metabolism [41]. Studies have demonstrated that t-DARPP also facilitates HER2 interaction with HSP90, stabilizing the receptor and further contributing to drug resistance [42, 43].

In addition, activation of the Wnt/β-catenin pathway promotes drug resistance in ADCs. The role of Wnt3 in trastuzumab resistance has been described by Y. Kim *et al*. Overexpression of Wnt3 leads to increased expression of β-catenin, increased growth rate and invasiveness, and trastuzumab resistance in these cells [44]. Furthermore, one study revealed EPHA5 deficiency promotes trastuzumab resistance in HER2-positive breast cancers by increasing cancer stem cell-like traits such as NANOG, CD133+, E-cadherin expression, and CD44+/CD24-/low phenotypes. This leads to higher tumor malignancy, linked to the upregulation of BCSC-related markers and activation of Notch1 and PTEN/AKT pathways [45]. Taken together, these parallel pathway activations represent a general mechanism contributing drug resistance signaling.

Payload related resistance

Beyond antigen loss or altered internalization, resistance to ADCs can stem from changes affecting the payload's activity or its intracellular processing. One key factor is drug efflux [22]. Overexpression of ATP-binding cassette (ABC) transporters such as ABCC2, ABCG2, and ABCB1 enhances the cytotoxic payload efflux of HER2+ cancer cells, reducing the intracellular concentration of DM1 and the efficacy of ADCs. Blocking these transporters can restore the sensitivity of T-DM1 [46-48]. Additionally, apoptosis resistance via LIFR-mediated STAT3 activation upregulates anti-apoptotic proteins (Bcl-xL, Bcl-2, Mcl-1), undermining therapeutic effect and offering a potential vulnerability through STAT3 inhibition [49]. Cell cycle dysregulation and alterations in DNA repair pathways are critical contributors to resistance against T-DM1 and related ADCs. T-DM1 induces mitotic arrest by disrupting microtubules, but cancer cells may circumvent this block [50, 51]. High levels of Polo-like kinase 1 (PLK1) can enable drug-resistant cells to bypass cell cycle arrest, while PLK1 inhibitors (such as volasertib) can restore sensitivity to T-DM1 in these cells [52]. It has been reported that reduced Cyclin B1 expression allows cells to bypass. T-DM1-induced mitotic arrest, promoting resistance [53, 54]. In addition, the overexpression of CDK12 has been linked to enhanced DNA repair and Wnt signaling, contributing to trastuzumab and ADC resistance [55]. In T-DXd-resistant models, CCNE1 overexpression disrupts cell cycle control, while its knockdown restores drug sensitivity [56]. Moreover, loss of SLX4 compromises DNA damage response, requiring higher ADC concentrations to achieve cytotoxic effects [57]. A recent 2024 study employing proteomic profiling revealed that HER2-ADC-resistant cells (T-DM1R and T-DXdR) exhibit broad upregulation of DNA damage response pathways, including homologous recombination, nonhomologous end joining, and checkpoint signaling. In HER2-ADC resistant cell lines, ATR, pATR, ATM, Chk1, Chk2 were observed to be upregulated compared to their parent cell lines [25].

Impaired lysosomal activity

ADCs rely on efficient intracellular trafficking and lysosomal degradation to release their cytotoxic payload. Following receptor-mediated internalization, ADCs are routed to lysosomes, where acidic pH and proteolytic enzymes facilitate linker cleavage and drug release. However, lysosomal dysfunction can block this critical step, as shown by studies indicating that HER2-positive breast cancer cells resistant to T-DM1 exhibited elevated lysosomal pH, impairing proteolytic activity and leading to reduced drug activation. These resistant cells retained normal HER2 expression and internalization, but failed to process T-DM1 effectively [58].

Another resistance mechanism involves impaired transport of payloads from lysosomes to the cytoplasm, particularly relevant for ADCs with non-cleavable linkers. Hamblett et al. identified SLC46A3 as a transporter required for the cytoplasmic translocation of maytansine catabolites. Loss of SLC46A3 resulted in payload accumulation within lysosomes and diminished cytotoxicity [59]. Additionally, T-DM1 relies on lysosomal proteolysis to release lysine-MCC-DM1, which is then transported to the cytoplasm to inhibit microtubule polymerization. However, reduced lysosomal acidity caused by decreased V-ATPase activity disrupts this process, leading to T-DM1 resistance [60].

Impaired cellular trafficking

ADCs selectively bind to tumor antigens and, upon internalization, release their cytotoxic payload within endosomes and lysosomes, inducing targeted cancer cell death. However, alterations in intracellular trafficking can undermine this process and contribute to drug resistance. In HER2-positive cancer cells, resistance to trastuzumab emtansine (T-DM1) has been linked to disruptions in endocytosis, particularly through caveolae-mediated pathways. Studies have shown that T-DM1-resistant NCI-N87 cells exhibit upregulation of Caveolin-1 (CAV1) and cavin-1, which are essential for caveolae formation. This may promote drug uptake while limiting lysosomal degradation, ultimately reducing T-DM1 efficacy [61]. Endophilin A2 (Endo II), a mediator of clathrin-independent endocytosis, has also been implicated in ADC sensitivity. Its downregulation disrupts HER2 internalization and diminishes the cytotoxic effects of both trastuzumab and T-DM1, as shown in HCC1954 and SKBR3 cell lines [62]. These findings suggest that Endo II deficiency contributes to resistance. Overall, alterations in intracellular trafficking influence drug resistance, underscoring the need for further investigation into these mechanisms.

Tumor microenvironment

The tumor immune microenvironment plays a critical role in cancer progression, metastasis, and response to therapy, including ADCs. It consists of stromal cells, cytokines, immune cells such as MDSCs, Tregs and TAM, which interact with cancer cells to influence treatment outcomes [63, 64]. Although the mechanisms underlying ADC resistance are intricate, they are typically associated with resistance to the payload or antibody [50]. Physical obstacles like the binding site barrier can cause resistance to the overall ADC complex [65]. A clinical trial reveals that Pertuzumab can help restore T-DM1 activity by blocking HER2 and HER3 dimerization, but combining this treatment only shows small improvements, making it hard to fully overcome this resistance [66]. When breast cancer cells develop resistance to ADCs, their metabolic pathways undergo reprogramming, shifting from reliance on endogenous fatty acid (FA) synthesis to the uptake of exogenous fatty acids. Studies have shown that HER2 is closely associated with endogenous FA synthesized by fatty acid synthase (FASN). Inhibition of the HER2 signaling pathway reduces FASN activity, leading to decreased FA synthesis and impaired cell proliferation [67]. In breast cancer, immunosuppressive cells contribute to a microenvironment that limits immune-mediated clearance and fosters resistance to trastuzumab and T-DM1. On the other hand, T cells, NK cells, and dendritic cells (DCs) can mount an anti-tumor response [68]. In HER2+ breast cancer, one key immune resistance pathway is the JAK1-STAT3 axis. High IL-6 levels trigger autocrine activation of JAK1-STAT3, promoting the expansion of breast cancer stem cells (BCSCs), which are linked to drug resistance and recurrence [69, 70]. Trastuzumab-treated HER2+ tumors show increased PD-L1 expression, driven by IFNγ secretion from T cells and NK cells. PD-L1 binds to PD-1 on immune cells, suppressing their activity and allowing tumors to escape immune destruction. This mechanism contributes to trastuzumab resistance [71-73]. These findings highlighting the PD-1/PD-L1 axis as a promising target. Moreover, patients with poor response to HER2-targeted therapies often exhibit increased expression of CD73 and elevated infiltration of immunosuppressive Tregs. These features contribute to an immune-excluded phenotype and resistance to ADCs like T-DM1 [74]. Some studies also show that combining T-DM1 with anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and PD-L1 treatment significantly improves efficacy compared to monotherapy approaches [75]. Additionally, long-term exposure to trastuzumab can alter cytokine expression. Genes like CCL5, CXCL10, and CXCL1 have been linked to resistance. In particular, CCL5 activates the ERK signaling pathway, which promotes trastuzumab resistance. Elevated CCL5 also attracts Tregs into the tumor microenvironment, reducing immune-mediated tumor clearance and lowering the pathological complete response (pCR) after neoadjuvant therapy [76, 77]. These findings suggest that trastuzumab resistance mechanisms may also contribute to T-DM1 or T-DXd resistance; however, more research is needed. The interplay between the tumor immune microenvironment and intracellular signaling pathways significantly influences resistance to HER2-targeted therapies such as trastuzumab and T-DM1.

Potential strategies to overcome ADC resistance in breast cancer

ADCs are an important class of cancer therapeutics, with several FDA-approved ADCs presently available for treating various cancers. Despite all the advancements in cancer therapy, inherent and acquired drug resistance continue to be major obstacles to successful treatment.

As previously discussed, resistance to ADC can arise through a variety of mechanisms. Therefore, the development of strategies to overcome ADC resistance requires multipronged strategies, including improved 1. ADC design optimization, 2. Combination Therapy, 3. Dual-Targeting Strategies, 4. Targeting Intracellular Processing. Potential strategies to overcome resistance to HER2-targeted ADCs are summarized in **Figure 3**.

ADC design optimization

The effectiveness of ADCs depends critically on the stability and cleavability of linkers. Selective payload release is ensured via cleavable linkers, which are sensitive to tumor-specific factors like pH or enzyme activity [78, 79]. T-DXd has superior antitumor activity as compared to TDM1, owing to its cleavable linker, higher DAR, and more potent payload [80]. Another strategy to develop new ADCs is to modify the linker to make it more hydrophilic and reduce MDR expression [48]. Optimizing ADC payloads to increase the bystander effect is crucial to overcoming tumor heterogeneity-related resistance. The neutral catabolite emission from DM4 indicates that the effect is dependent on the payload charge [81, 82]. Advancements in site-specific conjugated ADCs with defined drug-to-antibody ratios (DARs) have improved the therapeutic index and decreased off-target effects, which will help to overcome resistance [83]. ADC payload transport modeling quantifies the effectiveness of payload targeting untargeted tumor cells and indicates a range of bystander potential [84]. Although direct ADC action is more effective, we evaluated the tissue penetration of bystander payloads and found that bystander effects are important in cancer with mixed or antigen-negative expression [85].

Combination therapy

PI3K/AKT/mTOR pathway inhibitors: Combining ADCs with agents that target critical signaling pathways is another promising strategy. In HER2-positive breast cancer, PI3K/AKT/mTOR and MAPK pathway mutations contribute to resistance. Oncogenic mutations in PI3K/Akt/mTOR and MAPK pathways are strongly associated with resistance to HER2 inhibitors. Preclinical studies indicate that integrating PI3K inhibitors with HER2 inhibition is efficacious in PIK3CA-mutant HER2+ breast cancer. Similarly, in the ER-/HER2+ BC cell lines HCC1954 and UACC893, both of which had PIK3CA mutations, combination treatment with the PI3K inhibitors alpelisib or GDC-0077 (inavolisib) plus trastuzumab resulted in antiproliferative effects and significant apoptosis compared to single-drug therapies. p4EBP1 could be used as both a diagnostic and predictive biomarker for such a combination [86, 87]. Three types of PI3K inhibitors can be distinguished: Pan-PI3K inhibitors (Pan-PI3Ki) are effective against all PI3K1 isoforms. Dual PI3K/mTOR inhibitors (Dual PI3K/mTORi) target both PI3K and mTOR. Isoform-specific PI3K inhibitors (IS PI3Ki) target individual PI3K isoforms. This category includes specific inhibitors for the PI3Kα, PI3Kβ, and PI3Kδ isoforms [88, 89]. Allosteric AKT inhibitors bind to an allosteric location on AKT, altering its conformation and hence decreasing its activity. Similarly, mTOR inhibitors are rapamycin analogs that suppress mTOR by attaching to its intracellular receptor FKBP12. ATP-competitive mTOR kinase inhibitors (TORKi) compete with ATP for binding to the kinase domain of mTOR and hence decrease its activity. HER2 and EGFR co-expression or dimerization cause targeted treatment resistance [90, 91]. B2C4-MMAE targets both, which helps to circumvent resistance. Its effectiveness depends on the interaction between the two arms [91, 92]. Inhibition of BCL-2 and BCL-X_L via navitoclax/ABT-263 combined with TDM1 enhance its cytotoxicity in advance breast cancer model [93, 94].

DNA damaging agents: Clinical research shows that the combination of PARPi and chemotherapy stops DNA damage repair, induces apoptosis, and leads to better outcomes than chemotherapy alone (27). PARP inhibitors such as veliparib, olaparib, and Niraparib demonstrated effective outcomes when combined with platinum-based chemotherapy [95]. AZD5305 showed safety and effectiveness in BRCA-mutated TNBC during the phase 1/2 PETRA trial, achieving a 25% ORR and a 53% DCR.

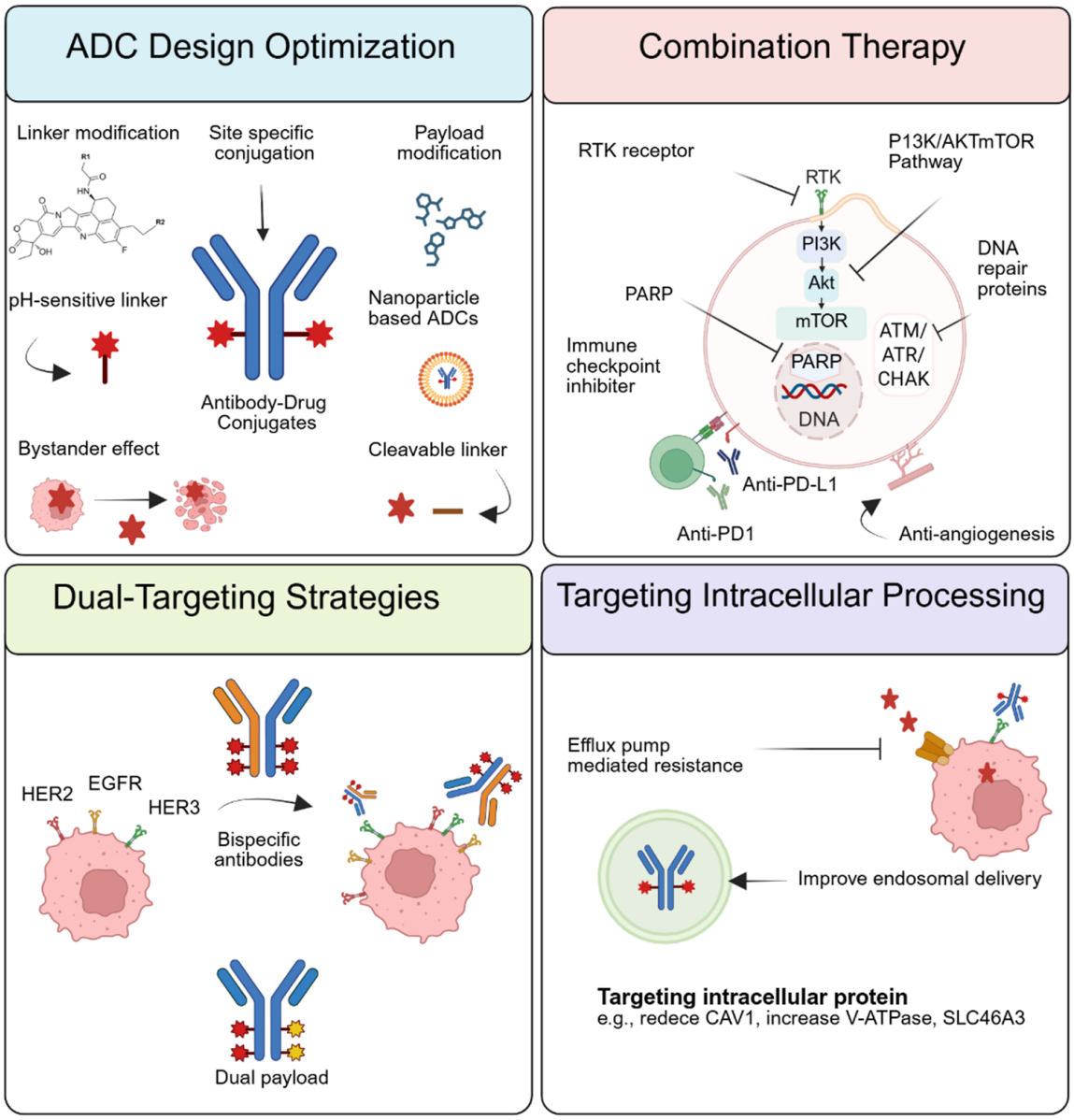

Figure 3. Potential Strategies to Overcome ADCs resistance in breast cancer. Schematic overview of therapeutic approaches to overcome resistance to HER2-targeted antibody-drug conjugates (ADCs), including trastuzumab, T-DM1, and T-DXd. Strategies are categorized into four major areas: (1) Optimization of ADCs design, including cleavable linkers, site-specific conjugation, payload modification, and enhancement of the bystander effect. (2) Combination therapy such as co-treatment with PI3K/AKT/mTOR pathway inhibitors and DNA damage response modulators to sensitize tumors and overcome oncogenic resistance. (3) Dual-targeting strategies utilizing bispecific ADCs that co-target HER2 and alternative receptors (e.g., EGFR) to improve internalization, overcome antigen escape, or incorporate dual payloads for broader cytotoxic effects. (4) Targeting intracellular processing, including modulation of intracellular trafficking, enhancement of lysosomal degradation, and improved payload release to increase cytotoxic efficacy.

While initial findings are encouraging, additional clinical trials are needed to assess the effectiveness of both combination and monotherapy in TNBC [96].

Although T-DM1 in combination with Docetaxel was effective, it resulted in serious side effects for almost 50% of the patients [97]. Evidence suggests that T-DXd combined with the next-generation PARP1 inhibitor AZD5305 showed preclinical activity in HRD and HR proficient models [98]. Preclinical findings indicate that T-DXd and elimusertib are a promising combination for HER2 ADC-resistant BC, supporting

the use of DNA repair inhibitors in treatment [25, 99]. HER2 stability in HER2+ cancer depends on HSP90, and its inhibition causes HER2 to degrade through a ubiquitin-dependent process [100].

Immune checkpoint therapy: Cancer immunotherapy employs cutting-edge technologies, including ICIs such as those targeting PD-1, PD-L1, CTLA-4, and CAR-T cell therapies. Combining ADC and IC1 treatments can boost the effectiveness of immunotherapy by increasing the recruitment of CD8+ T lymphocytes to tumor tissues [101]. ADCs combined Immunotherapies primarily targeting PD-1/PD-L1 and CTLA-4, driving immune effects via cell death, T cell infiltration, immune modulation, and dendritic cell maturation [102]. Recent clinical reports suggest Higher intra-tumoral CD8+ T cells predict better response to checkpoint therapy, highlighting the potential of combining immune-oncology drugs [103]. Müller et al. demonstrated that combining T-DM1 with anti CTLA-4/anti PD-1 was more effective than monotherapy in activating both innate and adaptive immunity [104]. In addition, combining ADCs with agents like pembrolizumab modulates immune infiltration and enhance cytotoxicity. Concurrent administration of pembrolizumab enhanced DS-8201 efficacy by the increase in CD3+ T cells in the blood and CD8+/CD4+ T cell ratio [105].

Anti-angiogenesis: In comparison to primary breast tumors or regional metastases, metastatic breast tumors now exhibit higher levels of a VEGF profile made up of 13 genes [106], its higher level associated with poor prognosis [107]. Novel bevacizumab-based ADCs with multifunctional theranostic capabilities demonstrated stable reactive oxygen generation, rapid drug release, and antigen-binding ability. They also exhibited antiangiogenesis activity and vascular normalization effects, providing a basis for further evaluation of antitumor activity in clinical trials [108]. This approach may hold therapeutic potential for breast cancer treatment.

Dual targeting strategies

To circumvent resistance from HER2 downregulation or receptor heterodimerization (e.g., with EGFR), bispecific ADCs are being developed. These ADCs can recognize and bind to two distinct antigens or epitopes, improving internalization and cytotoxicity. Bispecific antibody-drug conjugates target two distinct epitopes on a single cell. This dual-targeting method can improve ADC efficiency, and internalization accumulation at the tumor site [109]. e.g. B2C4-MMAE targets both HER2 and EGFR which help to overcome the downregulation resistance issue. Enhance efficient binding and elimination of tumors by targeting two separate antigens [92, 110].

Additionally, dual-payload ADCs (e.g., Tmab-vcMMAE-SMCC-DM1) exhibited had a superior cytotoxic effect than Trastuzumab alone. Indicating that combining multiple payloads can help to tackle treatment resistance and tumour recurrences [111]. HER3-targeting ADCs such as patritumab deruxtecan have also demonstrated improved immune infiltration and responsiveness to immune checkpoint therapy [112].

Targeting intracellular processing pathways

Several intracellular factors influence ADCs efficacy, particularly after internalization and lysosomal trafficking. Cathepsin B-cleavable ADCs like SYD985 induce cytotoxicity in tumors with low antigen expression or defective uptake, broadening the potential target spectrum [113-115]. In addition, ABC transporter upregulation was associated with resistance to DS-8201, Besides, RAB5A, a small GTPase that controls the vesicle fusion to early endosomes during clathrin-mediated endocytosis, has been implicated in ADC resistance [116]. Several studies show that RAB5A-HER2 interaction plays a significant role in T-DM1 sensitivity and its high expression leads to better outcomes [117, 118]. Hunter et al. recently suggested that the activity of V-ATPase in lysosomes can serve as a novel predictor of T-DM1 resistance [119]. The expression of SLC46A3, a lysosomal drug efflux transporter, may serve as an indirect predictor of resistance to ADCs [120]. Therefore, modulating expression of V-ATPase and SLC46A3 may overcome ADCs resistance. Taken together, these findings highlight the importance of intracellular trafficking, lysosomal activity, and transporter expression in determining ADC efficacy.

Conclusion

Despite the remarkable success of HER2-targeted ADCs like trastuzumab, T-DM1, and

T-DXd, ADCs resistance remains a major clinical hurdle. Tackling this challenge requires approaches, including rational ADC design, synergistic drug combinations, dual-targeting strategies, and interventions that target intracellular processing or microenvironmental barriers. Ongoing clinical trials (e.g., NCT-06533826, NCT04739761, NCT04538742, NCT0453993, NCT06956690, NCT04494425, NCT06827236, NCT06439693, NCT059509-45, NCT04829604, NCT04450732) are investigating combination plus monotherapy to overcome ADCs resistance in breast cancer, with a particular focus on HER2-positive breast cancer. This review summarizes the diverse mechanisms contributing to ADC resistance, discusses potential strategies for improving therapeutic improvement in HER2 positive breast cancer.

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Disclosure of conflict of interest

None.

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References

[1] Siegel RL, Miller KD, Wagle NS and Jemal A. Cancer statistics, 2023. CA Cancer J Clin 2023; 73: 17-48.

[2] Chen YF, Xu YY, Shao ZM and Yu KD. Resistance to antibody-drug conjugates in breast cancer: mechanisms and solutions. Cancer Commun (Lond) 2023; 43: 297-337.

[3] Albagoush SA, Zubair M and Limaiem F. Tissue evaluation for HER2 tumor marker. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.

[4] Rubin E, Shan KS, Dalal S, Vu DUD, Milillo-Naraine AM, Guaqueta D and Ergle A. Molecular targeting of the human epidermal growth factor receptor-2 (HER2) genes across various cancers. Int J Mol Sci 2024; 25: 1064.

[5] Bargmann CI, Hung MC and Weinberg RA. The neu oncogene encodes an epidermal growth factor receptor-related protein. Nature 1986; 319: 226-230.

[6] Schechter AL, Stern DF, Vaidyanathan L, Decker SJ, Drebin JA, Greene MI and Weinberg RA. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature 1984; 312: 513-516.

[7] Ferraro E, Drago JZ and Modi S. Implementing antibody-drug conjugates (ADCs) in HER2-positive breast cancer: state of the art and future directions. Breast Cancer Res 2021; 23: 84.

[8] Corti C, Giugliano F, Nicolò E, Ascione L and Curigliano G. Antibody-drug conjugates for the treatment of breast cancer. Cancers (Basel) 2021; 13: 2898.

[9] Carter P, Presta L, Gorman CM, Ridgway JB, Henner D, Wong WL, Rowland AM, Kotts C, Carver ME and Shepard HM. Humanization of an anti-p185HER2 antibody for human cancer therapy. Proc Natl Acad Sci U S A 1992; 89: 4285-4289.

[10] Fu Z, Li S, Han S, Shi C and Zhang Y. Antibody drug conjugate: the "biological missile" for targeted cancer therapy. Signal Transduct Target Ther 2022; 7: 93.

[11] Drago JZ, Modi S and Chandarlapaty S. Unlocking the potential of antibody-drug conjugates for cancer therapy. Nat Rev Clin Oncol 2021; 18: 327-344.

[12] Liu K, Li M, Li Y, Li Y, Chen Z, Tang Y, Yang M, Deng G and Liu H. A review of the clinical efficacy of FDA-approved antibody-drug conjugates in human cancers. Mol Cancer 2024; 23: 62.

[13] Vivekanandhan S and Knutson KL. Resistance to trastuzumab. Cancers (Basel) 2022; 14: 5115.

[14] Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, Pegram M, Oh DY, Diéras V, Guardino E, Fang L, Lu MW, Olsen S and Blackwell K; EMILIA Study Group. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012; 367: 1783-1791.

[15] Tong JTW, Harris PWR, Brimble MA and Kavianinia I. An insight into FDA approved antibody-drug conjugates for cancer therapy. Molecules 2021; 26: 5847.

[16] Nakada T, Sugihara K, Jikoh T, Abe Y and Agatsuma T. The latest research and development into the antibody-drug conjugate [fam-] trastuzumab deruxtecan (DS-8201a), for HER2 can-

cer therapy. Chem Pharm Bull (Tokyo) 2019; 67: 173-185.
[17] Ando M, Shimoi T, Imai T, Kojima Y, Sudo K and Yonemori K. Clinical outcome of treatment rechallenge with HER2-directed antibody-drug conjugate in metastatic breast cancer: a single-centered real-world experience. Eur J Cancer 2024; 200.
[18] Koster KL, Huober J and Joerger M. New antibody-drug conjugates (ADCs) in breast cancer - an overview of ADCs recently approved and in later stages of development. Explor Target Antitumor Ther 2022; 3: 27-36.
[19] Rugo HS, Bianchini G, Cortes J, Henning JW and Untch M. Optimizing treatment management of trastuzumab deruxtecan in clinical practice of breast cancer. ESMO Open 2022; 7: 100553.
[20] Bon G, Pizzuti L, Laquintana V, Loria R, Porru M, Marchiò C, Krasniqi E, Barba M, Maugeri-Saccà M, Gamucci T, Berardi R, Livi L, Ficorella C, Natoli C, Cortesi E, Generali D, La Verde N, Cassano A, Bria E, Moscetti L, Michelotti A, Adamo V, Zamagni C, Tonini G, Barchiesi G, Mazzotta M, Marinelli D, Tomao S, Marchetti P, Valerio MR, Mirabelli R, Russo A, Fabbri MA, D'Ostilio N, Veltri E, Corsi D, Garrone O, Paris I, Sarobba G, Giotta F, Garufi C, Cazzaniga M, Del Medico P, Roselli M, Sanguineti G, Sperduti I, Sapino A, De Maria R, Leonetti C, Di Leo A, Ciliberto G, Falcioni R and Vici P. Loss of HER2 and decreased T-DM1 efficacy in HER2 positive advanced breast cancer treated with dual HER2 blockade: the SePHER study. J Exp Clin Cancer Res 2020; 39: 279.
[21] Burris HA 3rd, Rugo HS, Vukelja SJ, Vogel CL, Borson RA, Limentani S, Tan-Chiu E, Krop IE, Michaelson RA, Girish S, Amler L, Zheng M, Chu YW, Klencke B and O'Shaughnessy JA. Phase II study of the antibody drug conjugate trastuzumab-DM1 for the treatment of human epidermal growth factor receptor 2 (HER2)-positive breast cancer after prior HER2-directed therapy. J Clin Oncol 2011; 29: 398-405.
[22] Loganzo F, Tan X, Sung M, Jin G, Myers JS, Melamud E, Wang F, Diesl V, Follettie MT, Musto S, Lam MH, Hu W, Charati MB, Khandke K, Kim KS, Cinque M, Lucas J, Graziani E, Maderna A, O'Donnell CJ, Arndt KT and Gerber HP. Tumor cells chronically treated with a trastuzumab-maytansinoid antibody-drug conjugate develop varied resistance mechanisms but respond to alternate treatments. Mol Cancer Ther 2015; 14: 952-963.
[23] Li G, Guo J, Shen BQ, Yadav DB, Sliwkowski MX, Crocker LM, Lacap JA and Phillips GDL. Mechanisms of acquired resistance to trastuzumab emtansine in breast cancer cells. Mol Cancer Ther 2018; 17: 1441-1453.
[24] Hu D, Lyu X, Li Z, Ekambaram P, Dongre A, Freeman T, Joy M, Atkinson JM, Brown DD, Cai Z, Carleton NM, Crentsil HE, Little J 4th, Kemp F, Klei LR, Beecher M, Sperinde J, Huang W, Joensuu H, Srinivasan A, Pogue-Geile KL, Wang Y, Feng H, Eli LD, Lalani AS, Zou J, Tseng GC, Bruno TC, Lee AV, Oesterreich S, Wolmark N, Allegra CJ, Jacobs SA, McAllister-Lucas LM and Lucas PC. p95HER2, a truncated form of the HER2 oncoprotein, drives an immunosuppressive program in HER2+ breast cancer that limits trastuzumab deruxtecan efficacy. Nat Cancer 2025; 6: 1202-1222.
[25] Lee J, Kida K, Koh J, Liu H, Manyam GC, Gi YJ, Rampa DR, Multani AS, Wang J, Jayachandran G, Lee DW, Reuben JM, Sahin A, Huo L, Tripathy D, Im SA and Ueno NT. The DNA repair pathway as a therapeutic target to synergize with trastuzumab deruxtecan in HER2-targeted antibody-drug conjugate-resistant HER2-overexpressing breast cancer. J Exp Clin Cancer Res 2024; 43: 236.
[26] Mosele MF, Lusque A, Dieras V, Deluche E, Ducoulombier A, Pistilli B, Bachelot T, Viret F, Levy C, Signolle N, et al. LBA1 Unraveling the mechanism of action and resistance to trastuzumab deruxtecan (T-DXd): biomarker analyses from patients from DAISY trial. Ann Oncol 2022; 33: S123.
[27] Dieras VC, André F, Lusque A, Pistilli B, Bachelot T, Pierga JY, Viret F, Levy C, Salabert L, Dalenc F, et al. 7P Trastuzumab deruxtecan (T-DXd) for advanced breast cancer (ABC) patients, regardless of HER2 status: a phase II study with biomarkers analysis (DAISY) - update analysis with longer follow-up. ESMO Open 2024; 9.
[28] Nagy P, Friedländer E, Tanner M, Kapanen AI, Carraway KL, Isola J and Jovin TM. Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. Cancer Res 2005; 65: 473-482.
[29] Price-Schiavi SA, Jepson S, Li P, Arango M, Rudland PS, Yee L and Carraway KL. Rat Muc4 (sialomucin complex) reduces binding of anti-ErbB2 antibodies to tumor cell surfaces, a potential mechanism for herceptin resistance. Int J Cancer 2002; 99: 783-791.
[30] Raina D, Uchida Y, Kharbanda A, Rajabi H, Panchamoorthy G, Jin C, Kharbanda S, Scaltriti M, Baselga J and Kufe D. Targeting the MUC1-C oncoprotein downregulates HER2 activation and abrogates trastuzumab resistance in breast cancer cells. Oncogene 2014; 33: 3422-3431.
[31] Shafi H, Astvatsaturyan K, Chung F, Mirocha J, Schmidt M and Bose S. Clinicopathological significance of HER2/neu genetic heterogeneity in HER2/neu non-amplified invasive breast

carcinomas and its concurrent axillary metastasis. J Clin Pathol 2013; 66: 649-654.
[32] Ocaña A, Amir E and Pandiella A. HER2 heterogeneity and resistance to anti-HER2 antibody-drug conjugates. Breast Cancer Res 2020; 22: 15.
[33] Mittendorf EA, Wu Y, Scaltriti M, Meric-Bernstam F, Hunt KK, Dawood S, Esteva FJ, Buzdar AU, Chen H, Eksambi S, Hortobagyi GN, Baselga J and Gonzalez-Angulo AM. Loss of HER2 amplification following trastuzumab-based neoadjuvant systemic therapy and survival outcomes. Clin Cancer Res 2009; 15: 7381-7388.
[34] Kovacs E, Zorn JA, Huang Y, Barros T and Kuriyan J. A structural perspective on the regulation of the epidermal growth factor receptor. Annu Rev Biochem 2015; 84: 739-764.
[35] Vecchi M, Baulida J and Carpenter G. Selective cleavage of the heregulin receptor ErbB-4 by protein kinase C activation. J Biol Chem 1996; 271: 18989-18995.
[36] Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012; 490: 61-70.
[37] Ghosh R, Narasanna A, Wang SE, Liu S, Chakrabarty A, Balko JM, González-Angulo AM, Mills GB, Penuel E, Winslow J, Sperinde J, Dua R, Pidaparthi S, Mukherjee A, Leitzel K, Kostler WJ, Lipton A, Bates M and Arteaga CL. Trastuzumab has preferential activity against breast cancers driven by HER2 homodimers. Cancer Res 2011; 71: 1871-1882.
[38] Zhang S, Huang WC, Li P, Guo H, Poh SB, Brady SW, Xiong Y, Tseng LM, Li SH, Ding Z, Sahin AA, Esteva FJ, Hortobagyi GN and Yu D. Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. Nat Med 2011; 17: 461-469.
[39] DiGiovanna MP, Stern DF, Edgerton SM, Whalen SG, Moore D 2nd and Thor AD. Relationship of epidermal growth factor receptor expression to ErbB-2 signaling activity and prognosis in breast cancer patients. J Clin Oncol 2005; 23: 1152-1160.
[40] Calabrich A, Fernandes Gdos S and Katz A. Trastuzumab: mechanisms of resistance and therapeutic opportunities. Oncology (Williston Park) 2008; 22: 1250-1258.
[41] Belkhiri A, Dar AA, Peng DF, Razvi MH, Rinehart C, Arteaga CL and El-Rifai W. Expression of t-DARPP mediates trastuzumab resistance in breast cancer cells. Clin Cancer Res 2008; 14: 4564-4571.
[42] Gu L, Waliany S and Kane SE. Darpp-32 and its truncated variant t-Darpp have antagonistic effects on breast cancer cell growth and herceptin resistance. PLoS One 2009; 4: e6220.
[43] Lenz G, Hamilton A, Geng S, Hong T, Kalkum M, Momand J, Kane SE and Huss JM. t-Darpp activates IGF-1R signaling to regulate glucose metabolism in trastuzumab-resistant breast cancer cells. Clin Cancer Res 2018; 24: 1216-1226.
[44] Kim Y, Bae YJ, Kim JH, Kim H, Shin SJ, Jung DH and Park H. Wnt/β-catenin pathway is a key signaling pathway to trastuzumab resistance in gastric cancer cells. BMC Cancer 2023; 23: 922.
[45] Li Y, Chu J, Feng W, Yang M, Zhang Y, Zhang Y, Qin Y, Xu J, Li J, Vasilatos SN, Fu Z, Huang Y and Yin Y. EPHA5 mediates trastuzumab resistance in HER2-positive breast cancers through regulating cancer stem cell-like properties. FASEB J 2019; 33: 4851-4865.
[46] Saleh K, Khoury R, Khalife N, Chahine C, Ibrahim R, Tikriti Z and Le Cesne A. Mechanisms of action and resistance to anti-HER2 antibody-drug conjugates in breast cancer. Cancer Drug Resist 2024; 7: 22.
[47] Takegawa N, Nonagase Y, Yonesaka K, Sakai K, Maenishi O, Ogitani Y, Tamura T, Nishio K, Nakagawa K and Tsurutani J. DS-8201a, a new HER2-targeting antibody-drug conjugate incorporating a novel DNA topoisomerase I inhibitor, overcomes HER2-positive gastric cancer T-DM1 resistance. Int J Cancer 2017; 141: 1682-1689.
[48] Kovtun YV, Audette CA, Mayo MF, Jones GE, Doherty H, Maloney EK, Erickson HK, Sun X, Wilhelm S, Ab O, Lai KC, Widdison WC, Kellogg B, Johnson H, Pinkas J, Lutz RJ, Singh R, Goldmacher VS and Chari RV. Antibody-maytansinoid conjugates designed to bypass multidrug resistance. Cancer Res 2010; 70: 2528-2537.
[49] Wang L, Wang Q, Gao M, Fu L, Li Y, Quan H and Lou L. STAT3 activation confers trastuzumab-emtansine (T-DM1) resistance in HER2-positive breast cancer. Cancer Sci 2018; 109: 3305-3315.
[50] Islam SS, Uddin M, Noman ASM, Akter H, Dity NJ, Basiruzzman M, Uddin F, Ahsan J, Annoor S, Alaiya AA, Al-Alwan M, Yeger H and Farhat WA. Antibody-drug conjugate T-DM1 treatment for HER2+ breast cancer induces ROR1 and confers resistance through activation of Hippo transcriptional coactivator YAP1. EBioMed 2019; 43: 211-224.
[51] Barok M, Joensuu H and Isola J. Trastuzumab emtansine: mechanisms of action and drug resistance. Breast Cancer Res 2014; 16: 209.
[52] Saatci Ö, Borgoni S, Akbulut Ö, Durmuş S, Raza U, Eyüpoğlu E, Alkan C, Akyol A, Kütük Ö, Wiemann S and Şahin Ö. Targeting PLK1 overcomes T-DM1 resistance via CDK1-dependent phosphorylation and inactivation of Bcl-2/xL in HER2-positive breast cancer. Oncogene 2018; 37: 2251-2269.

[53] Visconti R, Della Monica R and Grieco D. Cell cycle checkpoint in cancer: a therapeutically targetable double-edged sword. J Exp Clin Cancer Res 2016; 35: 153.
[54] Sabbaghi M, Gil-Gómez G, Guardia C, Servitja S, Arpí O, García-Alonso S, Menendez S, Arumi-Uria M, Serrano L, Salido M, Muntasell A, Martínez-García M, Zazo S, Chamizo C, González-Alonso P, Madoz-Gúrpide J, Eroles P, Arribas J, Tusquets I, Lluch A, Pandiella A, Rojo F, Rovira A and Albanell J. Defective cyclin B1 induction in trastuzumab-emtansine (T-DM1) acquired resistance in HER2-positive breast cancer. Clin Cancer Res 2017; 23: 7006-7019.
[55] Li H, Wang J, Yi Z, Li C, Wang H, Zhang J, Wang T, Nan P, Lin F, Xu D, Qian H and Ma F. CDK12 inhibition enhances sensitivity of HER2+ breast cancers to HER2-tyrosine kinase inhibitor via suppressing PI3K/AKT. Eur J Cancer 2021; 145: 92-108.
[56] Yamaguchi K, Ito M, Isobe T, Koreishi S, Taguchi R, Uehara K, Ueno S, Imajima T, Kitazono T, Tsuchihashi K, Ohmura H, Yoshihiro T, Tanoue K, Nishiyori S, Iwama E, Maeda T, Akashi K and Baba E. Impact of genomic alterations on efficacy of trastuzumab deruxtecan against human epidermal growth factor receptor-2-positive advanced gastric cancer. JCO Precis Oncol 2024; 8: e2300681.
[57] Shah S, Kim Y, Ostrovnaya I, Murali R, Schrader KA, Lach FP, Sarrel K, Rau-Murthy R, Hansen N, Zhang L, Kirchhoff T, Stadler Z, Robson M, Vijai J, Offit K and Smogorzewska A. Assessment of SLX4 mutations in hereditary breast cancers. PLoS One 2013; 8: e66961.
[58] Ríos-Luci C, García-Alonso S, Díaz-Rodríguez E, Nadal-Serrano M, Arribas J, Ocana A and Pandiella A. Resistance to the antibody-drug conjugate T-DM1 is based in a reduction in lysosomal proteolytic activity. Cancer Res 2017; 77: 4639-4651.
[59] Hamblett KJ, Jacob AP, Gurgel JL, Tometsko ME, Rock BM, Patel SK, Milburn RR, Siu S, Ragan SP, Rock DA, Borths CJ, O'Neill JW, Chang WS, Weidner MF, Bio MM, Quon KC and Fanslow WC. SLC46A3 is required to transport catabolites of noncleavable antibody maytansine conjugates from the lysosome to the cytoplasm. Cancer Res 2015; 75: 5329-5340.
[60] Wang H, Wang W, Xu Y, Yang Y, Chen X, Quan H and Lou L. Aberrant intracellular metabolism of T-DM1 confers T-DM1 resistance in human epidermal growth factor receptor 2-positive gastric cancer cells. Cancer Sci 2017; 108: 1458-1468.
[61] Sung M, Tan X, Lu B, Golas J, Hosselet C, Wang F, Tylaska L, King L, Zhou D, Dushin R, Myers JS, Rosfjord E, Lucas J, Gerber HP and Loganzo F. Caveolae-mediated endocytosis as a novel mechanism of resistance to trastuzumab emtansine (T-DM1). Mol Cancer Ther 2018; 17: 243-253.
[62] Chung YC, Chang CM, Wei WC, Chang TW, Chang KJ and Chao WT. Metformin-induced caveolin-1 expression promotes T-DM1 drug efficacy in breast cancer cells. Sci Rep 2018; 8: 3930.
[63] Saito S, Morishima K, Ui T, Hoshino H, Matsubara D, Ishikawa S, Aburatani H, Fukayama M, Hosoya Y, Sata N, Lefor AK, Yasuda Y and Niki T. The role of HGF/MET and FGF/FGFR in fibroblast-derived growth stimulation and lapatinib-resistance of esophageal squamous cell carcinoma. BMC Cancer 2015; 15: 82.
[64] Tharp KM, Kersten K, Maller O, Timblin GA, Stashko C, Canale FP, Menjivar RE, Hayward MK, Berestjuk I, Ten Hoeve J, Samad B, Ironside AJ, di Magliano MP, Muir A, Geiger R, Combes AJ and Weaver VM. Tumor-associated macrophages restrict CD8+ T cell function through collagen deposition and metabolic reprogramming of the breast cancer microenvironment. Nat Cancer 2024; 5: 1045-1062.
[65] Yang L, Bhattacharya A, Peterson D, Li Y, Liu X, Marangoni E, Robila V and Zhang Y. Targeted dual degradation of HER2 and EGFR obliterates oncogenic signaling, overcomes therapy resistance, and inhibits metastatic lesions in HER2-positive breast cancer models. Drug Resist Updat 2024; 74: 101078.
[66] Li S, Zhao X, Fu K, Zhu S, Pan C, Yang C, Wang F, To KKW and Fu L. Resistance to antibody-drug conjugates: a review. Acta Pharm Sin B 2025; 15: 737-756.
[67] Jin Q, Yuan LX, Boulbes D, Baek JM, Wang YN, Gomez-Cabello D, Hawke DH, Yeung SC, Lee MH, Hortobagyi GN, Hung MC and Esteva FJ. Fatty acid synthase phosphorylation: a novel therapeutic target in HER2-overexpressing breast cancer cells. Breast Cancer Res 2010; 12: R96.
[68] Guo LJ, Wu J, Lu W, Li J, Wang Y, Yang H and Wang TZ. Nanoparticles modulating the immune microenvironment in breast cancer treatment. Int J Nanomedicine 2025; 20: 1367-1382.
[69] Hartman ZC, Yang XY, Glass O, Lei G, Osada T, Dave SS, Morse MA, Clay TM and Lyerly HK. HER2 overexpression elicits a proinflammatory IL-6 autocrine signaling loop that is critical for tumorigenesis. Cancer Res 2011; 71: 4380-4391.
[70] Wei W, Tweardy DJ, Zhang M, Zhang X, Landua J, Petrovic I, Bu W, Roarty K, Hilsenbeck SG, Rosen JM and Lewis MT. STAT3 signaling is activated preferentially in tumor-initiating cells in claudin-low models of human breast cancer. Stem Cells 2014; 32: 2571-2582.

[71] Sun C, Mezzadra R and Schumacher TN. Regulation and function of the PD-L1 checkpoint. Immunity 2018; 48: 434-452.

[72] Yamashita K, Iwatsuki M, Yasuda-Yoshihara N, Morinaga T, Nakao Y, Harada K, Eto K, Kurashige J, Hiyoshi Y, Ishimoto T, Nagai Y, Iwagami S, Baba Y, Miyamoto Y, Yoshida N, Ajani JA and Baba H. Trastuzumab upregulates programmed death ligand-1 expression through interaction with NK cells in gastric cancer. Br J Cancer 2021; 124: 595-603.

[73] Loi S, Giobbie-Hurder A, Gombos A, Bachelot T, Hui R, Curigliano G, Campone M, Biganzoli L, Bonnefoi H, Jerusalem G, Bartsch R, Rabaglio-Poretti M, Kammler R, Maibach R, Smyth MJ, Di Leo A, Colleoni M, Viale G, Regan MM and André F; International Breast Cancer Study Group and the Breast International Group. Pembrolizumab plus trastuzumab in trastuzumab-resistant, advanced, HER2-positive breast cancer (PANACEA): a single-arm, multicentre, phase 1b-2 trial. Lancet Oncol 2019; 20: 371-382.

[74] Turcotte M, Allard D, Mittal D, Bareche Y, Buisseret L, José V, Pommey S, Delisle V, Loi S, Joensuu H, Kellokumpu-Lehtinen PL, Sotiriou C, Smyth MJ and Stagg J. CD73 promotes resistance to HER2/ErbB2 antibody therapy. Cancer Res 2017; 77: 5652-5663.

[75] Bianchini G, Pusztai L, Pienkowski T, Im YH, Bianchi GV, Tseng LM, Liu MC, Lluch A, Galeota E, Magazzù D, de la Haba-Rodríguez J, Oh DY, Poirier B, Pedrini JL, Semiglazov V, Valagussa P and Gianni L. Immune modulation of pathologic complete response after neoadjuvant HER2-directed therapies in the NeoSphere trial. Ann Oncol 2015; 26: 2429-2436.

[76] Zazo S, González-Alonso P, Martín-Aparicio E, Chamizo C, Luque M, Sanz-Álvarez M, Mínguez P, Gómez-López G, Cristóbal I, Caramés C, García-Foncillas J, Eroles P, Lluch A, Arpí O, Rovira A, Albanell J, Madoz-Gúrpide J and Rojo F. Autocrine CCL5 effect mediates trastuzumab resistance by ERK pathway activation in HER2-positive breast cancer. Mol Cancer Ther 2020; 19: 1696-1707.

[77] Force J, Howie LJ, Abbott SE, Bentley R, Marcom PK, Kimmick G, Westbrook K, Sammons SL, Parks M, Topping DL, Emerson R, Broadwater G, Hyslop T, Blackwell KL and Nair SK. Early stage HER2-positive breast cancers not achieving a pCR from neoadjuvant trastuzumab- or pertuzumab-based regimens have an immunosuppressive phenotype. Clin Breast Cancer 2018; 18: 410-417.

[78] Doronina SO, Toki BE, Torgov MY, Mendelsohn BA, Cerveny CG, Chace DF, DeBlanc RL, Gearing RP, Bovee TD, Siegall CB, Francisco JA, Wahl AF, Meyer DL and Senter PD. Development of potent monoclonal antibody auristatin conjugates for cancer therapy. Nat Biotechnol 2003; 21: 778-784.

[79] Caculitan NG, dela Cruz Chuh J, Ma Y, Zhang D, Kozak KR, Liu Y, Pillow TH, Sadowsky J, Cheung TK, Phung Q, Haley B, Lee BC, Akita RW, Sliwkowski MX and Polson AG. Cathepsin B is dispensable for cellular processing of Cathepsin B-cleavable antibody-drug conjugates. Cancer Res 2017; 77: 7027-7037.

[80] Ogitani Y, Hagihara K, Oitate M, Naito H and Agatsuma T. Bystander killing effect of DS-8201a, a novel anti-human epidermal growth factor receptor 2 antibody-drug conjugate, in tumors with human epidermal growth factor receptor 2 heterogeneity. Cancer Sci 2016; 107: 1039-1046.

[81] Johann F, Wöll S and Gieseler H. "Negative" impact: the role of payload charge in the physicochemical stability of auristatin antibody-drug conjugates. J Pharm Sci 2024; 113: 2433-2442.

[82] Li F, Emmerton KK, Jonas M, Zhang X, Miyamoto JB, Setter JR, Nicholas ND, Okeley NM, Lyon RP, Benjamin DR and Law CL. Intracellular released payload influences potency and bystander-killing effects of antibody-drug conjugates in preclinical models. Cancer Res 2016; 76: 2710-2719.

[83] Junutula JR, Raab H, Clark S, Bhakta S, Leipold DD, Weir S, Chen Y, Simpson M, Tsai SP, Dennis MS, Lu Y, Meng YG, Ng C, Yang J, Lee CC, Duenas E, Gorrell J, Katta V, Kim A, McDorman K, Flagella K, Venook R, Ross S, Spencer SD, Lee Wong W, Lowman HB, Vandlen R, Sliwkowski MX, Scheller RH, Polakis P and Mallet W. Site-specific conjugation of a cytotoxic drug to an antibody improves the therapeutic index. Nat Biotechnol 2008; 26: 925-932.

[84] Khera E, Cilliers C, Bhatnagar S and Thurber GM. Computational transport analysis of antibody-drug conjugate bystander effects and payload tumoral distribution: implications for therapy. Mol Syst Des Eng 2018; 3: 73-88.

[85] Khera E, Dong S, Huang H, de Bever L, van Delft FL and Thurber GM. Cellular-resolution imaging of bystander payload tissue penetration from antibody-drug conjugates. Mol Cancer Ther 2022; 21: 310-321.

[86] Chung WP, Huang WL, Lee CH, Hsu HP, Huang WL, Liu YY and Su WC. PI3K inhibitors in trastuzumab-resistant HER2-positive breast cancer cells with PI3K pathway alterations. Am J Cancer Res 2022; 12: 3067-3082.

[87] Fujimoto Y, Morita TY, Ohashi A, Haeno H, Hakozaki Y, Fujii M, Kashima Y, Kobayashi SS and Mukohara T. Combination treatment with a PI3K/Akt/mTOR pathway inhibitor overcomes

resistance to anti-HER2 therapy in PIK3CA-mutant HER2-positive breast cancer cells. Sci Rep 2020; 10: 21762.

[88] Glaviano A, Foo ASC, Lam HY, Yap KCH, Jacot W, Jones RH, Eng H, Nair MG, Makvandi P, Geoerger B, Kulke MH, Baird RD, Prabhu JS, Carbone D, Pecoraro C, Teh DBL, Sethi G, Cavalieri V, Lin KH, Javidi-Sharifi NR, Toska E, Davids MS, Brown JR, Diana P, Stebbing J, Fruman DA and Kumar AP. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Mol Cancer 2023; 22: 138.

[89] Debien V, Marta GN, Agostinetto E, Sirico M, Jacobs F, Molinelli C, Moreau M, Paesmans M, De Giorgi U, Santoro A, Taylor D, Duhoux FP, Botticelli A, Barchiesi G, Speranza I, Lambertini M, Wildiers H, Azambuja E and Piccart M. Real-world clinical outcomes of patients with stage I HER2-positive breast cancer treated with adjuvant paclitaxel and trastuzumab. Crit Rev Oncol Hematol 2023; 190: 104089.

[90] Lee HJ, Seo AN, Kim EJ, Jang MH, Kim YJ, Kim JH, Kim SW, Ryu HS, Park IA, Im SA, Gong G, Jung KH, Kim HJ and Park SY. Prognostic and predictive values of EGFR overexpression and EGFR copy number alteration in HER2-positive breast cancer. Br J Cancer 2015; 112: 103-111.

[91] Yonesaka K, Zejnullahu K, Okamoto I, Satoh T, Cappuzzo F, Souglakos J, Ercan D, Rogers A, Roncalli M, Takeda M, Fujisaka Y, Philips J, Shimizu T, Maenishi O, Cho Y, Sun J, Destro A, Taira K, Takeda K, Okabe T, Swanson J, Itoh H, Takada M, Lifshits E, Okuno K, Engelman JA, Shivdasani RA, Nishio K, Fukuoka M, Varella-Garcia M, Nakagawa K and Jänne PA. Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. Sci Transl Med 2011; 3: 99ra86.

[92] Zhang R, Su C, Jia Y, Xing M, Jin S and Zong H. Molecular mechanisms of HER2-targeted therapy and strategies to overcome the drug resistance in colorectal cancer. Biomed Pharmacother 2024; 179: 117363.

[93] Díaz-Rodríguez E, Gandullo-Sánchez L, Ocaña A and Pandiella A. Novel ADCs and strategies to overcome resistance to anti-HER2 ADCs. Cancers (Basel) 2021; 14: 154.

[94] Zoeller JJ, Vagodny A, Taneja K, Tan BY, O'Brien N, Slamon DJ, Sampath D, Leverson JD, Bronson RT, Dillon DA and Brugge JS. Neutralization of BCL-2/X(L) enhances the cytotoxicity of T-DM1 in vivo. Mol Cancer Ther 2019; 18: 1115-1126.

[95] Geyer CE, Sikov WM, Huober J, Rugo HS, Wolmark N, O'Shaughnessy J, Maag D, Untch M, Golshan M, Lorenzo JP, Metzger O, Dunbar M, Symmans WF, Rastogi P, Sohn JH, Young R, Wright GS, Harkness C, McIntyre K, Yardley D and Loibl S. Long-term efficacy and safety of addition of carboplatin with or without veliparib to standard neoadjuvant chemotherapy in triple-negative breast cancer: 4-year follow-up data from BrighTNess, a randomized phase III trial. Ann Oncol 2022; 33: 384-394.

[96] Yap TA, Im SA, Schram AM, Sharp A, Balmana J, Baird RD, Brown JS, Schwaederle M, Pilling EA and Moorthy G. Abstract CT007: PETRA: first in class, first in human trial of the next generation PARP1-selective inhibitor AZD5305 in patients with BRCA1/2, PALB2 or RAD51C/D mutations. Cancer Res 2022; 82: CT007.

[97] Martin M, Fumoleau P, Dewar JA, Albanell J, Limentani SA, Campone M, Chang JC, Patre M, Strasak A, de Haas SL, Xu J and Garcia-Saenz JA. Trastuzumab emtansine (T-DM1) plus docetaxel with or without pertuzumab in patients with HER2-positive locally advanced or metastatic breast cancer: results from a phase Ib/IIa study. Ann Oncol 2016; 27: 1249-1256.

[98] Wallez Y, Proia T, Leo E, Bradshaw L, Wilson Z, Owusu J, Cheraghchi-Bashi-Astaneh A, Staniszewska A, O'Connor M and Cosulich S. Activity and tolerability of combination of trastuzumab deruxtecan with the next generation PARP1-selective inhibitor AZD5305 in preclinical models. Cancer Res 2022; 82: 1142.

[99] Varadarajan K, Cruz Pico CX, Evans KW, Raso MG, Rizvi YQ, Zheng X, Chachad D, DiPeri TP, Wang B and Scott SM. Efficacy of ATR kinase inhibitor elimusertib monotherapy or combination in tumors with DNA damage response pathway and other genomic alterations. Mol Cancer Ther 2025; 24: 1402-1414.

[100] Citri A, Kochupurakkal BS and Yarden Y. The Achilles heel of ErbB-2/HER2: regulation by the Hsp90 chaperone machine and potential for pharmacological intervention. Cell Cycle 2004; 3: 51-60.

[101] Gerber HP, Sapra P, Loganzo F and May C. Combining antibody-drug conjugates and immune-mediated cancer therapy: what to expect? Biochem Pharmacol 2016; 102: 1-6.

[102] Lee HT, Lee SH and Heo YS. Molecular interactions of antibody drugs targeting PD-1, PD-L1, and CTLA-4 in immuno-oncology. Molecules 2019; 24: 1190.

[103] Principe N, Kidman J, Goh S, Tilsed CM, Fisher SA, Fear VS, Forbes CA, Zemek RM, Chopra A, Watson M, Dick IM, Boon L, Holt RA, Lake RA, Nowak AK, Lesterhuis WJ, McDonnell AM and Chee J. Tumor infiltrating effector memory antigen-specific CD8(+) T cells predict response to immune checkpoint therapy. Front Immunol 2020; 11: 584423.

[104] Müller P, Kreuzaler M, Khan T, Thommen DS, Martin K, Glatz K, Savic S, Harbeck N, Nitz U,

Gluz O, von Bergwelt-Baildon M, Kreipe H, Reddy S, Christgen M and Zippelius A. Trastuzumab emtansine (T-DM1) renders HER2+ breast cancer highly susceptible to CTLA-4/PD-1 blockade. Sci Transl Med 2015; 7: 315ra188.

[105] Jiang D, Xie B, Tang Y, Chen H, Hou X, Xu C, Shao D, Chen X, Gu X and Van L. Investigating trastuzumab-deruxtecan (DS-8201) resistance mechanisms using clinically relevant PDX models and combination strategies with anti-PD-1 antibody. Cancer Res 2025; 85: 1701.

[106] Hu Z, Fan C, Livasy C, He X, Oh DS, Ewend MG, Carey LA, Subramanian S, West R, Ikpatt F, Olopade OI, van de Rijn M and Perou CM. A compact VEGF signature associated with distant metastases and poor outcomes. BMC Med 2009; 7: 9.

[107] Linderholm B, Grankvist K, Wilking N, Johansson M, Tavelin B and Henriksson R. Correlation of vascular endothelial growth factor content with recurrences, survival, and first relapse site in primary node-positive breast carcinoma after adjuvant treatment. J Clin Oncol 2000; 18: 1423-1431.

[108] Li Y, Wang J, Liu T, Zhang J, Shan Y and Zhang J. Discovery of a novel ADC for multifunctional theranostics: from vascular normalization to synergistic therapy. Bioconjug Chem 2025; 36: 1079-1087.

[109] Huang H, Zhou Y, Shang C, Zhang Y and Shen Y. A novel anti-HER2/EGFR bispecific antibody-drug conjugate demonstrates promising anti-tumor efficacy and overcomes resistance to HER2- or EGFR-targeted ADCs. Invest New Drugs 2025; 43: 262-275.

[110] Weisser NE, Sanches M, Escobar-Cabrera E, O'Toole J, Whalen E, Chan PWY, Wickman G, Abraham L, Choi K, Harbourne B, Samiotakis A, Rojas AH, Volkers G, Wong J, Atkinson CE, Baardsnes J, Worrall LJ, Browman D, Smith EE, Baichoo P, Cheng CW, Guedia J, Kang S, Mukhopadhyay A, Newhook L, Ohrn A, Raghunatha P, Zago-Schmitt M, Schrag JD, Smith J, Zwierzchowski P, Scurll JM, Fung V, Black S, Strynadka NCJ, Gold MR, Presta LG, Ng G and Dixit S. An anti-HER2 biparatopic antibody that induces unique HER2 clustering and complement-dependent cytotoxicity. Nat Commun 2023; 14: 1394.

[111] McKertish CM and Kayser V. A novel dual-payload ADC for the treatment of HER2+ breast and colon cancer. Pharmaceutics 2023; 15: 2020.

[112] Haratani K, Yonesaka K, Takamura S, Maenishi O, Kato R, Takegawa N, Kawakami H, Tanaka K, Hayashi H, Takeda M, Maeda N, Kagari T, Hirotani K, Tsurutani J, Nishio K, Doi K, Miyazawa M and Nakagawa K. U3-1402 sensitizes HER3-expressing tumors to PD-1 blockade by immune activation. J Clin Invest 2020; 130: 374-388.

[113] Staudacher AH and Brown MP. Antibody drug conjugates and bystander killing: is antigen-dependent internalisation required? Br J Cancer 2017; 117: 1736-1742.

[114] Bernardes GJ, Casi G, Trüssel S, Hartmann I, Schwager K, Scheuermann J and Neri D. A traceless vascular-targeting antibody-drug conjugate for cancer therapy. Angew Chem Int Ed Engl 2012; 51: 941-944.

[115] Gébleux R, Stringhini M, Casanova R, Soltermann A and Neri D. Non-internalizing antibody-drug conjugates display potent anti-cancer activity upon proteolytic release of monomethyl auristatin E in the subendothelial extracellular matrix. Int J Cancer 2017; 140: 1670-1679.

[116] Barbieri MA, Roberts RL, Gumusboga A, Highfield H, Alvarez-Dominguez C, Wells A and Stahl PD. Epidermal growth factor and membrane trafficking. EGF receptor activation of endocytosis requires Rab5a. J Cell Biol 2000; 151: 539-550.

[117] Frittoli E, Palamidessi A, Marighetti P, Confalonieri S, Bianchi F, Malinverno C, Mazzarol G, Viale G, Martin-Padura I, Garré M, Parazzoli D, Mattei V, Cortellino S, Bertalot G, Di Fiore PP and Scita G. A RAB5/RAB4 recycling circuitry induces a proteolytic invasive program and promotes tumor dissemination. J Cell Biol 2014; 206: 307-328.

[118] Engebraaten O, Yau C, Berg K, Borgen E, Garred Ø, Berstad MEB, Fremstedal ASV, DeMichele A, Veer LV', Esserman L and Weyergang A. RAB5A expression is a predictive biomarker for trastuzumab emtansine in breast cancer. Nat Commun 2021; 12: 6427.

[119] Hunter FW, Barker HR, Lipert B, Rothé F, Gebhart G, Piccart-Gebhart MJ, Sotiriou C and Jamieson SMF. Mechanisms of resistance to trastuzumab emtansine (T-DM1) in HER2-positive breast cancer. Br J Cancer 2020; 122: 603-612.

[120] Kinneer K, Meekin J, Tiberghien AC, Tai YT, Phipps S, Kiefer CM, Rebelatto MC, Dimasi N, Moriarty A, Papadopoulos KP, Sridhar S, Gregson SJ, Wick MJ, Masterson L, Anderson KC, Herbst R, Howard PW and Tice DA. SLC46A3 as a potential predictive biomarker for antibody-drug conjugates bearing noncleavable linked maytansinoid and pyrrolobenzodiazepine warheads. Clin Cancer Res 2018; 24: 6570-6582.