

Antibody drug conjugates targeting HER2: Clinical development in metastatic breast cancer

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ABSTRACT

The identification of the HER2 alteration as an actionable oncogenic driver in breast cancer has propelled the development of HER-targeting monoclonal antibodies (mAb) such as trastuzumab and pertuzumab, which led to dramatic improvements in survival outcomes. Lately, the great strides made toward developing antibody-conjugation methods have led to the development of a new class of compelling compounds, the antibody-drug conjugates (ADCs) targeting HER2 which have profoundly transformed the treatment landscape of breast cancer. HER2-targeting ADCs, trastuzumab-emtansine and trastuzumab-deruxtecan, have improved the overall survival in the second and third-line settings with manageable adverse events. Other HER2-targeting ADCs using novel technological advances in the antibody, linker and/or payload conception have shown promising activity in preclinical and clinical studies and some of them are now being evaluated in larger clinical trials. Multiple challenges still impede the success of ADCs in breast cancer namely the lack of a comprehensive understanding of resistance mechanisms as well as the mechanisms of action of ADCs in special subgroups of patients such as those with low or ultra-low HER2 expression and patients with brain or leptomeningeal metastases (BM). In this framework, we review the approved indications and ongoing trials for HER2-targeting ADCs, across patient subgroups, including those with BM and discuss the associated potential mechanisms of action and resistance. Last, we provide an overview of the future perspectives involving HER2-targeting ADCs in breast cancer.

1. Introduction

Around twenty percent of patients with breast cancer overexpress the receptor tyrosine-protein kinase erbB-2, also known as human epidermal growth factor receptor 2 or HER2 [1]. Historically, women with HER2-breast cancer had a poor prognosis with significantly shortened disease-free survival and overall survival [2]. Translational research indicated that these tumors typically present an *ERBB2* amplification that leads to cellular proliferation and survival through HER2 dimerization with other tyrosine kinase receptors and subsequent activation of several signaling pathways, PI3K–AKT and RAS–MAPK [3]. As such, HER2-positive breast cancer (HER2+ BC) presented an oncogenic driver that provided investigators with the opportunity to target HER2 specifically. The active efforts in the nineties led to the development of trastuzumab, a humanized anti-HER2 monoclonal antibody, that interfered with HER2 signaling [4]. The proof of concept for this targeted therapy was confirmed two decades ago in a randomized trial showing an improvement in survival outcomes among patients with

metastatic HER2+ BC treated with the addition of trastuzumab to chemotherapy [5]. Pertuzumab is another humanized monoclonal antibody that targets a different extracellular subdomain of HER2 receptor (subdomain II) than the one bound by trastuzumab (subdomain IV). Its combination with trastuzumab and taxanes showed a survival benefit among patients with metastatic HER2+ BC and the corresponding pivotal trial supports the current standard of care in this patient population [6].

Over the last three decades, major improvements in antibody engineering, particularly in site-specific conjugation techniques along with the development of more stable linkers and highly potent cytotoxic have finally led to the development of antibody drug conjugates (ADCs) with the specific intent of delivering highly potent cytotoxic agents to cancer cells without affecting normal tissues [7]. Indeed, the initial clinical trials that evaluated ADCs in the '80s showed considerable toxicities without a clinical significant activity [8,9]. Lately, the remarkable specificity of HER2 antibodies has raised a novel interest in coupling them with potent cytotoxic drugs [10]. The technological improvements

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in the conception and execution of ADCs targeting HER2 led to the development of ado-trastuzumab emtansine (T-DM1) which became the first ADC to be approved for the treatment of a solid tumor [11]. The momentum of ADC development has since surged leading to increasingly effective HER2-targeting ADCs, with multiple further approvals over the past 3 years. Here, we provide a comprehensive overview of approved indications and ongoing trials for HER2-targeting ADCs, across patient subgroups, including those with BM and discuss the associated potential mechanisms of action and resistance along with the future developmental perspectives over the coming years.

2. Construct and mechanisms of action of ADCs targeting HER2

The structure of ADC comprises an antibody, a linker, and a cytotoxic payload. Monoclonal antibodies targeting HER2 constitute the backbone of ADCs for HER2-positive breast cancer; they are designed to minimize immunogenicity, while maintaining a high target affinity and properties of the naked antibody such as immune-mediating functions and tumor target modulation [12]. Humanized or chimeric immunoglobulin G (IgG), in general IgG1, is the most commonly-used antibody backbone for ADC engineering given their optimal solubility, long half-life, complement fixation and activation of antibody-dependent cell-mediated cytotoxicity [13]. Payloads are mainly cytotoxic agents that are highly potent at sub-nanomolar concentrations [14]. The linker used to bind the payload to the antibody must be stable in the circulation to deliver the payload directly to tumor cells and avoid its premature release in the bloodstream [15]. The first-generation ADCs were unstable in the circulation, led to systemic loss of the drug and showed a narrow therapeutic index due to the insufficient potency of the payload and the unpredictable systemic toxicity. A larger therapeutic index was obtained with the second- and third generation of ADCs that rely on innovative linkers, more potent payloads and site-specific conjugation to engineer homogenous ADCs with a well-defined drug-to-antibody ratio. Moreover, the use of humanized, instead of chimeric, antibody backbone has furtherly reduced ADC immunogenicity [16,17]. Table 1 summarizes the main technological advancements among the various ADC generations with examples for each category.

The exact mechanisms of action of ADCs have not been fully clarified and are likely related to the interplay of the monoclonal antibody, the linker and payload with the tumor cells and microenvironment [14] (see Fig. 1) The binding of the monoclonal antibody of the ADC to HER2 on the surface of cancer cells exerts an antitumor activity by modulating the signals that emanate from HER2 (PI3K–AKT–mTOR and RAS–MAPK pathways) and through Fc-mediated effector functions. Thereafter, the ADC-HER2 complex is internalized in a clathrin-coated early endosome and transported to lysosomes. Inside the lysosomes, ADCs with cleveable linkers will release the payload and those with non-cleavable linkers will break down the ADC and release the linker-payload complex [18]. Subsequently, the payload elicits antitumor activity through its classical mechanism of action within the targeted cell [14]. Depending on the linker and payload combination, the payload can be released within the extracellular space before or after the ADC internalization. In both cases, the payload can exert its activity in the neighboring cells, which may or may not express HER2. This wider drug delivery to tumor cells, known as the bystander effect, improved the activity of ADC in cancers with heterogeneous and/or low HER2 expression [19].

2.1. Ado-trastuzumab emtansine

Ado-trastuzumab emtansine is an ADC that combines trastuzumab to DM1, a cytotoxic microtubule inhibitor, through a thioether uncleavable linker [20]. A proof-of-concept phase II study has shown promising activity of single-agent T-DM1 in 112 patients with HER2-positive MBC who had progressed while receiving HER2-directed therapy [21]. The objective response rate (ORR) was 25.9% and the median progression-free survival (PFS) was 4.6 months (95% CI, 3.9–8.6

Table 1
Main technological advancements among the various ADC generations with examples for each category.

	Antibody backbone	Conjugation site	Linker	Payload	Drug-to-antibody ratio	Example
1st generation	Murin/chimeric antibodies	Chemical conjugation with natural amino-acids of antibody backbone (stochastic method)	Plasma instability	Anthracyclines, anti-metabolite/antifolate agents	High variability	BR96 antibody–doxorubicin conjugate
2nd generation	Humanized or fully human antibodies	Chemical conjugation with natural amino-acids of antibody backbone (stochastic method)	Higher plasma stability; majority non-cleavable linkers	Auristatins and maytansinoids	3–4	Trastuzumab emtansine (T-DM1)
3rd generation	Humanized or fully human antibodies	Selective introduction of reactive molecules (site-specific conjugation)	Higher plasma stability; majority cleavable linkers	Topo-1 inhibitors DNA-binding agents	6–8	Trastuzumab-Deruxtecan (T-DXd)

months). Retrospective central testing ($n = 74$) detected 21 patients that did not overexpress HER2; the median PFS was higher among patients with tumors that were HER2+ BC compared to those found not to overexpress HER2 (8.2 vs 2.6 months) [21]. The activity of T-DM1 was also confirmed in another phase II trial of 110 heavily pretreated patients and yielded an ORR of 34.5% and median PFS of 6.9 months [22].

The phase III trial TH3RESA compared T-DM1 to the treatment of physician choice in patients with HER2+ BC previously treated with trastuzumab and lapatinib [23,24]. Six hundred six heavily pretreated (median of 4 lines) patients were randomized to T-DM1 ($n = 404$) or standard therapy ($n = 198$). Albeit 47% of the control arm crossed over to the T-DM1 at progression, the median overall survival was significantly longer in the experimental arm (OS; 22.7 vs 15.8 months; HR 0.68, 95%CI 0.54–0.85; $p = 0.0007$). The most common grade 3 or greater adverse event (AE) with T-DM1 was Thrombocytopenia (19 [5%] vs three [2%]).

The phase III EMILIA trial assessed the efficacy of T-DM1 in comparison to lapatinib plus capecitabine in 991 patients with HER2+ BC previously treated with trastuzumab and taxanes [25,26]. Treatment with T-DM1 was associated with improved ORR (43.6% vs 30.8%), median PFS (9.6 vs 6.4 months; HR 0.65, 95%CI 0.55–0.77; $p < 0.001$) and median OS (29.9 vs 25.9 months; HR 0.75, 95%CI 0.64–0.88; $p < 0.001$). The most frequently reported T-DM1-related adverse events were thrombocytopenia (grade 3, 14%) and increased aspartate aminotransferase (grade 3, 5%). At that time, EMILIA firmly established T-DM1 as the recommended second-line treatment in metastatic HER2+ BC after the failure of frontline trastuzumab and taxane [27]. Around the same time, the phase III CLEOPATRA trial led to the approval of pertuzumab plus trastuzumab and taxane in the frontline treatment of metastatic HER2+ BC [28]. Translational research has previously shown that the addition of pertuzumab to trastuzumab downregulated HER2 expression at the membrane level which was associated with a lower T-DM1 efficacy [29]. Few retrospective studies have reported on the efficacy of TDM-1 in pertuzumab pretreated patients with conflicting results [30–39]. A meta-analysis of these studies showed similar one-year PFS for T-DM1 in the second-line and beyond in pertuzumab pretreated patients and those treated within the EMILIA trial [40].

The phase IIIB single-arm KAMILLA trial aimed to determine T-DM1 safety in a real-life setting of heavily pretreated patients with advanced HER2+ BC [41]. Among 2002 patients, 1321 patients (66.0%) had received more than prior treatments for metastatic BC, 373 were aged ≥ 65 years (18.6%), 890 had an ECOG performance status ≥ 1 (44.4%) and 398 patients (19.9%) had brain metastases. After a median follow-up of 20.6 months, the median PFS and OS were 6.9 (95%CI, 6.0–7.6) and 27.2 months (95%CI, 25.5–28.7), respectively. With increasing lines of prior advanced therapy (0–1 versus 4+), median PFS and OS decreased numerically from 8.3 to 5.6 months and from 31.3 to 22.5 months, respectively. Grade 3–5 adverse events occurred in 751 (37.5%) patients mainly including anaemia (3.0%), thrombocytopenia (2.7%) and fatigue (2.5%) [41].

T-DM1 was also evaluated in patients with previously untreated HER2+ BC and has demonstrated encouraging efficacy [42]. Compared to patients receiving upfront trastuzumab plus docetaxel ($n = 70$), those receiving frontline T-DM1 ($n = 67$) had higher ORR (64.2 vs 58%) and longer median PFS (14.2 vs 9.2 months) [42]. The phase III MARIANNE study was designed later and randomized 1095 patients into trastuzumab plus taxane, T-DM1, or T-DM1 plus pertuzumab [43]. The two T-DM1 containing experimental arms did not show statistically superior efficacy compared with trastuzumab plus taxane despite an *in vitro* synergistic activity between T-DM1 plus pertuzumab [43,44]. Interestingly, the patients who achieved an objective response had a numerically longer duration of response with T-DM1 compared to the control arm (20.7 vs 12.5 months). However, T-DM1 was no longer developed in the frontline treatment of patients with metastatic HER2+ BC.

2.2. Fam-trastuzumab deruxtecan

Fam-trastuzumab deruxtecan (T-DXd) is an ADC that combines trastuzumab to an exatecan derivative (DX-8951) a topoisomerase I inhibitor, through a cleavable tetrapeptide-based linker. In comparison to T-DM1, T-DXd has a higher drug-to-antibody ratio, membrane-permeable payload and a cleavable linker [45,46]. These pharmaceutical properties are essential features of the third generation ADCs and may account for a higher and broader activity of T-DXd. The phase I dose-escalation study of T-DXd enrolling 111 patients with HER2+ BC showed promising activity with an ORR of 59.5% [47]. The phase II DESTINY-Breast01 trial evaluated the efficacy of T-DXd in 184 patients with HER2+ BC who have received multiple prior lines of therapy (median of 6), including T-DM1⁴⁸. An objective response was achieved in 112 patients (60.9%). The median response duration and PFS were 14.8 and 16.4 months. The most common grade 3–5 adverse events were neutropenia, anemia and nausea reported in 20.7%, 8.7%, and 7.6%, respectively. Of particular interest, T-DXd was associated with a substantial risk of interstitial lung disease in 13.6% of the patients (grade 1 or 2, 10.9%; grade 3 or 4, 0.5%; and grade 5, 2.2%) [48]. Therefore, an independent adjudication committee (AC) was established since November 2017 to retrospectively review all potential ILD/pneumonitis cases related to T-DXd and specific guidelines for early detection and management of ILD were provided [49]. This study led to the FDA accelerated approval of T-DXd in patients with HER2+ BC who received ≥ 2 prior anti-HER2-based regimens [50].

Lately, the phase III DESTINY-Breast03 trial evaluated the efficacy of T-DXd in comparison to T-DM1 among 524 patients with HER2+ BC previously treated with trastuzumab and taxanes of whom around 60% received pertuzumab [51]. T-DXd was associated to a significantly improved ORR (79.7% vs 34.2%) and PFS (not reached vs 6.8 months; HR 0.28, 95%CI 0.22–0.37, $p < 0.001$) and a better OS (94.1 vs 85.9%; HR 0.55, 95%CI 0.36–0.86). Grade 3–5 adverse events were more common in the T-DXd arm (45.1 vs 39.8%) and mainly included neutropenia, thrombocytopenia, leukopenia and nausea reported in 19.1%, 7.0%, 6.6% and 6.6%, respectively. Interstitial lung disease was the most common treatment-emerging AE leading to the discontinuation of T-DXd and occurred in 10.5% of patients who were receiving T-DXd (grade 1, 2.7%; grade 2, 7%; grade 3, 0.8%). Based on these compelling results, T-DXd obtained FDA and EMA accelerated approval for the treatment of patients with metastatic HER2+ BC following frontline therapy with a trastuzumab-based regimen either in the metastatic setting or in the neoadjuvant or adjuvant setting and have developed disease recurrence during or within six months of completing therapy [52]. As far as concern Quality of life (QoL) assessment, the median time to deterioration of QLQ-C30 GHS did not show statistically significant differences (9.7 vs 8.3 months; HR 0.88, 95%CI 0.70–1.11) between the 2 arms; on the other hand patients receiving T-DXd had a longer median time to deterioration of EQ-5D-5L VAS (13.2 vs 8.5 months; HR 0.77, 95%CI, 0.61–0.98) [53].

Because preclinical evidences showed that upon T-DXd internalization the released payload may diffuse through the cell membrane and kill neighboring tumor cells regardless of target expression [54,55], a number of clinical trials explored the possible bystander effect of T-DXd, so including patients with HER2-low tumors (score of 1+ or 2+ on immunohistochemical (IHC) analysis and no demonstrated amplification on *in situ* hybridization) []. In the phase I dose-escalation trial, 19 of the 43 patients with low expression of HER2 had a response to T-DXd [57]. Interestingly, the phase II DAISY trial, enrolled 179 metastatic BC patients in three cohorts according to HER2 expression to receive T-DXd until progression or unacceptable toxicity [58]. Patients were heavily pretreated as 82% had received at least three prior lines of therapy, and 38% had received six or more of therapy. Among the 68 patients included in the HER2 high-expression cohort (IHC3+ or IHC2+/ISH+), the ORR was 69.1% and median PFS 11.1 months. The 73 patients in the HER2 low-expression cohort (IHC1+ or IHC2+/ISH– tumors) had an

ORR of 33.3% and median PFS of 6.7 months. In the HER2 null-expression cohort (IHC0+) including 38 patients, the ORR was 30.6% and median PFS 4.2 months [58]. The phase III Destiny-Breast04 trial evaluated the efficacy of T-DXd among pretreated patients with HER2-low BC who had received one or two previous lines of chemotherapy [59]. Overall, 557 patients were randomized to receive T-DXd or physician's choice of chemotherapy. In the hormone receptor-positive subgroup (n = 494), T-DXd yielded a longer PFS (10.1 vs 5.4 months; HR 0.51, 95%CI 0.40–0.64, $p < 0.001$) and OS (23.9 vs 17.5 months; HR 0.64, 95%CI 0.48–0.86, $p = 0.003$). These benefits were maintained in the global population, including patients with hormone receptor-negative BC with a median PFS of 9.9 months (vs 5.1 months; HR 0.50, 95%CI 0.40–0.63, $p < 0.001$) and OS of 23.4 months (vs 16.8 months; HR 0.64, 95%CI 0.49–0.84, $p = 0.001$). A lower rate of grade 3–4 AEs was observed in the T-DXd arm: 52.6% vs 67.4%, while ILD was reported in 12.1% of the patients who received T-DXd (grade 1, 3.5%; grade 2, 6.5%; grade 3, 1.3%, grade 5, 0.8%) [59].

Table 2 summarizes the construct design and the pivotal trials leading to HER2-targeting ADC approvals in BC.

2.3. Other HER2-targeting ADCs

The technological innovations in antibody, linker and payload design have led to the development of new HER2-targeted ADCs that are currently being evaluated in clinical trials and some of which have shown preliminary interesting results. Table 3 summarizes the construct design of these ADCs and the corresponding ongoing trials in BC.

Vic-trastuzumab duocarmazine (SYD985) is an ADC that combines trastuzumab to duocarmycin, a DNA-alkylating agent, via a cleavable linker [60,61]. Following a phase I trial where SYD985 showed notable clinical activity in heavily pretreated patients with HER2+ and HER2-low BC, the phase III TULIP trial randomized 431 HER2+ BC

patients who progressed after 2 or more HER2 targeted therapy to SYD985 or physician's choice of treatment. SYD985 yielded a longer median PFS (7.0 vs 4.9 months; HR 0.64, 95%CI 0.49–0.84; $p = 0.002$) and a trend for OS benefit (HR 0.83, 95%CI 0.62–1.09; $p = 0.153$). Ocular toxicity was the most common treatment-emergent adverse event, with grade 3 or higher keratitis and conjunctivitis (5.6 vs 0.0%) reported in 12.2 and 5.6% of patients [61]. Mitigation measures, such as prophylactic use of eye drops are being evaluated.

Disitamab Vedotin (RC48) targets HER2 via the humanized monoclonal antibody hertuzumab coupled to the monomethyl auristatin E (MMAE), a tubuline targeting agent, via a cleavable linker [62]. In comparison to trastuzumab, hertuzumab has a higher affinity for HER2 and more potent antibody-dependent cell-mediated cytotoxicity [63]. In the C001 CANCER dose escalation phase I trial enrolling 70 patients with HER2+ BC and 48 patients with HER2-low BC, the activity of RC48 was consistent independent of the HER2 expression. In the HER2-positive subgroup, RC48 1.5, 2.0, and 2.5 mg/kg doses yielded an ORR of 22.2%, 42.9%, and 40.0% and median PFS of 4.0, 5.7 and 6.3 months, respectively. In the HER2-low expressing subgroup, the ORR and median PFS were 39.6% and 5.7 months, respectively with RC48 2.0 mg/kg [64].

XMT-1522 consists of HT-19, a humanized monoclonal antibody that targets a different epitope of the HER2 than trastuzumab, linked to auristatin F-hydroxypropylamide (AF-HPA) via a cleavable linker. Pre-clinical trials showed an interesting activity of XMT-1522 xenograft mouse models with T-DM1 resistant HER2+BC and gastric cancer [65]. The development of this agent was discontinued due to commercial reasons in January 2019.

ALT-P7 encloses a trastuzumab variant combined with MMAE linked with a cleavable linker. The phase I trial enrolling 27 heavily pretreated patients with HER2+ BC progressive to at least two prior anti-HER2 therapies showed an ORR of 77% and median PFS of 6.2 months [66]. The reported adverse events included myalgia (33.3%), fatigue (25.9%), sensory neuropathy (22.2%), alopecia (22.2%), pruritus (22.2%) and neutropenia (22.2%).

ARX788 comprises a humanized HER-2 antibody combined to amebastatin (AS269), a tubulin inhibitor, using a unique amino acid-enabled conjugation technology and a non-cleavable linker which ensure slow release of pAF-AS269 and hence a lower systemic toxicity and an increased delivery to tumor cells. In vitro studies showed superior efficacy of ARX788 over T-DM1 in various HER2+ cell lines, including BC [67]. In the phase I ACE-BREAST01 and ACE-PAN tumor01 trials enrolling patients with various tumor types, ARX788 showed an ORR of 74% and 67% respectively [68]. The most common grade 3–4 adverse events were ocular adverse events (5.7%) and pneumonitis (4.3%) in the ACE-Breast-01 trial; pneumonitis (2.9%) and fatigue (2.9%) in the ACE-Pan tumor-01 trial. In the phase I trial enrolling only patients with HER + BC, the ORR was 65.5% and the median PFS was 17.0 months [69].

The currently ongoing ACE-Breast-03 (NCT04829604) is a single arm phase 2 study that aims to evaluate ARX788 activity and safety in patients with metastatic HER2 positive breast cancer who are resistant to T-DM1, T-DXd, and/or tucatinib-containing regimens.

PF-06804103 is composed of an anti-HER2 IgG1 antibody trastuzumab-derived conjugated to the Aur0101, a tubuline inhibitor, with a cleavable linker. Preliminary results from the phase I trial in heavily pretreated HER-2 positive breast cancer patients and gastro-esophageal cancer showed an ORR of 52.4% and adverse events were mainly arthralgia, neuropathy, myalgia and fatigue [70].

MRG002 combines MMAE to sugar-modified trastuzumab via a cleavable linker. In the single arm phase II trial (NCT04742153) enrolling 56 patients with HER2-low BC, the ORR was 34.7% and consistent in patients with IHC 1+ and IHC 2+ subgroups, as is 34.1% and 37.5% [71]. The reported adverse events included mostly neutropenia (53.6%), AST and ALT increase (46.4 and 32.1%), and alopecia (39.3%).

Table 2
Summary of the HER2-targeting ADC pivotal trials.

	Ado-trastuzumab emtansine [25,26]	Fam-trastuzumab deruxtecan [51,59]	
Design	Second-generation ADC	Third-generation ADC	
Antibody	Trastuzumab – Humanized IgG1 antibody	Trastuzumab – Humanized IgG1 antibody	
Linker	Uncleavable – thioether linker attached to random lysines	Cleavable – GGFG tetrapeptide-based linker enzymatically cleavable by lysosomal proteases	
Payload	DM1	Exatecan derivative	
DAR	3.5:1	8:1	
Pivotal trial	EMILIA trial	DESTINY-Breast03	DESTINY-Breast04
Regimen	T-DM1 3.6 mg/kg every 21 days	T-DXd 5.4 mg/kg every 21 days	T-DXd 5.4 mg/kg every 21 days
Population	Pre-treated trastuzumab plus taxane, HER2+ BC	Pre-treated trastuzumab plus taxane, HER2+ BC	Pre-treated trastuzumab plus taxane, HER2-low BC
Outcomes	Improved ORR, PFS and OS	Improved ORR and PFS	Improve ORR, PFS and OS
ORR	43.6%	79.7%	52.3%
PFS	9.6 (95%CI NR) months	Not reached (95% CI 18.5-not reached)	9.9 (95%CI 9.0–11.3) months ^a
OS	29.9 (95%CI 26.3–34.1) months	Not reached	23.4 (95%CI 20.0–24.8) months ^a

ADC: antibody drug conjugate; BC: breast cancer; DAR: drug-to-antibody ratio; GGFG: glycine-glycine-phenylalanine-glycine; NR: not reported; ORR: objective response rate; OS: overall survival; PFS: progression free survival.

^a Outcomes among patients with hormone receptor positive and negative breast cancer.

Table 3

Summary of the components of the approved and ongoing ADC in HER2+ BC.

	Antibody	Linker	Payload	DAR	Status ^a
Vic-trastuzumab duocarmazine	Trastuzumab	Cleavable	Duocarmycin (DNA alkylator)	2.8:1	Ongoing NCT04602117
Disitamab vedotin	Hertuzumab	Cleavable	Monomethyl auristatin E (Tubulin targeting agent)	4:1	Ongoing NCT05331326 NCT04400695 NCT03052634 NCT03500380
XMT-1522	HT-19	Cleavable	Auristatin F-hydroxypropylamide (Tubulin targeting agent)	12:1	No ongoing trials in BC
ALT-P7	Trastuzumab variant	Cleavable	Monomethyl auristatin E (Tubulin targeting agent)	2:1	No ongoing trials in BC
ARX788	Modified anti-Her2 antibody	Uncleavable	Auristatin analogue - Dolastatin (Tubulin targeting agent)	1.9:1	Ongoing NCT04829604, NCT05018676, NCT05018702
PF-06804103	Trastuzumab variant	Cleavable	Auristatin analogue - Aur0101 (Tubulin targeting agent)	4:1	No ongoing trials in BC
MRG002	Trastuzumab variant	Cleavable	Monomethyl auristatin E (Tubulin targeting agent)	3.8:1	Ongoing NCT04924699 NCT05263869 NCT04742153
A166	Trastuzumab variant	Cleavable	Duostatin 5 (Tubulin targeting agent)	2:1	Ongoing NCT05346328 NCT05311397 NCT03602079
ZW49	ZW25	Cleavable	Auristatin (Tubulin targeting agent)	NR	Ongoing NCT03821233
BDC-1001	Trastuzumab biosimilar	Uncleavable	TLR 7/8 agonist (Immune stimulant)	NR	Ongoing NCT04278144
T-PNU	Trastuzumab	Uncleavable	Anthracycline derivative - PNU-159682 (DNA topoisomerase II inhibitor)	4:1	No ongoing trials in BC
FS-1502	Trastuzumab	Uncleavable	Monomethyl Auristatin F (Tubulin targeting agent)	2:1	Ongoing NCT03944499
GQ1001	Trastuzumab	Intelligent Ligase dependent	Maytansinoid derivative - DM1 (Tubulin targeting agent)	2:1	Ongoing NCT04450732
HER2xPRLR bsADC	HER2xPRLR bsAb1	Uncleavable	Maytansinoid derivative - DM1 (Tubulin targeting agent)	3.3:1	No ongoing trials in BC

ADC: antibody-drug conjugate; BC: breast cancer; DAR: drug-to-antibody ratio; NR: not reported; TLR: toll-like receptor.

^a The ongoing trials include breast cancer only or in addition to other solid tumors.**Table 4**

Summary of the pivotal trials of ADC in breast cancer with main CNS exclusion criteria

Trial	CNS exclusions	ADC arm (N; n)	Comparator arm (N; n)	OS (ADC vs comparator arm) months ^a	PFS (ADC vs comparator arm) months ^a
TH3RESA [26]	Active or untreated CNS metastases or those whose CNS metastases were treated within 1 month of randomization	T-DM1 (N = 404; n = 44)	PCC (N = 198; n = 28)	22.7 vs 15.8 (HR 0.68; 95%CI 0.54–0.85)	6.2 vs 3.3 (HR 0.53; 95%CI 0.44–0.66)
EMILIA [25, 26,74]	Active or untreated CNS metastases or CNS metastases treated within 2 months of randomization	T-DM1 (N = 495; n = 45)	Capecitabine plus lapatinib (N = 496; n = 50)	29.9 vs 25.9 (HR 0.75; 95%CI 0.64–0.88)	9.6 vs 6.4 (HR 0.55; 95%CI 0.36–0.86)
DESTINY-Breast03 ⁵¹	Untreated or active CNS metastases	T-DXd (N = 261; n = 62)	T-DM1 (N = 263; n = 52)	NR vs NR (HR 0.55; 95%CI 0.36–0.86)	NR vs 6.8 (HR 0.28; 95%CI 0.22–0.37)
DESTINY-Breast04 ⁵⁹	Untreated or active CNS metastases	T-DXd (N = 373; n = 24)	PCC (N = 184; n = 8)	23.4 vs 16.8 (HR 0.64; 95%CI 0.49–0.84)	9.9 vs 5.1 (HR 0.50; 95%CI 0.40–0.63)

ADC: antibody-drug conjugate; CNS: central nervous system; N: Number of patients, n: patients with brain metastases; NA: not available; NR: not reached; OS: overall survival; PCC: physician choice of chemotherapy; PFS: progression-free survival.

**Not reported whether CNS toxicity occurred in the patients with brain metastases.

^a The OS and PFS reported in this table are the data of the overall population treated with ADC in each trial.

A166 targets HER2 via a monoclonal antibody similar to trastuzumab linked to duostatin-5 (MMAF derivative) via a stable protease cleavable linker. The phase I trial first-in-human enrolled 35 patients with different solid tumor types to evaluate the safety and efficacy of A166 [72]. Among 27 patients evaluable for efficacy, 7 had partial response and 9 stable disease (26 and 33%, respectively). Responses were seen only at the dose levels of 3.6 mg/kg and 4.8 mg/kg. The trial showed an ORR of 36% at efficacious dose levels and up to 100% in HER2+ patients regardless of histology (2 colorectal cancer, 1 BC and 1 non-small cell lung cancer). The treatment related adverse events include mainly ocular toxicities (80–83%) and decreased appetite

3. HER2-directed ADC and brain metastases

Considering the high prevalence of brain metastases (BMs) in patients with HER2 breast cancer, one of the main concerns is whether HER2-targeting ADCs would pass through the blood-brain barrier, despite their large size [73]. As such, the pivotal trials evaluating ADCs in HER2+ BC have excluded patients with untreated and/or unstable central nervous system metastases (Table 4). These trials mandated baseline brain imaging in neurologically asymptomatic patients and none of these trials stratified patients according to the BM status. The percentage of enrolled patients with BM was 12% (72 patients) in TH3RESA trial, 9.6% (95 patients) in EMILIA trial, 19.9% (398 patients)

in KAMILLA trial, 21.8% (114 patients) in DESTINY-Breast03 trial, and 5.6% (31 patients) in DESTINY-Breast04 trial; the MARIANNE trial did not report on the proportion of patients with BM [24,43,51,59,74–76].

The KAMILLA trial, which has enrolled the largest number of patients with BM, showed in this subgroup an overall response rate of 21%, PFS and OS of 5.5 (95%CI 5.3–5.6) and 18.9 months (95%CI 17.1–21.3), respectively [75]. In the small subgroup of patients with stable BM in TH3RESA trial, T-DM1 achieved better PFS (5.8 vs 2.9 months; HR 0.47, 95%CI 0.24–0.89) and yielded numerically longer OS (17.3 vs 12.6 months; HR 0.62, 95%CI 0.34–1.13) compared to physician's choice [23,24]. Similarly, the post hoc analysis of the EMILIA trial showed a statistically significant improvement in OS among patients treated with T-DM1 compared with capecitabine plus lapatinib (26.8 vs 12.9 months; HR 0.38, 95%CI 0.18–0.80, $p = 0.008$) [74]. Additionally, small studies have also reported meaningful responses in patients with BM treated with T-DM1 [77, 78].

The subgroup analysis of the phase II DESTINY-Breast01 trial has explored the activity of T-DXd among 24 HER2+ BC patients with a history of BM [79]. The efficacy outcomes of this subgroup were comparable to those in the total patient population with an ORR of 58.3% (vs 60.9%) and a median PFS of 18.1 months (vs 16.4 months). Among the 14 of 24 patients with information available on baseline BM diameter, the central nervous system response rate was 50%. The pattern of disease progression was similar in patients with and without BMs (33% [8 of 24 patients] vs 25% [40 of 160 patients]), however central nervous system progression may be more common in the patients with BMs (2 of 24 patients vs 2 of 160 patients) [79]. A small subgroup of patients with stable BM in DESTINY-Breast03 trial also showed promising activity for T-DXd in patients with BM [59]. In comparison to patients in the T-DM1 arm, those receiving T-DXd had longer PFS (15.0 vs 5.7 month; HR 0.38, 95%CI 0.23–0.64) [51]. The DESTINY-Breast04 trials did not report on the outcomes of patients with BM [59]. The phase II TUXEDO-1 trial included 15 patients with HER2+ BC and BM that were either newly diagnosed and untreated (6 patients) or had progressed after local therapy (9 patients) [80]. All patients were previously exposed to an anti-HER2-directed therapy including 9 patients having received T-DM1. Patients received treatment with T-DXd at 5.4 mg/kg every 3 weeks until disease progression or unacceptable toxicity. T-DXd yielded an intracranial ORR of 73.3%, a clinical benefit rate of 86.7%, median PFS of 14 months. The extra- and intracranial response rates were comparable (62.2% and 73.3%). Importantly, the quality of life and cognitive function were maintained and the safety profile did not report central nervous system-related adverse events [80]. The ongoing phase II DEBBRAH trial, is evaluating the efficacy of T-DXd in pretreated patients with HER2+ and HER2-low BC with stable, untreated, or progressing BMs and/or leptomeningeal carcinomatosis [81]. An analysis of the patients with HER2+ BC has shown an intracranial ORR of 46.2% (95%CI 19.2–74.9) on active BM. At data cutoff, all patients had intracranial progressive disease and one patient also had an extracranial progressive disease. The global quality-of-life was maintained without any improvement or degradation [81].

4. Perspectives

Clinically HER2+ BC has been considered a single tumor entity for a long time. To date, HER2 positivity is defined according to the initial HER2+ clinical trials of trastuzumab, either (1) by an intense and complete circumferential membrane staining in more than 10% of tumoral cells and/or (2) HER2 amplification using an ISH technique with a HER2/CEP17 ratio ≥ 2 and HER2 copy number ≥ 4 signals/cell [1]. Recently, the 3rd generation of HER2-targeting ADCs have put into question the categorical classification of HER2+ BC mainly with the results of DESTINY-Breast04 showing meaningful activity in patients with HER2-low expression [59]. Thus, the traditional HER2 testing assays (IHC and ISH) need to be accompanied by more sensitive methods that better quantify the levels of HER2 required for response to T-DXd or

other HER2-targeting ADCs [82].

Antibody-drug conjugates have definitely transformed the treatment landscape of HER2+ BC. The corresponding pivotal trials have shown a higher efficacy for ADC over standard treatment options however, primary and secondary resistances occur and tumors eventually progress. Resistance mechanisms may overcome HER2 signaling blockade, Fc-mediated immune response and payload-mediated cytotoxicity (Fig. 2). For T-DM1, resistance mechanisms with the strongest evidence relate to dysfunctional intracellular metabolism of the construct and subversion of DM1-mediated cytotoxicity [83]. Other resistance mechanisms involving alterations in ADC internalization, transit, drug catabolism and drug efflux were also reported in experimental models; insensitivity to HER2 signaling blockade is less supported [83]. T-DXd encloses a different linker-payload combination, presents a bystander effect and is extruded less efficiently by efflux transporters, which may account for its activity in patients that progressed during T-DM1 treatment [84]. However, additional resistance mechanisms might well arise and induce resistance to T-DXd. The translational studies within the DAISY trial have shown that primary resistance may be associated to a high prevalence of HER2 0 cells and to their spatial distribution or to ERBB2 hemizygous deletion. Secondary resistances involved a decrease in HER2 expression in two-third of the cases, although T-DXd uptake does not look reduced, and in some cases the occurrence of SLX4 mutation, which mediates resistance to the payload [85,85]. Understanding these resistance mechanisms can assist in treatment sequencing. For instance, cases with resistances due to payload target alterations would likely confer cross-resistance to another antibody linked to the same payload but not to the same antibody with a different payload. In cases with HER2 alterations, therapeutic sequencing with non-HER2 directed ADC linked to the same payload could achieve a therapeutic benefit. Moreover, understanding the resistance mechanisms will also guide ADC development and treatment combinations.

The new generation of ADCs can benefit from the advances in the antibody, linker and payload developments [86]. For instance, the trastuzumab component of HER2-targeting ADCs can be replaced by HER2 fragment antigen-binding fragments (Fabs) given their stability and higher internalization in cancer cells [87]. Bispecific antibodies can also be used in the ADC construct to facilitate the internalization of the ADC by targeting two different epitopes of HER2 or several HER antigens. ZW49 encloses a biparatopic antibody that recognizes the nonoverlapping epitopes in subdomains 2 and 4 of the HER2 ecto-domain, linked with site-specific conjugation to auristatin has shown promising activity in its phase I trial; noteworthy its intolerable toxicity at doses >0.3 mg/kg [88]. Another bispecific antibody, the HER2xPRLR bispecific ADC is composed of HER2xPRLR bsAb1 conjugated to DM1 via a noncleavable linker and has shown promising activity in breast cancer cells that coexpress HER2 and PRLR. Indeed, translational research has shown that HER2xPRLR bispecific ADC kills BC cells more effectively than HER2 ADC [89]. In addition, more potent payloads such as pyrrolobenzodiazepines, tubulysin, and immunomodulatory agents can be used. SBT6050 and BDC-1001 are two HER2-targeting immune targeting antibody conjugates that use Toll-like receptor 8 (TLR8) agonist payload to induce immune activation in HER2-expressing solid tumors [90–92]. T-PNU an ADC combining trastuzumab conjugated to a derivative of the highly potent anthracycline, PNU-159682, through a non-cleavable linker showed promising results in vivo on breast cancer cell lines resistant to T-DM1 and trastuzumab [93].

Several studies have evaluated ADC-based combinations with agents that are hypothesized to overcome the biological pathways implicated in ADC resistance. The addition of immune checkpoint inhibitors to ADC has shown synergistic activity in preclinical models with a marked increase in CTLA-4 and PD(L)-1 expression [94]. The phase II KATE2 trial randomized 330 HER2+ BC patients previously treated with trastuzumab plus a taxane to T-DM1 with or without atezolizumab, a PDL-1 inhibitor [95]. The addition of atezolizumab did not show a

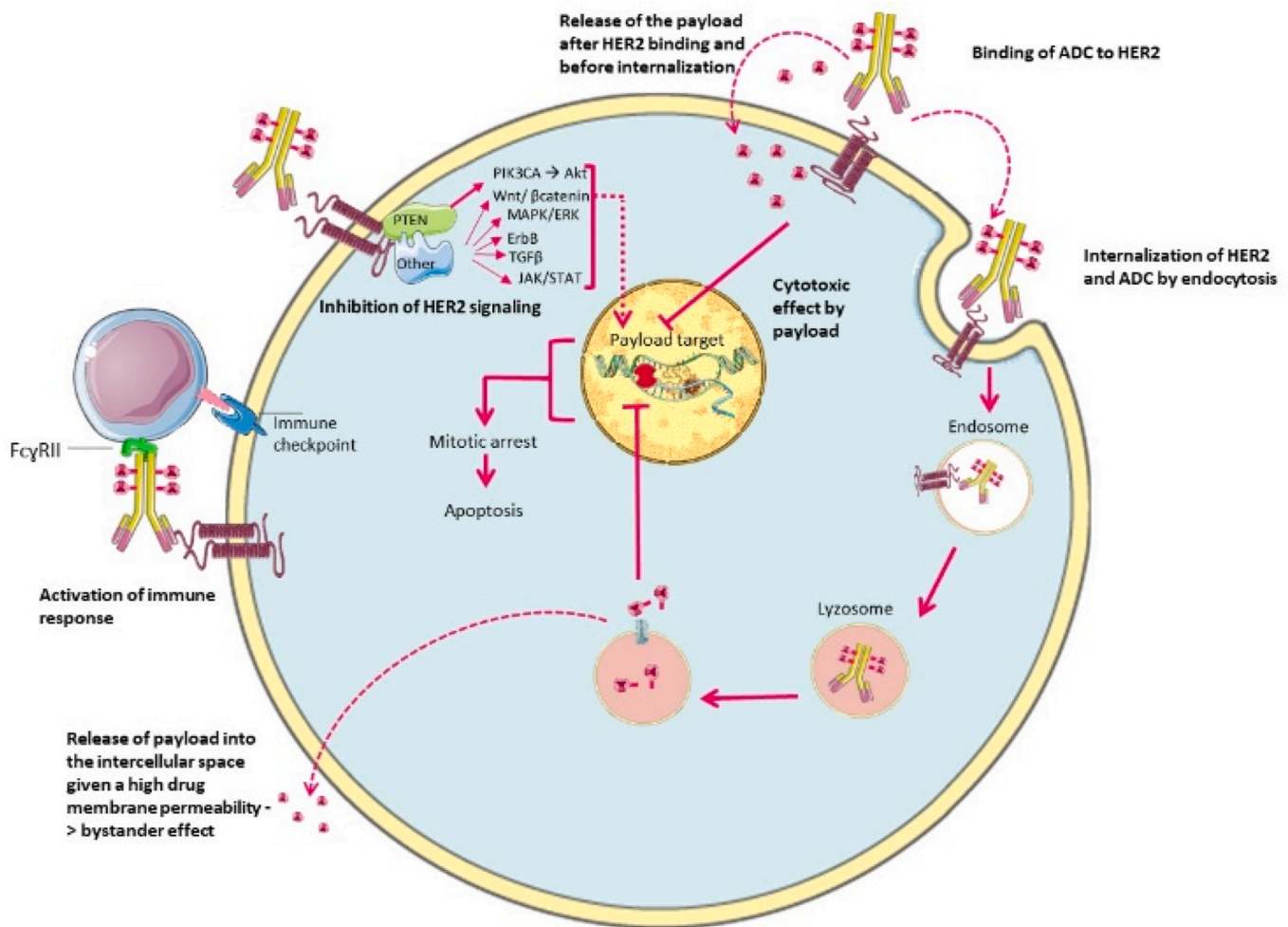


Fig. 1. Mechanisms of action of HER2-targeting ADC.

statistically significant improvement in median PFS (8.2 vs 6.8 months; HR 0.82, 95%CI 0.55–1.23, $p = 0.33$) and OS (not reached in the two arms; HR 0.74, 95%CI 0.42–1.30, $p =$ not reported). These outcomes remained not statistically significant in PDL-1 positive HER2+ BC (PFS HR 0.60; 95%CI 0.55–1.23 and OS, HR 0.55; 95%CI 0.22–1.38) [95,96]. The DS8201-A-U105 phase Ib trial evaluated the efficacy of nivolumab plus T-DXd among heavily pretreated patients with HER2+ and -low BC [97]. In the HER2+ cohort, the ORR was 65.6% and the median PFS 11.6 months; in the HER2-low cohort, the ORR was 50% and the median PFS of 37.5%. These findings were disappointingly in line with the aforementioned trials reporting on the efficacy of T-DXd monotherapy in HER2+ (ORR 60.9% and PFS 16.4 months) [98] and HER2-low BC (ORR 37.5% and PFS 6.7 months) [58]. The combination of T-DXd with another PD-1 inhibitor, pembrolizumab, is currently being evaluated in a phase Ib/II trial [99]. As pretreated patients with chemotherapy and HER2 blockade have a lower infiltration of cytotoxic T cells in metastatic sites [100], the phase Ib/II BEGONIA trial evaluated the efficacy and safety of durvalumab plus T-DXd in patients without previous therapy for stage IV BC. Eligible patients had HER2-low (IHC 2+/ISH-, IHC 1+/ISH-, or IHC 1+/ISH- untested) and hormone receptor-negative BC [101]. The initial results presented in ASCO 2021 meeting showed an activity regardless of PD-L1 expression and a promising efficacy with an ORR of 66.7%. The combination of ADC with other anticancer therapy is also being investigated. For instance, DESTINY-Breast08 (NCT04556773) is evaluating the combination of T-DXd to durvalumab plus paclitaxel, capivasertib, anastrozole, fulvestrant, capecitabine in patients with HER2-low BC. In a similar design, DESTINY-Breast07

(NCT04538742) is investigating the safety and efficacy of combining T-DXd to durvalumab, pertuzumab, paclitaxel, durvalumab plus paclitaxel, and tucatinib in HER2+ BC [102]. The phase II HER2CLIMB-04 trial (NCT04539938) is another ongoing study of particular interest that aims to evaluate the safety and antitumor activity of the combination T-DXd + tucatinib in pretreated patients with HER2+ BC, including those with active BM [103].

5. Conclusions

During the last decade, HER2-targeting ADCs have advanced dramatically and transformed the treatment landscape of breast cancer. The impact of ADCs on survival outcomes improved between the second and third generation of ADCs in HER2+ BC and showed unprecedented results in HER2-low BC. In the particular subgroup of HER2+ BC patients with BM, the reported findings on ADC among patients HER2+ BC and BM suggest that the presence of asymptomatic and/or treated BM does not attenuate the survival benefit of ADC in this population.

Many benefits have been achieved but multiple challenges still impede the success of ADCs. For instance, the efficacy of ADCs, namely T-DXd, was dampened in HER2-low compared to HER2+ BC. This suggests that the efficacy of HER2-targeting ADCs is contingent to the HER2 expression level and requires a more comprehensive understanding of the resistance mechanisms. The preliminary results of the DAISY trial have shed light on some aspects of these mechanisms and is expected to unveil more details in the near future. However, a reconsideration of the HER2 expression evaluation is probably warranted given that the

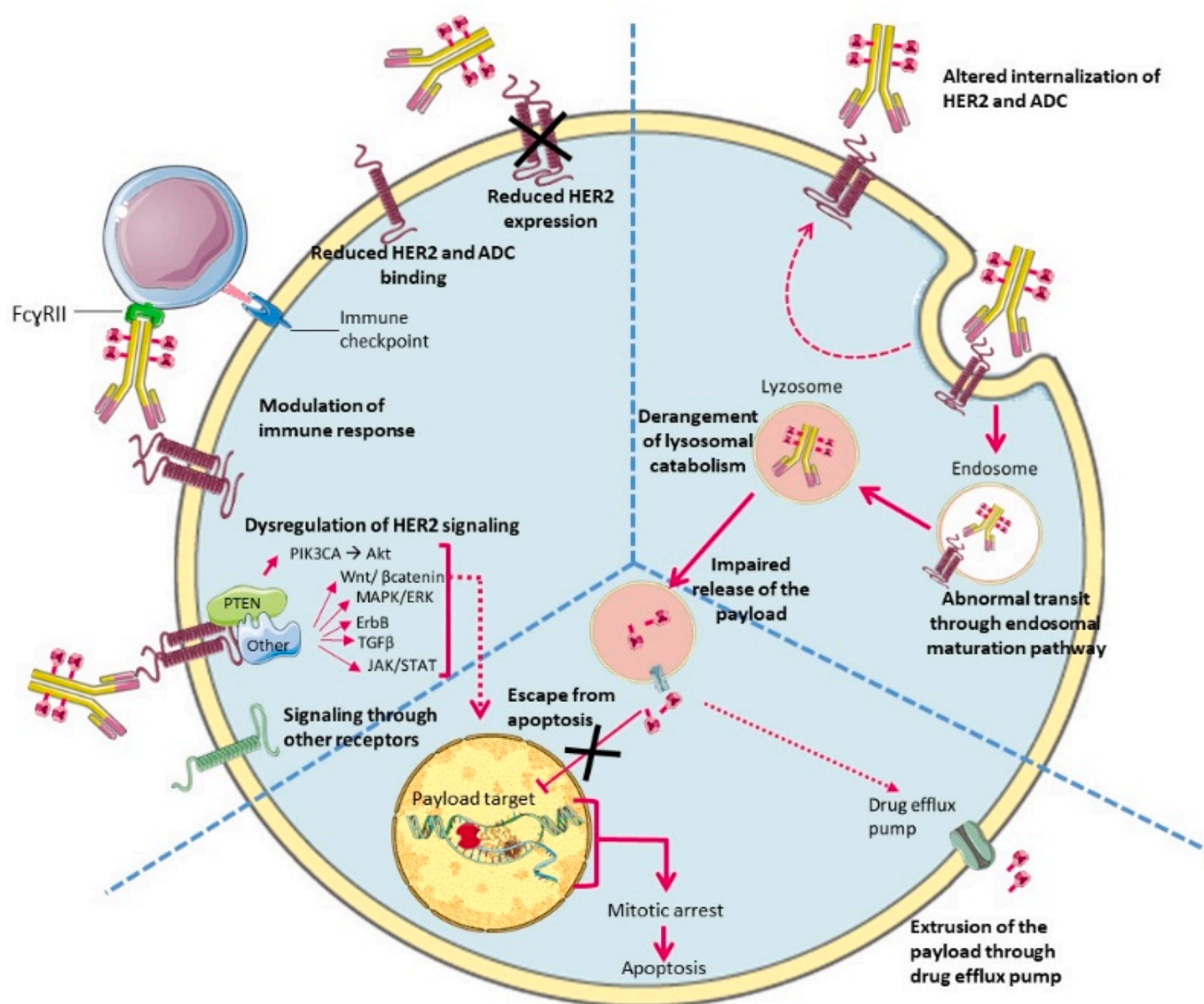


Fig. 2. Mechanisms of resistance of HER2-targeted antibody-drug conjugates.

spectrum of HER2 expression seems more of a continuum than a categorical variable. The current challenges for HER2-targeting ADCs in BC is to develop new treatment combinations and construct components that would overcome primary and secondary resistances to ADCs. Unfortunately, the initial trials combining HER2-targeting ADCs in BC were disappointing probably due to the selection of tumors in an immune-escape status; some ongoing trials are evaluating the combination in earlier lines of therapy and will be reported in the near future. The advances in the technologies used to engineer the different components of the ADC construct are moving forward and have led to certain upgrades in the ADC designs that are being tested in phase I trials. The rapid progress that has led to the present era of ADC is expected to continue and to rapidly overcome the current obstacles.

Author contributions

Paper concept and design: BP and ER; Review of the literature: ER, LR and BP; Data analysis and interpretation: ER, LR and BP; Manuscript editing and critical review: All authors; Approval of the final draft: All authors

Data availability statement

Not applicable

Declaration of competing interest

The other authors did not declare any conflict of interest.

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