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Review

Recent advances of antibody-drug conjugates in treating breast cancer with different HER2 status

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***Abstract*:** Despite the availability of multiple treatment options for breast cancer, challenges such as adverse events, drug resistance, and disease progression persist for patients. The identification of human epidermal growth factor receptor 2 (HER2) as an oncogenic driver in a subset of breast cancers, alongside the development of HER2-targeted therapies, has significantly improved the prognosis of HER2-amplified breast cancers. However, therapeutic options remain limited for HER2-overexpressing or HER2-negative breast cancers. In response to this gap, antibody-drug conjugates (ADCs) have emerged as a promising approach. ADCs combine the specificity of monoclonal antibodies with the cytotoxic effects of chemotherapy, which allows for the targeted delivery of a cytotoxic payload to cancer cells. ADCs have been used as adjuvant chemotherapeutic treatments and salvage therapies across various breast cancer subtypes, which have greatly improved the prognosis of breast cancer patients. Numerous ongoing clinical trials seek to optimize dosing strategies and identify patient populations that would benefit most from ADCs. This review presents an updated and comprehensive overview of emerging investigational ADCs for treating breast cancer patients with various HER2 subtypes. These ADCs are spearheading a new era in targeted cancer therapy, promising to innovate treatment paradigms for both HER2-positive and HER2-low breast cancers.

Keywords: antibody-drug conjugates, breast cancer, human epidermal receptor 2

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Introduction

Nowadays, breast cancer accounts for a growing fraction of malignant neoplastic diseases worldwide. According to the latest statistics from the National Cancer Center of China in 2022, the incidence rate of female breast cancer in all cancers ranks first among female cancers, while the mortality rate of all cancer types ranks fifth among women.[1] These highlighted the urgent need to implement more preventive measures and treatment options to confront this escalating health challenge. Breast cancers are classified into four molecular subtypes on the presence of hormone receptors (estrogen receptor (ER) and progesterone receptor (PR)), human epidermal growth factor receptor 2 (HER2), and Ki-67 levels in the tumor: luminal A (ER+, HER2−, high PR, and low Ki-67), luminal B (ER+, HER2−, low PR, and high Ki-67), triple-negative (ER−, PR−, HER2−), and HER2-positive (HER2+, any ER, any PR, and any Ki-67 level).[2]

HER2 is a receptor tyrosine protein kinase that belongs to the epidermal growth factor (EGF) receptor family, which also comprises epidermal growth factor receptor (EGFR), HER3 and HER4.[3] HER2 amplification drives tumorigenesis by multiple mechanisms, with approximately 15% of cancer cases exhibiting HER2 gene amplification. However, in approximately 50% of breast cancers, HER2 expression levels are absent or low.[3] Among these cases, triple-negative breast cancers (TNBCs) are associated with the worst prognosis.[2] Clinically approved methods for

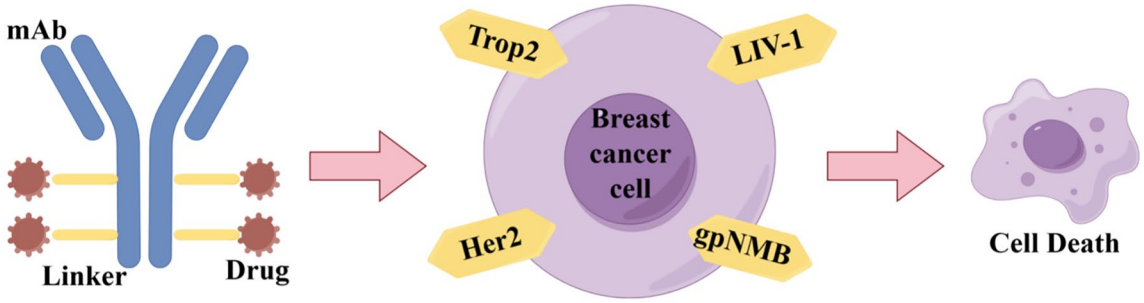

Figure 1. Cartoon representing the mechanism of action of ADCs in breast cancer.
Source: Figdraw.
ADC, antibody-drug conjugates.

detecting HER-2 expression include immunohistochemistry (IHC), in situ hybridization (ISH), and fluorescence in situ hybridization (FISH) assays,[4] which classify HER2 expression into three levels: HER-2 negative (IHC0), HER-2 low expression (IHC1+ or IHC2+/ISH−), and HER-2 positive (IHC2+/ISH+ or IHC3+).

Nowadays, the three subtypes of breast cancer are approached with tailored treatment strategies. Among these, HER2-positive breast cancer primarily benefits from HER2-targeted therapy as the optimal treatment option.[5] These treatment regimens include various antibody-drug conjugates (ADCs), such as T-DM1, DS-8201, and RC-48. Conversely, breast cancer patients with HER2 negative or low expression typically undergo common treatments such as surgery and radiotherapy, as specific therapeutic modalities for these subtypes are still lacking. However, ADCs have also provided therapeutic prospects to patients with breast cancer exhibiting low HER2 expression or HER2 negativity. This review focuses on providing a comprehensive overview of treatment options, supported by both preclinical and clinical evidence, for ADC drugs in breast cancer across various HER2 expression levels.

Antibody-drug conjugates

ADCs are composed of a monoclonal antibody, a chemical linker, and a cytotoxic payload covalently bound.[2] This structure provides ADC with the advantage of binding to specific targets and exerts cytotoxic effects. The stability of the linker connecting the monoclonal antibody and the cytotoxic drug is crucial for maintaining the integrity of ADCs.[3] ADCs leverage the specificity of antibodies to deliver potent cytotoxicity to tumor cells while protecting healthy cells from toxic agents. (Figure 1) The fundamental mechanism of ADCs involves the specific binding of monoclonal antibodies to tumor antigens on the cell surface, followed by the internalization of ADCs. This internalization releases the active cytotoxic agent into the cell's cytoplasm, leading to tumor cell death.[6,7] (Figure 2) Upon being released into the cell, the payload can potentially diffuse into neighboring cells, including those not targeted by the antigen, which contributes to non-antigen-dependent cytotoxicity in adjacent cells, a phenomenon known as the "bystander effect."[6,7] The intensity of this effect is influenced by factors such as the extent of ADC internalization, the type of linker, and the properties of the payload.[6] Chemical linkers are employed to attach the payload to a monoclonal antibody, preventing the premature release of cytotoxic agents.[7,8] These linkers can be classified as cleavable or non-cleavable, depending on whether they are cleaved within the cell. Cleavable linkers can be broken under certain conditions to enhance the "bystander effect" by allowing the drug to permeate neighboring cells and eliminate antibody-negative cells.[7,8] Conversely, non-cleavable linkers are degraded by proteasomes within the target cell.[3] The drug-antibody ratio (DAR), indicating the amount of payload attached to a monoclonal antibody, is a crucial parameter for evaluating the effectiveness and potential toxicity of ADCs.[6,8]

ADCs have been utilized across a range of cancer types. For instance, ADC drugs such as sacituzumab govitecan, trastuzumab, and enfortumab-vedotin have shown promise in treating metastatic prostate cancer.[9] Trophoblast cell-surface antigen 2 (TROP2)-targeting ADCs are widely used in clinical due to TROP2 expression in both squamous and non-squamous lung adenocarcinomas.[10]

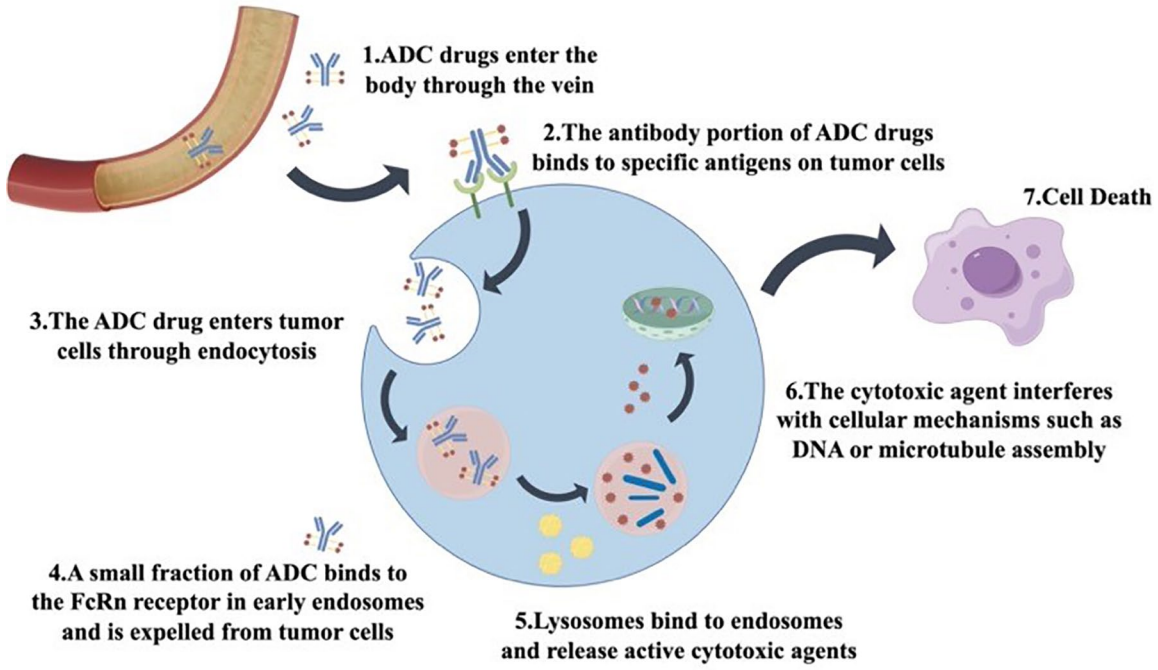

Figure 2. The step-by-step mechanism of ADC in vivo.
Source: Figdraw.
ADC, antibody-drug conjugates.

Enfortumab-vedotin, an ADC, has notably enhanced treatment outcomes for patients with metastatic urothelial cancer who have undergone a median of third-line therapy.[11] In breast cancer, anti-HER2 ADCs like T-DM1 and Trastuzumab deruxtecan (T-DXd) are highly effective, playing crucial roles in both preoperative and postoperative treatment across various breast cancer types.[2,11]

Now many ADC drugs for breast cancer with different HER2 expressions have completed some clinical trials and been put into clinical use (Table 1); There are also many ADC drugs undergoing related trials (Table 2). This review specifically summarizes the clinical trials of ADC drugs for breast cancer with different HER2 expressions.

Preclinical and clinical trials of ADC drugs for the treatment of breast cancer with different HER2 statuses

Therapeutic strategies utilizing ADCs for HER2-negative (IHC0) breast cancer

Sacituzumab govitecan (IMMU-132). IMMU-132 is an ADC that targets trophoblast cell-surface antigen 2 (Trop2). It combines a humanized anti-Trop2 monoclonal antibody with the active irinotecan metabolite, SN-38, via a hydrolyzable CL 2A junction.[12] Trop2 is frequently overexpressed in various cancers, including TNBC. In April 2020, the Food and Drug Administration (FDA) approved sacituzumab govitecan for the treatment of patients with unresectable locally advanced or metastatic TNBC who have received two or more prior systemic therapies.[13] Results from an multicenter open-label randomized phase III clinical trial (NCT02574455) demonstrated that sacituzumab govitecan achieved a median overall survival (OS) of 11.8 months (95% confidence interval (CI), 10.5–13.8) in TNBC (triple negative is defined as $<1\%$ expression for ER and PR and negative for HER2 by in situ hybridization) patients, compared to 6.9 months (95% CI, 5.9–7.7) with chemotherapy. The risk ratio was 0.51 (95% CI, 0.41–0.62), indicating a more favorable prognosis with the sacituzumab govitecan compared to the TPC (eribulin, capecitabine, gemcitabine, or vincristine) monotherapy.[14]

Glembatumumab vedotin (CDX-011). Glembatumumab vedotin (CDX-011) is an ADC consisting of a fully human monoclonal antibody targeting the tumor-specific antigen glycoprotein NMB (gpNMB) and the potent

Table 1. Completed clinical trials on breast cancer of different HER2 types.

Drug name	Target	Research object	Experimental staging and ID	Endpoint
T-DXd	HER2	HER2 + BC (breast cancer)	NCT03523585, DB-02	PFS
		HER2 + MBC after targeted therapy	NCT03248492, DB-01	PFS
		HER2-low expression MBC	III (DB-04)	PFS, OS
		HER2 + MBC	NCT03529110, DB-03	PFS
		HER2 + MBC	IIIb/IV (NCT04739761\DB-12)	
T-DM1	HER2	HER2 + BC	NCT00679211, NCT00509769, NCT01419197, NCT00943670, NCT00875979	PFS, OS
		HER2 + MBC combined with neratinib		ORR
		HER2 + MBC combined with Docetaxel/ pertuzumab	Ib/IIa (NCT009348560)	PFS, ORR
		HER2 + MBC combined with Cdk 4/6 inhibitor Ribociclib	Ib/II (NCT02657343)	PFS
		Early BC of neoadjuvant HER2+ after anthracycline chemotherapy	NCT01196052	Safe dose
IMMU-132	Trop-2	TNBC	III (NCT02574455)	OS
SGN-LIV 1A	LIV-1	Metastatic TNBC	I (SGNLVA-001)	ORR, DCR
CDX-011	gpNMB	TNBC		ORR, PFS
SYD985	HER2	HER2+/low expression MBC	I	ORR, PFS
		HER2 + MBC	TULIP	PFS, OS
		HER2 + MBC	I	ORR
		HER2+/HER2 low expression BC combined with paclitaxel	ISPY-P1.01	
ARX788	HER2	HER2 + BC after anti-HER2 treatment	I (ACE-Breast-01)	DCR, PFS
XMT-1522	HER2	HER2 + MBC	Ib (NCT02952729)	DCR
RC-48 ADC	HER2	HER2 low expression BC after MTA treatment		PFS
		HER2 + MBC after treatment	Ib/II (NCT03052634)	objective response rate, DCR, CBR
		HER2 + MBC		
A166	HER2	HER2 + MBC	I (NCT03602079)	objective response rate, DCR
ALT-P7	HER2	HER2 + MBC after treatment with trastuzumab	I (NCT03281824)	
PF-06804103	HER2	HER2+/HER2– solid tumor	I (NCT032847230)	Safe dose
FS-1502	HER2	HER2 + MBC	I (NCT03944499)	Safe dose

CBR, clinical benefit rate; DCR, disease control rate; gpNMB, glycoprotein NMB; HER2, human epidermal growth factor receptor 2; MBC, metastatic breast cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; T-DXd, trastuzumab deruxtecan; TNBC, triple-negative breast cancer.

Table 2. Ongoing or recruiting or discontinued clinical trials on breast cancer of different HER2 types.

Drug name	Target	Research object	Experimental staging and ID	Study status
T-DXd	HER2	HR+/HER2 low expression BC	III (DB-06)	Active
		HER2–MBC	DB-15 (NCT05950945)	Active
		HER2 low expression MBC	NCT04556773	Active
		Recurrent after chemotherapy/HER2 low expression MBC	DB-respond HER2-low Europe	Recruiting
		HER2 low expression MBC	TUXeD0-4	Not yet recruiting
		Metastatic TNBC	II	Not yet recruiting
		HER2+ MBC combined with other anticancer drugs	NCT04538742, DB-07	Active
		HER2+ MBC combined with Patuzumab	III (DB-09)	Active
		HER2 low expression MBC combined EZH1/2 inhibitor Valemetostat	Ib (NCT05633979)	Recruiting
		HER2+ BC combined with THP/ddAC-THP	III (NCT05113215, DB-11)	Recruiting
		Residual invasive HER2+ BC after neoadjuvant therapy	NCT04622319, DB-05	Active
		New adjuvant therapy of HER2 low expression BC combined with Anastrozole	II	Recruiting
T-DM1	HER2	Elderly breast cancer	II (NCT03587740)	Active
		HER2+ MBC combined with DZd1516	I (NCT04509596)	Active
		HER2+ MBC combined with Atezolizumab	NCT04740918	Active
		HER2+ MBC combined with Vinorelbine	I/II (NCT02658084)	Terminated
		HER2+ BC combined with Poziotinib	NCT03429101	Terminated
		Suspicious HER2 expression in BC with neoadjuvant therapy	NCT02725541	Terminated
DS-1062a	Trop-2	HR+/HER2– BC	I (NCT03401385)	Active
		HR+/HER2– BC	III (NCT05104866)	Active
		TNBC	I/II (NCT05460273)	Active
		TNBC with PD-L1+	III (NCT06103864)	Recruiting
		TNBC or HR low expression/HER2– BC	III (NCT06112379)	Recruiting
		metastatic TNBC	II (NCT05866432)	Recruiting
		metastatic TNBC Combined with PD-1/PD-L1 inhibitors	NCT05374512	Active
		TNBC after neoadjuvant therapy	NCT056229585	Recruiting

(Continued)

Table 2. (Continued)

Drug name	Target	Research object	Experimental staging and ID	Study status
SYD985	HER2	HER2+ MBC	III(NCT03262935)	Active
		HER2+ BC combined with Niraparib, Adriamycin, and Cyclophosphamide	NCT04235101	Active
ARX788	HER2	HER2 low expression	NCT05018676	Recruiting
		HER2 low expression MBC	II (NCT06224673)	Not yet recruiting
		HER2+ MBC	II/III (CTR 20201708)	Recruiting
		HER2+ MBC after T-DXd treatment	II (NCT04829604, NCT05018702)	Recruiting
		HER2+ BC for neoadjuvant therapy combined with Pyrotinib maleate and TCbHP	II/III (NCT05426486)	Recruiting
RC-48ADC	HER2	HER2+ BC	II (NCT05134519)	Not yet recruiting
		HER2+ MBC	II	Active
		HER2+ advanced liver MBC	III (NCT02500380)	Active
		HER2 low expression MBC	NCT05831878, NCT04400695	Recruiting
		HR+/HER2 low expression MBC	NCT05904964	Recruiting
		HER2+ MBC	II (NCT05331326)	Recruiting
		HER2+ BC combined with Pyrotinib	III (NCT06278870)	Recruiting
		HR+/HER2 low expression MBC	II (NCT06105008)	Not yet recruiting
		HR-/HER2 low expression MBC	II (NCT06000033)	Not yet recruiting
		Neoadjuvant treatment of HER2+ BC combined with Pertuzumab/Ripalimab	II (NCT06178159)	Recruiting
		Neoadjuvant treatment of HER2 low expression BC combined with AK105	NCT05726175	Not yet recruiting
MRG002	MMAE	HER2+ MBC	II (NCT047442135)	Recruiting
		HER2+ MBC	NCT04924699	Recruiting
		HER2+ MBC after previous Trastuzumab/TKIs treatment	II (NCT05263869)	Recruiting
A166	HER2	HER2+ MBC	II (NCT05346328)	Not yet recruiting
BDC-1001	HER2	HER2+ MBC after treatment with Trastuzumab combined with Patuzumab	II (NCT05954143)	Recruiting
		HER2+ MBC combined with Nivolumab	I/II (NCT04278144)	Recruiting
ZW49	HER2	HER2+ MBC	I (NCT03821233)	Active
GQ1001	HER2	HER2+ BC	I (NCT04450732)	Recruiting
		HER2+ MBC combined with Pyrotinib	Ib/II (NCT05575804)	Recruiting
FS-1502	HER2	HER2+ MBC	III (NCT05757048)	Recruiting

CBR, clinical benefit rate; HER2, human epidermal growth factor receptor 2; MBC, metastatic breast cancer; ORR, objective response rate; T-DXd, trastuzumab deruxtecan; TKI, tyrosine kinase inhibitor; TNBC, triple-negative breast cancer.

microtubule inhibitor monomethyl auristatin E (MMAE).[15] In an unplanned analysis of the CR 011-CLN-20 study, an overall response rate (ORR) of 18% (5/28) was observed in TNBC patients treated with CDX-011, while no remissions were seen in 11 TNBC patients treated with investigator's choice (IC). Among TNBC patients with high tumor expression of pNMB, the ORR was 40% (4/10) in the CDX-011 group and 0% (0/6) in the IC group. These results suggest that CDX-011 might improve progression-free survival (PFS) and OS outcomes in TNBC patients. However, in a randomized multicenter pivotal study evaluating the efficacy and safety of CDX-011 in patients with advanced gpNMB-overexpressing TNBC, no significant differences were found in median PFS (2.8 months in the capecitabine group vs 2.9 months in the CDX-011 group, *p*-value 0.761) or OS (8.7 months in the capecitabine group vs 8.9 months in the CDX-011 group, *p*-value 0.726), when compared to capecitabine treatment. All TNBC patients in this trial were negative for HER2 expression.[15]

Ladiratuzumab vedotin (SGN-LIV 1A). SGN-LIV 1A consists of three key components: a monoclonal antibody targeting LIV-1, a protease-cleavable liker, and a microtubule-disrupting agent with monomethyl auristatin E (MMAE) as the payload.[16] In ongoing early-stage clinical trials for breast cancer patients, the phase I dose-escalation SGNLVA-001 trial demonstrated an ORR of 32% and a disease control rate (DCR) of 64% in individuals with metastatic TNBC treated with SGN-LIV 1A. The median PFS was 11.3 weeks. Additionally, in patients with second-line refractory TNBC, SGN-LIV 1A achieved an ORR of 28% at a 1.25 mg/kg dose, indicating promising therapeutic efficacy.[16] All TNBC patients in this trial were negative for HER2 expression.

Datopotamab deruxtecan (DS-1062a). DS-1062a, an ADC, utilizes a monoclonal antibody targeting humanized immunoglobulin G1 (IgG1), coupled with a tumor-specific, stable tetratricopeptide-based cleavable linker. This ADC delivers a potent cytotoxic topoisomerase I (Topo-I) inhibitor payload to TROP2-expressing tumor cells. DS-1062a's payload features a short systemic half-life, minimizing systemic toxicity and inducing bystander tumor effects upon payload release.[17] Preclinical studies demonstrated DS-1062a's efficacy in inducing DNA damage and apoptosis in TROP2-high tumor models, including breast cancer. Moreover, evidence indicates that Topo-I inhibitors, such as DS-1062a, can enhance the efficacy of immune checkpoint inhibitors. Preclinical studies suggest that the enhancement might be achieved by boosting the antitumor immune response triggered by the delivered DXd payload. Therefore, combining DS-1062a with immunotherapy shows promise as an avenue for further investigation.[18]

A phase I clinical trial, TROPION-PanTumor 01 (NCT 03401385), assesses the efficacy and safety of DS-1062a in patients with metastatic hormone receptor-positive and HER2-negative breast cancer, showing a favorable safety profile and remarkable antitumor activity in heavily pretreated patients. Among 41 patients, DS-1062a achieved an objective remission rate of 27%, including 11 confirmed partial remissions, and an DCR of 85%. These results highlight the promising efficacy of DS-1062a in breast cancer, particularly in individuals with a history of extensive prior treatments.[17,19]

The main results of the TROPION-Breast01 were announced at the ESMO conference in 2024. Patients were randomly assigned to Datopotamab deruxtecan (Dato-DXd) ($n = 365$) or Investigator's Choice of Chemotherapy (ICC) ($n = 367$). Dato-DXd significantly reduced the risk of progression or death versus ICC (PFS by Blinded Independent Central Review (BICR) hazard ratio, 0.63 (95% CI, 0.52–0.76); $p < 0.0001$). Consistent PFS benefit was observed across subgroups. Although OS data were not mature, a trend favoring Dato-DXd was observed (HR, 0.84 (95% CI, 0.62–1.14)). The rate of grade $\geqslant 3$ treatment-related adverse events (TRAEs) with Dato-DXd was lower than ICC (20.8% vs 44.7%). The most common TRAEs (any grade; grade $\geqslant 3$) were nausea (51.1%; 1.4%) and stomatitis (50%; 6.4%) with Dato-DXd and neutropenia (grouped term, 42.5%; 30.8%) with ICC. Results support Dato-DXd as a novel treatment option for patients with inoperable/metastatic HR+/HER2- breast cancer who have received one to two previous lines of chemotherapy in this setting.[20]

The latest progress of I-SPY 2.2 (all patients' HER2 expression were negative in the experimental results) was reported at the ASCO conference in 2024, with a focus on the efficacy of Dato-DXd monotherapy or combination therapy with Durva neoadjuvant therapy. Among the 103 patients in the Dato-DXd monotherapy cohort, 33 (32%)

underwent early surgery; among the 106 patients in the Dato-DXd + Durva cohort, 35 (33%) underwent early surgery. The "assessment criteria" for whether neoadjuvant therapy can undergo surgery are based on baseline, magnetic resonance imaging at weeks 3 and 12, and biopsy at week 12 to determine whether predict residual cancer burden (preRCB) has been achieved. In the neoadjuvant therapy of Dato-DXd + Durva, the rate of pathologic complete response (pCR) of immune+ patients reached 43% (20/40), while the modeled pCR rate was as high as 65%, both exceeding the threshold (40%). In addition, the pCR rate and model pCR rate of TNBC patients also reached 33% and 44%, respectively. In terms of drug treatment, Dato-DXd, as a new generation of ADC, has shown positive efficacy and safety in the field of advanced HR+/HER2− and TNBC. The results of this study demonstrate that in the neoadjuvant therapy of Dato-DXd monotherapy or combination immunotherapy, the overall pCR rate is higher in response predictive subtype (RPS) of immune+ patients. Furthermore, the model pCR of Dato-DXd + Durva in immune+ patients is as high as 65%, and the efficacy data of this new adjuvant therapy without chemotherapy is very encouraging. It can be said that Dato-DXd + Durva has reached the "graduation threshold," which is expected to bring new adjuvant treatment benefits to immune+ breast cancer patients.[21]

At present, many trials (NCT05104866; Tropion-Breast02, NCT05374512; NCT05460273; NCT05866432) are under way to evaluate the efficacy of DS-1062a combined chemotherapy plus PD-1/PD-L1 inhibitor in the treatment of locally advanced or metastatic HER2 negative breast cancer patients.[17,22] In addition, relevant experiments (NCT06103864; Tropion-Breast03, NCT05629585; NCT06112379) on the combination of DS-1062a and different chemotherapy drugs are currently being recruited.

Trastuzumab deruxtecan (DS-8201a). T-DXd is an innovative ADC targeting HER2, featuring a tetrapeptide-based junction that enhances stability in the patient's bloodstream and minimizes systemic toxicity. This junction is engineered to be cleaved by lysosomal enzymes, releasing the payload and allowing it to penetrate neighboring cells through the target cell membrane, which potentially enables bystander killing and enhances clinical efficacy. T-DXd has been approved by the FDA for the treatment of metastatic HER2-positive or low-level HER2 breast cancer.[23] A multinational open-label clinical trial DESTINY-Breast 15 (NCT05950945) is ongoing to evaluate the benefits of T-DXd in patients with inoperable and/or advanced HER2-negative breast cancer. Samples collected in this study were confirmed HER2 IHC 1+ or IHC 2+/ISH− (HER2-low) status or HER2 IHC 0 status. Results from the trial are not yet available.

Therapeutic strategies utilizing ADCs for HER2 low-expression (IHC1+ or IHC2+/ISH−) breast cancer

Trastuzumab deruxtecan (DS-8201a). In the DESTINY-Breast 04 trial, a phase III randomized, open-label study focusing on HER2 low-expressing metastatic breast cancer (MBC), T-DXd significantly improved median PFS (10.1 months for T-DXd vs 5.4 months for chemotherapy group, $p < 0.0001$) and OS (23.9 months for T-DXd vs 17.5 months for chemotherapy, $p = 0.003$) compared to physician's choice of chemotherapy (TPC). These results led to the FDA approval of T-DXd for HER2 low-expressing MBC.[8,24,25] Other trials (DESTINY-Breast 06, NCT04781909; DESTINY-Breast 08, NCT04556773) on T-DXd compared with traditional chemotherapy or combined chemotherapy for MBC with low HER2 expression are ongoing. Some ongoing studies (NCT05633979; DESTINY-Breast Respond HER2-low Europe; TUXeDO-4) on T-DXd combined with chemotherapy or immunotherapy are recruiting participants.

Trastuzumab duocarmazine (SYD985). Trastuzumab duocarmazine (SYD985) is a HER2-targeting ADC based on trastuzumab, incorporating a cleavable linker and duocarmazine (vc-seco-duba) payload. This novel payload, DUBA, induces DNA alkylation and damage in both dividing and non-dividing cells. Upon linker cleavage by proteases, duba is released into the tumor microenvironment, promoting a bystander effect that extends its activity against tumor cells with low HER2 expression.[25] Indications in preclinical trials, SYD985 exhibited superior activity compared to T-DM1 in two HER2 low-expression cell lines (MDA-MB-175-VII and ZR-75-1). These findings were further validated in vivo, where SYD985 demonstrated efficacy in HER2 low-expressing breast cancer xenograft models, whereas T-DM1 lost its effectiveness. Furthermore, the bystander-killing effect of SYD985 was observed in co-culture experiments involving HER2-expressing cells (SKBR3 and MDA-MB-175-VII) and HER2-negative cells

(NCI-H520).[3] These preclinical data further supported the efficacy of SYD in HER2 low-expressing breast cancer.

Preliminary data from a phase I extension cohort study of SYD985 in heavily treated MBC patients, including those with HER2 positive and HER2 low-expression, showed an ORR of 33% and a median PFS of 9.4 months. In HER2 low-expressing MBC, including hormone receptor-positive ($N = 32$) and triple-negative ($N = 17$) subtypes, ORRs were 27% and 40%, respectively. Overall, SYD985 demonstrates safety, and efficacy in this setting.[26] These findings indicate a notable improvement in ORR with SYD985 for patients with refractory HER2-negative breast cancer, despite the small sample size and multiple prior treatment lines.[7]

Disitamab vedotin (RC-48 ADC). RC-48 utilizes MMAE as its payload to inhibit microtubule protein polymerization, thereby exerting significant mitotic inhibition.[27] It targets distinct epitopes of the HER2 receptor and exhibits greater molecular affinity for HER2 compared to trastuzumab.[25] Preclinical and clinical studies have shown RC-48's efficacy in both HER2 positive and HER2 low-expressing advanced breast cancer.[27] In a clinical case report, two patients with HER2 low-expressing breast cancer, previously treated with MTAs were re-treated with RC-48. Both achieved partial remission with PFS durations of 13.5 and 9 months, respectively.[27] This underscores RC-48's potential in subsequent treatment in HER2 low-expressing breast cancer. Several ongoing clinical trials (NCT05831878;NCT04400695;NCT05904964) are investigating RC-48-ADC in advanced breast cancer cases with low HER2 expression. Several other trials (NCT05726175; NCT06105008; NCT06000033) are under way to evaluate the adjuvant or neoadjuvant treatment of RC-48 combined chemotherapy or immunotherapy, likes pembrolizumab (AK105) or trenbolizumab, for breast cancer with low HER2 expression.

ARX788. ARX788 is a next-generation, site-specific anti-HER2 ADC, comprising a humanized HER2-targeted monoclonal antibody linked with the cytotoxic microtubule protein inhibitor Amberstatin (AS269), utilizing Ambrx's unnatural amino acid doping technology platform. It is characterized by homogeneity and high stability, aiming to improve therapeutic outcomes while minimizing off-target toxicity through enhanced drug delivery efficiency to tumor cells, thus widening the therapeutic window and optimizing targeted efficacy.[28] Preclinical studies focusing on HER2 low-expression and T-DM1-resistant breast cancer demonstrated that ARX788 has the superior in vitro and in vivo efficacy compared to T-DM1, particularly in tumors with low HER2 expression. ARX788 induced tumor regression even in a HER2 IHC1+/FISH-negative model, aligning with in vitro findings, which highlighted its potential effectiveness in patients with low HER2 expression.[28] Currently, a clinical trial (NCT05018676) is enrolling patients with HER2 low-expression breast cancer to evaluate ARX788. Furthermore, a phase II open-label study (NCT06224673) is in the pre-production stage to investigate ARX788 for treating locally advanced unresectable/MBC with HER2 low expression.

MRG002. MRG002 is a recombinant humanized monoclonal antibody (MAB802) conjugated with MMAE via a cleavable valine-citrulline linker. MAB802 is a recombinant humanized monoclonal antibody, which is structurally similar to trastuzumab but with higher fucosylation and reduces antibody-dependent cellular cytotoxicity (ADCC) activity to minimize the impact on immune cells. MRG002 has an average DAR of 1.38, indicating the amount of MMAE attached to each antibody molecule.[29] A multicenter, non-randomized, open-label phase II clinical trial (NCT04742153) is currently enrolling patients to assess the efficacy and safety of MRG002 in HER2-low expressing locally advanced or MBC.

MEDI4276. MEDI4276 is an ADC featuring a double complementary-site tetravalent monoclonal antibody targeting two distinct HER2 epitopes. Similar to pertuzumab, it inhibits recombinant modulin-b1-stimulated HER2/HER3 receptor phosphorylation in cancer cells. Constructed from 39S, MEDI4276 links the scFv of trastuzumab to the amino-terminal end of the 39SIgG1 heavy chain, creating a structure with two antigen-binding units on each arm that interacts with two different epitopes on the extracellular domain of HER2.[30] In vitro assay demonstrated MEDI4276's strong antitumor activity in HER2 low-expression cell lines (MCF7-GTU and ZR-75-1), whereas T-DM1 showed no efficacy. MEDI4276 also induced tumor regression in multiple HER2 low-overexpressing xenograft models.[3]

XMT-1522. XMT-1522 is an innovative anti-HER2 ADC featuring a human IgG1 anti-HER2

monoclonal antibody (HT-19) that targets HER2 structural domain IV at a distinct binding site from trastuzumab, avoiding competition for HER2 binding.[31] In cellular assays, XMT-1522 demonstrated superior tumor growth inhibition compared to T-DM1 in a HER2 low-expressing breast cancer xenograft model. Additionally, combining XMT-1522 with an anti-PD-1 monoclonal antibody enhanced antitumor efficacy, leading to complete responses in vivo. Sequential administration of XMT-1522 followed by the checkpoint inhibitor further increased complete remission frequency.[3]

Therapeutic strategies utilizing ADCs for HER2-positive (IHC3+ or IHC2+/ISH+) breast cancer

Trastuzumab emtansine (T-DM1). Trastuzumab emtansine (T-DM1) is a medetomidine derivative comprising an antibody conjugated to the cytotoxic agent DM1, linked to trastuzumab through a stabilized thioether junction. It has been approved by FDA for second-line use in HER2-positive advanced breast cancer patients previously treated with trastuzumab.[32] In a phase III study (NCT00829166), researchers randomly assigned patients with HER2-positive advanced breast cancer, who had previously been treated with trastuzumab and a taxane, to T-DM1 or lapatinib plus capecitabine. Among 991 randomly assigned patients, Trastuzumab Emtansine (T-DM1) significantly improved median PFS (9.6 months for T-DM1 vs 6.4 months for lapatinib plus capecitabine, $p < 0.001$) and median OS (30.9 months for T-DM1 vs 25.1 months for lapatinib plus capecitabine, $p < 0.001$). The ORR was higher with T-DM1 (43.6% vs 30.8% with lapatinib plus capecitabine; $p < 0.001$); results for all additional secondary end points favored T-DM1. These results all indicate that the ADC T-DM1, as compared with lapatinib plus capecitabine, significantly improved PFS and OS among patients with HER2-positive MBC who had previously received trastuzumab and a taxane. The benefit was observed regardless of the line of therapy in patients with metastatic disease and was seen in patients with a disease-free interval of less than 6 months after completion of trastuzumab-based therapy in the adjuvant or neoadjuvant setting.[33] The outcomes of numerous other trials (NCT00679211, NCT00509769, NCT01419197, NCT00943670, etc.) have shown improved PFS and OS rates in HER2-positive breast cancer patients treated with T-DM1 compared to conventional chemotherapy.[34]

A safety trial (NCT01196052) for T-DM1 neoadjuvant therapy for HER2-positive early breast cancer post-anthracycline-based chemotherapy indicated that 82.4% of 148 patients completed the 1-year program of HER2-directed therapy. Grade 3 and 4 adverse events occurred in 38.5% and 2.7% of patients, respectively. About 95% of patients receiving T-DM1 combination radiotherapy completed ⩾95% of planned radiotherapy with ⩽5 days' delay. Only four patients (2.7%) experienced an asymptomatic LVEF decline, leading to T-DM1 discontinuation in one case. These findings suggest that the T-DM1 regimen after anthracycline-based chemotherapy is feasible and well-tolerated in HER2-positive patients, supporting the need for a phase III clinical trial in further.[35]

In a phase Ib/II dose-escalation study combining T-DM1 with neratinib for metastatic HER2-positive breast cancer treatment, 19 patients demonstrated evaluable remissions. The best responses included 3 cases of complete remission lasting 364, 510, and 969+ days, 9 cases of partial remission, 2 cases of stable disease, and 5 cases of disease progression.[32] These findings suggest that the combination of T-DM1 with neratinib is more effective than either drug alone for treating metastatic HER2-positive breast cancer. The other three similar trials (NCT00934856; NCT00875979; NCT02657343) have the same results with regard to the efficacy of T-DM1 combined with chemotherapy or immunotherapy in patients with metastatic/advanced breast cancer.[36,37]

Other trials investigating combination therapy with T-DM1 in HER2-positive breast cancer patients have faced challenges. A phase I/II clinical trial evaluating vincristine alongside T-DM1 in HER2-positive MBC (NCT02658084) was halted due to low enrollment and toxicity concerns. Similarly, a study on T-DM1 neoadjuvant therapy for HER2-suspect breast cancer (NCT02725541) was discontinued due to financial constraints. Several other clinical trials involving T-DM1 have also been halted for comparable reasons. Although many trials have been stopped for various reasons, there are still many ongoing trials (NCT03587740; NCT04509596; NCT04740918) of T-DM1 in the treatment of HER2-positive breast cancer.

Trastuzumab deruxtecan (DS-8201a). The DESTINY-Breast 01 trial (NCT03248492) investigated the efficacy of T-DXd in HER2-positive

advanced breast cancer patients previously treated with HER2-targeted therapies. Among 184 patients, 112 responded (60.9%; 95% CI, 53.4–68.0). The median follow-up was 11.1 months (range, 0.7–19.9). The median duration of remission was 14.8 months (95% CI, 13.8–16.9), and PFS was 16.4 months (95% CI, 12.7–not reached). This study highlighted T-DXd's durable antitumor activity with manageable side effects in HER2-positive MBC.[38] The DESTINY-Breast 02 trial (NCT03523585) and the DESTINY-Breast 03 trial (NCT03529110) all showed the same results.[39] The results were announced at the 2024 European Society for Medical Oncology (ESMO) Annual Meeting, which of the phase IIIb/IV DESTINY-Breast12 (NCT04739761) study investigated T-DXd in patients with HER2+ mBC. In this study, patients (stable/active brain metastases (BMs) ($n=263$) and no BMs ($n=241$)) treated with one or more prior anti-HER2-based regimens received T-DXd (5.4 mg/kg). The experimental results indicate that in the BMs cohort, 12-month PFS was 61.6% (95% CI: 54.9–67.6), and 12-month CNS PFS was 58.9% (95% CI: 51.9–65.3). In the non-BMs cohort, ORR was 62.7% (95% CI: 56.5–68.8). Grade 3 or higher adverse events occurred in 51% (BMs cohort) and 49% (non-BMs cohort) of patients. Investigator reported interstitial lung disease/pneumonitis occurred in 16% (grade ⩾3: 3%) of patients with BMs and 13% (grade ⩾3: 1%) of patients without BMs. These data show substantial and durable overall and intracranial activity for T-DXd, supporting its use in previously treated patients with HER2+ mBC irrespective of stable/active baseline BMs.[40]

Additionally, DESTINY-Breast05 (NCT04622319) and DESTINY-Breast11 (NCT05113251) are two ongoing trials of T-DXd neoadjuvant therapy for HER2-positive breast cancer patients. Several trials (DESTINY-Breast07 trial, NCT04538742; DESTINY-Breast09 trial, NCT04784715) on the efficacy of DS-8201a combined with various chemotherapy drugs in the treatment of HER-positive breast cancer patients are ongoing.

Trastuzumab duocarmazine (SYD985). In cellular assays, SYD985 demonstrated similar potency to T-DM1 in HER2-positive breast cancer cell lines (SKBR3) and trastuzumab-resistant breast cancer cell lines (UACC-893). Moreover, in a phase I study of SYD985, which assessed its toxicity profile and anticancer activity in comparing HER2-positive and HER2 low-expressing breast cancers, 16 out of 48 patients with HER2-positive MBC achieved objective remission (33%, 95% CI, 20.4–48.4). This indicates that SYD985 exhibits comparable antitumor effects on HER2-positive breast cancer as T-DM1.[3,7] A randomized phase III clinical trial is currently underway (NCT03262935), comparing the outcomes of patients with locally advanced or metastatic HER2-positive breast cancer after pretreatment with SYD985 and physician-recommended T-DM1. Results of this ongoing phase III clinical trial will be announced in the future.[26]

A multicenter, open-label, randomized clinical trial (TULIP) compared the efficacy and safety of SYD985 with physician's choice of chemotherapy (including lapatinib, Capecitabine, Trastuzumab, Vinorelbine, and Eribulin) in patients with HER2-positive unresectable locally advanced or MBC. The analysis demonstrated a significant improvement in median PFS (SYD985 vs conventional chemotherapy group: 7.0 vs 4.9, p-value 0.002). However, there is no significant difference in median OS between the SYD985 group (21.0 months) and the conventional chemotherapy group (19.5 months), with a p-value of 0.236. This highlights that SYD985 offers a longer PFS compared to clinically conventional chemotherapy regimens.[25] Other ongoing trials (ISPY-P1.01; NCT04235101) are investigating the combination of T-DM1 with paclitaxel or PARP inhibitor Niraparib, with doxorubicin and cyclophosphamide, for HER2-positive breast cancer.[7]

PF-06804103. PF-06804103 is an anti-HER2 ADC drug that links the auristatin microtubule inhibitor Aur 0101 to an engineered cysteine in the anti-HER2 antibody backbone using a valine-citrulline junction. Preclinical studies indicate PF-06804103's superior effectiveness compared to T-DM1 in breast cancer models, and its ability to overcome T-DM1 resistance.[41] A phase I dose-escalation study (NCT03284723) to evaluate the safety and tolerability of PF-06804103 in patients with HER2-positive and -negative solid tumors has been completed, and the trial consisted of two aspects, dose-escalation (P1) and dose extension (P2). In P1, patients with HER2+ breast cancer were treated with PF-06804103 at doses of 0.15–5.0 mg/kg (Q3W). In P2, patients with HER2+ or HER2-low (IHC1+ or IHC2+/ISH−) breast cancer were treated with PF-06804103 at doses of 3.0 or 4.0 mg/kg Q3W. A total of nine patients enrolled in the P1 group were treated with the

PF-06804103 regimen, of which four patients (two each in the 3.0 and 4.0 mg/kg groups) experienced dose-limiting toxicity, mostly grade 3. The safety and efficacy results showed a dose-response relationship. In the P1 group, 2 patients (2/79, 2.5%) achieved complete remission, and 21 patients (21/79, 26.6%) had partial remission. In the P2 group, HER2-positive patients had a higher ORR compared with patients with hormone receptor-positive and HER2-low breast cancer (3.0 mg/kg: 16.7% [2/12) vs 10.0% [1/10); 4.0 mg/kg: 47.4% [9/19) vs 27.3% [3/11)). This study demonstrated that PF-06804103 has significant antitumor activity and a dose-related safety profile on HER2-positive breast cancer patients.[42]

Disitamab vedotin (RC-48 ADC). In an open-label, multicenter phase Ib/II pilot study of RC-48 (NCT03052634) in pretreated patients with metastatic HER2-positive breast cancer, findings from the phase Ib segment showed promising results. Among the 30 enrolled patients (6 IHC 2+/ISH+, 24 IHC 3+) in the 1.5 and 2.0 mg/kg cohorts, 19 patients (63%) had received prior treatment with a HER2-targeted agent, and 16 patients (53%) had undergone at least 3 prior chemotherapy regimens in a metastatic setting. The study exhibited an objective response rate of 37% (11 partial responses), a DCR of 97% (29/30), and a clinical benefit rate of 47% (14/30). The objective response rates for the 1.5 and 2.0 mg/kg cohorts were 27% and 47%, respectively.[3] These findings suggest a higher clinical benefit with the 2.0 mg/kg dose of RC-48. These outcomes were consistent with results from a separate trial assessing the effectiveness of RC-48 in HER2-positive MBC.[7] In addition, a number of RC-48 trials (NCT03500380, NCT06168227, NCT03052634, NCT05134519, and NCT05331326) among HER- positive local advanced or MBC patients are ongoing. Two more trials (NCT06278870, NCT06178159) are recruiting patients to evaluate RC-48 combined with pilotinib or combined with pertuzumab and/or tropizumab for treating HER2-positive breast cancer.

ARX788. A phase I study (ACE-Breast-01) evaluating the safety, pharmacokinetics, and antitumor activity of ARX788 in patients with HER2-positive MBC who had received prior anti-HER2 therapy showed promising antitumor activity. The study reported an objective response rate of 65.5%, a DCR of 100%, and a median PFS of 17.02 months (95% CI, 10.09–none). On December 18, 2020, the FDA approved ARX788 as monotherapy for the treatment of advanced or metastatic HER2-positive breast cancer.[8,43] Several trials (NCT04829604, NCT05018702, CTR 20201708, and ZMC-ARX788-211) investigating ARX788 in HER2-positive breast cancer are currently recruiting participants.[43] Moreover, a phase II–III neoadjuvant study (NCT05426486) is enrolling patients to investigate ARX788 in combination with pilotinib maleate compared to TCBHP (trastuzumab, pertuzumab, doxorubicin, and carboplatin) in HER2-positive breast cancer patients.

XMT-1522. A study showcasing the significant anticancer potential of XMT-1522 against HER2-positive T-DM1-resistant breast cancer utilized both in vitro and in vivo models of T-DM1-sensitive and T-DM1-resistant breast and gastric cancers. The findings revealed that XMT-1522 effectively suppressed the growth of T-DM1-resistant cells in cellular assays, which was corroborated by in vivo experiments where XMT-1522 eradicated breast cancer xenografts. Moreover, a phase Ib trial (NCT02952729) evaluating dose escalation and amplification of XMT-1522 in patients with advanced HER2-expressing breast cancer and other advanced tumors has concluded. Preliminary results indicate an overall DCR of 83%, observed in 5 out of 6 patients, with a DCR of 25% (3 out of 12) reported in patients treated with doses below 16 mg/m.[27]

A166. A166 is an ADC targeting HER2, composed of the cytotoxic agent Duostatin-5, an anti-microtubule drug, conjugated to a humanized anti-HER2 antibody via a stable, protease-cleavable valine-guanine linker. This linker remains stable in the bloodstream and is selectively cleaved by lysosomal histone proteases, which are upregulated in cancer cells. This design prevents the premature release of toxin molecules outside the tumor cells, thereby reducing systemic toxicity.[44]

In a phase I trial of A166 in advanced HER2-expressing solid tumors (NCT03602079), 58 patients with HER2-positive breast cancer were treated with A166. The trial reported an objective response rate of 70.7% (41/58; 95% CI, 57.3–81.9) and a DCR of 81.0% (47/58; 95% CI, 68.6–90.1). All responding patients had received prior treatment with trastuzumab ± pertuzumab. Among them, 39 out of 55 patients (70.9%) had received at

least one prior anti-HER2 tyrosine kinase inhibitor (TKI). These results underscore the potent antitumor activity of A166, particularly in heavily pretreated HER2-positive MBC, with an objective response rate of 73.9% and a median PFS exceeding 12 months.[44] This study lays a solid foundation for further investigations of A166. Another phase II trial (NCT05346328) evaluating the efficacy of A166 injection in patients with HER2-positive refractory unresectable locally advanced or MBC is currently in the pre-production stage.

ALT-P7 (HM2-MMAE). ALT-P7, a novel HER2-targeting ADC, was developed by coupling a trastuzumab variant with MMAE. Upon binding to HER2 and internalizing, ALT-P7 releases MMAE which binds to microtubulin, inhibiting its polymerization and causing G2/M phase arrest and apoptosis.[45] A phase I open-label, dose-escalation study (NCT03281824) was conducted to assess the safety, tolerability, and pharmacokinetics of ALT-P7 treatment in patients with HER2-positive MBC who have previously received trastuzumab therapy. This study has been completed, but specific results are currently unavailable.[3]

MRG002. Results from a preclinical trial evaluating MRG002, a novel HER2-targeting ADC drug, indicated a favorable toxicity profile and significant antitumor activity of MRG002 against HER2-positive solid tumors.[29] A clinical trial (NCT04924699) is currently recruiting participants to investigate the use of MRG002 for treating HER2-positive non-locally resectable advanced or MBC. Additionally, another ongoing open-label, multicenter, single-arm phase II study (NCT05263869) aims to assess the efficacy and safety of MRG002 in patients with advanced HER2-positive breast cancer who have previously received treatment with trastuzumab and TKIs.

BDC-1001. BDC-1001 combines a biosimilar of trastuzumab with a TLR7/8 agonist using a non-cleavable linker. It activates antigen-presenting cells while maintaining antibody-mediated effector functions like ADCC. Preclinical research has revealed that BDC-1001 exerts a robust and enduring immune-mediated antitumor effect in xenograft models resistant to HER2-targeted therapy.[46] Another trial (NCT05954143) is currently recruiting patients to evaluate BDC-1001 as monotherapy or in combination with pertuzumab for HER2-positive MBC patients previously received T-DXd. A phase I/II study (NCT04278144) evaluates the utility of BDC-1001 as single-agent or in combination with Nivolumab for HER2-positive breast cancer.

Zanidatamab zovodotin (ZW49). ZW49 comprises a humanized IgG1 bispecific antibody, Zanidatamab, linked to a novel auristatin derivative through a protease-cleavable linker.[47] Preclinical investigations have indicated that ZW49 is internalized more rapidly by HER2-expressing cells compared to monospecific trastuzumab ADC. Moreover, it demonstrates potent antitumor activity in HER2-positive breast cancer cell lines and patient-derived xenograft models.[47] A phase I clinical trial (NCT03821233) is currently underway to evaluate ZW49 in patients with locally advanced or metastatic HER2-expressing cancers, including breast cancer.

GQ1001. GQ1001 represents an innovative ADC crafted by GeneQuantum Healthcare, designed to target HER2-positive solid tumors. It comprises a humanized anti-HER2 antibody coupled with DM1.[48] A pioneering, multicenter, phase I clinical trial with an open-label approach is underway to assess intravenous GQ1001 in adult patients with advanced HER2-positive solid tumors, including breast cancer (NCT04450732). Additionally, a phase Ib/II study is enrolling participants to investigate the efficacy of GQ1001 in combination with pyrotinib for HER2-positive MBC (NCT05575804).

FS-1502. FS-1502 is an innovative ADC targeting HER2, comprising a humanized anti-HER2 antibody, a β-glucuronidase cleavable junction, and the microtubule inhibitor monomethyl auristatin-F, connected through site-specific coupling with a DAR of 2. Both in vitro and in vivo studies have shown FS-1502's superior efficacy over T-DM1 in a HER2-positive breast cancer model. Initial findings suggest FS-1502's favorable tolerability and promising antitumor activity against HER2-expressing cancers.[49]

Preliminary findings from a multicenter, open-label, single-arm phase I clinical trial assessing the dose-expansion phase of FS-1502 for locally advanced or metastatic HER2-positive breast cancer (NCT03944499), demonstrated clinically significant efficacy of FS-1502 at doses of 2.3 mg/kg and higher.[49] A multicenter, open-label, randomized, controlled phase III clinical trial is currently underway to compare the efficacy and safety of FS-1502 versus T-DM1 in patients

suffering from HER2-positive unresectable locally advanced or MBC (NCT05755048). Recruitment for this study is ongoing.

MEDI4276. In vitro assay demonstrated that MEDI 4276 is over 10 times more potent than T-DM1 in the HER2-overexpressing cell line SKBR-3. In addition, MEDI 4276 showed efficacy in the T-DM1-resistant HER2-positive JIMT-1 cell line. An in vivo model illustrated that treatment with MEDI 4276 led to complete remission in all treated animals, with animals remaining tumor-free for 120 days post-treatment cessation. In contrast, T-DM1 only resulted in tumor arrest, with rapid tumor regrowth observed after cessation of treatment.[3]

Prospects and challenges in the development of ADCs

Prospects for ADCs development

Currently, the standard treatment for HER2+ breast cancer patients involves a combination of pertuzumab and trastuzumab. Pertuzumab binds to the HER2 receptor, inducing apoptosis, and inhibiting tumor growth. Trastuzumab enhances this effect by targeting different sites on the HER2 receptor. However, pertuzumab is associated with more cardiotoxic side effects. Patients with HER2- or HER2 low expression are less responsive to this targeted therapy. ADC drugs, such as T-DM1, offer a solution by combining HER2-targeting agents with cytotoxins, which are delivered directly to tumor cells via the HER2 receptor. The cytotoxin in T-DM1 damages tumor cell DNA, leading to apoptosis and providing a unique therapeutic approach for patients resistant to other medications.

Additionally, T-DXd and SYD985 exhibit a notable "bystander effect," offering an alternative treatment for some patients with HER2- or HER2-overexpressing breast cancers when targeted therapies are either unavailable or ineffective. Currently, ADC drugs are categorized into three generations. First-generation ADC drugs (e.g., Gitolizumab) are primarily utilized in acute myeloid leukemia treatment. These drugs often encounter severe toxic reactions in clinical settings, highlighting the need for improved toxicity control and targeting. Second-generation ADC drugs (e.g., T-DM1 and vibritumomab) exhibit enhanced targeting and better toxicity control compared to first-generation ADCs. However, prolonged use can lead to significant drug resistance and adverse effects such as liver injury and neurotoxicity. Third-generation ADCs (e.g., venetoclaximab and gosatuzumab) demonstrate further advancements in targeting, toxicity control, and efficacy, showing promise in specific cancers like TNBC. Nevertheless, their complexity and high production costs pose challenges for manufacturing and widespread clinical adoption.

Challenges and issues in the development of ADCs

Despite the promising potential of ADCs in cancer therapy, their research, development, and clinical application still encounter significant hurdles.

The presence of drug resistance and strategies for its management. Despite promising initial results of various ADC drug clinical trials, long-term treatments have revealed emerging drug resistance reactions. Given the complex structure of ADCs, resistance may be related to changes in antigen expression, ADC processing, and the chemotherapy payload.[50]

There are many related drug resistance targets, including human EGFR family (HER2, EGFR, HER3), Trophoblast Cell-Surface Antigen 2, Cluster of differentiation target antigen, Nectin-4 (a type I transmembrane cell adhesion molecule belonging to the Nectin family, which promotes the proliferation, differentiation, migration, and invasion of tumor cells by activating the PI3K/AKT pathway), B7H3 (an important member of the immune checkpoint family, which has a co-inhibitory effect, allowing tumors to evade immune responses and inhibiting NK cells-mediated cytotoxicity), TF (a transmembrane glycoprotein with procoagulant activity).[51]

Taking HER2 as an example, many researchers have found that downregulation or loss of target antigen expression on tumor cells is a major factor leading to the inability of ADCs to recognize and bind to target cells.[52] Based on retrospective research data, it was found that the efficacy of T-DM1 decreased after the combination therapy of trastuzumab/pertuzumab.[51] The SePHER study further revealed that compared to late-stage patients receiving only first-line treatment with trastuzumab, first-line treatment with trastuzumab combined with pertuzumab (a dual-targeted therapy) downregulated HER2 expression

on tumor cells and may mediate resistance to second-line HER2-ADC (T-DM1) (mPFS: 10.0 vs 6.0 m, $p = 0.03$).[53] In summary, the expression status of target antigens is significantly correlated with drug resistance in ADC-targeted therapy. When optimizing treatment strategies, the impact of antigen heterogeneity on mediating ADC resistance should also be considered.[51]

ADC drugs enter cells through endocytosis, while lysosomes effectively degrade ADC drugs to release their effects. Therefore defects in the endocytic pathway can prevent ADCs from entering cells, while impaired lysosomal function or reduced lysosomal protein hydrolysis activity can prevent effective degradation of ADCs.[54]

Tumor resistance to the payload itself can also lead to the development of ADCs resistance. The relevant experiments first discovered the role of parallel genomic changes in mediating ADC (such as SG) resistance mechanisms in antibodies and payload targets through RNA and whole exome sequencing of patient samples before and after treatment.[55]

In addition, like many other cancer chemotherapy drugs, continuous exposure of tumor cells to ADC can exert selective pressure, leading to the acquisition of drug-resistant mutations in the genome. For example, treatment with T-DM1 can lead to loss of phosphatase and tensin homolog (PTEN) and activating mutations in the gene encoding the PI3K catalytic subunit (PIK3CA), resulting in structural activation of the PI3K/AKT pathway and thus generating resistance.[51]

Researchers are continually exploring novel approaches to counter ADC drug resistance, such as expanding the range of drug targets and combining therapies like immunotherapy to address resistance and enhance efficacy. For instance, in breast cancer patients resistant to trastuzumab, T-DM1 employs a monoclonal antibody to bind to the HER2 receptor, inhibit its signaling pathway, and deliver a cytotoxin directly to tumor cells, effectively increasing toxin concentration and effectively killing HER2+ cells. Preclinical trials also indicate the efficacy of SYD985 and BDC-1001 in treating trastuzumab-resistant breast cancer. Similarly, the sequential administration of ARX788, MEDI4276, and XMT-1522 demonstrates clinical benefits when T-DM1 resistance arises, with ARX788 being effective against T-DXd-resistant breast cancer as well. This approach, termed "ADC sequential therapy," involves the sequential use of different ADC drugs.

Sequential therapy using ADC drugs (ADC after ADC) is a topic of intense clinical discussion. While this strategy appears promising, caution is necessary to prevent potential cross-resistance issues between ADC drugs related to their targets/antibodies and drug delivery mechanisms. Therefore, ADC drugs with alternative targets/antibodies or drug delivery mechanisms should be chosen for sequential use. Furthermore, determining the optimal sequencing of HER2 ADCs and Trop-2 ADCs among currently available ADCs is a pressing issue that requires resolution in the ongoing development of ADC drugs. Concerning HER2-positive MBC, current evidence on the optimal ADC-sequencing is primarily about T-DXd, which demonstrated therapeutic value when used post-T-DM1. Conversely, data are limited about the reverse sequence. Similarly, in HER2-negative MBC, recent studies evaluated the sequential use of Sacituzumab Govitecan and T-DXd, which was associated with poor responses.[56]

Integrating combination therapy strategies alongside sequential therapy can enhance the efficacy of ADC drugs. One promising approach involves combining ADC drugs with TKIs. TKIs, as small molecules targeting drugs capable of penetrating the blood-brain barrier, hold promise for breast cancer patients with BM. Studies have shown that combining T-DM1 with small-molecule TKIs not only improves efficacy but also exhibits therapeutic activity against intracranial tumors. Determining optimal combination doses is crucial for the safe implementation of ADC and TKI combination therapy in clinical settings. In summary, rational ADC combination therapy strategies are actively pursued by scientists. Numerous studies on ADC combination therapy are ongoing, with the expectation that more combination programs will enhance efficacy and prognosis for a broader range of patients in the future.[57]

In regard to ADC drug combinations with chemotherapy, an increasing amount of preclinical and clinical data demonstrates varying degrees of success, offering valuable insights to steer future drug development efforts. Stronger evidence supports the antitumor activity of ADC and chemotherapy combinations, but their advancement will

likely require significant optimization of ADC characteristics and careful selection of tumor types and accompanying chemotherapeutic agents to ensure tolerability.[58]

Furthermore, the cytotoxic payload of ADC drugs can synergize with immunotherapy by releasing tumor antigens, thereby heightening the sensitivity of immunotherapy and potentially enhancing antitumor effects.[58,59] This synergy results from mechanisms such as inducing immunogenic cell death, promoting dendritic cell maturation, boosting T-lymphocyte infiltration, and reinforcing immune memory and expression of immunomodulatory proteins. The integration of immunotherapy with ADC drugs is emerging in clinical practice. Both preclinical data and initial clinical findings showcase augmented antitumor activity through this combination strategy.[60–63] However, combination therapies involving ADC drugs with TKIs, immunologic agents, and chemotherapeutic drugs still warrant further exploration and development in the future.

Adverse effects of ADCs and their management. Although ADC drugs can offer the tumor-targeting effect of "high selectivity + potent killing" with high efficiency and low toxicity, their safety management is of paramount importance. Common side effects of ADC drugs include cytotoxic side effects (myelosuppression, hepatotoxicity, nephrotoxicity, etc.), allergic reactions, immune reactions, neurological side effects, digestive system side effects, and cardiovascular system side effects. The different ADC drugs may show different side effects in different patients, immune response, neurological side effects, digestive system side effects, and cardiovascular system side effects. The severity of these side effects can vary among different ADC drugs and patients, for example, TDM1 is associated with a relatively mild incidence of nausea, vomiting, and diarrhea.

These side effects typically arise from multiple mechanisms. Myelosuppression, liver, and kidney damage are primarily due to the cytotoxicity of ADC drugs, affecting not only the targeted tumor cells but also surrounding normal tissue cells, leading to toxicity. Furthermore, the metabolism of these cytotoxic substances in the body can generate active metabolites that contribute to liver and kidney damage. Immune and allergic reactions are primarily triggered by the antibody component of ADC drugs, which activates the body's immune response and impedes the effective targeting of tumor cells, thereby impacting therapeutic outcomes. The varied side effects in other systems result from the combined cytotoxic effects and the antibody component's ability to induce apoptosis and alter intracellular signaling pathways in tumor cells.

At present, effective management of these side effects involves preventive strategies, continuous monitoring during treatment, and personalized treatment approaches. Prior to treatment, a comprehensive assessment of the patient's health is crucial to mitigate high-risk factors, allowing for the adjustment of drug regimens and dosages accordingly. During treatment, close monitoring of hematological parameters and imaging studies enables early detection and management of adverse reactions. Prophylactic administration of anti-allergic drugs may be considered for patients susceptible to allergic reactions. Personalized treatment plans are implemented based on individual-specific conditions. Particular attention is given to managing interstitial pneumonitis, a serious adverse reaction characterized by chest tightness and shortness of breath. Prompt medical intervention and timely CT imaging are crucial for such cases. However, subtle symptoms like chest tightness might be overlooked, underscoring the need for heightened clinical monitoring. Ensuring drug safety is critical in the selection process, emphasizing patient-centric management and long-term follow-up to ensure the safe administration of ADCs. In summary, by adopting meticulous safety measures and management strategies, the clinical efficacy of ADCs can be maximized while minimizing adverse effects.

Summary

ADCs are potent anticancer agents combining monoclonal antibody specificity with chemotherapeutic potency. ADC targeting HER2 exhibited high DARs and bystander-killing effects, even in HER2 low-expressing tumors, revolutionizing breast cancer management across HER2 subtypes. A burgeoning array of ADCs, such as IMMU-132, SGN-LIV 1A, and SYD985, are advancing through preclinical and early clinical phases, showing encouraging outcomes in breast cancer treatment. While all ADCs exhibited different levels of hematologic and hepatic toxicity, ongoing trials will provide deeper insights into their impact on treatment intensity and compliance. Despite encountering challenges such as adverse effects, drug

resistance, production costs, and other hurdles, advancements in research instill confidence in their future development.

Declarations

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Not applicable.

Consent for publication
Not applicable.

Author contributions
Yue Qiu: Data curation; Formal analysis; Writing – original draft.

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Linlin Lu: Project administration; Supervision.

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