Breast Cancer: Targets and Therapy

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REVIEW

Advances in Antibody-Drug Conjugates in the Treatment of HER2-Positive Breast Cancer

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Abstract: Although targeted drugs improved the therapeutic effect of HER2-positive breast cancer, the long-term prognosis was still poor. In this regard, the research and development of antibody-drug conjugates (ADCs) came into being and made a lot of progress. ADCs had the characteristics of both chemotherapeutic agents and targeted agents by combining chemotherapeutic agents and targeted agents through a linker. It not only had a strong anti-tumor effect on HER2-positive breast cancer, but also had certain anti-tumor effects on HER2-low and even HER2-negative patients. In addition, the clinical researches of ADCs combined with immune checkpoint inhibitors (ICIs) therapy had also made a great breakthrough. This review aimed to summarize the clinical progress of ADCs, in particular the two drugs approved by the US Food and Drug Administration (FDA) for HER2-positive metastatic breast cancer as well as to summarize the current status of ADCs in combination with ICIs.

Keywords: breast cancer, HER2-positive, antibody-drug conjugates, immunotherapy, advances

Introduction

Breast cancer (BC) had become the most common malignant tumor in women worldwide, accounting for approximately 11.7% of new cancer cases in 2020, which seriously endangered women's health and became the main cause of female death.[1,2] HER2 (human epidermal growth factor receptor-2) is an important biomarker of breast cancer and a member of the ERBB family of tyrosine kinase receptors. It is a transmembrane protein with tyrosine protein kinase activity, which consists of three parts: extracellular ligand binding domain, single strand transmembrane domain and intracellular protein tyrosine kinase domain.[3] When HER-2 binds to the ligand, it activates tyrosine kinase activity mainly by inducing receptor dimerization and autophosphorylation of cytoplasmic tyrosine kinase. The types of variation in HER2 include overexpression, mutation and amplification.[4] At present, the only cancers involved in routine detection and treatment of HER2 targets are gastric cancer and breast cancer. The HER2 criteria used were the same, that is, combined with HER2 protein overexpression and gene amplification to determine HER2 positive.[5] In short, if the immunohistochemistry (IHC) result is 3+, it is judged as positive; if it is 0 or 1+, it is judged as negative; if the IHC result is 2+, it is determined by detecting HER2 gene amplification using in situ hybridization (ISH) technique. The so-called HER2 low expression specifically refers to the subtypes IHC1+ and IHC2+/ISH-. Among them, the HER2-positive patients accounted for 20–25% of the total and were considered a poor prognostic factor in the past. However, with the evolution of anti-HER2 treatment drugs, the treatment pattern of HER2-positive advanced breast cancer was constantly rewritten.[6–8] Among them, monoclonal antibody drugs such as trastuzumab, pertuzumab and inituximab mainly target the extracellular binding domain of HER2 and block the heterodimerization of HER2 with other HER receptors by binding HER2, thus slowing down the growth of tumor.[9] Small molecular tyrosine kinase inhibitors (TKI), including Afatinib, Neratinib, Pyrotinib, Lapatinib and Tucatinib, target the intracellular

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tyrosine kinase region of HER2 to block signal transduction by inhibiting phosphorylation.[10] Despite achieving the breakthrough, nearly all patients with HER2-positive metastatic breast cancer (MBC) progressed eventually on anti-HER2 therapy due to the resistance.[11] For the patients with low-level expression of HER2 which is nearly 50% of BC patients, targeted drugs were not effective.[12] As a result, currently, the treatment of HER2-positive BC is still a difficult problem and a great challenge all over the world.

One strategy is to apply antibody-drug conjugates (ADCs), improving the therapeutic index by combining the targeted specificity of monoclonal antibodies with the anti-tumor ability of cytotoxic drugs. ADCs are composed of three elements, respectively, a monoclonal antibody to identify tumor cells and internalize the entire ADC complex via endocytosis, a cytotoxic agent (also called "payload") to kill tumor cells, and a linker to attach the cytotoxic agent to the antibody. The above three parts are vital for designing an effective ADC. Another factor influencing the therapeutic index of an ADC is the drug-to-antibody ratio (DAR), specifically the average number of payloads linked to each antibody. Too small means the treatment may less effective, and too many may make side effects harder to tolerate.

The incidence of HER-2 overexpression was the highest in breast cancer, and decreased successively in gastric cancer and colon cancer.[13] This allowed some ADCs to treat other cancers besides BC. For example, T-DM1 was effective in BC, colorectal cancer, gastric cancer, non-small cell lung cancer, and so on, but it had been approved only for the treatment of HER2+ breast cancer. With a particular emphasis placed on the two FDA-recommended drugs, this study will mainly provide the latest advances of ADCs in the treatment of HER2-positive BC, offering an overview of ADC combined immunotherapy.

ADCs Approved by FDA for HER2-Positive BC

Trastuzumab Emtansine (T-DM1)

T-DM1 was a classic ADC, which was the first ADC approved by the FDA for the treatment of HER2-positive MBC in 2013, specifically for the treatment of patients who had received trastuzumab and a taxane. The payload is DM1, which is a derivative of maytansine. Maytansine is a potent inhibitor of tubulin polymerization, which is stable and can be adequately soluble in the aqueous milieu of the antibody. The antibody is trastuzumab. Per antibody molecule links with 3.5 maytansinoid molecules on average, and the conversion of trastuzumab into an ADC significantly enhanced its cell-killing power.[14] Trastuzumab and DM1 are covalently linked via a stable thioether linker (N maleimidomethyl) cyclohexane-1-carboxylate, which is considered to limit the exposure of normal tissue to DM1, thus restricting its toxicity.[15] The cytotoxicity of T-DM1 may vary with the concentration of DM1 accumulated in cancer cells. High intracellular concentration will lead to rapid apoptosis, lower concentration will lead to cell transport damage and mitotic disaster, and the lowest concentration will lead to poor response to T-DM1.[16]

Approval of this drug was based on the date of the phase III EMILIA study which investigated the efficacy of T-DM1 versus capecitabine and lapatinib in patients with HER2-positive advanced breast cancer (ABC). T-DM1 was more effective, showing an improvement in progression-free survival (PFS) (9.6 vs 6.4 months; HR 0.65, 95% CI 0.55 to 0.77) and median overall survival (OS) (29.9 vs 25.9 months; HR 0.75, 95% CI 0.64 to 0.88).[17]

TH3RESA was a phase III study that evaluated the efficacy and safety of T-DM1 in patients with progressive HER2-positive BC after trastuzumab-based and lapatinib-based therapy for advanced disease and previously treated with a taxane. T-DM1 significantly improved PFS and objective response, with a favorable safety profile in comparison with the physician's choice (median PFS: 6.2 vs 3.3 months). The study demonstrated that even after several lines of previous therapy (median of four previous regimens), the use of a more effective HER2-directed therapy can contribute to meaningful clinical benefits.[18] It was the study MARIANNE that supported T-DM1 to be the first-line treatment for patients with HER2-positive MBC who were deemed unsuitable for taxane-based therapy. The MARIANNE was a phase III study, which was designed to assess the efficacy and safety of T-DM1 and T-DM1 plus pertuzumab compared with trastuzumab plus taxane in those with HER2-positive ABC, and no prior therapy for advanced disease (n=1095 patients). T-DM1 showed non-inferior, but not superior, efficacy and better tolerability when compared with a taxane plus trastuzumab for first-line treatment. Median PFS was 13.7 months in the trastuzumab plus taxane group, 14.1 months in the T-DM1 group, and 15.2 months in the T-DM1 plus pertuzumab group.[19]

The treatment of HER2-positive MBC after initial HER2-directed therapy, T-DM1 continued to be a significant medical need by the study of TDM4374g and TDM4258g. Moreover, the trials also showed that the therapeutic effect of T-DM1 was related to HER2 expression levels. Like the results of TDM4374g, the objective response rate (ORR) per independent central review (IRF) in patients with at least median HER2 expression was 42.9% (95% CI 26.3 to 60.6) with median PFS being 8.0 months (95% CI 5.4 months to N/E). ORR in patients with less than median HER2 expression was 38.2% (95% CI 22.2 to 56.4), and median PFS was 6.2 months (95% CI 3.9 to 12.3).[20,21]

The risk of recurrence or death of invasive BC was 50% lower with T-DM1 than with trastuzumab for those with residual invasive early breast cancer (EBC) after neoadjuvant chemotherapy plus HER2-targeted therapy recently based on the results of KATHERINE, a phase III study. A lower rate of invasive disease occurred in the T-DM1 group than in the trastuzumab group (12.2% vs 22.2%). The percentage of patients in the T-DM1 group who were estimated to be free of invasive disease at 3 years was 88.3% and 77.0% in the trastuzumab group. Invasive disease-free survival was significantly higher among those who received T-DM1 than among those who received trastuzumab (HR 0.50, 95% CI 0.39 to 0.64, P<0.001).[22]

T-DM1 is efficient and well-tolerated while some challenges remain. In the USA, T-DM1 carried black box warnings for hepatotoxicity, cardiac toxicity, and embryo-fetal toxicity.[23] The lack of payload bystander effects limits utility in tumors with heterogeneous HER2 expression. Resistance can be caused due to defects in intracellular trafficking and increased expression of drug transporters MDR1 and MRP1.[24]

Trastuzumab Deruxtecan (T-DXd; DS-8201; Enhertu; AZD4552; Fam-Trastuzumab Deruxtecan-Nxki)

T-DXd was the second ADC approved by FDA to treat patients with HER2-positive, unresectable or metastatic BC following two or more prior anti-HER2-based regimens in December 2019 and was officially approved as a second-line drug on May 4, 2022. The payload of T-DXd is DXd, which is a derivative of DX-8951. DX-8951 is a more potent topoisomerase I inhibitor than SN-38, which can bind to topoisomerase I-DNA cleavable complexes and stabilize them, resulting in the induction of double-strand DNA breaks and apoptosis.[25,26] At the same time, because DXd is a topoisomerase I inhibitor, which is different from the mechanism of chemotherapy drugs commonly used in the treatment of breast cancer, cross-drug resistance can be effectively avoided. The antibody is a humanized HER2-targeted antibody that has the same amino structure as trastuzumab. Each antibody binds to eight DXd molecules, so T-DXd has a higher DAR than T-DM1, thus allowing it to deliver more payload molecules to targeted tumor cells. The novel enzyme-cleavable linker remains stable in plasma, which is cleaved by proteases known as lysosomal cathepsins once in the cell, causing the release of the cytotoxic drug.[27] After optimization, the hydrophobicity of T-DXd connectors is greatly reduced and the stability is enhanced. Basic studies showed that after 21 days of treatment, the drug release rate of T-DXd in human plasma was only 2.1%, while that of T-DM1 was 18.4% after 4 days. In addition, the connectors are specifically cleaved by lysosomal enzymes highly expressed in tumor cells, such as cathepsin B and L, ensuring the stability of T-DXd in systemic circulation and limited systemic toxicity.[28,29] And the half-life of DXd is short (based on animal data, it is about 1.37h in systemic circulation), which helps to minimize the miss toxicity of T-DXd.

The phase I DS8201-A J101 study and the phase II DESTINY-Breast01 study provided strong validation of the breakthrough efficacy of DS-8201 in the late-line treatment of HER2-positive breast cancer. However, the application of DS-8201 was not limited to the back line, and its performance in the field of second-line treatment was even more commendable. DS8201-A-J101 was the first-in-human trial of T-DXd. T-DXd showed preliminary anti-tumor activity in patients with HER2-positive and previously treated with T-DM1. The study showed that the confirmed ORR was 59.5% (95% CI 49.7 to 68.7).[30,31] The phase II trial of DESTINY-Breast01 showed that T-DXd was significant and sustained in patients with HER2-positive MBC who progressed on or after T-DM1. With a median duration of follow-up of 20.5 months, patients treated with T-DXd achieved a confirmed ORR (by ICR) of 61.4% (95% CI 54.0 to 68.5) with a median duration of response (DOR) of 20.8 months (95% CI 15.0 to NE).[32,33] Based on the earlier results from the trial, T-DXd had been approved for posterior line therapy in patients with HER2-positive BC by the FDA.[34] T-DXd replaced T-DM1 as the preferred second-line drug to treat HER2-positive MBC according to DESTINY-Breast03. DESTINY-Breast03

was a phase III trial that assessed the efficacy and safety of T-DXd vs T-DM1 in patients with HER2-positive MBC previously treated with trastuzumab and taxane and met the primary endpoint. T-DXd had a superior PFS and ORR. The PFS assessed by the researchers achieved an unprecedented breakthrough, with a median PFS of 25.1 months, more than three times that of the T-DM1 group. The percentage of those who were alive without disease progression at 12 months was 75.8% for T-DXd and 34.1% for T-DM1.[35] For overall patients, confirmed ORR with T-DXd was 79.7% and 34.2% with T-DM1. Subgroup analysis showed that all subgroups of patients with HR status, pertuzumab treated, baseline visceral disease, previous treatment lines, and brain metastasis could obtain more significant PFS from the late second-line treatment of T-DXd than that of the T-DM1 group. And T-DM1 had a manageable safety profile.[36] Under this background, T-DXd continues to expand its territory. DESTINY-Breast09 research is moving towards first-line therapy. DESTINY-Breast11 study challenges the neoadjuvant therapy of PH dual-target combined chemotherapy. DESTINY-Breast05 study launches an impact on the adjuvant therapy stage of high-risk HER2-positive breast cancer patients after neoadjuvant therapy. There is much to look forward to.

Because of the cleavage of connectors and the high membrane permeability of DXd, DS-8201 not only has cytotoxicity against targeted tumor cells, but also plays a strong bystander effect on nearby tumor cells.[28] Based on this, DS-8201 may be effective in the treatment of heterogeneous tumors, as well as in tumor cells with low expression of HER2. On August 5th, T-DXd was approved by the FDA for the treatment of ABC with low expression of HER2, which became the first targeted therapy for BC with low expression of HER2 in the world. DESTINY-Breast04 was a randomized controlled Phase 3 study that established the first targeted therapy for breast cancer with low HER2 expression.[37] The study included breast cancer patients with low HER2 expression who received first-or second-line treatment, and who were randomly assigned to receive T-DXd or physician-selected chemotherapy. The main endpoint was PFS in patients with positive hormone receptor (HR). In the general population (regardless of HR status), T-DXd significantly prolonged PFS (9.9 vs 5.1 months; HR 0.5, $P < 0.001$) and OS (23.4 vs 16.8 months; HR 0.64, $P = 0.0010$) compared with the physician-selected chemotherapy (TPC), and the significant benefits of PFS (10.1 vs 5.4 months; HR 0.51, $P < 0.001$) and OS (23.4 vs 16.8 months; HR 0.64, P=0.0010) could also be seen in HR+ patients. Patients with HR were in an exploratory study population, with a smaller sample size, but we could also saw benefits from PFS (8.5 vs 2.9 months; HR 0.46) and OS (18.2 vs 8.3 months; HR 0.48). A Ib trial (NCT03523572) was conducted to investigate the efficacy of T-DXd in combination with an anti-PD-1 antibody (nivolumab) in patients with HER2-expressing MBC or advanced urothelial cancer. Interim results for the BC cohorts had been presented and revealed efficacy. Respectively, the confirmed ORR was 59.4% and 37.5% in the HER2-positive and HER2-low cohorts.[38] Durvalumab (a humanized monoclonal IgG1 antibody against PD-L1) plus T-DXd also showed promising early safety and efficacy in triple-negative breast cancer (TNBC) by a study (NCT03742102) and the confirmed ORR was 100% (4/4).[39] The reason for its efficacy to treat HER2-low BC may be the bystander effect. DXd is cell membrane permeable, and thus it may enter nearby cells, even those without strong HER2 expression.[40] However, the lower limit of low expression of HER2 is still controversial. The DAISY study explored the efficacy of DS8201 in BC with different HER2 expression status. The results suggested that about 30% of patients with HER2 IHC0 could still benefit from DS8201 treatment. Therefore, some questions are worth thinking about: whether patients with lower HER2 expression can also benefit from ADC drug therapy? Can patients with lower HER2 expression also be classified as people with low HER2 expression?

Side effects of T-DXd were more commonly associated with adverse reactions to cytotoxic chemotherapy, such as cytopenia, nausea, vomiting, diarrhea, and hair loss.[41] A high incidence of interstitial lung disease was observed in early trials while this appears to be manageable in most patients treated with glucocorticoids.[41] In addition, drug resistance of T-DXd was also inevitable At present, the exploratory analysis of DAISY studies on T-DXd suggested that the primary drug resistance may be related to the hemizygotic deletion mutation of ERBB2 gene on chromosome 17, and the SLX4 mutation may induce secondary drug resistance in T-DXd. At the same time, the decrease of HER2 expression level was also observed in patients with T-DXd drug resistance, but the specific mechanism of drug resistance needed further clinical verification. Other trials that study the synergy in combination with other therapies are ongoing, such as DESTINY-Breast07/08/09, BEGONIA, and HER2CLIMB-04.

The updated data and ongoing trials of the T-DXd are shown in Tables 1 and 2, respectively.

Table 1 Trials of T-DXd That Have Updated Date

Study (NCT Number)	Population	Setting	Experimental Arm	Primary End Points	Secondary End Point	Adverse Reactions ≥ Grade 3
DS8201-A-J101 (NCT02564900)[31]	HER2-low MBC	No prior HER2-targeted therapy	T-DXd	cORR (ICR): 37.0% cORR (by investigator): 44.4%	cDCR (ICR): 47% cDCR (by vestigator): 45% mDOR: 10.4 months mPFS: 11.1 months mOS: 29.4 months	63.00%
DS8201-A-J101 (NCT02564900)[74]	HER2+ MBC	Prior T-DM1	T-DXd	cORR (by investigator): 59.5%	CR: 3% PR: 57% SD: 34% PD: 5% cDCR: 93.7% mTTR: 1.6 months mDOR: 20.7 months mPFS: 22.1 months	50.00%
DS8201-A-U105 (NCT03523572)[75]	HER2-expressing MBC or advanced urothelial cancer	-	T-DXd in combination with nivolumab	cORR (HER2+ cohort, by ICR): 59.4% cORR (HER2-low cohort, by ICR): 37.5%	DCR (HER2+ cohort): 90.6% DCR (HER2-low cohort): 75.0% mPFS (HER2+ cohort): 8.6 months mPFS (HER2-low cohort): 6.3 months	43.80%
DESTINY-Breast01 (NCT03248492)[34,76]	HER2+ MBC	Prior T-DM1	T-DXd	cORR (by ICR): 61.4%	mDOR: 20.8 months mPFS: 19.4 months mOS: 24.6 months DCR: 97.3% CR: 6.5% PR: 54.9% SD: 35.9% PD: 1.6%	-

Other ADCs Under Development

There are many HER2-targeted ADCs under development. This review will discuss some ADCs, which have different compositions.

Trastuzumab Duocarmazine (SYD985)

SYD985 is the second-generation ADC and has obtained fast-track designation from FDA to treat patients with HER2-positive MBC who have progressed during or after at least two different HER2-target treatment regimens. The antibody is trastuzumab, binding to a potent duocarmycin payload via a cleavable linker (VC-Seco-DUBA).[42] Wim Dokter et al predicted that its membrane-permeable nature and the cleavable linker in SYD985 allow for significant bystander effects and may lead to the successful treatment of tumors where not all tumor cells express high levels of HER2.[43] A phase I trial has shown efficacy on HER2-positive, HER2-low, and triple-negative MBC whose ORR were 33%, 27%, and 40% respectively.[44] SYD985 displays an unusual activity on models resistant to T-DM1, suggesting that it is an efficient drug to overcome resistance to T-DM1.[45] TULIP is a phase III trial involving 437 patients with HER2-positive MBC who had received at least two regimens or T-DM1 for MBC. SYD985 was more effective than the physician's choice, with the

Table 2 Trials of T-DXd That is on Going

Study (NCT-number)	Population	Setting	Experimental Arm	Control Arm	Primary End Points	Secondary End Point
DESTINY-Breast02 (NCT03523585)[77]	HER2+ MBC	On or after T-DM1	T-DXd	Investigator's choice	PFS (by BICR)	OS ORR DOR PFS (by investigator) CBR
DESTINY-Breast03 (NCT03529110)[78,79]	HER2+ MBC	Prior trastuzumab and a taxane	T-DXd	T-DM1	PFS (by BICR)	PFS (by investigator) cORR DOR OS
DESTINY-Breast04 (NCT03734029)[80]	HER2-low MBC	Prior chemotherapy	T-DXd	Investigator's choice	PFS (by BICR)	PFS (by investigator) OS ORR DOR (by BICR) DOR (by investigator)
DESTINY-Breast05 (NCT04622319)[81]	Primary BC with residual invasive disease	After neoadjuvant therapy	T-DXd	T-DM1	IDFS (by nvestigator)	OS IDFS DRFI BMFI
- NCT04553770[82]	HER2-low, HR+ EBC	After neoadjuvant therapy	T-DXd ± anastrozole	-	pCRrate	Incidence of adverse events Molecular changes in tumor biomarkers ORR Biomarker analyses
DESTINY-Breast06 (NCT04494425)[83]	HR+, HER2-low MBC	≥ 2 prior lines of endocrine therapy have not received chemotherapy or any anti-HER2 therapy for metastatic disease	T-DXd	Investigator's choice of chemotherapy (paclitaxel, nab-paclitaxel, or capecitabine)	PFS (HER2-low, by BICR)	PFS (HER2 low, by investigator) PFS (intent-to-treat, by BICR) OS (HER2 low) OS (intent-to-treat) ORR (by BICR) ORR (by investigator) DOR (by BICR) DOR (by investigator)
DESTINY-Breast07 (NCT04538742)[84]	HER2+ MBC	Part 1: ≥2 lines of treatments Part 2: No prior treatment for MBC	T-DXd or in combination with other anti-cancer agents	-	AEs SAEs	ORR PFS PFS2 DOR OS
DESTINY-Breast08 (NCT04556773)[85]	HER2-low MBC	-	T-DXd in combination with other anti-cancer agents	-	AEs SAEs	ORR DOR PFS (by investigator) OS
DESTINY-Breast09 (NCT04784715)[86]	HER2+ MBC	No prior chemotherapy or HER2-targeted therapy for MBC	Arm A (T-DXd + placebo) Arm B (T-DXd + pertuzumab) Arm C (THP)	-	PFS (by BICR)	PFS (by investigator) OS ORR DOR PFS2

(*Continued*)

Table 2 (Continued).

Study (NCT-number)	Population	Setting	Experimental Arm	Control Arm	Primary End Points	Secondary End Point
TUXEDO-1 (NCT04752059)[87]	HER2+, newly diagnosed or progressing BM	Progressing BM with radiological progression after prior local therapy, prior exposure to trastuzumab and pertuzumab	T-DXd	-	RR (intracranial, by RANO):83.3%	RR (extracranial) PFS OS Safety Quality-of-life
DEBBRAH (NCT04420598)[88]	(1) HER2+ MBC with non-progressing BM (2) HER2+ or HER2-low MBC with asymptomatic BM (3) HER2+ MBC with progressing BM (4) HER2-low MBC with progressing BM (5) HER2+ or HER2-low MBC with LMC	(1) Prior radiotherapy and/or surgery (2) Prior untreated (3) Prior local treatment (4) Prior local treatment (5) Not required	T-DXd	-	PFS OS ORR	Additional efficacy outcome Safety
HER2CLIMB-04 (NCT04539938)[89]	HER2+ MBC	Prior 2 or more anti-HER2-based regimens	T-DXd + tucatinib	-	cORR	DOR PFS DCR OS
DS8201-A-U106 (NCT04042701)[90]	HER2+/HER2-low MBC	Prior T-DM1/Prior have exhausted treatments that can confer any clinically meaningful benefit	T-DM1 + pembrolizumab	-	cORR	DOR DCR PFS TTR OS
HER2-PREDICT (NCT04257162)[91]	Metastatic/advanced cancer	Treated with T-DXd	T-DXd		HER2 mRNA cut-point predictive of T-DXd response	

median PFS for the SYD985 cohort being 7.0 months (95% CI 5.4–7.2), and the physician's choice was 4.9 months (95% CI 4.0–5.5).[46]

XMT-1522

An ADC XMT-1522 has a humanized anti-HER2 antibody (HT-19) conjugated to 12–15 molecules of the payload AF-HPA (an auristatin derivative). XMT-1522 is more potent than T-DM1 in vitro, showing early signs of anti-tumor activity in a phase I trial to treat patients with HER2-expressing breast, lung, and gastric tumors.[47,48] The disease control rate (DCR) was 83% (5/6) for patients at a dose of 16 or 21.3 mg/m2, and 25% (3/12) in patients treated at doses less than 16 mg/m2, providing that drug efficacy may be related to dose.[48] In addition, in preclinical studies, XMT-1522 was effective on T-DM1 resistant models of breast cancer and gastric cancer.[49]

A166

A166 is an ADC composed of monomethyl auristatin F derivative conjugated to trastuzumab via a stable protease-cleavable valine citrulline linker. The first-in-human study of A166 demonstrated its efficacy as DCR was 59% at the dose levels of 3.6 mg/kg and 4.8 mg/kg.[50] This study is expected to be completed in December 2022. A study in China revealed that tumor response was evaluable in 6 of 19 patients at the same doses.[51]

RC48-Adc

RC48-ADC refers to a novel ADC conjugating a monomethyl auristatin E derivative (MMAE) with a humanized anti-HER2 antibody. A dose-escalation phase I study to treat HER-positive MBC revealed that, at doses≥1.5 mg/kg, partial response (PR) was 57.1% (8/14) and stable disease (SD) was 28.6% (4/14). ORR was 72.7% for 11 trastuzumab-pretreated patients.[52] A phase Ib trial evaluated RC48-ADC in patients with HER2-positive MBC. The DCR was 96.7% (29/30) and the clinical benefit rate (CBR) was 46.7% (14/30). It was more effective in patients who had not been treated with trastuzumab with a higher ORR (57.1% vs 33.3%). Moreover, no grade ≥4 AE was observed.[53] A phase II trial is performed to compare with capecitabine/lapatinib in HER2-positive BC (NCT03500380). Meanwhile, a phase III trial is also planned in HER2-low BC (NCT04400695).

MM-302

MM-302 is a HER2-targeted antibody-liposomal doxorubicin conjugate. Although efficacy was shown in phase I trial,[54] the phase II trial named HERMIONE failed to find any clinical benefit of MM-302 plus trastuzumab versus TPC (chemotherapy of physician's choice plus trastuzumab) with HER2-positive MBC.[55]

MEDI4276

The payload in MEDI4276 (an ADC) is MMETA, a tubulin-based microtubule inhibitor, which via the maleimidocaproyl linker to a HER2-bispecific antibody targeting two different epitopes on HER2. MEDI4276 showed cytotoxic effects on HER2-positive tumor cells in vitro containing T-DM1 resistant cells,[56] and another first-in-human, phase I study also confirmed that. There was one complete response (0.5 mg/kg) and two partial responses (0.6 and 0.75 mg/kg). All had prior treatment with trastuzumab, pertuzumab, and T-DM1 for BMC.[57]

PF-06804103

PF-06804103 is an ADC with anti-HER2 immunoglobulin G1, which is conjugated with a cleavable linker to the cytotoxic agent auristatin microtubule inhibitor Aur0101. A phase I trial in HER2-positive BC and gastric cancer exhibited anti-tumor activity, and preliminary ORR in the patients (≥3mg/kg) was 52.4% (11/21).[58]

ARX788

ARX788 is an ADC with a humanized HER2 targeting mAb conjugated to a cytotoxic tubulin inhibitor, Amberstatin (AS269).[59] A phase I study of ARX788 generated a preliminary anti-tumor effect and safety in Chinese patients undergoing HER2-positive MBC. The ORR was 31% (13/42) and 42% (5/12) at a dose of 1.3 mg/kg. In addition, treatment-related serious adverse events were not observed.[60] And ARX-788 is investigated in an ongoing phase I trial (NCT03255070).[61]

ALT-P7 (HM2/MMAE)

ALT-P7 is an ADC, in which the trastuzumab bio better HM2 is conjugated to monomethyl auristatin E (MMAE). The first efficacy results from a phase I trial of 27 patients with HER2-positive MBC were presented with a DCR of 77.3% (17/22) with the median PFS being 6.2 months (95% CI 2.5–9.9) at doses from 2.4 to 4.8mg/kg.[62]

DHES0815A (Anti-HER2/PBD-MA)

In the ADC DHES0815A, a monoclonal HER2 targeting antibody conjugates to pyrrolobenzodiazepine monoamide (PBD-MA). A Phase I study of DHES0815A in patients with HER2-positive BC has a primary completion date while no published data is available.[61]

BAT8001

BAT8001 is a novel HER2-targeting ADC composed of a trastuzumab biosimilar conjugated to the drug-linker Batansine. In a phase I study, BAT8001 showed anti-tumor activity in HER2-positive BC with a reported ORR of 41.4% (95% CI 23.5–61.1) with DCR being 82.8% (95% CI 64.2–94.2).[63]

The payloads, antibodies, and associated clinical trials for the above-mentioned drugs are documented in Table 3.

ADCs Combine with Immunotherapy

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of immunogenic cancers by enabling the priming and infiltration of T-cells into the tumor microenvironment, promoting cytotoxic signalling pathways and affecting tumor cytolysis.[64] However, BC tumors had been considered immunologically quiescent historically, having intrinsic resistance

Table 3 The Approved and Investigational Anti-HER2 ADCs

Drug	ADC main parts 1) Monoclonal antibody 2) Payload 3) Linker	Drug-antibody ratio (DAR)	Study (NCT-number)	Population and setting	Efficacy	Adverse events grade≥3
Trastuzumab emtansine	1) DM1 2) Trastuzumab 3) N-maleimidomethyl cyclohexane-1-carboxylate (MCC)	3.5[92]	EMILIA (NCT00829166)	HER2+ MBC; Prior trastuzumab and a taxane	PFS: 9.6 months[17] mOS: 29.9 months	Diarrhoea Fatigue AST increased ALT increased Anaemia Peripheral neuropathy Hypokalaemia Neutropenia Thrombocytopenia
Trastuzumab deruxtecan	1) DXd 2) A humanized HER2-targeted antibody 3) Cysteine-maleimide	8	DESTINY-Breast01 (NCT03248492)	HER2+ MBC; Prior Trastuzumab emtansine	cORR: 61.4% DOR: 20.8 months mPFS: 19.4 months mOS: 24.6 months DCR: 97.3%[33]	Anemia Decreased lymphocyte count Fatigue Vomiting Constipation Decreased appetite Diarrhea Aopecia Hematologic Interstitial lung disease[93]
SYD985	1) Trastuzumab 2) Duocarmycin 3) Vc-seco-DUBA	2[42]	TULIP (NCT03262935)	HER2+ MBC; ≥2 previous regimens or T-DM1	mPFS (by blinded central review): 7.0 months mPFS (by investigator): 6.9 months months OS: Unknown ORR: No significant difference HRQoL: No significant difference	Eye disorders Respiratory disorders
			-(NCT02277717)	HER2+/HER2 low/Triple-negative MBC; Prior ≥ 3 HER2-targeting regimens	ORR (HER2+): 33% PFS (HER2+): 9.4 months ORR (HER2-low): 27% ORR (Triple-negative): 40%	Neutropenia Cnjunctivitis
XMT-1522	1) HT-19 2) AF-HPA 3) Dolaflexin ADC platform[48]	10~15	- (NCT02952729)	HER2+ MBC; Prior all standard of care therapies	DCR (at dose of 16 or 21.3 mg/m2): 83% (5/6) DCR (at doses less than 16 mg/m2): 25% (3/12)	No

(*Continued*)

Table 3 (Continued).

Drug	ADC main parts 1) Monoclonal antibody 2) Payload 3) Linker	Drug-antibody ratio (DAR)	Study (NCT-number)	Population and setting	Efficacy	Adverse events grade≥3
A166	1) Trastuzumab 2) Monomethyl auristatin F derivative 3) Valine citrulline	Unknown	- (NCT03602079)	HER2+/HER2-amplified MBC;-	PD: 41% (11/27) SD: 33% (9/27) PR: 26% (7/35)	Unknown
RC48-ADC	1) Hertuzumab 2) Monomethyl auristatin E (MMAE) 3)maleimidocaproyl(mc)[52]	4	- (NCT02881138)	HER2+ MBC;-	PR: 57.1% (8/14) SD: 28.6% (4/14)	Leucopenia AST elevation Neutropenia
			- (NCT03052634)	HER2+ MBC; Prior trastuzumab/ chemotherapy	DCR: 96.7% (29/30) CBR (1.5 mg/kg): 26.7% (8/30) CBR (2.0mg/kg): 46.7% (14/30) ORR (trastuzumab-naive): 57.1% (17/30) ORR (trastuzumab-pretreated): 33.3% (10/30)	Neutropenia Leucopenia AST elevation ALT elevation
MM-302	1) A HER2-targeted antibody 2) Liposomal doxorubicin 3) Unknown	Unknown	- (NCT01304797)	HER2+ MBC;-	RR (MM-302 ≥ 30 mg/m2): 12% mPFS: 7.6months mPFS(MM-302 + trastuzumab + cyclophosphamide): 10.6 months	Constipation Cough Decreased appetite Diarrhea Dyspnea Fatigue Nausea Neutropenia Stomatitis Vomiting Cardiac failure
			HERMIONE (NCT02213744)	HER2+ MBC; Prior trastuzumab, and anthracycline-naive	Failed to find any clinical benefit	Unknown
MEDI4276	1) A tubulysin-based microtubule inhibitor (MMETA) 2) A bivalent biparatopic antibody 3) Engineered cysteines with a maleimide-bearing mc-Lys protease cleavable linker	4[94]	- (NCT02576548)	HER2+ breast or gastric cancer; Prior trastuzumab, pertuzumab, and T-DM1, either alone or in combination	CR: (0.5 mg/kg; breast): 1/43 PR: (0.6 /0.75mg/kg; breast): 2/43 SD: 28% (12/43)	Increased AST Increased ALT Diarrhea Increased blood bilirubin
PF-06804103	1) Aur0101 2) Anti-HER2 immunoglobulin G1 antibody 3) Anti-HER2 immunoglobulin G1 antibody	4[95]	- (NCT03284723)	HER2+ BC or HER2+ GC; Prior HER2-targeted therapy	ORR (≥ 3mg/kg): 52.4% (11/21)	Arthralgia Neuropathy Myalgia Fatigue Osteomuscular pain

(*Continued*)

Table 3 (Continued).

Drug	ADC main parts 1) Monoclonal antibody 2) Payload 3) Linker	Drug-antibody ratio (DAR)	Study (NCT-number)	Population and setting	Efficacy	Adverse events grade≥3
ARX788	1) A cytotoxic tubulin inhibitor, Amberstatin (AS269) 2) A humanized HER2 targeting mAb 3) pAF-hydroxylamine-PEG4	2[96]	- (NCT03255070)	HER2+ MBC; Prior pertuzumab and other HER2-directed therapies	Trial is ongoing	Trial is ongoing
			- (NCT02512237)	HER2+ MBC; rior a HER2 targeting therapy	Unknown	Unknown
ALT-P7	1) Monomethyl auristatin E (MMAE) 2) trastuzumab biobetter HM2 3) Cysteine-maleimide	2[62]	- (NCT03281824)	HER2+ MBC; Prior ≥ 2 anti-HER2 treatment	DCR: 77.3% (17/22) Median PFS: 6.2 months	Febrile neutropenia Thrombocytopenia Hyperbilirubinemia Myalgia Hyponatremia
DHES0815A	1) Pyrrolo benzodiazepine monoamide (PBD-MA) 2) A monoclonal HER-2 targeting antibody 3) Unknown	Unknown	- (NCT03451162)	HER2+ MBC; Prior ≥ 2 chemotherapy regimens	cORR: 86% (10/14) cCRR (at dose of 1.2 mg/kg): 7% (1/14)[97]	Blepharitis Eye pain Photophobia
BAT8001	1) A maytansine derivative 2) A trastuzumab biosimilar 3) Cysteine-3AA	Unknown	- (NCT04189211)	HER2+ MBC;-	ORR: 41.4% (12/29) DCR: 82.8% (24/29)	thrombocytopenia aspartate aminotransferase increased γ-glutamyl transferase increased alanine aminotransferase increased diarrhea

to ICIs. A key strategy to overcome resistance to ICIs in BC is to develop combination immunotherapy that eliminates immunosuppressive cells and increases infiltration and activation of T-cells, thereby transforming the tumor from immune unresponsive to immune responsive.[65] And the activity of an ADC can be further enhanced by adding an ICI. Chemotherapy causes the death of cancer cells, which promotes cancer cell antigen release and presentation. HER2-targeted drugs can kill tumor cells by the immune-mediated mechanism of antibody-dependent cellular cytotoxicity (ADCC). The two points support the potential of ADCs combined with immune checkpoint therapy in treating HER2-positive breast cancer.

A preclinical study showed that the combination of T-DM1 with anti-CTLA-4/PD-1 immunotherapy was considered effective in treating HER2-positive BC due to increased T-cell infiltration in tumor tissue, proliferation and signalling in the mice tumor model.[66] In most relevant clinical trials, eligible patients had MBC previously treated with trastuzumab and taxane. An immunotherapy combination strategy added PD1/PD-L1 inhibitors in combination with T-DM1. A phase Ib trial (NCT03032107) enrolled a similar cohort of 20 patients and showed that T-DM1 plus the PD-1 inhibitor pembrolizumab was safe and tolerable. ORR was 20% with a median PFS of 9.6 months, and there were no grade 4 or above adverse events.[67] In a phase Ib trial called GO29831 (NCT02605915), increased in PD-L1-expressing tumor-infiltrating immune cells were observed in both HER2+ EBC and MBC with the combination of atezolizumab (a PD-L1 inhibitor) and T-DM1.[68] However, a phase II study named KATE2 (NCT02924883) demonstrated that T-DM1 plus atezolizumab did not significantly improve PFS, which increased the incidence of adverse events. In the PD-L1-positive

subgroup, the atezolizumab group had more PFS events than the placebo group (27 vs 18). This suggested that the benefit of the combination of PD-L1 inhibitors may be limited to PD-L1-positive patients.[69] KATE3 (NCT04740918) was a phase III trial, which investigated the efficacy and safety of T-DM1 in combination with atezolizumab or placebo in patients undergoing HER2-positive and PD-L1-positive MBC.[70] Another immunotherapy combination strategy added the CD137-specific agonistic antibody utomilumab in combination with T-DM1 or trastuzumab. A phase IB/II clinical trial is ongoing and has not yet reported results (NCT03364348). There are also trials about T-DXd combined immunotherapy. A Ib trial (NCT03523572) was conducted to investigate the efficacy of T-DXd in combination with an anti-PD-1 antibody (nivolumab) in patients with HER2-expressing MBC or advanced urothelial cancer. Interim results for the BC cohorts have been presented and revealed efficacy. Respectively, the confirmed ORR was 59.4% and 37.5% in the HER2-positive and HER2-low cohorts.[38] In addition, a trial (NCT04042701) in combination with pembrolizumab (a PD-1 inhibitor) is ongoing.[71]

Other ADCs that have not been approved by the FDA also have studies to explore the efficacy of the combination with immunotherapy. For instance, RC48-ADC combined with PD-1/PD-L1 immune checkpoint inhibition (pembrolizumab/atezolizumab) significantly enhanced HER2-positive tumor suppression and antitumor immunity in PD-1 transgenic mice.[72] Apart from that, immune-stimulating antibody conjugates (ISACs) are also under development.[73]

Summary and Prospect

In the field of breast cancer, ADC had changed the treatment landscape of HER2-positive although it was previously thought to be incurable. ADCs were relatively new class of anticancer biologics with highly targeted properties and had at least three advantages. Firstly, they broaden the indications. They have been developed for the treatment of HER2-positive BC, trastuzumab sensitive and resistant. It is of note that some ADCs exert therapeutic effects on HER2-low and even TNBC. Secondly, ADC combines the advantages of antibodies and cytotoxic drugs to make it more effective by specifically recognizing tumor antigens. Meanwhile, the accumulation of the conjugated drug in tumor cells can be achieved. Chemotherapy drugs need to be combined with targeted drugs to be most effective, and targeted drugs can prevent chemotherapy resistance. Thirdly, this target-dependent activation allows selective cytotoxicity to cancer cells, thereby significantly lowering systemic side effects. ADCs combined with immunotherapy provide a new idea for the treatment of HER2-positive BC. More than 100 ADCs are currently in various stages of development. ADC is generally well tolerated, but each ADC has adverse effects that require special attention, such as T-DM1-related thrombocytopenia and T-DXD-related ILD. Antigenic selection, mechanism of drug action, chemical properties of linkers and coupling sites are important determinants of ADC-related adverse reactions.The rapid development of biotechnology has promoted the optimal selection of cytotoxins and linkers, and the effectiveness of the new generation of ADCs has greatly increased, but the risk/benefit ratio is still a factor that must be measured in clinical decisions.It is believed that ADCs will exert a further role in the treatment of BC in the future. We hope that this review gives useful information to physicians in the area.

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Disclosure

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