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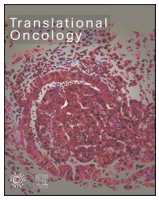

Research progress of antibody-drug conjugates therapy for HER2-low expressing gastric cancer

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ABSTRACT

Gastric cancer (GC) is a highly fatal malignant tumor in the world. Most of the patients are in an unresectable state when they have symptoms. Systemic treatment is the primary treatment for advanced patients. Among them, the Human epidermal growth factor receptor 2 (HER2) is an important therapeutic target. With the continuous optimization of chemotherapeutic drugs and chemotherapy regimens, the prognosis of some HER2-positive GC patients has been greatly improved. However, the needs of GC patients with a low level of HER2 expression still need to be met. Several targeted drugs against human epidermal growth factor receptor 2 emerged in recent years, including Antibody-drug Conjugates (ADC), novel humanized anti-HER2 monoclonal antibodies, and Tyrosine kinase inhibitors (TKI). As an important breakthrough in treating HER2-positive GC, ADC became one of the fastest-growing anti-tumor drugs. Some drugs also showed an anti-tumor effect on GC with low expression of HER2. It may also be the key to the treatment of low expression of HER2 GC in the future. This article mainly reviews several promising ADC drugs for the treatment of HER2 low-expression GC and related trials.

Introduction

GC is one of the most important cancers worldwide. It has the fifth-highest morbidity and the fourth-highest mortality globally [1]. Early GC has a good prognosis. However, most patients are in the advanced or metastatic stage when diagnosed with GC. With the continuous optimization of chemotherapy drugs and chemotherapy regimens, the survival time of patients with advanced GC has been significantly prolonged. The median overall survival time (mOS) barely exceeds a year, and it is only 10–12 months. The 5-year survival rate is even worse; only 32% of patients will survive over five years [2]. For patients who have lost the opportunity of surgery, non-operative treatment is an efficient way to prolong life. HER2-positive GC accounts for about 20% of human GC cases and about 11% of advanced GC cases [3–5]. Anit-HER2 ADC is an increasingly promising therapy for HER2-positive cancer.

As one of the fastest-growing antineoplastic drugs, the studies of ADC were the breakthroughs in the tumor treatment area, especially for HER2-positive GC treatment. Whereas ADC is hopeful for HER2-positive GC, there is still a lack of targeted therapy for low expression of HER2, which accounts for the most advanced GC. Clinical treatment of ADC can effectively treat homogeneous and high-expression HER2-positive GC. However, the performance of ADC with low expression of HER2 is not always satisfactory. In recent years, people have paid more attention to the low HER2 expression of GC, looking forward to developing new antineoplastic drugs.

HER2-low expressing gastric cancer

The human epidermal growth factor receptor (HER) family is composed of four members, including EGFR (HER1 or ErbB1), HER2 (ERBB2), and HER3 (ERBB3) and HER4 (ERBB4). It plays a central role in regulating cell proliferation, survival, differentiation, and migration [6]. The overexpression of the HER family is considered to be associated with many types of tumors. Each member contains an extracellular domain (consisting of four subdomains), a single transmembrane helix, and an intracellular kinase domain that interacts with signal molecules [7,8]. They become active through iso-or ectopic dimerization promoted by ligand binding [9–11]. HER2 is always in the "open" conformation of ligand binding. It is the only hybrid member in the family that can bind

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to any other family member dimer [12]. HER2 is a protooncogene located on chromosome 17 that encodes a 185-kDa transmembrane protein [13]. Dimerization leads to the phosphorylation of tyrosine residues in the intracellular protein kinase domain, which initiates a variety of signal pathways, leading to cell division, proliferation, differentiation, and anti-apoptosis signals [8], resulting in the invasive growth of tumors. The activation of HER2 is involved in about 1/5 of human GC cases [3,4]. It is a unique subgroup of overexpression of HER2 receptors and can be used as a target for targeted therapy of HER2 [5]. In recent years, with the research and development of tumor molecular biology, it has been found that the positive expression of the HER2 receptor is associated with the poor prognosis of GC [3]. It is considered a new biomarker diagnostic factor and the preferred target for cancer therapy, bringing tumor treatment into a new era [13–16]. Inhibiting HER2 activity with monoclonal antibodies, ADC, or small molecular kinase inhibitors are common methods for the treatment of HER2-positive GC. Although these treatments targeting HER2 improve overall survival, they might be ineffective in some cases. In most cases, the expression level of HER2 was low [7]. According to the clinical guidelines, the HER2 status is mainly assessed by using a combination of immunohistochemistry (IHC, to evaluate HER2 protein expression levels) and gene amplification using fluorescence *in situ* hybridization (FISH,to detect the mutation status of the HER2 gene) or another *in situ* hybridization (ISH, to assess HER2 gene status) [17,18]. Patients are HER2-negative if the IHC score is 0 or 1+, HER2-equivocal if the score is 2+, and HER2- positive if the score is 3+. The HER2 status of patients with HER2-equivocal IHC scores should be additionally examined by FISH or other ISH methods. FISH-positive/ISH-positive and FISH-negative/ISH-negative tumors are then classified as HER2-positive and -negative, respectively. Patients are HER2-high if IHC3+ or IHC2+/ISH+, HER2-low if IHC 1+ or IHC 2+/ISH-. According to the literature and previous studies, the proportion of low expression of HER2 (IHC2+/FISH-) was more than 40%−60% of GC patients; although the level of HER2 was higher than normal tissues, these patients did not meet the currently available treatment conditions [19]. Trastuzumab was approved more than 20 years ago, which led to substantial progress in targeted therapy for HER2 [20,21]. However, there are no treatments specifically for HER2-low gastric cancer, and effective therapies are needed.

In recent years, some studies have reported the anti-tumor effect of new ADC-targeted anti-HER2 drugs on HER2-positive GC, and some of them also showed anti-tumor effects on GC with low expression of HER2.

Anti-HER2 antibody-drug conjugate

ADC is a targeted drug based on macromolecules, mainly composed of three parts: a targeting agent, a linker, and a payload. The primary function of the antibody part is to selectively bind antigens on the target cells, thus concentrating cytotoxins on the tumor site. The antibody triggers the effective internalization of the antibody-antigen complex by selectively binding to the target antigen to exert its anti-tumor effect [22]. Linkers commonly include breakable linkers and unbreakable linkers. The function of linkers is coupling toxins to antibodies, maintaining the stability of drugs, and avoiding off-target toxicity. At the same time, after effectively internalized, cytotoxic drugs should release cytotoxic drugs quickly to kill tumor cells [23]. Payload is the critical component of ADC that mediates the anti-tumor effect and impacts vital cellular processes to kill tumor cells [24]. Typically, ADCs release payloads in a biologically active form intracellularly after lysosomal degradation [25]. The payloads used for ADCs mainly include microtubule inhibitors and DNA damaging drugs. Microtubule inhibitors destroy microtubule assembly during mitosis; thus, it has relative selectivity for rapidly proliferating tumor cells. DNA damaging drugs play a cytotoxic role through nucleic acid chain breakage, alkylation, or cross-linking induced by DNA double helix grooves; thereby inhibiting tumor cell proliferation [26,27]. In addition, other types of cytotoxic drugs, such as RNA polymerase inhibitors and RNA splice inhibitors, are still under development. ADCs use the targeting of antibodies to specifically recognize antigens on the surface of target cells, enter cells through endocytosis mediated by grid protein, and release cytotoxic drugs through cleavage or enzymolysis to kill target cells (Fig. 1). Due to ADCs' unique composition, their drug design, pharmacokinetics, pharmacodynamics, efficacy, adverse reactions, and drug resistance mechanism are different from other medications. ADCs showed great potential in anti-tumor therapy and attracted wide attention, even becoming the hot spot of great research.

On the one hand, with the continuous optimization of HER2 targeting ADCs, some HER2-positive GC patients have benefited from it. Up to October 2022, the Food and Drug Administration (FDA) of the United States approved listing two anti-HER2 targeted ADCs. In addition, three anti-HER2 targeted ADCs were on the market (Table 1) worldwide. At the same time, many HER2-targeted ADCs are in clinical trials (Table 2), and some showed potential for treating GC with low HER2 expression, which will be further expanded below.

Trastuzumab deruxtecan

Trastuzumab deruxtecan (T-Dxd, or DS-8201a) is a second-generation ADC developed to overcome the GC-specific challenges for HER2 targeted therapy. It is composed of trastuzumab, a restriction endonuclease linker, and a new DNA topoisomerase I inhibitor (Drutecan). Trastuzumab modulates the expression of topoisomerase I in extracellular vesicles released by HER2-positive cancer cells. The combination of topoisomerase inhibitor DXd with trastuzumab shows a potential anti-tumor effect [28]. On this basis, the bystander effect of T-DXd can further kill nearby cancer cells to enhance the killing effect of tumors. Its high drug-to-antibody ratio (about 8) and short half-life ensure that the DXd payload is effectively delivered to the cells expressing HER2. This advantage increases the cytotoxic effect while minimizing systemic exposure to limit off-target toxicity in normal cells [29]. In addition, the payload has high membrane permeability, which allows a cytotoxic effect on tumor cells close to the target cells, regardless of their HER2 expression levels.

In the study of the patient-derived xenograft (PDX) model, T-DXd exhibited a great potential to inhibit the growth of low expression of HER2 and trastuzumab- or trastuzumab emtansine (T-DM1)-insensitive HER2-positive cancer cells [28–30]. These preclinical studies showed that T-DXd had good activity and a significant anti-tumor effect in cells with low expression of HER2. These characteristics allow targeting tumors with the heterogeneous expression of HER2. The expression level of HER2 in tumors has been proven to affect the efficacy of T-DXd. Further studies had shown that the effective rate of patients with HER2 (IHC3+) in GC was higher than that of patients with HER2 (IHC2+ and ISH+) low expression, with a respective objective response of 58% and 29% [31]. However, due to the small sample size of this study, concrete conclusions cannot be drawn. So far, no data shows why this happens [32].

DISTY-Gastric01(ClinicalTrials.gov identifier: NCT03329690) was a multicenter, open-label, multi-cohort phase II clinical study to evaluate the efficacy and safety of DS-8201 as a third-line and posterior-line treatment for HER2-positive gastric or gastroesophageal junction cancer patients. Forty-five patients diagnosed with gastric/GEJ adenocarcinoma confirmed by central laboratory testing and had not received anti-HER2 therapy are involved in the study. Twenty-one of them are categorized into cohort 1 (IHC 2+/ISH), and the rest (24) are in cohort 2 (IHC1+). All patients with cohort 1 and cohort 2 received T-DXd 6.4 mg/kg by intravenous infusion once every three weeks, as determined by previous pharmacokinetic, efficacy, and safety data until the occurrence of disease progression, withdrawal of consent, or unacceptable adverse events [33]. The experimental results showed that the objective response rate (ORR) of cohort 1 was 26.3%, and the median

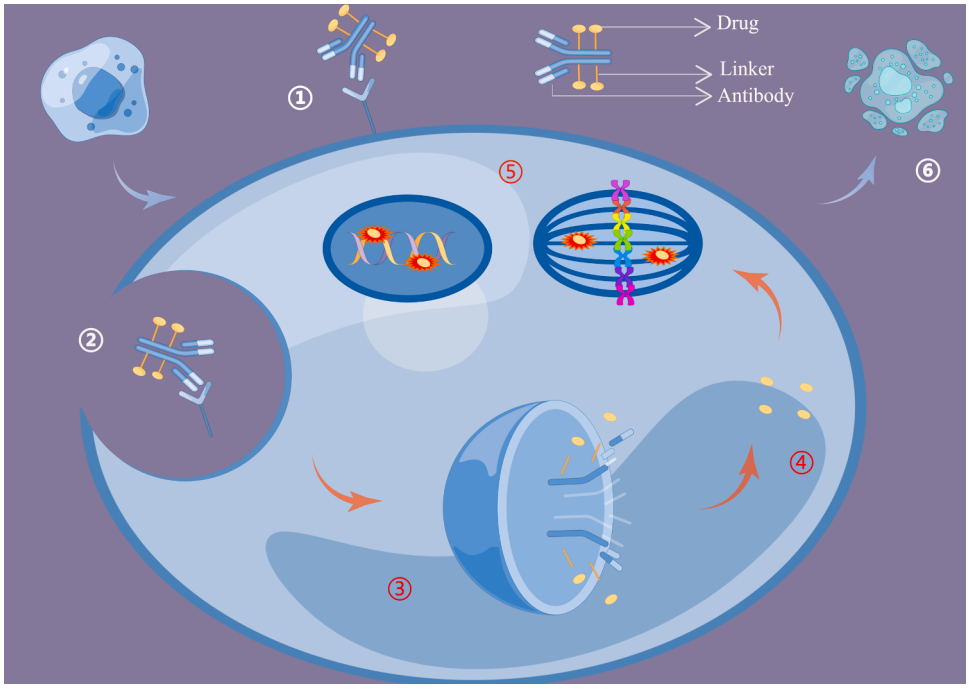

Fig. 1. The action mechanism of ADC
The action mechanism of ADC is divided into six steps: ①ADC binds to antigens on target cells; ②ADC-antigen complex is internalized by endocytosis; ③ADC is degraded in lysosomes; ④cytotoxic loads (drugs) release and play a role;⑤DNA or microtubule disruption;⑥target cell apoptosis.

Table 1
Anti-HER2 targeted ADC drugs on the market around the world
Note:data updated to October, 2022.

Serial number	First time on the market	Trade name	Common name	Target /antibody subtype	Small molecular drug	Linker	First approved indication	Country approved for the first time	Pharmaceutical company
1	2013.2	Kadcyla	Trastuzumab emtansine	HER2/IgG1	DM1	Non-cleavable thioether linker	Metastatic breast cancer	America	Roche
2	2019.12	Enhertu	Trastuzumab deruxtecan	HER2/ IgG1	Deruxtecan (Dxd)	Cleavable tetrapep-tid (Gly-Gly Phe-Gly)	Metastatic breast cancer	America	AstraZen-eca/ Daiichi Sankyo
3	2021.6	Aidixi	Disitamab vedotin	HER2	MMAE	Cleavable MC-Val Cit-PAB linker	Gastric cancer	China	RongchangCo.,Ltd

Table 2
Data on the results of trials related to selected ADCs in the review.

Study	Trial group	Trial design	Primary endpoints	Secondary endpoints	Adverse reactions greater than or equal to grade 3
DESTINY Gastric01 (NCT03329690)	Patients with HER2-positive advanced gastric or gastroesophageal junction adenocarcinoma	Trastuzumab deruxtecan; chemotherapy	mORR: 51% vs. 14%	mOS: 12.5 vs. 8.4 months; mPFS: 5.6 vs. 3.5 months	Grade 3 or higher TRAEs were decreased neutrophil count (51% vs. 24%), anemia (38% vs. 23%), and decreased white cell count (21% vs. 11%).
RC48 (NCT03556345)	Patients with HER2-positive locally advanced or metastatic gastric or gastroesophageal junction	RC48-ADC	mORR: 24.8%	mPFS: 4.1 months; mOS: 7.9 months	Grade 3 or higher AEs occurred in 36.0%, and the RC48-related AEs were mainly decreased neutrophil count (3.2%).
ARX788 (CTR20211583)	Patients with HER2-positive advanced gastric/ gastroesophageal junction adenocarcinoma	ARX788	ongoing	ongoing	ongoing
MRG002 (NCT05141747)	Patients with HER2-positive/HER2-low locally advanced or metastatic gastric/ gastroesophageal junction cancer	MRG002	ongoing	ongoing	ongoing
PF-06804103 (NCT03284723)	Patients with HER2 positive and negative breast and gastric cancer	PF-06804103; PF-06804103 + Palbociclib +Letrozole	ongoing	ongoing	ongoing

mORR, median objective response rate; mOS, median overall survival; mPFS, median progression-free survival; TRAEs, treatment-related adverse events.

progression-free survival (mPFS) and mOS were 4.4 and 7.8 months, respectively [34]. The ORR of cohort 2 was 9.5%, and the mPFS and mOS were 2.8 and 8.5 months [35]. 70.0% of patients in cohort 1 and 79.2% in cohort 2 had treatment-emergent adverse events (TEAEs) of grade 3 or higher. There were no drug-related TEAEs associated with death. Safety results in the exploratory HER2-low cohorts were consistent with those of the primary cohort, and overall, T-DXd demonstrated a manageable safety profile [35].

These studies showed that T-DXd had a specific therapeutic effect on patients with low expression of HER2 and may identify a new therapeutic target. It suggested that there was potential to redefine HER2 expression to enlarge the beneficiary population and that more rigorous and sensitive HER2 testing methods are needed. However, the sample size of this study was small, which needs to be supported by more research data. In the future, it is anticipated that the results of some clinical trials will pave the way for T-DXd to the therapy of HER2 low expression GC.

RC48

RC48 (Hertuzumab-VC-MMAE) is a new generation of ADC composed of humanized HER2-specific monoclonal antibody (hertuzumab) and microtubule inhibitor (sea rabbit toxin derivative MMAE) [36]. Hertuzumab has a higher affinity to other HER2-targeted antibodies, indicating a more effective binding [37]. Compared with trastuzumab, Hertuzumab has higher HER2-specific affinity and stronger antibody-dependent cell-mediated cytotoxicity (ADCC) activity *in vitro* [38]. RC48 shows a significant bystander effect with a cuttable linker, and the payload spread to adjacent cells [38,39]. MMAE plays a multifunctional role in inhibiting tubulin polymerization, leading to effective tumor regression [40]. Both *in vitro* and *in vivo* experiments confirmed that RC48 can selectively deliver MMAE to target tumor tissues. In general, RC48 exerts its anti-proliferation effect by blocking HER2 signal pathways such as PI3K/AKT/mTOR and MAPK signal pathways, induces cell cycle arrest in G2/M phase by microtubule depolymerization, and exerts its anti-tumor effect by inducing cell apoptosis [41]. Preclinical studies showed that in the NCI-N87 xenograft model, RC48-ADC had stronger anti-tumor activity than monotherapy of trastuzumab, hertuzumab, MMAE, and combination treatment of hertuzumab and MMAE [42]. A phase I study of RC48-ADC administered intravenously to subjects with HER2-positive in advanced malignant solid tumors (ClinicalTrials.gov identifier: NCT02881190) showed that RC48 had tolerable toxicity and significant efficacy in HER2-positive solid tumors, especially in GC with low HER2 expression. Among them, patients with GC (HER2:IHc2+/FISH- vs. IHc2+/FISH+ vs. IHC3+) had different therapeutic effects (ORR:35.7%vs.20%vs.13.6%), suggesting that RC48 also improved the survival benefit of patients with low expression of GC in HER2 [43]. A phase II study (ClinicalTrials.gov identifier: NCT03556345) showed that patients with advanced or metastatic HER2-positive gastric or gastroesophageal junction cancer demonstrated the desired results (ORR: 24.8%, mPFS: 4.1 months, mOS: 7.9 months). It is worth mentioning that, the ORR of RC48-ADC in patients with HER2 IHC2+/FISH- (1/6, 16.7%) was lower than the ORR that in HER2-positive patients (20/76, 26.3%) [44]. These studies showed that RC48 had significant anti-tumor activity and tolerable safety in patients with HER2+ GC, including those with HER2 low expression GC patients. Based on the above experimental results, RC48 had been conditionally listed by the State Cancer Administration of China in June 2021 for the treatment of locally advanced or metastatic GC (including gastroesophageal adenocarcinoma) [45,46].

ARX788

ARX788 is an anti-HER2 ADC composed of humanized HER2-targeted monoclonal antibodies, which showed superior anti-tumor effects in patient-derived xenograft breast cancer and GC models with low HER2 expression and trastuzumab emtansine-resistant [47]. Due to the off-target toxicity caused by the instability of the first-generation ADCs, their therapeutic effect was commonly limited. The new generation of site-specific anti-HER2 ADC ARX788 was developed to solve the problems encountered in the first generation of ADCs. ARX788 was commonly superior to T-DM1 in xenograft models with the high- and low-HER2 two subgroups and significantly inhibits tumor growth. Compared with T-DM1 through a group of cancer cell lines *in vitro*, ARX788 usually showed stronger activity *in vitro* and *in vivo* [47]. It is worth mentioning that ARX788 had no activity in normal cardiomyocytes. PDX model confirmed that ARX788 had strong anti-tumor activity in HER2 positive and HER2 low expression tumors and the T-DM1 drug resistance model. ARX788 treatment was well tolerated in all efficacy studies, and no significant weight loss was observed [48]. A Phase 1 multicenter, dose-expansion study (ChinaDrugTrials.org.cn: CTR20190639) showed that the confirmed ORR of ARX788 was 37.9%. The mPFS and mOS were 4.1 and 10.7 months, respectively. Only 13.3% of patients experienced grade 3 adverse events related to ARX788, and no grade 4 or 5 adverse events occurred. The study showed that ARX788 was well tolerated and associated with anti-tumor activity in participants with metastatic HER2-positive gastric/gastroesophageal junction adenocarcinoma [49]. Based on these encouraging results, ARX788 was awarded as an orphan drug by FDA in March 2021 for second-line treatment of HER2-positive GC and gastroesophageal junction cancer. A randomized controlled, open-label phase 2/3 study to evaluate the efficacy of ARX788 as second-line treatment with HER2-positive advanced G/GEJ adenocarcinoma globally is ongoing (Chinadrugtrials.org.cn: CTR20211583).

LCB-ADC

LCB-ADC (Biosciences-ADC) is a new type of HER2 targeting ADC composed of trastuzumab and monomethyl ADC. Its highlight is the well-designed cuttable connector. The ultimate goal of the connector is to allow effective drugs to be released to target cancer cells while maintaining a stable connection between antibodies and drugs during circulation. Since the efficacy and systemic toxicity of ADCs depend on the stability of the linker, it plays a vital role in the safety of ADC [50]. There are three members in the LCB-ADC family, which can be divided into LCB-ADC1, LCB-ADC2, and LCB-ADC3, according to the different linker types. The linker of LCB-ADC1 is PEG-3, and the drug-to-antibody ratio (DAR) is 2. The linkers of LCB-ADC2 and LCB-ADC3 are PEG-3, 3, 3 and PEG-6, 6, 3, and their DRA are both 4. The payloads of LCB-ADC1, LCB-ADC2, and LCB-ADC3 are all MMAF. Compared with T-DM1, LCB-ADC contains a cuttable connector that aims to achieve higher efficacy through various mechanisms. At the same time, LCB-ADC showed a higher cytotoxic potency than T-DM1 and had a higher rate of G2/M phase arrest [51]. In animal studies, LCB-ADC significantly inhibited tumor growth in N87 xenograft models with high expression of HER2 compared with trastuzumab or T-DM1. In particular, LCB-ADC showed an excellent inhibitory effect on tumor growth in a PDX model of HER2-positive GC and T-DM1-resistant models such as HER2 low-expressing JIMT-1 xenograft tumor and PDX. PDX models prepared with human GC tissues treated with T-DM1 or LCB-ADCs showed that tumor growth was inhibited by 103%, 99%, and 111% by T-DM1, LCB-ADC1, and LCB-ADC2, respectively [51]. It is noteworthy that T-DM1 did not have any effect in the HER (2+) GC PDX model. Moreover, LCB-ADCs displayed robust therapeutic efficacy and showed sufficient antitumor potency.

The results showed that LCB-ADC with a well-designed linker had higher efficacy and more excellent biological stability than its ADC counterpart. It showed promising potential for treating GC with low HER2 expression, which needs further study. LCB-ADC1 is being investigated in phase 1 clinical trial (ClinicalTrials.gov identifier: NCT03944499).

MRG002

MRG002 comprises a humanized anti-HER2 IgG1 mAb, a valine-citrulline linker, and the microtubule disrupting MMAE, and the DAR is 3.8 [52]. MRG002 showed an antigen-binding affinity similar to trastuzumab, but the ADCC activity was significantly decreased [52,53]. MRG002 showed tumor regression in both high- and low-expression HER2 *in vivo* xenograft models [53]. In preclinical studies, MRG002 showed anti-tumor solid activity in breast and stomach xenograft models with different levels of HER2 expression. In the mouse xenograft models, the efficacy of MRG002 was also better than that of trastuzumab and T-DM1. In addition, the combination of MRG002 and anti-PD-1 antibody enhanced the anti-tumor activity significantly [53]. Phase I studies of MRG002 as a single drug, are underway in patients with recurrent/refractory solid tumors, including breast, gastric, and salivary adenocarcinomas (Chinadrugtrials.org.cn: CTR20181778 and ClinicalTrials.gov identifier: NCT04941339). At the same time, various phase II trials were studying the efficacy of MRG002 in treating multiple malignant tumors with positive HER2 or low HER2. For example, a study of MRG002 in treating HER2-positive/HER2-low locally advanced or metastatic gastric/gastroesophageal junction cancer is ongoing (ClinicalTrials.gov identifier: NCT05141747). These studies are expected to bring hope to MRG002 treating GC patients with low HER2 expression.

PF-06804103

PF-06804103 is an anti-HER2 ADC that combines new design elements. It has a broader treatment window that improved its efficacy profiles compared to T-DM1. PF-06804103 has a protease cleavable valine-citrulline linker with an auristatin analog, Auristatin-0101, to maximize payload delivery efficiency. This linker-payload combination allows PF-06804103 to kill HER2-expressing cells and neighboring cells regardless of HER2 expression. Studies have shown that PF-06804103 had broader anti-tumor activity than T-DM1 in a group of cell-line xenografts and PDXs with low and heterogenous HER2 expression patterns [54]. However, PF06804103 treatment led to persistent tumor regression, which continued even after stopping administration. This showed that PF-06804103 can still maintain its efficacy in relapsed or refractory T-DM1. The N87 tumor-bearing mice were treated with PT-DM1 every week until the tumor recurred, and the treatment was switched to PF-06804103. After the first two doses of PF-06804103, all recurrent PT-DM1 tumors subsided and shrunk throughout PF-06804103 treatment. Therefore, PF-06804103 overcomes the resistance to PT-DM1 and could be an effective method for the treatment of patients with recurrent or refractory T-DM1. To verify the effectiveness of PF06804103 on tumors expressed by HER2, the *in vivo* activity of PF-06804103 was evaluated in a group of gastric tissues expressing HER2. Moreover, the study showed that compared with PT-DM1, PF-06804103 caused tumor regression continuously. In the waterfall analysis of a set of test models, the PDX model group of GC showed that the ORR values of PF-06804103 and PT-DM1 were 3/3 (100%) and 0/3 (0%) [54]. PF-06804103 showed promising *in vivo* activity in the non-breast PDX model and might provide clinical benefits in the indication of GC expressing HER2.

In summary, PF-06804103 enhanced the efficacy of HER2-positive GC models, including PDX with low and heterologous HER2 expression patterns. The drug resistance of T-DM1 obtained *in vitro* and *in vivo* was overcome, the safety was improved, and the toxicity was reduced by enhancing the stability of ADC. PF-06804103 is currently conducting a clinical study (ClinicalTrials.gov identifier: NCT03284723).

HER2 evaluation in gastric cancer

Recently, the clinical guidelines considered HER2 weak express and incomplete staining as HER2 negative [17,18]. The existing HER-2 detection methods(IHC, ISH, and FISH) can not quantify HER2 expression. Some patients with very low HER2 expression (<10% of tumor cells) may be considered HER2 negative, which results in these patients not being eligible candidates for valuable treatment options. Therefore, more rigorous and sensitive HER2 detection methods are needed in the future, such as standardizing and quantifying the HER2 expression.

To address those issues, several novel strategies have been developed for HER-2 evaluation in recent years. For example, chromogenic ISH (CISH), with less time-consuming, less expensive, and easier to identify invasive components, was developed as an alternative assay for FISH [55,56]. It uses a peroxidase enzyme-labeled probe for chromogenic detection using diaminobenzidine [55]. Meanwhile, some new methods (HER2-V2, MLPA, NGS, RISH) are also in clinical trials and have yet to be approved by FDA [57–61]. They showed good superiority: more accurate patient stratification, fast and inexpensive, need a tiny amount of DNA, and identify mutations in HER2 segments [59–63].

Conclusion

This review summarized the related clinical trials of ADCs for HER2-low expression GC and various coupling/ligation strategies for constructing this new class of molecules (Table 3). As a backbone therapy for patients with HER2+ GC, ADCs have attracted much attention, even becoming the hot spot of great research and providing an empirical basis for anti-HER2 therapeutics. In the future, with more advanced HER2 detection technology, ADCs will provide a better prognosis for HER2-low GC patients.

Translational oncology

The following information is required for submission. Please note that failure to respond to these questions/statements will mean your submission will be returned . If you have nothing to declare in any of these categories then this should be stated.

Please state any conflicts of interest

All authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

There are no conflicts of interest.

Please state any sources of funding for your research

All sources of funding should be declared as an acknowledgement at the end of the text . Authors should declare the role of study sponsors, if any, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

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Consent

Studies on patients or volunteers require ethics committee approval and fully informed written consent which should be documented in the paper.

Authors must obtain written and signed consent to publish the case report from the patient (or, where applicable, the patient's guardian or next of kin) prior to submission. We ask Authors to confirm as part of the submission process that such consent has been obtained, and the manuscript must include a statement to this effect in a consent section at the end of the manuscript, as follows: "Written informed consent was

Table 3
Selected ADCs in the review.

Name	Target	mAb	Linker	Payload	Efficacy
DS-8201a	HER2	IgG1 mAb	Cleavable tetrapeptide linker	DXd	mORR: 51%; mOS: 12.5 months
RC48	HER2	Hertuzumab	Cleavable dipeptide linker	MMAE	mORR: 24.8%; mPFS: 4.1 months; mOS: 7.9 months
PF-06804103	HER2	Anti-HER2 mAb	Valine-citrulline linker ('vc0101')	MMAE	–
ARX-788	HER2	Anti-HER2 mAb (ARX269)	Non-cleavable linker conjugated to pAcF	MMAF	ORR: 37.9%; mPFS: 4.1 months; mOS:10.7 months;
LCB-ADC	HER2	Anti-HER2 mAb	Cleavable PEG linker	MMAF	–
MRG002	HER2	Anti-HER2 mAb	Cleavable vc linker	MMAE	–

mAb, monoclonal antibody; mORR, median objective response rate; mOS, median overall survival; mPFS, median progression-free survival;ORR, objective response rate.

obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request".

Patients have a right to privacy. Patients' and volunteers' names, initials, or hospital numbers should not be used. Images of patients or volunteers should not be used unless the information is essential. for scientific purposes and explicit permission has been given as part of the consent. If such consent is made subject to any conditions, the Editor in Chief must be made aware of all such conditions.

Even where consent has been given, identifying details should be omitted if they are not essential. If identifying characteristics are altered to protect anonymity, such as in genetic pedigrees, authors should provide assurance that alterations do not distort scientific meaning and editors should so note.

No clinical trials or animal studies were conducted.

CRediT authorship contribution statement

Minghui Yu: Conceptualization, Writing – original draft, Writing – review & editing, Methodology, Formal analysis. **Yangyueying Liang:** Writing – review & editing, Methodology, Formal analysis. **Longhui Li:** Writing – review & editing, Methodology, Formal analysis. **Lu Zhao:** Writing – review & editing, Methodology, Formal analysis. **Fanming Kong:** Conceptualization, Writing – review & editing, Writing – original draft.

Declaration of Competing Interest

There are no conflicts of interest.

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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References

[1] H. Sung, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 71 (3) (2021) 209–249.
[2] S.K. Clinton, et al., The world cancer research fund/american institute for cancer research third expert report on diet, nutrition, physical activity, and cancer: impact and future directions, J. Nutr. 150 (4) (2020) 663–671.
[3] C. Gravalos, A. Jimeno, HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target, Ann. Oncol. 19 (9) (2008) 1523–1529.
[4] M. Tanner, et al., Amplification of HER-2 in gastric carcinoma: association with Topoisomerase IIalpha gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab, Ann. Oncol. 16 (2) (2005) 273–278.
[5] Y.J. Bang, et al., Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial, Lancet 376 (9742) (2010) 687–697.
[6] M. London, E. Gallo, Epidermal growth factor receptor (EGFR) involvement in epithelial-derived cancers and its current antibody-based immunotherapies[J], Cell Biol. Int. 44 (6) (2020) 1267–1282.
[7] J. Yu, et al., Antibody-drug conjugates targeting the human epidermal growth factor receptor family in cancers, Front. Mol. Biosci. 9 (2022), 847835.
[8] V. Oganesyan, et al., Structural insights into the mechanism of action of a biparatopic anti-HER2 antibody, J. Biol. Chem. 293 (22) (2018) 8439–8448.
[9] H. Ushiro, S. Cohen, Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A-431 cell membranes, J. Biol. Chem. 255 (18) (1980) 8363–8365.
[10] A.B. Schreiber, T.A. Libermann, I. Lax, Y. Yarden, J. Schlessinger, Biological role of epidermal growth factor-receptor clustering. Investigation with monoclonal anti-receptor antibodies, J. Biol. Chem. 258 (2) (1983) 846–853.
[11] I. Chung, R. Akita, R. Vandlen, D. Toomre, J. Schlessinger, I. Mellman, Spatial control of EGF receptor activation by reversible dimerization on living cells, Nature 464 (7289) (2010) 783–787.
[12] A. Arkhipov, Y. Shan, E.T. Kim, R.O. Dror, D.E. Shaw, Her2 activation mechanism reflects evolutionary preservation of asymmetric ectodomain dimers in the human EGFR family, Elife 2 (2013) e00708.
[13] H.M. Shepard, G.D. Lewis, J.C. Sarup, B.M. Fendly, D. Maneval, J. Mordenti, I. Figari, C.E. Kotts, M.A. Palladino Jr, A Ullrich, Monoclonal antibody therapy of human cancer: taking the HER2 protooncogene to the clinic, J. Clin. Immunol. 11 (3) (1991) 117–127.
[14] J. Watanabe, et al., Safety evaluation of trastuzumab emtansine in japanese patients with HER2-positive advanced breast cancer, In vivo 31 (3) (2017) 493–500 (Brooklyn).
[15] C. Gravalos, A. Jimeno, HER-2 in gastric cancer: a new prognostic factor and a novel therapeutic target, Ann. Oncol. 19 (9) (2008) 1523–1529.
[16] Y. Yonemura, et al., Evaluation of immunoreactivity for erbB-2 protein as a marker of poor short term prognosis in gastric cancer, Cancer Res. 51 (3) (1991) 1034–1038.
[17] A.N. Bartley, et al., HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: guideline from the college of american pathologists, american society for clinical pathology, and the american society of clinical oncology, J. Clin. Oncol. 35 (4) (2017) 446–464.
[18] J.A. Ajani, et al., Gastric cancer, version 2.2022, NCCN clinical practice guidelines in oncology, J. Natl. Compr. Cancer Netw. 20 (2) (2022) 167–192.
[19] C.B. Moelans, A.N. Milne, F.H. Morsink, et al., Low frequency of HER2 amplification and overexpression in early onset gastric cancer, Cell. Oncol. 34 (2011) 89–95 (Dordr).
[20] C.L. Sawyers, Herceptin: a first assault on oncogenes that launched a revolution, Cell 179 (1) (2019) 8–12.
[21] A. Woelderink, D. Ibarreta, M.M. Hopkins, E. Rodriguez-Cerezo, The current clinical practice of pharmacogenetic testing in Europe: TPMT and HER2 as case studies, Pharmacogenomics J. 6 (1) (2006) 3–7.
[22] M. Abdollahpour-Alitappeh, M. Lotfinia, T. Gharibi, J. Mardaneh, B. Farhadihosseinabadi, P. Larki, B. Faghfourian, K.S. Sepehr, K. Abbaszadeh-Goudarzi, G. Abbaszadeh-Goudarzi, et al., Antibody-drug conjugates (ADCs) for cancer therapy: strategies, challenges, and successes, J. Cell. Physiol. 234 (2019) 5628–5642.
[23] J. Lu, F. Jiang, A. Lu, G. Zhang, Linkers having a crucial role in antibody-drug conjugates, Int. J. Mol. Sci. 17 (4) (2016) 561.

[24] Y. Jin, S. Edalatian Zakeri, R. Bahal, A.J. Wiemer, New technologies bloom together for bettering cancer drug conjugates, Pharmacol. Rev. 74 (3) (2022) 680–711.
[25] V. Kostova, et al., The Chemistry Behind ADCs, Pharmaceuticals 14 (5) (2021), 442.
[26] S. Yaghoubi, M.H. Karimi, M. Lotfinia, T. Gharibi, M. Mahi-Birjand, E. Kavi, F. Hosseini, K. Sineh Sepehr, M. Khatami, N. Bagheri, M. Abdollahpour-Alitappeh, Potential drugs used in the antibody-drug conjugate (ADC) architecture for cancer therapy, J. Cell. Physiol. 235 (1) (2020) 31–64.
[27] J. Anderl, H. Faulstich, T. Hechler, M. Kulke, Antibody-drug conjugate payloads, Methods Mol. Biol. 1045 (2013) 51–70.
[28] A. Indini, E. Rijavec, F. Grossi, Trastuzumab deruxtecan: changing the destiny of her2 expressing solid tumors, Int. J. Mol. Sci. 22 (9) (2021) 4774.
[29] Y. Ogitani, et al., DS-8201a, A Novel HER2-targeting ADC with a novel DNA topoisomerase I inhibitor, demonstrates a promising antitumor efficacy with differentiation from T-DM1, Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 22 (20) (2016) 5097–5108.
[30] Y. Ogitani, K. Hagihara, M. Oitate, H. Naito, T. Agatsuma, Bystander killing effect of DS-8201a, a novel anti-human epidermal growth factor receptor 2 antibody-drug conjugate, in tumors with human epidermal growth factor receptor 2 heterogeneity, Cancer Sci. 107 (7) (2016) 1039–1046.
[31] K. Shitara, Y.J. Bang, S. Iwasa, N. Sugimoto, M.H. Ryu, D. Sakai, H.C. Chung, H. Kawakami, H. Yabusaki, J. Lee, K. Saito, Y. Kawaguchi, T. Kamio, A. Kojima, M. Sugihara, K. Yamaguchi, DESTINY-Gastric01 Investigators, Trastuzumab deruxtecan in previously treated HER2-positive gastric cancer, New Engl. J. Med. 382 (25) (2020) 2419–2430.
[32] K. Shitara, E. Baba, K. Fujitani, E. Oki, S. Fujii, K. Yamaguchi, Discovery and development of trastuzumab deruxtecan and safety management for patients with HER2-positive gastric cancer, Gastric Cancer Off. J. Int. Gastric Cancer Assoc. Jpn. Gastric Cancer Assoc. 24 (4) (2021) 780–789.
[33] K. Shitara, H. Iwata, S. Takahashi, K. Tamura, H. Park, S. Modi, J. Tsurutani, S. Kadowaki, K. Yamaguchi, S. Iwasa, K. Saito, Y. Fujisaki, M. Sugihara, J. Shahidi, T. Doi, Trastuzumab deruxtecan (DS-8201a) in patients with advanced HER2-positive gastric cancer: a dose-expansion, phase 1 study, Lancet Oncol. 20 (6) (2019) 827–836.
[34] K. Yamaguchi, et al., Trastuzumab deruxtecan (T-DXd; DS-8201) in patients with HER2-low, advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma: results of the exploratory cohorts in the phase II, multicenter, open-label DESTINY-Gastric01 study, Ann. Oncol. 31 (2020) S899–S900.
[35] K. Yamaguchi, et al., Trastuzumab deruxtecan in anti-human epidermal growth factor receptor 2 treatment-naive patients with human epidermal growth factor receptor 2-low gastric or gastroesophageal junction adenocarcinoma: exploratory cohort results in a phase II trial, J. Clin. Oncol. (2022). JCO2200575. Advance online publication.
[36] Y. Xu, et al., Phase I study of the recombinant humanized anti-HER2 monoclonal antibody-MMAE conjugate RC48-ADC in patients with HER2-positive advanced solid tumors, Gastric Cancer Off. J. Int. Gastric Cancer Assoc. Jpn. Gastric Cancer Assoc. 24 (4) (2021) 913–925.
[37] G. Rinnerthaler, S.P. Gampenrieder, R. Greil, HER2 directed antibody-drug-conjugates beyond T-DM1 in breast cancer, Int. J. Mol. Sci. 20 (5) (2019) 1115.
[38] H. Li, et al., An anti-HER2 antibody conjugated with monomethyl auristatin E is highly effective in HER2-positive human gastric cancer, Cancer Biol. Ther. 17 (4) (2016) 346–354.
[39] X. Yao, J. Jiang, X. Wang, C. Huang, D. Li, K. Xie, et al., A novel humanized Anti-HER2 antibody conjugated with MMAE exerts potent anti-tumor activity, Breast Cancer Res. Treat. 153 (1) (2015) 123–133.
[40] N. Wang, Q. Mei, Z. Wang, L. Zhao, D. Zhang, D. Liao, J. Zuo, H. Xie, Y. Jia, F. Kong, Research progress of antibody-drug conjugate therapy for advanced gastric cancer, Front. Oncol. 12 (2022), 889017.
[41] Z. Chen, J. Yuan, Y. Xu, C. Zhang, Z. Li, J. Gong, Y. Li, L. Shen, J. Gao, From AVATAR mice to patients: RC48-ADC exerted promising efficacy in advanced gastric cancer with HER2 expression, Front. Pharmacol. 12 (2022), 757994.
[42] H. Li, C. Yu, J. Jiang, C. Huang, X. Yao, Q. Xu, An Anti-HER2 antibody conjugated with monomethyl Auristatin E Is highly effective in HER2-positive human gastric cancer, Cancer Biol. Ther. 17 (2016) 346–354.
[43] Y. Xu, Y. Wang, J. Gong, et al., Phase I study of the recombinant humanized anti-HER2 monoclonal antibody-MMAE conjugate RC48-ADC in patients with HER2-positive advanced solid tumors, Gastric Cancer 24 (2021) 913–925.
[44] Z. Peng, T. Liu, J. Wei, A. Wang, Y. He, L. Yang, et al., Efficacy and safety of a novel anti-HER-2 therapeutic antibody RC48 in patients with HER2-overexpressing, locally advanced or metastatic gastric or gastroesophageal junction cancer: a single-arm phase II study, Cancer Commun. 41 (11) (2021) 1173–1182.
[45] Z. Peng, T. Liu, J. Wei, A. Wang, Y. He, L. Yang, et al., Efficacy and safety of a novel anti-HER2 therapeutic antibody RC48 in patients with HER2-overexpressing, locally advanced or metastatic gastric or gastroesophageal junction cancer: a single-arm phase II study, Cancer Commun. 41 (11) (2021) 1173–1182.
[46] E.D. Deeks, Disitamab vedotin: first approval, Drugs 81 (16) (2021) 1929–1935.
[47] M. Barok, V. Le Joncour, A. Martins, J. Isola, M. Salmikangas, P. Laakkonen, H. Joensuu, ARX788, a novel anti-HER2 antibody-drug conjugate, shows anti-tumor effects in preclinical models of trastuzumab emtansine-resistant HER2-positive breast cancer and gastric cancer, Cancer Lett. 473 (2020) 156–163.
[48] L. Skidmore, S. Sakamuri, N.A. Knudsen, A.G. Hewet, S. Milutinovic, W. Barkho, S. L. Biroc, J. Kirtley, R. Marsden, K. Storey, I. Lopez, W. Yu, S.Y. Fang, S. Yao, Y. Gu, F. Tian, ARX788, a Site-specific Anti-HER2 antibody-drug conjugate, demonstrates potent and selective activity in HER2-low and T-DM1-resistant Breast and gastric cancers, Mol. Cancer Ther. 19 (9) (2020) 1833–1843.
[49] Y. Zhang, et al., Phase 1 multicenter, dose-expansion study of ARX788 as monotherapy in HER2-positive advanced gastric and gastroesophageal junction adenocarcinoma, Cell Rep. Med. 3 (11) (2022), 100814.
[50] A. Beck, et al., The next generation of antibody-drug conjugates comes of age, Discov. Med. 10 (53) (2010) 329–339.
[51] S.H. Shin, Y.H. Park, S.S. Park, E.J. Ju, J. Park, E.J. Ko, D.J. Bae, S.Y. Kim, C. W. Chung, H.Y. Song, S.J. Jang, S.Y. Jeong, S.Y. Song, E.K. Choi, An elaborate new linker system significantly enhances the efficacy of an HER2-antibody-drug conjugate against refractory HER2-positive cancers, Adv. Sci. 8 (23) (2021), e2102414 (Weinh).
[52] H. Li, et al., Preclinical evaluation of MRG002, a novel HER2-targeting antibody-drug conjugate with potent antitumor activity against HER2-positive solid tumors, Antib. Ther. 4 (3) (2021) 175–184.
[53] J. Yu, T. Fang, C. Yun, X. Liu, X. Cai, Antibody-drug conjugates targeting the human epidermal growth factor receptor family in cancers, Front. Mol. Biosci. 9 (2022), 847835.
[54] Graziani, et al., PF-06804103, a site-specific Anti-HER2 antibody-drug conjugate for the treatment of HER2-expressing breast, gastric, and lung cancers, Mol. Cancer Ther. 19 (10) (2020) 2068–2078.
[55] F. Penault-Llorca, M. Bilous, M. Dowsett, W. Hanna, R.Y. Osamura, J. Rüschoff, M van de Vijver, Emerging technologies for assessing HER2 amplification, Am. J. Clin. Pathol. 132 (4) (2009) 539–548.
[56] M.B. Lambros, R. Natrajan, J.S. Reis-Filho, Chromogenic and fluorescent *in situ* hybridization in breast cancer, Hum. Pathol. 38 (8) (2007) 1105–1122.
[57] D.E. Tacha, et al., A multi-method analysis of c-erbB2/HER2 in FFPE tissue: correlation of a novel and rapid *in situ* hybridization procedure, HER2 CISH and IHC, Cancer Res. 69 (24) (2009) 826s. -826s.
[58] M. Moutafi, C.J. Robbins, V. Yaghoobi, A.I. Fernandez, S. Martinez-Morilla, V. Xirou, Y. Bai, Y. Song, P. Gaule, J. Krueger, K. Bloom, S. Hill, D.C. Liebler, R. Fulton, D.L. Rimm, Quantitative measurement of HER2 expression to subclassify ERBB2 unamplified breast cancer, Lab. Investig. 102 (10) (2022) 1101–1108.
[59] Z. Su, B. Ning, H. Fang, H. Hong, R. Perkins, W. Tong, L. Shi, Next-generation sequencing and its applications in molecular diagnostics, Expert Rev. Mol. Diagn. 11 (3) (2011) 333–343.
[60] M.L. Metzker, Sequencing technologies - the next generation, Nat. Rev. Genet. 11 (1) (2010) 31–46.
[61] D. Lipson, Next-generation sequencing of FFPE breast cancers demonstrates high concordance with FISH in calling HER2 amplifications and commonly detects other clinically relevant genomic alterations, Cancer Res. 72 (2012) 1730–1731.
[62] G.M. Frampton, et al., Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing, Nat. Biotechnol. 31 (11) (2013) 1023–1031.
[63] L. Bernet, et al., mRNA *in situ* hybridization (HistoSonda): a new diagnostic tool for HER2-status in breast cancer-a multicentric Spanish study, Diagn. Mol. Pathol. Am. J. Surg. Pathol. Part B 21 (2) (2012) 84–92.