Advances in the Application of Antibody-Drug Conjugates in HER2 Low-Expression Gastric Cancer (Postprint)

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Abstract

Gastric cancer (GC) is one of the most heterogeneous and aggressive malignant tumors of the digestive system. Traditional chemotherapeutic agents and human epidermal growth factor receptor 2 (HER2)-targeted drugs such as trastuzumab still have drawbacks in the treatment of GC, including high incidence of drug resistance, significant toxic side effects, and poor patient tolerance. Therefore, the development of more effective anti-GC drugs is imperative. Currently, novel HER2-targeted drugs are continuously being developed, but they are ineffective or develop resistance in some cases, which is related to low HER2 expression in certain GC cells. HER2-low expression (HER2 IHC 1+ or IHC 2+/ISH-) accounts for approximately 40%~60% of all types, but in clinical practice, such patients are still reported as HER2-negative GC. Therefore, accurate detection of HER2 expression status is crucial for identifying patients who may benefit from trastuzumab therapy. The emergence of antibody-drug conjugates (ADCs) has provided new therapeutic options for HER2-positive GC, and with their precise and efficient anti-tumor effects, they are expected to replace traditional GC chemotherapy in the future. Recent studies have found that ADCs may have potential anti-tumor activity in HER2-low expression GC, and relevant clinical trials are evaluating their efficacy and safety in the treatment of HER2-low expression GC. This article provides a review of the application and latest research progress of ADCs in HER2-low expression GC patients in the era of targeted therapy, and discusses the challenges faced by HER2-targeted ADCs in their application and development process.

Full Text

Advances in the Application of Antibody-Drug Conjugates in Gastric Cancer with Low HER2 Expression

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Abstract

Gastric cancer (GC) is one of the most heterogeneous and aggressive malignant tumors of the digestive system. Traditional chemotherapy drugs and HER2-targeted agents such as trastuzumab still suffer from high rates of drug resistance, significant toxic side effects, and poor patient tolerance. Therefore, developing more effective anti-GC drugs is imperative. While novel HER2-targeted therapies continue to emerge, they are often ineffective or induce resistance in some cases, which is related to low HER2 expression in certain GC cells. HER2 low expression (HER2 IHC 1+ or IHC 2+/ISH-) accounts for approximately 40%-60% of all GC types, yet these patients are still reported as HER2-negative in clinical practice. Accurate detection of HER2 expression status is thus crucial for identifying patients who may benefit from trastuzumab therapy. The emergence of antibody-drug conjugates (ADCs) provides a new therapeutic option for HER2-positive GC, and their precise and efficient antitumor effects may enable them to replace traditional chemotherapy in the future. Recent studies have found that ADCs may possess potential antitumor activity in HER2 low-expression GC, and relevant clinical trials are evaluating their efficacy and safety in this population. This review summarizes the application and latest research progress of ADCs in HER2 low-expression GC patients in the era of targeted therapy and discusses the challenges faced in the development and clinical application of HER2-targeted ADCs.

Keywords: Stomach neoplasms; Gastric cancer; Human epidermal growth factor receptor 2; Low HER2 expression gastric cancer; Antibody-drug conjugates; Molecular targeted therapy; Review

1 HER2 Low-Expression GC

Gastric cancer is a common malignant tumor of the digestive system. Although surgical and chemotherapeutic approaches have significantly prolonged survival in advanced GC patients, the overall prognosis remains poor. Human epidermal growth factor receptor 2 (HER2) plays an important role in the biological behavior and pathogenesis of advanced GC and represents a critical target in systemic therapy for advanced disease [1]. Located on chromosome 17, HER2 encodes a 185 kDa transmembrane protein whose dimerization leads to phosphorylation of tyrosine residues in the intracellular protein kinase domain, activating downstream MAPK and PI3K/Akt/mTOR pathways. This results in abnormal division, proliferation, differentiation, and anti-apoptotic signals in GC cells, promoting invasive growth [2]. Approximately 15%-20% of patients exhibit abnormal HER2 activation or overexpression, which is significantly associated with poor prognosis [3].

Although monoclonal antibodies (mAbs) or small-molecule targeted drugs can inhibit HER2 expression and improve overall survival, they are ineffective in some cases, reportedly due to low HER2 expression [2]. The ToGA study established trastuzumab as first-line therapy for advanced HER2-positive GC, yet subsequent clinical research on HER2-positive GC targeted therapy failed to achieve substantial progress until the emergence of ADCs, which represented another major breakthrough following ToGA. Furthermore, HER2-targeted ADCs combined with immunotherapy have demonstrated remarkable efficacy with manageable safety profiles in some advanced HER2-positive solid tumors [4], suggesting great potential for ADC-immunotherapy combinations in advanced GC. However, HER2 low-expression GC, as a special form of HER2-positive GC, is associated with suboptimal outcomes from HER2-targeted therapy due to lower HER2 copy numbers [5]. The GASTBY study found that trastuzumab emtansine (T-DM1) was not superior to paclitaxel in HER2 low-expression gastric/gastroesophageal junction (G/GEJ) cancer. Therefore, the need for effective targeted drugs for HER2 low-expression GC remains unmet, and treatment options are still limited.

The implementation of biomarker testing, particularly immunohistochemistry (IHC) and/or molecular detection of HER2/ERBB2 expression status, is crucial for determining therapeutic benefits in advanced GC patients. HER2 status is primarily assessed by IHC and fluorescence in situ hybridization (FISH) or in situ hybridization (ISH) [6], while real-time fluorescent quantitative PCR (qRT-PCR) offers a rapid, sensitive, and high-throughput method for HER2 gene amplification detection. Additionally, when tissue availability is limited or biopsy is not feasible, comprehensive genomic profiling via validated next-generation sequencing (NGS) under chemiluminescent immunoassay (CLIA) conditions can identify ERBB2 amplification [7]. According to guidelines from the College of American Pathologists, American Society of Clinical Pathology, and American Society of Clinical Oncology, HER2 negativity is defined as IHC score 0 or 1+, equivocal expression as IHC 2+, and positivity as IHC 3+. Patients with IHC

2+ should undergo further testing with FISH or ISH. Based on FISH+/ISH+ and FISH-/ISH- results, patients are classified as HER2-positive or -negative, respectively, with IHC 3+ or IHC 2+/ISH+ representing high HER2 expression and IHC 1+ or IHC 2+/ISH- representing low HER2 expression.

Unlike in breast cancer, the prognostic significance of HER2 expression levels in GC remains unclear. A retrospective analysis of 157 early-stage GC patients by YANG et al. [8] found that approximately 31.8% had HER2 low expression, which was more common in elderly patients (70% vs. 49.3%, P=0.021) and associated with higher Ki-67 indices, potentially facilitating tumor cell proliferation. KIM et al. [9] demonstrated that low HER2 amplification index (AI) was associated with trastuzumab resistance and poor prognosis. Compared to patients with HER2 AI <5, those with HER2 AI $ $5 showed significantly higher objective response rates (58.8% vs. 21.4%, P=0.002) and longer median progression-free survival (PFS: 9.5 vs. 4.0 months, P<0.001) and overall survival (OS: 20.8 vs. 11.1 months, P=0.001). Thus, low HER2 copy number correlates with high resistance rates and suboptimal outcomes from HER2-targeted therapy in GC. All patients should undergo HER2 status assessment before HER2-targeted therapy, with repeat testing considered in advanced or metastatic GC. Currently, no effective treatments exist for HER2 low-expression GC, highlighting the urgent need for novel drug development.

2 HER2-Targeted ADCs for HER2 Low-Expression GC

ADCs are molecularly targeted drugs based on macromolecules, primarily composed of a mAb, a linker, and a cytotoxic payload. The mAb selectively binds to antigens on target cells, delivering the payload precisely to cancer cells and triggering intracellular internalization to exert antitumor effects. Linkers are classified as cleavable or non-cleavable, serving to conjugate the toxin to the mAb while maintaining drug stability and preventing off-target toxicity [10]. The payload is the key component of ADCs, mediating antitumor effects by disrupting critical cellular processes [11]. Common payloads include microtubule inhibitors and DNA-damaging agents. Microtubule inhibitors disrupt microtubule assembly during mitosis, exhibiting relative selectivity for rapidly proliferating tumor cells. DNA-damaging agents induce nucleic acid strand breaks, alkylation, or cross-linking through DNA double-helix grooves to exert cytotoxic effects and inhibit tumor cell proliferation [12]. Other novel cytotoxic agents, such as RNA polymerase and splicing inhibitors, are under development.

By perfectly combining the specificity of mAbs with the potency of cytotoxic drugs, ADCs offer precise tumor-killing properties and reduced off-target tissue toxicity compared to conventional small-molecule chemotherapeutics. Therefore, ADCs represent a major focus in future antitumor therapy, with broad prospects in novel target drug design, next-generation ADC development, and combination strategies. As one of the fastest-growing antitumor agents, trastuzumab deruxtecan (T-DXd) achieved an objective response rate (ORR) of 51.3% as third-line therapy in GC patients, with median OS exceeding

12 months, making it the first ADC approved for advanced HER2-positive GC. In China, based on the C008 study, the National Medical Products Administration approved RC48 for third-line or later treatment of advanced HER2-overexpressing GC in June 2021, filling the gap in post-line anti-HER2 therapy for advanced GC [13].

3 Application and Latest Research Progress of ADCs in HER2 Low-Expression GC

With improvements in site-specific conjugation technology, discovery of novel payloads, and breakthroughs in antibody modification, HER2-positive GC patients continue to benefit from ADC therapy. As of 2022, three HER2-targeted ADCs have demonstrated efficacy in GC treatment. Numerous clinical studies have found that T-DXd and RC48 may have potential therapeutic effects in HER2 low-expression GC (Table 1). Meanwhile, multiple HER2-targeted ADCs are in various stages of clinical investigation (Table 2), with some showing potential for treating HER2 low-expression GC.

3.1 T-DXd and RC48 as Promising Therapies for HER2 Low-Expression GC

3.1.1 T-DXd T-DXd is a second-generation ADC composed of trastuzumab conjugated via a tetrapeptide linker to deruxtecan (DXd), a novel DNA topoisomerase I inhibitor. Trastuzumab can modulate topoisomerase I expression in extracellular vesicles released by HER2-positive tumor cells, and the combination of DXd with trastuzumab demonstrates potential antitumor effects. The cleavable tetrapeptide linker confers a significant "bystander effect," enhancing tumor cell killing. With a high drug-to-antibody ratio (DAR) of approximately 8 and a short half-life, T-DXd ensures efficient delivery of DXd to HER2-overexpressing cells, significantly increasing antibody-dependent cellular cytotoxicity (ADCC) while minimizing systemic exposure and off-target toxicity [14]. Additionally, DXd' s high membrane permeability enables T-DXd to exert ADCC effects on tumor cells adjacent to target cells regardless of HER2 expression levels. In patient-derived xenograft (PDX) models, T-DXd significantly inhibited the growth of HER2 low-expression tumors and HER2-positive tumors resistant to trastuzumab or T-DM1 [15-16], demonstrating good activity and significant antitumor effects in HER2 low-expression cells.

DESTINY-Gastric01 is a multicenter, open-label phase II study (NCT03329690) [17] evaluating T-DXd as third-line or later therapy for HER2-positive G/GEJ cancer. The main cohort included patients with high HER2 expression (IHC 3+ or IHC 2+/ISH+) who had received prior trastuzumab, while exploratory cohorts enrolled HER2 low-expression patients without prior trastuzumab (Cohort 1: IHC 2+/ISH-; Cohort 2: IHC 1+). Data presented at the 2020 ESMO annual meeting showed that in the HER2 low-expression exploratory cohorts, Cohort 1 achieved an ORR of 36.8%, median duration of response (DOR) of 7.6

months, median OS of 7.8 months, and median PFS of 4.4 months. Cohort 2 showed an ORR of 19%, median DOR of 12.5 months, median OS of 8.5 months, and median PFS of 2.8 months. Grade 3 or higher treatment-emergent adverse events (TEAEs) occurred in 70.0% of patients in Cohort 1 and 79.2% in Cohort 2. The safety profile in exploratory cohorts was consistent with the main cohort, and overall, T-DXd demonstrated manageable safety [19]. These results indicate that T-DXd is effective in HER2 low-expression GC and may represent a new therapeutic target. Building on its excellent efficacy in advanced HER2-positive GC, the EPOC2003 study will explore T-DXd monotherapy in the neoadjuvant setting for HER2-positive GC patients, including a main cohort (HER2-positive: IHC 3+ or IHC 2+/ISH+) and an exploratory cohort (HER2 low-expression: IHC 1+ or IHC 2+/ISH- and HER2-ECD >11.6 g/L), potentially providing new guidance for perioperative use in HER2 low-expression GC [20].

3.1.2 RC48 RC48 is a third-generation ADC composed of humanized hertuzomab conjugated via a maleimidocaproyl-valine-citrulline-p-aminobenzylcarbamate (MC-Val-Cit-PABC-PNP) linker to monomethyl auristatin E (MMAE), a microtubule protein inhibitor. Hertuzomab exhibits higher HER2 affinity and stronger ADCC activity compared to trastuzumab. After internalization, RC48 selectively delivers MMAE to target cells [21], exerting antiproliferative effects by blocking HER2 signaling and related pathways such as PI3K/AKT/mTOR and MAPK, inducing G2/M cell cycle arrest through microtubule depolymerization, and triggering apoptosis. The enzymatic cleavability of MC-Val-Cit-PABC-PNP enables RC48 to kill non-targeted cancer cells, demonstrating stronger ADCC activity in cell line-derived xenograft (CDX) models of trastuzumab- and lapatinib-resistant tumors [22].

RC48 also inhibits HER2 low-expression GC. LI et al. [23] treated a human CDX model established from NCI-N87 cells with RC48 and found it exhibited stronger anticancer activity than trastuzumab, hertuzomab, MMAE monotherapy, or hertuzomab combined with MMAE. JIN et al. [24] treated HER2 low-expression cell lines with different RC48 doses and found that RC48 had 48-hour and 72-hour IC50 values of 9.449 mg/L and 6.056 mg/L, respectively, against MGC803 cells, and 48-hour IC50 values of 24.20 mg/L, 331.90 mg/L, and 35.32 mg/L against MKN7, MKN45, and HGC27 cells, respectively, with 72-hour IC50 values of 66.48 mg/L, 63.44 mg/L, and 26.43 mg/L. Additionally, some HER2 low-expression patients achieved partial response (PR) after RC48 treatment.

A phase I study (NCT02881190) [25] of RC48 in subjects with HER2-positive advanced solid tumors demonstrated that RC48 was effective and had a manageable safety profile in HER2 low-expression GC. Furthermore, RC48 efficacy (ORR: 35.7% vs. 20% vs. 13.6%) correlated negatively with HER2 expression levels (HER2: IHC 2+/FISH- vs. IHC 2+/FISH+ vs. IHC 3+). However, XU et al. [26] found that RC48 treatment outcomes in HER2 IHC 2+/FISH- patients (ORR 35.7%) were similar to those in IHC 2+/FISH+ (ORR 20%) and IHC

3+ (ORR 13.6%) patients. Another phase II study (NCT03556345) reported similar results, showing significant RC48 efficacy in progressive or metastatic HER2-positive G/GEJ cancer (ORR: 24.8%; median PFS: 4.1 months; median OS: 7.9 months). Importantly, RC48 was also effective in HER2 low-expression (IHC 2+/FISH-) patients (ORR: 16.7%) [21]. These studies demonstrate significant antitumor activity of RC48 in HER2-positive GC patients, including those with HER2 low expression. Based on these results, the China Food and Drug Administration approved RC48 in June 2021 for treating locally advanced or metastatic G/GEJ cancer patients with HER2 overexpression who have received at least two prior systemic chemotherapies [27]. Additional studies are exploring RC48 combined with tegafur for first-line treatment of advanced HER2 low-expression (IHC 2+/ISH-) GC and RC48 combined with PD-1 inhibitors and capecitabine in neoadjuvant GC therapy.

3.2 Latest Research Progress of HER2-Targeted ADCs in HER2 Low-Expression GC

3.2.1 ARX788 ARX788 comprises a humanized trastuzumab conjugated to the microtubule protein inhibitor Amberstatin (AS269) using non-natural amino acid site-specific conjugation technology, resulting in strong drug homogeneity but a relatively low DAR of approximately 2. After entering tumor cells, ARX788 releases Amberstatin to inhibit mitosis, induce cell cycle arrest, and cause cell death. The non-cleavable linker conjugated to p-acetylphenylalanine (pAcF) overcomes the off-target toxicity of previous-generation ADCs [31].

SKIDMORE et al. [32] evaluated ARX788 in GC PDX models, finding that multiple treatments with 2-3 mg/kg ARX788 significantly inhibited tumor cell proliferation in four HER2 low-expression GC PDX models. Although ARX788' s non-cleavable linker and poor membrane permeability of the payload limit its bystander effect and antitumor activity, ARX788 induced tumor regression in HER2 low-expression PDX models, with uniform HER2 staining observed in tumor cell populations by IHC. Consistent with in vitro data, aberrant HER1 expression on IHC 2+ cells may enable ARX788 to release sufficient payload to kill tumor cells. Therefore, ARX788 is a homogeneous, stable, and effective ADC that may be effective against HER2 low-expression GC. BAROK et al. [31] found that ARX788 inhibited the growth of trastuzumab-resistant GC cells and showed stronger anticancer activity than T-DM1 in both HER2 high-expression and low-expression PDX subgroups. Notably, ARX788 showed no activity in normal cardiomyocytes. SKIDMORE et al. [32] also demonstrated ARX788' s antitumor activity in HER2 low-expression GC and T-DM1-resistant PDX models, with good tolerability and no significant weight loss observed in mice. These preclinical studies provide data support and theoretical basis for subsequent clinical research on this novel HER2-targeted ADC.

In a phase II multicenter dose-expansion study (CTR20190639) [33], ARX788 treatment achieved an ORR of 37.9%, with median PFS and OS of 4.1 months and 10.7 months, respectively. Only 13.3% of patients experienced ARX788-

related grade 3 adverse events (AEs). Thus, ARX788 demonstrates good efficacy and safety in locally advanced/metastatic HER2-positive G/GEJ cancer patients regardless of HER2 expression level. Based on these findings, ARX788 received FDA orphan drug designation in 2021 for second-line treatment of HER2-positive G/GEJ cancer (including HER2 low expression). The global ACE-Gastric-02 phase II/III clinical study (CTR20211583) [34] of ARX788 in HER2-positive G/GEJ cancer patients is ongoing and expected to yield promising results.

3.2.2 LCB-ADC (LegoChem Biosciences-ADC) LCB-ADC is a novel class of HER2-targeted ADCs composed of trastuzumab and monomethyl auristatin F (MMAF) connected through an elaborately designed cleavable linker. The key feature is the novel cleavable linker, which allows precise release of the cytotoxic payload to targeted tumor cells while maintaining stable connection between antibody and drug in circulation. The efficacy and safety of LCB-ADC are critically important [35]. LCB-ADCs are categorized into LCB-ADC1, LCB-ADC2, and LCB-ADC3 based on different linkers. LCB-ADC1 uses a polyethylene glycol-3 (PEG-3) linker with a DAR of 2, while LCB-ADC2 and LCB-ADC3 use PEG-3,3,3 and PEG-6,6,3 linkers, respectively, both with a DAR of 4. All three LCB-ADCs use MMAF as the payload. Compared to T-DM1, LCB-ADCs feature cleavable linkers designed to achieve higher anticancer activity through various mechanisms. LCB-ADCs also demonstrate stronger ADCC effects and higher G2/M arrest rates than T-DM1 [36]. In CDX models of HER2 low-expression GC (JIMT-1 cells) and T-DM1-resistant models, LCB-ADCs showed tumor-suppressive effects. Treatment of human GC tissue PDX models with T-DM1 or LCB-ADCs revealed tumor growth inhibition (TGI) rates of 103%, 99%, and 111% for T-DM1, LCB-ADC1, and LCB-ADC2, respectively. Notably, T-DM1 showed no effect in HER2 (IHC 2+) GC PDX models, whereas LCB-ADCs demonstrated potent therapeutic efficacy with sufficient antitumor potency. These results indicate that LCB-ADCs with elaborately designed cleavable linkers exhibit superior anticancer activity and excellent biological stability compared to T-DM1, showing great potential for HER2 low-expression GC treatment, though further research is needed. An open-label, single-arm phase I clinical study (NCT03944499) [37] is evaluating the efficacy, safety, and tolerability of LCB-ADC1 in HER2-positive (including IHC 1+, IHC 2+/FISH-) solid tumor patients.

3.2.3 MRG002 MRG002 comprises a glyco-engineered trastuzumab conjugated to MMAE via a valine-citrulline (Val-Cit) linker with a DAR of 3.8 [38]. MRG002 exhibits HER2 affinity similar to trastuzumab but has reduced ADCC activity due to high fucosylation of its mAb component, minimizing impact on immune cells [38-2]. Animal studies have shown that MRG002 induces tumor regression in HER2 low-expression CDX models [2]. In preclinical studies, MRG002 demonstrated antitumor activity in breast and gastric xenograft models with varying HER2 expression levels, showing superior effi-

cacy to trastuzumab and T-DM1 monotherapy in mouse xenograft models. Additionally, MRG002 significantly enhanced the antitumor activity of anti-PD-1 mAbs, suggesting that MRG002 combined with anti-PD-1 mAbs is a potential GC treatment strategy [2].

A phase I study of MRG002 as a single agent (NCT04941339) [39] is underway in HER2-positive recurrent or refractory GC patients. Meanwhile, various phase II trials are investigating MRG002 efficacy in HER2-positive or HER2-low multiple malignancies. For example, a study of MRG002 in HER2 low-expression locally advanced or metastatic G/GEJ cancer (NCT05141747) [40] is ongoing, offering hope for HER2 low-expression GC patients.

3.2.4 PF-06804103 PF-06804103 is a novel HER2-targeted ADC with a wider therapeutic window and broader indications than T-DM1. It consists of an enzyme-cleavable valine-citrulline (vc) linker conjugated to an auristatin analog (auristatin-0101), maximizing delivery of auristatin-0101 to HER2-positive cells. The vc linker combined with auristatin-0101 endows PF-06804103 with several characteristics: (1) the novel auristatin-0101 and vc linker combination enables killing of HER2-positive neighboring cells regardless of HER2 expression level; (2) site-specific cysteine conjugation overcomes the off-target toxicity of traditional ADCs; and (3) PF-06804103 enhances therapeutic efficacy in HER2 low-expression GC while overcoming acquired T-DM1 resistance [41]. Therefore, PF-06804103 exhibits good stability, favorable pharmacokinetic parameters, and low off-target toxicity.

Animal studies found that in CDX models of HER2 low-expression cells (N87 cells) treated with PT-DM1 until tumor recurrence, switching to PF-06804103 resulted in tumor shrinkage or regression after two treatment cycles. PF-06804103 thus demonstrates more significant antiproliferative activity than T-DM1 in HER2 low-expression CDX models, inducing durable tumor regression that persisted even after treatment discontinuation. This suggests PF-06804103 can maintain stable plasma concentrations in recurrent or T-DM1-resistant GC and may be an effective treatment for recurrent or refractory T-DM1-resistant disease. Preliminary results from a dose-escalation phase I study (NCT03284723) [42] showed an ORR of 52.4% when PF-06804103 dose was $ $3 mg/kg. In a waterfall plot analysis of HER2 low-expression GC PDX models, PF-06804103 treatment achieved an ORR of 89%, suggesting potential clinical benefit in HER2 low-expression GC.

In addition to the above agents, multiple novel HER2-targeted ADCs including XMT-2056, SYD985, BAT8001, and ZW49 are in various stages of clinical development, with their efficacy and safety in HER2 low-expression GC also attracting attention (Table 2).

4 Challenges in ADC Application and Development

As one of the fastest-developing anti-GC targeted drugs, ADCs face three major challenges in application and development: (1) Improving tumor cell uptake of ADCs is a key bottleneck limiting their development [47]. The efficacy of HER2-targeted ADCs primarily depends on high HER2 expression on tumor cell surfaces to ensure effective internalization and payload release. However, over 50% of GC cells express low HER2 levels, limiting the efficacy of existing ADCs. Therefore, enhancing ADC uptake by HER2-positive tumor cells, particularly HER2 low-expression GC cells, is crucial [48]. (2) Off-target toxicity is a major cause of ADC clinical trial failure, related to multiple factors including the mAb, linker, cytotoxic payload, and reduced cell surface antigen expression [47]. Thus, further optimization of ADC components is essential to minimize off-target toxicity. (3) Drug resistance is another obstacle to overcome. Ineffective ADC internalization and lysosomal trafficking or degradation may be primary mechanisms of T-DM1 resistance [49-50]. Reduced HER2 expression, emergence of truncated HER2 forms, and HER2 gene mutations are also associated with ADC resistance [51-52]. Recently, a novel HER2 mutant subtype, ERBB2Δ16, was confirmed to be associated with trastuzumab resistance. WANG et al. [53] found that patients with high ERBB2Δ16/HER2 ratios had significantly shorter PFS and OS when receiving trastuzumab, and Cox proportional hazards analysis confirmed that high ERBB2Δ16 expression was significantly associated with poor prognosis in GC patients. The ERBB2Δ16/HER2 ratio may thus represent a novel prognostic indicator for GC patients receiving molecular targeted therapies such as trastuzumab.

5 Summary and Outlook

HER2 low-expression accounts for 40%-60% of all GC types, yet no effective therapeutic agents have been developed, making the research and development of highly effective and low-toxicity anti-HER2 low-expression GC drugs a major focus. The clinical characteristics, molecular patterns, and treatment strategies of HER2 low-expression GC differ from HER2-negative or HER2-high-expression GC. Since the early 20th century, researchers have used various targeted drugs in clinical studies to identify effective treatment strategies for HER2 low-expression GC subgroups, but none achieved substantial progress. Traditional HER2-positive GC targeted agents such as trastuzumab, lapatinib, and T-DM1 show suboptimal efficacy in HER2 low-expression GC. The emergence of ADCs like T-DXd and RC48 has demonstrated good efficacy and safety in clinical trials for HER2 low-expression GC. Concurrently, clinical trials of novel HER2-targeted ADCs including ARX788, MRG002, and SYD985 are underway and expected to yield promising results.

Future directions for HER2-targeted ADCs include: First, exploring novel antibody-drug recombination strategies to improve cancer cell delivery and lysosomal trafficking, such as bispecific antibodies and antibodies conjugated with cell-penetrating peptides to enhance targeting and lysosomal delivery. Sec-

ond, continued improvement in ADC design is needed. Next-generation ADCs should incorporate more precise humanized mAbs, more efficient cytotoxic payloads, stable yet enzymatically cleavable linkers, and novel conjugation technologies to maximize therapeutic efficacy, reduce off-target toxicity, and expand indications. Third, clinical and translational approaches will play key roles in improving the therapeutic window of ADCs. Combination therapies may also enhance ADC efficacy and reduce resistance. Additionally, optimizing patient selection and developing new methods for monitoring HER2 response signals will improve the therapeutic index of HER2-targeted ADCs.

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References

[1] LONDON M, GALLO E. Epidermal growth factor receptor (EGFR) involvement in epithelial-derived cancers and its current antibody-based immunotherapies [J]. Cell Biol Int, 2020, 44(6): 1267-1282. DOI: 10.1002/cbin.11340.

[2] YU J F, FANG T, YUN C Y, et al. Antibody-drug conjugates targeting the human epidermal growth factor receptor family in cancers [J]. Front Mol Biosci, 2022, 9: 847835. DOI: 10.3389/fmolb.2022.847835.

[3] GRAVALOS C, JIMENO A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target [J]. Ann Oncol, 2008, 19(9): 1523-1529. DOI: 10.1093/annonc/mdn169.

[4] ZHOU L, XU H Y, LI S M, et al. Study RC48-C014: Preliminary results of RC48-ADC combined with toripalimab in patients with locally advanced or metastatic urothelial carcinoma [J]. J Clin Oncol, 2022, 40(6_{suppl}): 515. DOI: 10.1200/jco.2022.40.6_{suppl}.515.

[5] ZHANG L L, HAMDANI O, GJOERUP O, et al. ERBB2 copy number as a quantitative biomarker for real-world outcomes to anti-human epidermal growth factor receptor 2 therapy in advanced gastroesophageal adenocarcinoma [J]. JCO Precis Oncol, 2022, 6: e2100330. DOI: 10.1200/PO.21.00330.

[6] AJANI J A, D' AMICO T A, BENTREM D J, et al. Gastric cancer, version 2.2022, NCCN clinical practice guidelines in oncology [J]. J Natl Compr Canc Netw, 2022, 20(2): 167-192. DOI: 10.6004/jnccn.2022.0008.

[7] YUN T, WANG S N, JIANG B, et al. Significance of detection of the HER2 gene and PD-1/PD-L1 in gastric cancer [J]. J Oncol, 2020, 2020: 8678945. DOI: 10.1155/2020/8678945.

[8] YANG T, XU R, YOU J H, et al. Prognostic and clinical significance of HER-2 low expression in early-stage gastric cancer [J]. BMC Cancer, 2022, 22(1): 1168. DOI: 10.1186/s12885-022-10262-7.

[9] KIM C, LEE C K, CHON H J, et al. PTEN loss and level of HER2 amplification is associated with trastuzumab resistance and prognosis in HER2-positive gastric cancer [J]. Oncotarget, 2017, 8(69): 113494-113501. DOI: 10.18632/oncotarget.23054.

[10] ABDOLLAHPOUR-ALITAPPEH M, LOFTINIA M, GHARIBI T, et al. Antibody-drug conjugates (ADCs) for cancer therapy: strategies, challenges, and successes [J]. J Cell Physiol, 2019, 234(5): 5628-5642. DOI: 10.1002/jcp.27419.

[11] JIN Y M, EDALATIAN ZAKERI S, BAHAL R, et al. New technologies bloom together for bettering cancer drug conjugates [J]. Pharmacol Rev, 2022, 74(3): 680-711. DOI: 10.1124/pharmrev.121.000499.

[12] YAGHOUBI S, KARIMI M H, LOFTINIA M, et al. Potential drugs used in the antibody-drug conjugate (ADC) architecture for cancer therapy [J]. J Cell Physiol, 2020, 235(1): 31-64. DOI: 10.1002/jcp.28967.

[13] Chinese Society of Clinical Oncology Guidelines Working Committee. CSCO guidelines for diagnosis and treatment of gastric cancer-2022 [M]. Beijing: People' s Medical Publishing House, 2022.

[14] INDINI A, RIJAVEC E, GROSSI F. Trastuzumab deruxtecan: changing the destiny of HER2 expressing solid tumors [J]. Int J Mol Sci, 2021, 22(9): 4774. DOI: 10.3390/ijms22094774.

[15] OGITANI Y, AIDA T, HAGIHARA K, 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 [J]. Clin Cancer Res, 2016, 22(20): 5097-5108. DOI: 10.1158/1078-0432.CCR-15-2822.

[16] OGITANI Y, HAGIHARA K, OITATE M, et al. 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 [J]. Cancer Sci, 2016, 107(7): 1039-1046. DOI: 10.1111/cas.12966.

[17] ClinicalTrials.gov. DS-8201a in human epidermal growth factor receptor 2 (HER2)-expressing gastric cancer [DESTINY-Gastric01] [EB/OL]. (2020-11-27) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT03329690.

[18] YAMAGUCHI K, BANG Y J, IWASA S, et al. 1422MO 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 [J]. Ann Oncol, 2020, 31: S899-900. DOI: 10.1016/j.annonc.2020.08.1928.

[19] YAMAGUCHI K, BANG Y J, IWASA S, 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]. J Clin Oncol, 2023, 41(4): 816-825. DOI: 10.1200/JCO.22.00575.

[20] Phase 2 study of trastuzumab deruxtecan in the neoadjuvant treatment for patients with HER2-positive gastric and gastroesophageal junction adenocarcinoma (EPOC2003). 2022 ASCO. Abstract TPS4161.

[21] PENG Z, LIU T S, WEI J, 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 [J]. Cancer Commun, 2021, 41(11): 1173-1182. DOI: 10.1002/cac2.12214.

[22] CHEN Z H, YUAN J J, XU Y Y, et al. From AVATAR mice to patients: RC48-ADC exerted promising efficacy in advanced gastric cancer with HER2 expression [J]. Front Pharmacol, 2021, 12: 757994. DOI: 10.3389/fphar.2021.757994.

[23] LI H W, YU C, JIANG J, et al. An anti-HER2 antibody conjugated with monomethyl auristatin E is highly effective in HER2-positive human gastric cancer [J]. Cancer Biol Ther, 2016, 17(4): 346-354. DOI: 10.1080/15384047.2016.1139248.

[24] JIN Y B, CAI Q, JI J, et al. Antibody-drug conjugate RC48 for HER2 low-expression gastric cancer cell lines [J]. J Clin Oncol, 2023, 28(1): 1-7. DOI: 10.3969/j.issn.1009-0460.2023.01.001.

[25] ClinicalTrials.gov. Study of RC48-ADC administered intravenously in subjects with HER2-positive advanced malignant solid tumors [EB/OL]. (2020-11-27) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT02881190.

[26] XU Y Y, WANG Y K, GONG J F, 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 [J]. Gastric Cancer, 2021, 24(4): 913-925. DOI: 10.1007/s10120-021-01168-7.

[27] National Medical Products Administration. Conditional approval of RC48 for injection [EB/OL]. (2021-06-09) [2023-04-15]. https://www.nmpa.gov.cn/yaowen/ypjgyw/202106090854521

[28] ClinicalTrials.gov. Phase 2 study of T-DXd in the neoadjuvant treatment for patients with HER2 positive gastric and GEJ adenocarcinoma [EB/OL]. (2020-11-27) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT05034887.

[29] ClinicalTrials.gov. A study of RC48-ADC in local advanced or metastatic gastric cancer subjects with the overexpression of HER2 [EB/OL]. (2022-01-13) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT03556345.

[30] ClinicalTrials.gov. First-line treatment with RC48 plus tislelizumab and S-1 (RCTS) in advanced gastric cancer (RCTS) [EB/OL]. (2022-10-19) [2023-04-15].

https://clinicaltrials.gov/ct2/show/NCT05586061.

[31] BAROK M, JONCOUR V L, MARTINS A, et al. 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 [J]. Cancer Lett, 2020, 473: 156-163. DOI: 10.1016/j.canlet.2019.12.037.

[32] SKIDMORE L, SAKAMURI S, KNUDSEN N A, et al. 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 [J]. Mol Cancer Ther, 2020, 19(9): 1833-1843. DOI: 10.1158/1535-7163.MCT-19-1004.

[33] ZHANG Y, QIU M Z, WANG J F, et al. Phase 1 multicenter, dose-expansion study of ARX788 as monotherapy in HER2-positive advanced gastric and gastroesophageal junction adenocarcinoma [J]. Cell Rep Med, 2022, 3(11): 100814. DOI: 10.1016/j.xcrm.2022.100814.

[34] Clinicaltrials. Search list detail. Study of ARX788 in HER2-positive advanced gastric and gastroesophageal junction adenocarcinoma patients [EB/OL]. (2021-07-12) [2023-04-15]. http://www.chinadrugtrials.org.cn/CTR20211583.

[35] EDUPUGANTI V V S R, TYNDALL J D A T D A, GAMBLE A B. Self-immolative linkers in prodrugs and antibody drug conjugates in cancer treatment [J]. Recent Pat Anticancer Drug Discov, 2021, 16(4): 479-497. DOI: 10.2174/1574892816666210509123 4.

[36] SHIN S H, PARK Y H, PARK S S, et al. An elaborate new linker system significantly enhances the efficacy of an HER2-antibody-drug conjugate against refractory HER2-positive cancers [J]. Adv Sci, 2021, 8(23): e2102414. DOI: 10.1002/advs.202102414.

[37] ClinicalTrials.gov. Phase 1 study of FS-1502 in patients with HER2 expressed advanced solid tumors and breast cancer [EB/OL]. (2022-05-20) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT03944499.

[38] LI H, ZHANG X, XU Z Y, et al. Preclinical evaluation of MRG002, a novel HER2-targeting antibody-drug conjugate with potent antitumor activity against HER2-positive solid tumors [J]. Antib Ther, 2021, 4(3): 175-184. DOI: 10.1093/abt/tbab017.

[39] ClinicalTrials.gov. A study of MRG002 in the treatment of patients with HER2-positive advanced solid tumors [EB/OL]. (2021-12-03) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT04941339.

[40] ClinicalTrials.gov. A study of MRG002 in the treatment of HER2-positive/HER2-low locally advanced or metastatic gastric/gastroesophageal junction cancer [EB/OL]. (2022-02-23) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT05141747.

[41] GRAZIANI E I, SUNG M, MA D S, et al. PF-06804103, a site-specific anti-HER2 antibody-drug conjugate for the treatment of HER2-expressing breast,

gastric, and lung cancers [J]. Mol Cancer Ther, 2020, 19(10): 2068-2078. DOI: 10.1158/1535-7163.MCT-20-0237.

[42] ClinicalTrials.gov. PF-06804103 dose escalation in HER2 positive and negative (negative only in part 2) solid tumors [EB/OL]. (2022-03-18) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT03284723.

[43] ClinicalTrials.gov. A dose-escalation, expansion study of ARX788, in advanced solid tumors subjects with HER2 expression (ACE-Pan Tumor 01) [EB/OL]. (2023-04-10) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT03255070.

[44] ClinicalTrials.gov. A study of MRG002 in patients with HER2-positive advanced solid tumors and locally advanced or metastatic gastric/gastroesophageal junction (GEJ) cancer [EB/OL]. (2022-05-06) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT04492488.

[45] ClinicalTrials.gov. A study of XMT-2056 in advanced/recurrent solid tumors that express HER2 [EB/OL]. (2023-03-15) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT05514717.

[46] ClinicalTrials.gov. ISPY-P1.01: Evaluating the safety of weekly paclitaxel with trastuzumab duocarmazine (SYD985) in patients with metastatic cancer [EB/OL]. (2023-03-24) [2023-04-15]. https://clinicaltrials.gov/ct2/show/NCT04602117.

[47] MAHALINGAIAH P K, CIURLIONIS R, DURBIN K R, et al. Potential mechanisms of target-independent uptake and toxicity of antibody-drug conjugates [J]. Pharmacol Ther, 2019, 200: 110-125. DOI: 10.1016/j.pharmthera.2019.04.008.

[48] YU M H, LIANG Y, LI L H, et al. Research progress of antibody-drug conjugates therapy for HER2-low expressing gastric cancer [J]. Transl Oncol, 2023, 29: 101624. DOI: 10.1016/j.tranon.2023.101624.

[49] GARCÍA-ALONSO S, OCAÑA A, PANDIELLA A. Trastuzumab emtansine: mechanisms of action and resistance, clinical progress, and beyond [J]. Trends Cancer, 2020, 6(2): 130-146. DOI: 10.1016/j.trecan.2019.12.010.

[50] DÍAZ-RODRÍGUEZ E, GANDULLO-SÁNCHEZ L, OCAÑA A, et al. Novel ADCs and strategies to overcome resistance to anti-HER2 ADCs [J]. Cancers, 2021, 14(1): 154. DOI: 10.3390/cancers14010154.

[51] BON G, PIZZUTI L, LAQUINTANA V, et al. Loss of HER2 and decreased T-DM1 efficacy in HER2 positive advanced breast cancer treated with dual HER2 blockade: the SePHER Study [J]. J Exp Clin Cancer Res, 2020, 39(1): 279. DOI: 10.1186/s13046-020-01740-4.

[52] GINZEL J D, ACHARYA C R, LUBKOV V, et al. HER2 isoforms uniquely program intratumor heterogeneity and predetermine breast cancer trajectories during the occult tumorigenic phase [J]. Mol Cancer Res, 2021, 19(10): 1699-1711. DOI: 10.1158/1541-7786.MCR-21-0215.

[53] WANG S, ZHAO Y Z, SONG Y G, et al. ERBB2Δ16 expression in HER2 positive gastric cancer is associated with resistance to trastuzumab [J]. Front

Oncol, 2022, 12: 855308. DOI: 10.3389/fonc.2022.855308.

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