

# Development, efficacy and side effects of antibody-drug conjugates for cancer therapy (Review)

TE SUN<sup>1,2</sup>, XUELI NIU<sup>1,2</sup>, QING HE<sup>1,2</sup>, MIN LIU<sup>1,3</sup>, SHUAI QIAO<sup>1,2</sup> and RUI-QUN QI<sup>1,2</sup>

<sup>1</sup>Department of Dermatology, The First Hospital of China Medical University;

<sup>2</sup>Key Laboratory of Immunodermatology, National Joint Engineering Research Center for Theranostics of Immunological Skin Diseases, Ministry of Education and NHC; <sup>3</sup>Institute of Respiratory Disease, The First Hospital of China Medical University, Shenyang, Liaoning 110001, P.R. China

Received October 15, 2022; Accepted March 22, 2023

DOI: 10.3892/mco.2023.2643

**Abstract.** Antibody-drug conjugates (ADCs) are anticancer drugs that combine cytotoxic small-molecule drugs (payloads) with monoclonal antibodies through a chemical linker and that transfer toxic payloads to tumor cells expressing target antigens. All ADCs are based on human IgG. In 2009, the Food and Drug Administration (FDA) approved gemtuzumab ozogamicin as the initial first-generation ADC. Since then, at least 100 ADC-related projects have been initiated, and 14 ADCs are currently being tested in clinical trials. The limited success of gemtuzumab ozogamicin has led to the development of optimization strategies for the next generation of drugs. Subsequently, experts have improved the first-generation ADCs and have developed second-generation ADCs such as ado-trastuzumab emtansine. Second-generation ADCs have higher specific antigen levels, more stable linkers and longer half-lives and show great potential to transform cancer treatment models. Since the first two generations of ADCs have served as a good foundation, the development of ADCs is accelerating, and third-generation ADCs, represented by trastuzumab deruxtecan, are ready for wide application. Third-generation ADCs are characterized by strong pharmacokinetics and high pharmaceutical activity, and their drug-to-antibody ratio mainly ranges from 2 to 4. In the past decade, the research prospects of ADCs have broadened, and an increasing number of specific antigen targets and mechanisms of cytotoxic drug release have been discovered and studied. To date, seven ADCs have been approved by the FDA

for lymphoma, and three have been approved to treat breast cancer. The present review explores the function and development of ADCs and their clinical use in cancer treatment.

## Contents

1. Introduction
2. First generation of ADCs: Gemtuzumab ozogamicin (GO)
3. Second-generation ADCs
4. Third-generation ADCs
5. Conclusions and future perspectives

## 1. Introduction

*The origin of antibody-drug conjugates (ADCs).* Cancer ranks first in mortality worldwide, followed by heart disease (1). Many cancer therapies have been developed, including surgery, chemotherapy, radiotherapy and monoclonal antibodies (mAbs). Although radiotherapy and chemotherapy inhibit tumor growth to help reduce the risk of cancer recurrence, these methods also exert off-target effects and kill nontargeted cells with different degrees of toxicity, and therefore, patients must tolerate an unbalanced immune system (2). The first mAb therapy was developed 40 years ago and was found to attenuate most side effects of traditional treatments. As the forefront of cancer therapeutics with numerous specific agents, mAbs work effectively *in vivo*, protect the host from multisystem damage and promise a long-term disease-free state (3). The concept of mAbs originates from hybridoma cells. These antibodies are produced by B-cell clones that are highly homogeneous and directed against only a specific epitope (4). However, the use of mouse mAbs as therapeutics is restricted by their short half-life, limited effective function and high risk of systemic exposure and toxicity (5). Monoclonal antibody technology was further studied in subsequent decades by combining cytotoxic drugs with antibodies through a chemical linker to decrease toxicity while increasing the efficacy of the payloads and expanding the potential therapeutic window. These new drugs, termed antibody-drug conjugates (ADCs), are biological agents in which a cytotoxic drug is conjugated to a mAb through a linker (6).

---

*Correspondence to:* Professor Rui-Qun Qi or Mrs. Shuai Qiao, Department of Dermatology, The First Hospital of China Medical University, 155 Nanjing Bei Street, Shenyang, Liaoning 110001, P.R. China

E-mail: xiaoqiliumin@163.com

E-mail: qs197708@163.com

**Key words:** antibody-drug conjugates, antibody, payload, linker, gemtuzumab ozogamicin, ado-trastuzumab emtansine, trastuzumab deruxtecan

**ADC components.** ADCs include three basic components that are joined through bioconjugation: the antibody, the linker and the payload. Compared with normal cells, ADC target antigens are expressed at remarkably high levels on the surface of tumor cells, which allows the antibodies to effectively bind antigens and cytotoxic drugs to exert their functions. Poor antibody selectivity or the presence of the antigen in normal tissues promotes the delivery of cytotoxic drugs to normal cells, resulting in targeted toxicity. When selecting antigens for ADC development, the following three types of antigens must be distinguished: 1. antigens that are not internalized during antibody binding; 2. antigens that are internalized during antibody binding but are rapidly transferred to the cell membrane; and 3. antigens that are internalized when antibodies are not rapidly transferred. Only the third type of antigen is a valuable target for ADC development (7). Therefore, the appropriate selection of antibodies is particularly important in the development of ADCs (8).

**Antibodies** As the ‘compass’ of ADCs, antibody design has attracted considerable attention. Regardless of their investigation in clinical trials or during development, all antibodies in ADCs are human IgG due to their multiple native sites for conjugation and ability to be modified (9). The molecular weight of the antibody should be considered during selection. A high antibody molecular weight slows the diffusion rate, which is not conducive to effective penetration of target cells. Low bioavailability prevents the antibody from penetrating the capillary inner cortex and the extracellular space. However, if the molecular weight of the antibody is too low, its half-life in the body is reduced, which may lead to easy removal (10). Antibodies consist of two dominant fragments: the antigen-binding (Fab) fragment and the crystallizable region (Fc). The Fab is responsible for antigen recognition, and the Fc accepts Fc $\gamma$  receptors (Fc $\gamma$ RI, Fc $\gamma$ RIIA, Fc $\gamma$ RIIB, Fc $\gamma$ RIIC, Fc $\gamma$ RIIA and Fc $\gamma$ RIII), which are distributed differently in cells. Differences in the Fc region result in different capacities to bind antigens and activate various effector functions, including antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC). Thus, when selecting a suitable antibody for an ADC, the type of IgG and cellular distribution of the targets should be considered (11). IgG is classified into four subclasses (IgG1, IgG2, IgG3 and IgG4) based on the number of interchain disulfide bonds, the binding affinity of the Fc region and the length of the hinge region (12). Although IgG3 functions best in fixing complement and has the highest binding affinity for Fc $\gamma$ Rs, this subclass is excluded from use in antitumor therapeutic antibodies due to its short half-life and polymorphic nature, which lead to instability and nonspecific recognition (13). The other subclasses are selected depending on the biological functions and types of target cells.

**Linker** The linker, which tethers the antibody to the cytotoxic drug via a covalent bond, determines the mechanism and location of payload release. In addition, the linker determines the dose at which drugs act on tumor cells, termed the drug-to-antibody ratio (DAR), which is measured by ultraviolet/visible (UV/VIS) spectroscopic analysis (14). Generally, the DAR is the number of payloads conjugated to the antibody. Three conjugation methods have been developed: i) conjugation with

lysine exposed on the surface of the antibody; ii) reduction of cysteine in the interchain disulfide bonds; and iii) site-specific conjugation technologies (including site-specific glycan conjugation, cysteine engineering, incorporation of unnatural amino acids (UAAs) and conjugation of short peptide tags to drug linkers) (15). Among them, lysine conjugation involves many sites, and 0-8 small-molecule toxins can be conjugated to each antibody (i.e., a DAR range of 1-8); therefore, ADC products produced through lysine conjugation in the same batch have high heterogeneity in quality. Cysteine conjugation has relatively controllable conjugation sites because of the fixed positions of the disulfide bonds, but the number of connections is affected by the degree of disulfide bond reduction. When all four interchain disulfide bonds are completely reduced, each antibody can carry eight toxins; when they are partially reduced, products with DARs of 2, 4, 6 and 8 may be generated. Therefore, the cysteine conjugation products have significantly greater homogeneity than the lysine conjugation products. Site-specific conjugation mainly relies on biotechnology to introduce linker sites on the monoclonal antibody, which enables precise site and stoichiometric control. To incorporate UAAs, an orthogonal tRNA/aminoacyl-tRNA synthetase pair is used to replace the amber codon TAG in the monoclonal antibody with p-acetyl phenylalanine (pAcF). Small-molecule toxic drugs are then conjugated to pAcF, and the amount of pAcF represents the amount of drug conjugated (16). With advances in ADC conjugation methods, the problem of heterogeneous DARs in ADCs has been gradually solved. Two broad properties of the linker have been identified, and each retains the homogeneity and stability of ADCs under appropriate conditions (17). The linker is a critical safeguard that maintains the stability of the cytotoxic payload and protects physiological functions by eliminating excess toxicity before internalization. After transport through the blood to localize at tumor sites, removal of the linker accelerates cytotoxic drug release, which is a unique advantage over mAbs (18). Linkers are classified into two groups: cleavable and noncleavable. Cleavable linkers are used more often and have a wider range of applications. In the classical endocytosis pathway, ADCs are delivered into lysosomes, where the cleavable linker can be cleaved by a high concentration of hydrolytic enzymes. Likewise, the cleavable linker responds to acid, reducible disulfides and other exogenous stimuli (19). The noncleavable linker does not actively release payload molecules, and the linker only liberates the payload conjugated to an amino acid residue after the mAb is degraded completely by cytosolic and lysosomal proteases (20).

**Payload** The payload is essentially a cytotoxic molecule. As an influential factor of ADCs, they are regarded as a ‘warhead’ that kills target cells (21). Currently, ADC payloads investigated in clinical trials are classified into two categories (antimitotic or DNA damaging) according to their mechanism (22). The main antimitotic payloads include auristatins [monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF)] and maytansinoids [emtansine (DM1), DM3 and DM4], which bind microtubules to inhibit their aggregation, block cell cycle progression and subsequently induce tumor cell apoptosis. The main DNA-damaging payloads include camptothecin analogs (including the exatecan derivative DXd and the irinotecan

metabolite SN-38), which lead to DNA lysis and cell death by binding to the minor groove of the DNA double helix and cause DNA breakage by inhibiting topoisomerase I (TOPOI). Two core characteristics are considered when oncologists select a qualified payload. First, the efficiency of the payload is determined not only by restrictions related to its distribution in tumor tissues and the number of target antigens on the cell surface but also by linker metabolism and the efficiency of internalization and delivery. These limitations can result in the delivery of an insufficient toxin dose to kill tumor cells, and the inherent cytotoxic potency of a payload must be maintained at an extremely high level (23). Finally, the structure of the payload should contain a functional group that is also suitable to bind the antibody. Moreover, the payload must have the ability to dissolve in aqueous solution similar to the intracellular environment and remain stable in plasma to ensure a long antibody half-life.

*ADCs in clinical use.* ADCs are a promising therapeutic method (24). Since the first ADC was approved by the Food and Drug Administration (FDA) in 2009, at least 100 ADCs have been investigated, and 14 ADCs have been tested in clinical trials (25,26). Although ADCs have faced many clinical failures in the past, advances in humanized monoclonal antibodies, site-specific conjugation protocols, various potent cytotoxic payloads with different mechanisms of action and adaptable linker technologies have improved the properties and alleviated the side effects of previous generations, thereby accelerating the clinical use of ADCs. ADCs have significantly contributed to the future of immuno-oncology by allowing new technologies and biomarker selection strategies (27).

## 2. First generation of ADCs: Gemtuzumab ozogamicin (GO)

*The structure of GO.* The monoclonal antibodies used in early ADCs were of murine origin, which led to immune responses and the production of human anti-mouse antibodies (HAMAs). Since the development of GO, a recombinant humanized IgG4 kappa antibody has been used as the antibody in ADCs. GO, also known by the trade name Mylotarg, was the first ADC drug designed for the treatment of acute myeloid leukemia (AML) (28). GO is a recombinant humanized IgG4 kappa antibody that binds to CD33<sup>+</sup>, which is expressed on the surface of over 90% of blast cells in patients with AML; the antibody is conjugated to the drug via a hydrazone linker (29). The toxic substance (N-acetyl gamma calicheamicin) in GO binds to lysine (Lys) in the head of the antibody through the acid-cleavable linker 4-(4-acetylphenoxy) butanoic acid (AcBut). The average DAR of GO ranges from 2 to 3. When the antibody recognizes CD33, the GO-CD33 complex is internalized, which results in the release of a highly cytotoxic drug (calicheamicin) that causes DNA damage in cells. Calicheamicin induces cytotoxicity by binding to DNA and generating DNA double-strand breaks (30). The binding of GO is followed by internalization of the GO-CD33 complex, after which the highly cytotoxic DNA-strand-breaking calicheamicin compound is released intracellularly, leading to DNA damage and cell death (31).

*GO in clinical trials.* Initially, GO was indicated for patients aged > 60 years who experienced a first relapse of CD33<sup>+</sup> AML and who were not treated with any other ADC and for newly diagnosed patients with AML (aged 18 to 60 years) (32,33). However, GO did not pass the final clinical trials and did not produce a sufficient benefit of longer survival time. In addition, the instability of the linker led to the release of the bound drug within 48 h, and approximately half of the antibodies were unconjugated, which resulted in a high risk of side effects (prolonged thrombocytopenia). The highly hydrophobic nature of calicheamicin, its lack of specificity and its tendency to be eliminated prompted the withdrawal of GO from the commercial market in 2010 (34). Subsequently, oncologists changed to a lower dose and schedule of GO and used it with the traditional chemotherapeutic agents daunorubicin and cytarabine (DA) in 2017 (35). Notably, each medicine has its own dose in different courses (36). In addition to combination therapies, GO can be used as a single agent for patients with CD33<sup>+</sup> AML, and different dosages are used for different patient groups. For patients under 2 years old, 3 mg/m<sup>2</sup> is administered on days 1, 4 and 7. Patients with relapsed or refractory disease and patients who have been diagnosed but have not received any treatment receive 6 mg/m<sup>2</sup> on day 1 and 3 mg/m<sup>2</sup> on day 8. If the disease has not progressed, 2 mg/m<sup>2</sup> is injected on the first day of the week every four weeks (37,38). Moreover, patients with higher CD33 positivity are reported to be more sensitive to GO.

*Shortcomings of first-generation ADCs.* The first-generation ADCs represented by GO have several problems. First, due to insufficient drug efficacy, the drug concentration in the blood is lower than the therapeutically effective concentration, and the low expression of the target antigen leads to limited drug delivery; as a result, the intracellular drug concentration is not sufficient to kill cancer cells. Second, the disulfide bonds in the linkers are easily broken, releasing free ozogamicin into the blood and subsequently causing hepatotoxicity. Third, first-generation ADCs have a weak tumor-targeting effect and a low antigen-binding rate. Fourth, the cytotoxic small molecules in first-generation ADCs are only nonselectively linked to the cysteine or lysine residues of antibodies, and cytotoxin loading is not accurately controlled. Linker instability triggers systemic toxicity, which is the key problem limiting the application of ADCs (39).

## 3. Second-generation ADCs

*Progress achieved in second-generation ADCs.* Compared with first-generation ADCs, the greatest advantage of second-generation ADCs is the introduction of a more stable antibody-drug conjugation method and stronger cytotoxicity. For example, brentuximab vedotin has been approved by the FDA to treat CD30<sup>+</sup> anaplastic large cell lymphoma and Hodgkin's lymphoma, and its therapeutic effect on cutaneous T-cell lymphoma and peripheral T-cell lymphoma is being verified in phase III clinical trials (40,41). Brentuximab vedotin carries the antimetabolic agent MMAE and is linked to IgG1 through the protease cleavable linker Val-Cit (42,43). Inotuzumab ozogamicin is also used to treat lymphoma, and although it initially targeted patients with CD22<sup>+</sup> ALL, it was

later confirmed to have the potential to treat non-Hodgkin's lymphoma and Hodgkin's lymphoma (44). Inotuzumab ozogamicin consists of an acid-cleavable linker coupling an IgG4 monoclonal antibody and a calicheamicin derivative. Next, this article introduces a representative second-generation ADC, ado-trastuzumab emtansine (T-DM1), in detail.

*The structure of T-DM1.* Breast cancer (BC) is a dominant malignant disease that results in the death of women worldwide (45). Some patients with BC (15-30%) exhibit overexpression of the HER2 human epidermal growth factor receptor, which is associated with disease development (46,47). T-DM1 was a second-generation ADC that was developed for HER2-positive patients and included the monoclonal antibody trastuzumab, which was initially approved in 2013 (48). The previous generation was hampered by numerous pharmacological and safety considerations, but in contrast, T-DM1 shows better cell-mediated cytotoxicity and greater affinity for tumor cells, which may reduce irrelevant damage to healthy cells (49). The antibody of this ADC is IgG1, which carries the antimetabolic maytansinoid DM1 as the payload. DM1 is a derivative of maytansine, which controls cellular division by potently inhibiting tubulin polymerization, resulting in apoptosis (50). The antibody and payload are bound by a noncleavable linker, N-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate, and a DAR of 3.5 has been confirmed (51).

*T-DM1 in clinical trials and side effects.* The approval of T-DM1 by the FDA/European Medicines Agency (EMA) as a second-line treatment for HER2-positive breast cancer was based principally on three pivotal phase III trials-EMILIA, TH3RESA and MARIANNE (52). In the EMILIA trial, T-DM1 was superior to the traditional chemotherapy regimen tested (capecitabine plus lapatinib) in terms of progression-free survival (PFS; 9.6 vs. 6.4 months) and overall survival (OS; 30.9 vs. 25.1 months) (53). Patients in the T-DM1 arm also showed a higher response rate (43.6% vs. 30.8%;  $P < 0.001$ ) and fewer grade 3-4 adverse events (AEs) (41% vs. 57%). Other indices, such as quality of life (QoL), confidence interval (CI) and duration of response (DoR), were improved with T-DM1 treatment (54). In the TH3RESA trial, the patients were divided into groups receiving T-DM1 or physician's choice (PC). Similar to the EMILIA trial, OS and PFS were significantly improved in patients treated with T-DM1, and the pharmacokinetics of T-DM1 in the TH3RESA trial suggested that patients treated with a dosage of 3.6 mg/kg T-DM1 every 3 weeks experienced a benefit compared with PC (55). These two clinical trials shifted T-DM1 to a second-line treatment choice (56). In the MARIANNE trial, patients with HER2-positive metastatic breast cancer (MBC) who were not previously treated were randomized to receive T-DM1 plus placebo or pertuzumab and a trastuzumab-taxane combination. To compare T-DM1 (with or without pertuzumab) with trastuzumab plus taxane, subgroups were tested for the following biomarkers: HER2 and HER3 mRNA expression levels; HER2 staining intensity; phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) status; phosphatase and tensin homolog (PTEN) H-score; and PTEN protein expression level (57). However, no significant differences were observed between these two groups in the levels of biomarkers related to the HER2 pathway.

In conclusion, the median OS was similar across groups, and T-DM1 remains reserved as a second-line treatment because its actual efficacy is unclear (58). One of the most important side effects observed during the course of T-DM1 treatment is thrombocytopenia, which can lead to a reduction in drug dosage and even withdrawal (59). The guideline recommends that T-DM1 be suspended until a platelet (PLT) count  $\geq 75,000/\text{mm}^3$  is observed, at least for patients with grade 3 thrombocytopenia, while the dosage should be less than the previous dosage after the PLT count recovers for patients with grade 4 thrombocytopenia ( $\text{PLT} < 25,000/\text{mm}^3$ ) (60). This adverse reaction results from Fc receptor engagement, which inhibits megakaryocyte differentiation because of internalization by a macropinocytosis pathway independent of Fc $\gamma$ RIIA therapeutics (61).

#### 4. Third-generation ADCs

*Progress achieved in third-generation ADCs.* In the past decade, the experience obtained with first- and second-generation ADCs has paved the way for the development of third-generation ADCs. Third-generation ADCs utilize the site-specific binding of small-molecule drugs to monoclonal antibodies, which improves the stability and pharmacokinetics and increases the drug release rate without causing additional systemic toxicity or producing unbound monoclonal antibodies. Compared with second-generation ADCs, third-generation ADCs have more optimized linkers and conjugation mechanisms and thus a wider therapeutic window. The use of site-specific conjugation ensures the reproducibility of the DAR and efficacy of third-generation ADCs, improves their therapeutic index and maximizes their killing effect on tumor cells with low target expression. Polatuzumab vedotin targets CD79b and has been used to treat diffuse large B-cell lymphoma. This ADC is formed by linking the cleavable linker Val-Cit to IgG1 and MMAE mAb (62). Sacituzumab govitecan is an anti-trophoblast cell-surface antigen 2 (anti-Trop-2) ADC linked to the topoisomerase inhibitor SN-38 through a proprietary hydrolyzable linker. This ADC is used for the treatment of triple-negative breast cancer (TNBC). Although its DAR is as high as 7.6, its tolerance and efficiency are also high (63). In addition, enfortumab vedotin, which targets nectin-4, is used to treat refractory metastatic urothelial cancer (64). Belantamab mafodotin, which targets BCMA, is used to treat relapsed or refractory multiple myeloma (RRMM) (65). Table I summarizes three generations of ADCs which have been approved by FDA for treating cancers. Next, this review will introduce a representative third-generation ADC, trastuzumab deruxtecan (T-DXd/DS8201), in detail.

*The structure of T-DXd/DS8201.* In 2020, T-DXd was approved and became the second ADC for HER2<sup>+</sup> therapy (66). Similar to T-DM1, T-DXd was also designed for HER2-positive patients. T-DXd consists of an anti-HER2 humanized monoclonal IgG1 (MAAL-9001) with the same amino acid sequence as trastuzumab. Unlike T-DM1, which carries DM1, T-DXd is conjugated to seven or eight molecules of an exatecan derivative, DXd (topoisomerase I inhibitor), per molecule of the anti-HER2 monoclonal antibody through a novel linker-payload system (67). In addition to payload, T-DXd is superior to T-DM1 in many ways, including possessing a

Table I. Approved antibody-drug conjugates for clinical use by U.S Food and Drug Association (17).

Generation	Antibody-drug conjugate	Target antigens	Linker type	Payload	Indication
First generation	Gemtuzumab ozogamicin	CD33	The cleavable linker, 4-(4-acetylphenoxy) butanoic acid (acbut)	Calicheamicin	Acute myeloid leukemia
Second generation	Brentuximab vedotin	CD30	The cleavable linker, Val-Cit	MMAE	Relapsed Hodgkin lymphoma and systemic, anaplastic large cell lymphoma
Second generation	Inotuzumab ozogamicin	CD22	The cleavable linker, acbut	Calicheamicin	Acute lymphoblastic leukemia
Second generation	Ado-trastuzumab emtansine	HER2	The non-cleavable linker, N-succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate	DM1	HER2 <sup>+</sup> Breast cancer
Third generation	Polatuzumab vedotin	CD79b	The cleavable linker, Val-Cit	MMAE	Large B-cell lymphoma
Third generation	Sacituzumab govitecan	TROP-2	The cleavable linker, CL2A	SN-38	Triple-negative breastcancer
Third generation	Enfortumab vedotin	NECTIN-4	The cleavable linker, Val-Cit	MMAE	Metastatic urothelial cancer
Third generation	Belantamab mafodotin	BCMA	The non-cleavable linker, MC	MMAF	Relapsed or refractory multiple myeloma
Third generation	Trastuzumab deruxtecan	HER2	The cleavable linker, tetrapeptide	DXd	HER2 <sup>+</sup> breast cancer
Third generation	Vadastuximab talirine	CD33	The cleavable linker, Val-Cit	PBD dimer	Acute myeloid leukemia

short half-life and a wider therapeutic window; in addition, neither T-DXd nor T-DM1 increases the cytotoxic effect, and they do not expose normal cells to the drug through off-target toxicity (68). Moreover, T-DXd provides a new capability, the ‘bystander killing effect’, as it kills tumor cells in close proximity to the targeted cells by releasing deruxtecan into intercellular spaces to attack neighboring tumor cells, regardless of the HER2 expression level (69).

*T-DXd in clinical trials and side effects.* Three phase III clinical trials are ongoing (NCT03523585, NCT03529110 and NCT03734029) (70). The first two studies reported that T-DXd is most effective for patients with HER2<sup>+</sup> BC who were either treated with the combination of a mAb (trastuzumab) and traditional chemotherapy or with a mAb (trastuzumab) and a taxane, followed by T-DM1 (71). T-DXd has also been applied to treat HER2-low MBC, an indication that was identified in the third trial (72,73). Another trial further revealed that T-DXd is effective in patients with MBC with low HER2 expression. In this clinical trial, patients with low HER2 expression were randomly divided into two groups that received T-DXd or the PC of chemotherapy. Patients in the T-DXd arm experienced significantly longer PFS and OS than those who received the PC of chemotherapy. Furthermore, another study determined a recommended dose by evaluating the efficacy and safety of

different doses of T-DXd (74). Exposure-efficacy modeling showed better exposure and response rates and longer PFS after treatment with a higher dose (71). Additionally, key AEs, including interstitial lung disease, nausea and myelosuppression, were also related to treatment with a higher dose. To achieve a balance between safety and efficacy, and after the optimal dose was calculated, 5.4 mg per kilogram was recommended (75). In addition to BC, T-DXd is efficacious against gastric cancers that express HER2. T-DXd has already been approved by the FDA and is recommended in the Japanese Gastric Cancer Association treatment guidelines as the third-line treatment for HER2-positive gastric cancer (76). The trial method was similar to that for BC, and the primary outcome was the objective response, which was criticized by the independent central review. Some indices, including OS, DoR, PFS, confirmed response (response persisting  $\geq 4$  weeks) and safety, were also considered (77). Compared with standard therapies, T-DXd therapy resulted in significant improvements in response and OS. Apart from BC and gastric cancer, ongoing clinical trials are being conducted to determine the efficacy of T-DXd alone or in combination against other solid tumors, such as HER2-expressing colorectal cancer and HER2-expressing or HER2-mutated non-small cell lung cancer (NSCLC), for which the FDA has granted accelerated approval (78). The limited outcomes revealed that T-DXd has promising anti-

tumor activity with an acceptable safety profile in patients with HER2-expressing or HER2-mutant solid tumors who have received extensive pretreatment, especially patients with HER2-mutant NSCLC (79). In addition to NSCLC, T-DXd also showed preliminary activity against HER2-expressing colorectal cancer [9]. In the clinical trial, all patients injected with 5.4 mg/kg experienced at least one AE to different extents during treatment. In addition to some common AEs, such as nausea and myelosuppression, some severe AEs including hematological and pulmonary diseases [e.g., diffuse parenchymal lung disease (DPLD)] are common (80). A study in monkeys explored the relationship between dose and DPLD severity. Since the histopathological features in the monkeys were similar to those of DPLD associated with anticancer drugs in patients, the results from this study are translatable to humans. In conclusion, the risk of T-DXd-induced AEs depends on both a higher dose and the dosing frequency (81). Physicians should confirm that patients do not have pulmonary diseases, irrespective of the grade, and appropriate measures should be implemented before prescribing T-DXd to avoid fatal AEs (82).

## 5. Conclusions and future perspectives

Since a single medication, regardless of whether it is standardized chemotherapy or mAb treatment, may exert off-target effects, the use of ADCs in clinical cancer treatment has gradually become a mainstream method. After decades of research and clinical trials, ADCs have been updated to the third generation through optimization of intrinsic activity parameters and development of mAbs and linkers. The previous conjugation methods and selection of cytotoxic drugs have been improved, while the selection of the composition and mode of component binding are gradually becoming more mature through further exploration. The payload is released in the plasma where it remains stable and highly effective. In addition, the dose of cytotoxin that reaches the tumor site may be controlled by novel linkers.

However, further development of third-generation ADCs can still occur, and researchers are focused on the development of targets with dual-specificity and on drugs that carry two different payloads. The former refers to the design of an ADC that targets two different sites on one antigen to accelerate lysosomal aggregation and load delivery. The latter refers to ADCs that carry two drugs; by accurately controlling the ratio of the two drugs, the payload can be delivered synergistically and effectively to cancer cells to reduce the rate of drug resistance. In addition to improving the ADC release mechanism, oncologists are also seeking to identify additional biomarkers. The accurate identification of biomarkers improves the immunogenicity of targeted cells, increases tumor specificity and protects normal cells from attack, which enables ADCs to have higher efficacy in cancer treatment. Finally, optimizing the dosage and reducing drug side effects and drug resistance, which are also ultimate challenges that must be overcome for these drugs to be used in the clinic, are crucial to ensure the safety and wide application of ADCs. In addition to their use as single agents, the combination of ADCs with monoclonal antibodies or chemotherapeutic drugs has also attracted substantial attention. Combination medications may reduce the risk of hematologic and pulmonary diseases and liver damage resulting from ADCs. If ADCs bind

tumors and their pharmacological activity is maximized, the therapeutic effects on tumors will be substantially enhanced.

## Acknowledgements

Not applicable.

## Funding

This study was funded and supported by the National Natural Science Foundation of China (grant no. 82173401).

## Availability of data and materials

Not applicable.

## Authors' contributions

TS and RQ conceived and designed the study. TS wrote the first manuscript. XN, QH, ML, SQ and RQ revised the content and grammar of the manuscript. RQ, ML and SQ provided guidance and correction. ML, RQ and SQ acquired funding. All authors have read and agreed to the published version of the manuscript. Data sharing is not applicable.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

- Mun EJ, Babiker HM, Weinberg U, Kirson ED and Von Hoff DD: Tumor-treating fields: A fourth modality in cancer treatment. *Clin Cancer Res* 24: 266-275, 2018.
- Schirmmacher V: From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment (review). *Int J Oncol* 54: 407-419, 2019.
- Remer M, White A, Glennie M, Al-Shamkhani A and Johnson P: The use of anti-CD40 mAb in cancer. *Curr Top Microbiol Immunol* 405: 165-207, 2017.
- Wolska-Washer A and Robak T: Safety and tolerability of antibody-drug conjugates in cancer. *Drug Saf* 42: 295-314, 2019.
- Ovacik M and Lin K: Tutorial on monoclonal antibody pharmacokinetics and its considerations in early development. *Clin Transl Sci* 11: 540-552, 2018.
- Hafeez U, Parakh S, Gan HK and Scott AM: Antibody-drug conjugates for cancer therapy. *Molecules* 25: 4764, 2020.
- Hoffmann RM, Mele S, Cheung A, Larcombe-Young D, Bucaite G, Sachouli E, Zlatareva I, Morad HOJ, Marlow R, McDonnell JM, *et al*: Rapid conjugation of antibodies to toxins to select candidates for the development of anticancer antibody-drug conjugates (ADCs). *Sci Rep* 10: 8869, 2020.
- Sievers EL and Senter PD: Antibody-drug conjugates in cancer therapy. *Annu Rev Med* 64: 15-29, 2013.
- Abdollahpour-Alitappeh M, Lotfinia M, Gharibi T, Mardaneh J, Farhadhosseiniabadi B, Larki P, Faghfourian B, Sepehr KS, Abbaszadeh-Goudarzi K, Abbaszadeh-Goudarzi G, *et al*: Antibody-drug conjugates (ADCs) for cancer therapy: Strategies, challenges, and successes. *J Cell Physiol* 234: 5628-5642, 2019.

10. Goulet DR and Atkins WM: Considerations for the design of antibody-based therapeutics. *J Pharm Sci* 109: 74-103, 2020.
11. Yu J, Song Y and Tian W: How to select IgG subclasses in developing anti-tumor therapeutic antibodies. *J Hematol Oncol* 13: 45, 2020.
12. Vidarsson G, Dekkers G and Rispens T: IgG subclasses and allotypes: From structure to effector functions. *Front Immunol* 5: 520, 2014.
13. Hoffmann RM, Coumbe BGT, Josephs DH, Mele S, Ilieva KM, Cheung A, Tutt AN, Spicer JF, Thurston DE, Crescioli S and Karagiannis SN: Antibody structure and engineering considerations for the design and function of antibody drug conjugates (ADCs). *Oncoimmunology* 7: e1395127, 2017.
14. Wakankar A, Chen Y, Gokarn Y and Jacobson FS: Analytical methods for physicochemical characterization of antibody drug conjugates. *MAbs* 3: 161-172, 2011.
15. Chudasama V, Maruani A and Caddick S: Recent advances in the construction of antibody-drug conjugates. *Nat Chem* 8: 114-119, 2016.
16. Lyu Z, Kang L, Buuh ZY, Jiang D, McGuth JC, Du J, Wissler HL, Cai W and Wang RE: A switchable site-specific antibody conjugate. *ACS Chem Biol* 13: 958-964, 2018.
17. Drago JZ, Modi S and Chandralapaty S: Unlocking the potential of antibody-drug conjugates for cancer therapy. *Nat Rev Clin Oncol* 18: 327-344, 2021.
18. Lu J, Jiang F, Lu A and Zhang G: Linkers having a crucial role in antibody-drug conjugates. *Int J Mol Sci* 17: 561, 2016.
19. Bargh JD, Isidro-Llobet A, Parker JS and Spring DR: Cleavable linkers in antibody-drug conjugates. *Chem Soc Rev* 48: 4361-4374, 2019.
20. Tsuchikama K and An Z: Antibody-drug conjugates: Recent advances in conjugation and linker chemistries. *Protein Cell* 9: 33-46, 2018.
21. Pahl A, Lutz C and Hechler T: Amanitins and their development as a payload for antibody-drug conjugates. *Drug Discov Today Technol* 30: 85-89, 2018.
22. McCombs JR and Owen SC: Antibody drug conjugates: Design and selection of linker, payload and conjugation chemistry. *AAPS J* 17: 339-351, 2015.
23. Yaghoubi S, Karimi MH, Lotfinia M, Gharibi T, Mahi-Birjand M, Kavi E, Hosseini F, Sineh Sepehr K, Khatami M, Bagheri N and Abdollahpour-Alitappeh M: Potential drugs used in the antibody-drug conjugate (ADC) architecture for cancer therapy. *J Cell Physiol* 235: 31-64, 2020.
24. Fu Z, Li S, Han S, Shi C and Zhang Y: Antibody drug conjugate: The 'biological missile' for targeted cancer therapy. *Signal Transduct Target Ther* 7: 93, 2022.
25. Manabe S and Yamaguchi Y: Antibody glycoengineering and homogeneous antibody-drug conjugate preparation. *Chem Rec* 21: 3005-3014, 2021.
26. Jin Y, Schladetsch MA, Huang X, Balunas MJ and Wiemer AJ: Stepping forward in antibody-drug conjugate development. *Pharmacol Ther* 229: 107917, 2022.
27. Ponziani S, Di Vittorio G, Pitari G, Cimini AM, Ardini M, Gentile R, Iacobelli S, Sala G, Capone E, Flavell DJ, *et al*: Antibody-drug conjugates: The new frontier of chemotherapy. *Int J Mol Sci* 21: 5510, 2020.
28. Kantarjian HM, Kadia TM, DiNardo CD, Welch MA and Ravandi F: Acute myeloid leukemia: Treatment and research outlook for 2021 and the MD Anderson approach. *Cancer* 127: 1186-1207, 2021.
29. Ali S, Dunmore HM, Karres D, Hay JL, Salmonsson T, Gisselbrecht C, Sarac SB, Bjerrum OW, Hovgaard D, Barbachano Y, *et al*: The EMA review of mylotarg (gemtuzumab ozogamicin) for the treatment of acute myeloid leukemia. *Oncologist* 24: e171-e179, 2019.
30. McKertish CM and Kayser V: Advances and limitations of antibody drug conjugates for cancer. *Biomedicines* 9: 872, 2021.
31. Chari RV, Miller ML and Widdison WC: Antibody-drug conjugates: An emerging concept in cancer therapy. *Angew Chem Int Ed Engl* 53: 3796-3827, 2014.
32. Petersdorf SH, Kopecky KJ, Slovak M, Willman C, Nevill T, Brandwein J, Larson RA, Erba HP, Stiff PJ, Stuart RK, *et al*: A phase 3 study of gemtuzumab ozogamicin during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. *Blood* 121: 4854-4860, 2013.
33. Yu B and Liu D: Gemtuzumab ozogamicin and novel antibody-drug conjugates in clinical trials for acute myeloid leukemia. *Biomark Res* 7: 24, 2019.
34. Fatobene G, Souza GR, Rodrigues CA, Novis YAS and Rocha V: Gemtuzumab ozogamicin is efficacious in attaining complete remission in relapsed/refractory acute leukemia prior to hematopoietic cell transplant: A case series. *Hematol Transfus Cell Ther* 43: 532-536, 2021.
35. Hitzler J and Estey E: Gemtuzumab ozogamicin in acute myeloid leukemia: Act 2, with perhaps more to come. *Haematologica* 104: 7-9, 2019.
36. Lambert J, Pautas C, Terré C, Raffoux E, Turlure P, Caillot D, Legrand O, Thomas X, Gardin C, Gogat-Marchant K, *et al*: Gemtuzumab ozogamicin for de novo acute myeloid leukemia: Final efficacy and safety updates from the open-label, phase III ALFA-0701 trial. *Haematologica* 104: 113-119, 2019.
37. Kayser S and Levis MJ: Updates on targeted therapies for acute myeloid leukaemia. *Br J Haematol* 196: 316-328, 2022.
38. Schlenk RF, Paschka P, Krzykalla J, Weber D, Kapp-Schwoerer S, Gaidzik VI, Leis C, Fiedler W, Kindler T, Schroeder T, *et al*: Gemtuzumab ozogamicin in NPM1-mutated acute myeloid leukemia: Early results from the prospective randomized AMLSG 09-09 phase III study. *J Clin Oncol* 38: 623-632, 2020.
39. Baron J and Wang ES: Gemtuzumab ozogamicin for the treatment of acute myeloid leukemia. *Expert Rev Clin Pharmacol* 11: 549-559, 2018.
40. Horwitz S, O'Connor OA, Pro B, Trümper L, Iyer S, Advani R, Bartlett NL, Christensen JH, Morschhauser F, Domingo-Domenech E, *et al*: The ECHELON-2 Trial: 5-Year results of a randomized, phase III study of brentuximab vedotin with chemotherapy for CD30-positive peripheral T-cell lymphoma. *Ann Oncol* 33: 288-298, 2022.
41. Horwitz SM, Scarisbrick JJ, Dummer R, Whittaker S, Duvic M, Kim YH, Quagliano P, Zinzani PL, Bechter O, Eradat H, *et al*: Randomized phase 3 ALCANZA study of brentuximab vedotin vs physician's choice in cutaneous T-cell lymphoma: Final data. *Blood Adv* 5: 5098-5106, 2021.
42. Chen R, Herrera AF, Hou J, Chen L, Wu J, Guo Y, Synold TW, Ngo VN, Puverel S, Mei M, *et al*: Inhibition of MDR1 overcomes resistance to brentuximab vedotin in Hodgkin lymphoma. *Clin Cancer Res* 26: 1034-1044, 2020.
43. Di Girolamo A, Albanesi M, Sinisi A, Nettis E, Di Bona D, Caiaffa MF and Macchia L: Rapid desensitization for brentuximab vedotin (Adceteris<sup>®</sup>) allergy: A case report. *Clin Mol Allergy* 16: 22, 2018.
44. Chu Y, Zhou X and Wang X: Antibody-drug conjugates for the treatment of lymphoma: Clinical advances and latest progress. *J Hematol Oncol* 14: 88, 2021.
45. Jatoi I and Pinsky PF: Breast cancer screening trials: Endpoints and overdiagnosis. *J Natl Cancer Inst* 113: 1131-1135, 2021.
46. Hamilton E, Shastry M, Shiller SM and Ren R: Targeting HER2 heterogeneity in breast cancer. *Cancer Treat Rev* 100: 102286, 2021.
47. Derakhshani A, Rezaei Z, Safarpour H, Sabri M, Mir A, Sanati MA, Vahidian F, Gholamiyan Moghadam A, Aghadokht A, Hajiasgharzadeh K and Baradaran B: Overcoming trastuzumab resistance in HER2-positive breast cancer using combination therapy. *J Cell Physiol* 235: 3142-3156, 2020.
48. Cesca MG, Vian L, Cristóvão-Ferreira S, Pondé N and de Azambuja E: HER2-positive advanced breast cancer treatment in 2020. *Cancer Treat Rev* 88: 102033, 2020.
49. Dosio F, Brusa P and Cattel L: Immunotoxins and anticancer drug conjugate assemblies: The role of the linkage between components. *Toxins (Basel)* 3: 848-883, 2011.
50. Lombardi J, Lory P, Martin N, Mayeur D, Combret S, Grandvilllemin A, Boulay C and Schmitt A: Trastuzumab-emtansine induced pleural and pericardial effusions. *J Oncol Pharm Pract* 27: 2041-2044, 2021.
51. Theocharopoulos C, Lialios PP, Gogas H and Zogas DC: An overview of antibody-drug conjugates in oncological practice. *Ther Adv Med Oncol* 12: 1758835920962997, 2020.
52. Okines A, Irfan T, Khabra K, Smith I, O'Brien M, Parton M, Noble J, Stanway S, Somaiah N, Ring A, *et al*: Development and responses of brain metastases during treatment with trastuzumab emtansine (T-DM1) for HER2 positive advanced breast cancer: A single institution experience. *Breast J* 24: 253-259, 2018.
53. Hardy-Werbin M, Quiroga V, Cirauqui B, Romeo M, Felip E, Teruel I, Garcia JJ, Erasun C, España S, Cucurull M, *et al*: Real-world data on T-DM1 efficacy-results of a single-center retrospective study of HER2-positive breast cancer patients. *Sci Rep* 9: 12760, 2019.

54. Pondé N, Aftimos P and Piccart M: Antibody-drug conjugates in breast cancer: A comprehensive review. *Curr Treat Options Oncol* 20: 37, 2019.
55. Chen SC, Quartino A, Polhamus D, Riggs M, French J, Wang X, Vadhavkar S, Smitt M, Hoersch S, Strasak A, *et al*: Population pharmacokinetics and exposure-response of trastuzumab emtansine in advanced breast cancer previously treated with  $\geq 2$  HER2-targeted regimens. *Br J Clin Pharmacol* 83: 2767-2777, 2017.
56. Larionov AA: Current therapies for human epidermal growth factor receptor 2-positive metastatic breast cancer patients. *Front Oncol* 8: 89, 2018.
57. Perez EA, de Haas SL, Eiermann W, Barrios CH, Toi M, Im YH, Conte PF, Martin M, Pienkowski T, Pivot XB, *et al*: Relationship between tumor biomarkers and efficacy in MARIANNE, a phase III study of trastuzumab emtansine  $\pm$  pertuzumab versus trastuzumab plus taxane in HER2-positive advanced breast cancer. *BMC Cancer* 19: 517, 2019.
58. Perez EA, Barrios C, Eiermann W, Toi M, Im YH, Conte P, Martin M, Pienkowski T, Pivot XB, Burris HA III, *et al*: Trastuzumab emtansine with or without pertuzumab versus trastuzumab with taxane for human epidermal growth factor receptor 2-positive advanced breast cancer: Final results from MARIANNE. *Cancer* 125: 3974-3984, 2019.
59. Liu F, Ke J and Song Y: T-DM1-induced thrombocytopenia in breast cancer patients: New perspectives. *Biomed Pharmacother* 129: 110407, 2020.
60. Krop IE, Kim SB, González-Martín A, LoRusso PM, Ferrero JM, Smitt M, Yu R, Leung AC and Wildiers H; TH3RESA study collaborators: Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA): A randomised, open-label, phase 3 trial. *Lancet Oncol* 15: 689-699, 2014.
61. Li BT, Michelini F, Misale S, Cocco E, Baldino L, Cai Y, Shifman S, Tu HY, Myers ML, Xu C, *et al*: HER2-mediated internalization of cytotoxic agents in ERBB2 amplified or mutant lung cancers. *Cancer Discov* 10: 674-687, 2020.
62. Tilly H, Morschhauser F, Sehn LH, Friedberg JW, Trněný M, Sharman JP, Herbaux C, Burke JM, Matasar M, Rai S, *et al*: Polatuzumab vedotin in previously untreated diffuse large B-cell lymphoma. *N Engl J Med* 386: 351-363, 2022.
63. Bardia A, Tolaney SM, Punie K, Loirat D, Oliveira M, Kalinsky K, Zelnak A, Aftimos P, Dalenc F, Sardesai S, *et al*: Biomarker analyses in the phase III ASCENT study of sacituzumab govitecan versus chemotherapy in patients with metastatic triple-negative breast cancer. *Ann Oncol* 32: 1148-1156, 2021.
64. Chu CE, Sjöström M, Egusa EA, Gibb EA, Badura ML, Zhu J, Koshkin VS, Stohr BA, Meng MV, Pruthi RS, *et al*: Heterogeneity in NECTIN4 expression across molecular subtypes of urothelial cancer mediates sensitivity to enfortumab vedotin. *Clin Cancer Res* 27: 5123-5130, 2021.
65. Lonial S, Lee HC, Badros A, Trudel S, Nooka AK, Chari A, Abdallah AO, Callander N, Sborov D, Suvannasankha A, *et al*: Longer term outcomes with single-agent belantamab mafodotin in patients with relapsed or refractory multiple myeloma: 13-Month follow-up from the pivotal DREAMM-2 study. *Cancer* 127: 4198-4212, 2021.
66. Dahlgren D and Lennernäs H: Antibody-drug conjugates and targeted treatment strategies for hepatocellular carcinoma: A drug-delivery perspective. *Molecules* 25: 2861, 2020.
67. Takegawa N, Tsurutani J, Kawakami H, Yonesaka K, Kato R, Haratani K, Hayashi H, Takeda M, Nonagase Y, Maenishi O and Nakagawa K: [fam-] trastuzumab deruxtecan, antitumor activity is dependent on HER2 expression level rather than on HER2 amplification. *Int J Cancer* 145: 3414-3424, 2019.
68. Indini A, Rijavec E and Grossi F: Trastuzumab deruxtecan: Changing the destiny of HER2 expressing solid tumors. *Int J Mol Sci* 22: 4774, 2021.
69. Aoki M, Iwasa S and Boku N: Trastuzumab deruxtecan for the treatment of HER2-positive advanced gastric cancer: A clinical perspective. *Gastric Cancer* 24: 567-576, 2021.
70. Andrikopoulou A, Zografos E, Lontos M, Koutsoukos K, Dimopoulos MA and Zagouri F: Trastuzumab deruxtecan (DS-8201a): The latest research and advances in breast cancer. *Clin Breast Cancer* 21: e212-e219, 2021.
71. Yin O, Iwata H, Lin CC, Tamura K, Watanabe J, Wada R, Kastrissios H, AbuTarif M, Garimella T, Lee C, *et al*: Exposure-response relationships in patients with HER2-positive metastatic breast cancer and other solid tumors treated with trastuzumab deruxtecan. *Clin Pharmacol Ther* 110: 986-996, 2021.
72. Xu Z, Guo D, Jiang Z, Tong R, Jiang P, Bai L, Chen L, Zhu Y, Guo C, Shi J and Yu D: Novel HER2-targeting antibody-drug conjugates of trastuzumab beyond T-DM1 in breast cancer: Trastuzumab deruxtecan (DS-8201a) and (Vic-)trastuzumab duocarmazine (SYD985). *Eur J Med Chem* 183: 111682, 2019.
73. Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, Tsurutani J, Ueno NT, Prat A, Chae YS, *et al*: Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. *N Engl J Med* 387: 9-20, 2022.
74. Modi S, Saura C, Yamashita T, Park YH, Kim SB, Tamura K, Andre F, Iwata H, Ito Y, Tsurutani J, *et al*: Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. *N Engl J Med* 382: 610-621, 2020.
75. Modi S, Park H, Murthy RK, Iwata H, Tamura K, Tsurutani J, Moreno-Aspitia A, Doi T, Sagara Y, Redfern C, *et al*: Antitumor activity and safety of trastuzumab deruxtecan in patients with HER2-low-expressing advanced breast cancer: Results from a phase Ib study. *J Clin Oncol* 38: 1887-1896, 2020.
76. Kotani D and Shitara K: Trastuzumab deruxtecan for the treatment of patients with HER2-positive gastric cancer. *Ther Adv Med Oncol* 13: 1758835920986518, 2021.
77. Shitara K, Bang YJ, Iwasa S, Sugimoto N, Ryu MH, Sakai D, Chung HC, Kawakami H, Yabusaki H, Lee J, *et al*: Trastuzumab deruxtecan in previously treated HER2-positive gastric cancer. *N Engl J Med* 382: 2419-2430, 2020.
78. Perez J, Garrigós L, Gion M, Jänne PA, Shitara K, Siena S and Cortés J: Trastuzumab deruxtecan in HER2-positive metastatic breast cancer and beyond. *Expert Opin Biol Ther* 21: 811-824, 2021.
79. Tsurutani J, Iwata H, Krop I, Jänne PA, Doi T, Takahashi S, Park H, Redfern C, Tamura K, Wise-Draper TM, *et al*: Targeting HER2 with trastuzumab deruxtecan: A dose-expansion, phase I study in multiple advanced solid tumors. *Cancer Discov* 10: 688-701, 2020.
80. Meric-Bernstam F, Hurwitz H, Raghav KPS, McWilliams RR, Fakih M, VanderWalde A, Swanton C, Kurzrock R, Burris H, Sweeney C, *et al*: Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): An updated report from a multicentre, open-label, phase 2a, multiple basket study. *Lancet Oncol* 20: 518-530, 2019.
81. Kumagai K, Aida T, Tsuchiya Y, Kishino Y, Kai K and Mori K: Interstitial pneumonitis related to trastuzumab deruxtecan, a human epidermal growth factor receptor 2-targeting Ab-drug conjugate, in monkeys. *Cancer Sci* 111: 4636-4645, 2020.
82. Shitara K, Baba E, Fujitani K, Oki E, Fujii S and Yamaguchi K: Discovery and development of trastuzumab deruxtecan and safety management for patients with HER2-positive gastric cancer. *Gastric Cancer* 24: 780-789, 2021.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.