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# *Trastuzumab Deruxtecan: Molecular Design, Clinical Applications, Challenges, and Future Directions*

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***Abstract.*** Human epidermal growth factor receptor 2 (HER2) dysregulation contributes to the progression of breast, gastric, lung, and other solid tumors, yet conventional HER2-targeted therapies remain limited by tumor heterogeneity and acquired resistance. Trastuzumab deruxtecan (T-DXd), a HER2-targeted antibody-drug conjugate (ADC), combines trastuzumab with a cleavable tetrapeptide linker and the topoisomerase I inhibitor DXd. Its high drug-to-antibody ratio and membrane-permeable payload support potent cytotoxicity and a bystander effect in tumors with heterogeneous HER2 expression. Clinical trials have demonstrated benefit in HER2-positive and HER2-low breast cancer, HER2-positive gastric cancer, and selected HER2-altered non-small cell lung cancers, although evidence maturity and biomarker criteria vary among tumor types. Interstitial lung disease/pneumonitis, systemic toxicity, spatial and temporal HER2 heterogeneity, and multifactorial resistance continue to constrain the therapeutic index. This review examines the molecular design, mechanism of action, clinical applications, and limitations of T-DXd and evaluates antibody engineering, site-specific conjugation, linker and payload optimization, biomarker-guided selection, and mechanism-informed treatment strategies for next-generation HER2-targeted ADCs.

***Keywords:*** HER2, antibody-drug conjugates, trastuzumab deruxtecan, therapeutic resistance

## 1. Introduction

Human Epidermal Growth Factor Receptor 2 (HER2, also known as ERBB2) is a member of the epidermal growth factor receptor (EGFR) family and plays a role in the regulation of cell proliferation, differentiation, survival and migration. HER2 amplification or overexpression occurs in about 15-20% of breast cancers and 10-20% of gastric and gastroesophageal junction cancers, and has also been found in colorectal, lung, endometrial, and biliary tract cancers [1, 2]. Aberrant activation of HER2 promotes continuous downstream signals through the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) and mitogen-activated protein kinase (MAPK) pathways, thus promoting the proliferation, migration, metastasis and apoptosis evasion of tumour cells. Before the introduction of HER2-targeted therapy, HER2-positive cancer was generally associated with a poor prognosis and early recurrence. Therefore, further increases in the accuracy, effectiveness and safety of HER2-targeted therapies are needed clinically.

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Antibody-drug conjugates (ADCs) are now the leading form of HER2-targeted therapy in precision oncology. ADCs chemically link a highly specific monoclonal antibody to a strong cytotoxic agent for selective delivery of the agent to cancer cells expressing antigens. Trastuzumab deruxtecan (T-DXd; DS-8201a; Enhertu®) is a second-generation HER2-targeted ADC that consists of trastuzumab, a cleavable tetrapeptide linker, and DXd, a potent topoisomerase I inhibitor derived from exatecan. After HER2 binding, T-DXd is internalised and transported to lysosomes; there, linker cleavage releases membrane-permeable DXd. T-DXd has a relatively high drug-to-antibody ratio (DAR; around 8) and a strong bystander effect compared with earlier HER2-targeted ADCs, and thus can distribute the drug to neighbouring HER2-low or antigen-heterogeneous tumour cells. Together, these attributes offer a mechanism for the activity of tumours at different levels and patterns of HER2 expression.

Although T-DXd has shown some good clinical results, it is not free from defects. Interstitial Lung Disease (ILD)/pneumonitis is a serious side effect that can be fatal if not identified and dealt with in time. Myelosuppression, gastrointestinal adverse effects and fatigue are also relatively common during treatment. In addition, intrinsic and acquired resistance may be caused by HER2 downregulation, impaired ADC internalization, lysosomal dysfunction, altered linker processing, activation of compensatory signalling pathways, and reduced payload sensitivity. Tumor heterogeneity and off-tumor exposure further reduce the maintenance of a good therapeutic window. Thus, the clinical effect of T-DXd is related to the target selectivity, systemic exposure, payload release and tumour susceptibility.

Therefore, many efforts have been made to optimise T-DXd and other HER2-targeted ADCs. Antibody engineering aims to enhance tumour-specific recognition and reduce binding to normal tissues. To achieve a more uniform dispersion of DAR and enhance plasma stability, some site-specific modification techniques have been developed, including THIO-MAB engineering, enzymatic conjugation, and glycan-based conjugation. Linker engineering aims to balance the stability of circulation with efficient intracellular payload release. Payload development explores alternative mechanisms to improve potency, reduce systemic toxicity, and may address the problem of resistance. In short, the above two strategies are to enhance the anti-cancer effect and reduce the side effect.

This paper reviews the molecular mechanism, clinical applications and problems related to trastuzumab deruxtecan, and introduces recent developments in the four main areas of optimisation for HER2-targeted ADCs: targeting strategies, conjugation technology, linker design and payload engineering. Based on the latest preclinical and clinical data, this review presents new ways to improve the safety, selectivity and therapeutic effect of T-DXd, and offers an idea for the rational design of next-generation HER2-targeted ADCs.

## 2. Molecular structure, mechanism of action and design rationale of T-DXd

DXd (DS-8201a) is a HER2-targeted ADC consisting of an anti-HER2 monoclonal antibody, a cleavable tetrapeptide-based linker, and DXd, a potent topoisomerase I inhibitor. The antibody part is selective for HER2; the linker regulates the release of the intracellular payload, and DXd has cytotoxicity. A typical design feature of T-DXd is a relatively high DAR of around 8; thus, more payload molecules can be carried per antibody molecule without significantly altering the pharmacokinetic characteristics [3, 4]. The Molecular Structure and Main Design Features of T-DXd are shown in Figure 1.

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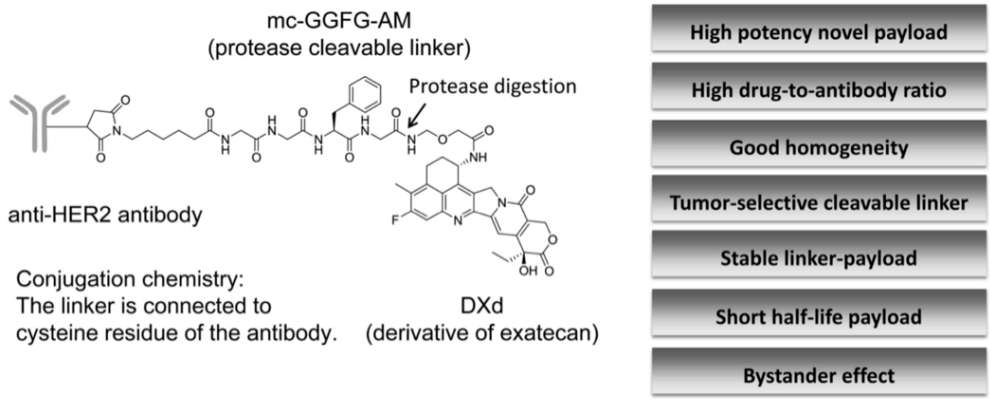



Figure 1. Structure and key features of T-DXd [4]

After administration, T-DXd first binds to HER2 on the tumor-cell surface and is subsequently internalized through receptor-mediated endocytosis. The ADC is delivered through the endosomal pathway to the lysosomes, and there, lysosomal enzymes such as cathepsins cleave the tetrapeptide linker to release the active DXd payload. The Design aims to maintain plasma stability and enable the release of the payload after ADC internalisation.

DXd inhibits topoisomerase I after release, causes damage to DNA during DNA replication, and thus induces tumour cell death. DXd is released from a target cell and can diffuse to neighbouring cells to induce a "bystander effect". This feature of T-DXd can mediate the killing of nearby tumour cells with low or heterogeneous levels of HER2 without requiring direct uptake by all tumour cells. The high DAR, efficient intracellular release, potent topoisomerase I inhibition, and membrane permeability of DXd thus provide a mechanistic basis for the activity of T-DXd in the context of tumors with heterogeneous HER2 expression [3, 4].

## 3. Clinical applications and therapeutic progress of T-DXd

T-DXd has shown good clinical results in many HER2-overexpressing cancers, including HER2-positive breast cancer and tumors with low or heterogeneous HER2 expression. DESTINY-Breast03 showed that T-DXd improved progression-free survival and overall survival compared with trastuzumab emtansine (T-DM1) in patients with unresectable or metastatic HER2-positive breast cancer who had previously received trastuzumab and a taxane, and was thus approved as a first-line treatment [5]. Activity also includes HER2-low breast cancer, which is operationally defined as immunohistochemistry (IHC) 1+ or IHC 2+/in situ hybridization-negative disease in DESTINY-Breast04. In that trial, T-DXd improved progression-free survival and overall survival compared with physician's-choice chemotherapy; thus, HER2 expression below the conventional positive threshold may still be suitable for clinical application of ADC targeting [6].

T-DXd is also effective for non-breast cancer indications. DESTINY-Gastric01 improved the response rate and overall survival of patients with previously treated HER2-positive gastric or gastroesophageal junction cancer compared to chemotherapy, and thus supported its use in a heavily pretreated population [7]. Assessment of HER2 in gastric cancer is more difficult due to its spatial heterogeneity; therefore, careful selection of biopsy sites and pathological analysis are required. T-DXd has shown anti-tumour effects in non-small cell lung cancer (NSCLC) with activating HER2 mutations and in some HER2-overexpressing tumours. Patient selection for NSCLC may therefore

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need to consider both HER2 protein expression and molecular detection of HER2 alterations based on differences in tumour biology [7].

Besides breast, gastric and lung cancers, T-DXd has also been studied in other HER2-overexpressing solid tumors, such as colorectal, biliary tract and endometrial cancers. Based on the above research, T-DXd may be suitable for various other cancers, especially those with HER2 amplification or other alterations to the HER2 gene. However, the evidence from these studies is less mature than that for breast and gastric cancer, as many of the studies are small, non-randomised and use different criteria for HER2 assessment. Therefore, more prospective studies are needed to identify suitable biomarkers, patients who are likely to benefit from this treatment, and other applications of T-DXd in various HER2-expressing solid tumors [8].

## 4. Safety, resistance mechanisms, and clinical challenges of T-DXd

T-DXd has shown good clinical results; however, there are also problems of treatment-related toxicity, acquired resistance and biological heterogeneity. The problems are due to the complexity of ADCs and involve coordinated function of antibodies, linkers, DARs and cytotoxic drugs. Therefore, the next step in development will be to improve the therapeutic index by reducing toxicity while maintaining the anti-cancer effect.

### 4.1. Major adverse events and their clinical management

The two serious adverse events of T-DXd in clinical practice are interstitial lung disease (ILD) and pneumonitis. Although many cases are mild and can be treated successfully, serious or fatal diseases may still occur; thus, early diagnosis and treatment are necessary. The clinical features of T-DXd-related ILD/pneumonitis are not distinct from those of infection, tumour progression and other pulmonary diseases. Therefore, the problems should be addressed in time. Systematic assessment of respiratory symptoms should be conducted, along with imaging surveillance and multidisciplinary consultation in cases of suspected ILD/pneumonitis. Patient education is also necessary; otherwise, early symptoms of cough, dyspnoea or new-onset respiratory problems may not be recognised in time to prevent severe illness. Management should correspond to the severity of ILD/pneumonitis, and according to the clinical grade and management guidelines [9], treatment interruption or discontinuation and corticosteroid-based treatment are required.

T-DXd may also have other systemic side effects, such as blood disorders, nausea, vomiting, loss of appetite and fatigue. These toxicities may be due to whole-cell exposure of the intact ADC or released payloads, and their mechanisms are not necessarily the same. High-potency ADC payloads are likely to damage normal cells by off-target effects or other reasons during systemic circulation [10]. Safety monitoring should therefore extend to ILD/pneumonitis. Periodically monitor blood counts, gastrointestinal symptoms, nutrition and general health to adjust the dosage of medications and reduce unnecessary harm. Generally speaking, early detection and suitable separation for prompt treatment are needed to keep within the range of therapy.

### 4.2. Intrinsic and acquired resistance mechanisms

Resistance to T-DXd can be caused by disruptions at various stages of the ADC pathway. As an activity-dependent mechanism, for this HER2 recognition, internalisation, intracellular trafficking, linker cleavage and payload release to occur, all stages must be intact. Dynamic loss or reduction of HER2 expression will decrease available binding sites and limit ADC binding. Resistance may also

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be associated with altered DNA damage repair, tumour microenvironment remodelling and immune evasion; therefore, it cannot be attributed solely to changes in HER2 expression.

Impaired uptake of the ADC or altered trafficking to the lysosome may lead to reduced processing and release of the cytotoxic ADC payload. Therefore, a defective lysosome is unable to increase the intracellular level of DXd. Changes in linker processing or drug-release pathways may also prevent the formation of an active cytotoxic agent from the intact ADC. The two reasons above are particularly relevant because, for the ADC to be effective, it needs to reach the target cell and be activated inside that cell [10, 11].

Resistance can also occur downstream of payload release. T-DXd is a topoisomerase I inhibitor, and altered DNA damage response or repair pathways may reduce the sensitivity of tumour cells to the resulting cytotoxic stress. Compensatory survival signals and alterations in the tumour microenvironment can also help tumour cells survive after exposure to the intracellular payload. Therefore, the problem of acquired resistance is complex and includes changes in the biology of target cells, alterations to intracellular regulation, DNA damage response, and tumour cell adaptation. Mechanistic diversity provides a rationale for the development of next-generation ADCs with alternative targets, linkers or payloads, and for exploring mechanism-informed combination strategies after progression on T-DXd.

### 4.3. Tumor heterogeneity and limitations of the therapeutic index

HER2 heterogeneity is also a problem for T-DXd. HER2 expression differs among patients, varies in different tumour locations within the same patient, and is not uniform among individual tumour cells. The space and time of these changes may affect ADC binding, uptake and release. The bystander effect of T-DXd may help to address heterogeneity in antigen expression, but only partially, as the payload released by T-DXd can affect neighbouring cells without fully resolving the variable antigen expression [10]. Alterations in HER2 expression during disease progression or after treatment may also participate in acquired resistance [11].

Targeted delivery is also employed outside of tumours at the same time. ADCs and released payloads can reach normal tissues through non-specific uptake or target expression in healthy tissues, and released drugs are also distributed systemically. Thus, the high potency of the ADC payload can be used to balance sufficient tumour exposure with reduced systemic exposure [10]. The Goal of T-DXd optimisation is still to enhance the therapeutic index. To improve tumour-selective delivery, enhance linker stability, reduce premature payload release and achieve more homogeneous ADCs, various strategies can be employed; however, improvements in one design parameter may introduce defects in others [12, 13].

## 5. Optimization strategies and future directions for HER2-targeted ADCs

### 5.1. Optimization of targeting molecules and conjugation technologies

Antibody parts help to improve the effectiveness of ADCs. Optimisation is modification of antibody affinity, epitope recognition, valency, molecular form and uptake behaviour. The best antibody may not be the one with the highest affinity; it may be a relatively low-affinity antibody that can still be taken up by the cells. Generally speaking, the antibody form may affect tumour penetration, antibody pharmacokinetics and interaction with non-tumoural tissues; therefore, it should be considered in conjunction with the selection of the linker and payload and not taken in isolation.

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The other place to improve is the conjugation technology. Conventional stochastic conjugation can produce populations of ADC molecules with different DAR species and thus exhibit various pharmacokinetic properties, efficacies and toxicities. Site-specific conjugation aims to introduce the payload molecule at a specific site on the antibody to produce a more uniform product with better control over its DAR and molecular characteristics [10, 13]. Controlled conjugation can also be achieved by other technologies such as THIOMAB, SMARTag, microbial transglutaminase-mediated conjugation and glycan remodeling [13]. Although the above technologies can improve the homogeneity and stability of products, their pharmacokinetic and safety advantages for all ADC constructs based on site-specificity have not been fully demonstrated.

However, it should not be assumed that an increase in DAR will always be optimal. Although many payload molecules on antibodies enhance cytotoxicity, they may also increase hydrophobicity; thus, they can aggregate, be poorly cleared and cause systemic toxicity. Therefore, control of DAR and its distribution is necessary in ADC engineering. A site-specific method can be used to build an explicit DAR and thus improve batch-to-batch and molecule-to-molecule consistency.

### 5.2. Linker and payload engineering

The linker is used to connect antibodies and payloads; thus, it helps maintain the stability of the complex in the bloodstream during systemic circulation and can be released effectively inside cells. Non-cleavable linkers generally require the degradation and intracellular processing of the antibody to generate an active metabolite carrying the payload; on the other hand, cleavable linkers can be triggered by various intracellular environments, such as proteases, acidity or reduction. Therefore, for the problem of plasma stability, intracellular delivery, payload characteristics and bystander effects, one of the four approaches must be chosen.

For T-DXd and other ADCs, premature linker cleavage will increase the systemic exposure of the cytotoxic payload, and overly stable linkers may inhibit intracellular release and thus reduce efficacy. Therefore, Linker Design will affect pharmacokinetics, toxicity, payload release and therapeutic effect. Recently, the direction of engineering efforts has been to improve plasma stability, optimise release triggers, modify spacer structures for hydrophilicity control, and promote efficient intracellular release. The above ways can help to regulate systemic stability and the release of tumour-targeting materials simultaneously [12].

Payload engineering is another way to address the deficiencies of current ADCs. Although highly potent cytotoxic agents are effective against rapidly dividing cancer cells, resistance to a specific mechanism of action may limit their prolonged effect. Development of payloads with alternative mechanisms can thus help address payload-specific resistance and support sequential ADC treatment, provided that clinically relevant cross-resistance is absent or small [11]. In addition, the whole ADC should consider the characteristics of the payload in terms of potency, membrane permeability and susceptibility to resistance mechanisms, etc., and not consider these factors separately.

### 5.3. Integrated multi-parameter design and translational development

The next generation of HER2-targeted ADCs will probably be based on combined optimisation rather than enhancement of a single molecular component. Affinity and Epitopes, Conjugation Sites, DAR, Linker Stability, Payload Potency, and Intracellular Release are all interconnected. For example, increasing the payload load may enhance potency but will alter hydrophobicity and pharmacokinetics; extending the stability of the linker will prevent premature release but may

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reduce intracellular payload release. Therefore, ADC development should be based on the systematic optimisation of the entire molecular system.

Biomarker-guided patient selection will also be employed. HER2 expression is not a fixed marker, and both its location and time of occurrence can be different due to treatment. Combine quantitative HER2 assessment with other biological markers of ADC sensitivity or resistance to improve patient stratification. In patients who develop T-DXd resistance, ADCs with other targets or payloads can be used, or HER2-directed therapy combined with other therapeutic methods may be employed according to the resistance mechanism found in each clinical case [11].

The ultimate goal of the development of next-generation HER2-targeted ADCs is to improve the therapeutic index by enhancing tumour-selective delivery and reducing systemic exposure to the active ingredient. Progress in site-specific conjugation, linker chemistry, antibody engineering and payload diversification have provided several routes for the construction of this system [10-13]. The next generation of ADCs will be employed as a whole-molecule system, and individually, antibodies, linkers, DARs and payloads will not be designed. The above multi-parameter approach can be used to select patients who are more likely to respond to HER2-targeted ADCs, improving the safety profile and extension of the antitumour effect through mechanistic understanding of resistance.

## 6. Conclusion

HER2-targeting ADCs, such as T-DXd, have expanded the treatment options for HER2-positive cancers. A high DAR, a tumor-cell-internalised cleavable linker, a highly potent DXd payload and a bystander effect are combined to improve the ability of T-DXd to target tumours with heterogeneous HER2 expression. Clinical evidence is relatively strong for HER2-positive and HER2-low breast cancer, HER2-positive gastric cancer, and some HER2-altered NSCLC. However, for other HER2-overexpressing solid tumors, the current results should be considered as supporting data for therapy rather than definitive proof of clinical benefit due to small sample sizes, early-stage development and heterogeneous study designs of available studies.

Although it has good clinical efficacy, the therapeutic prospects of T-DXd are limited by interstitial lung disease/pneumonitis, haematological and gastrointestinal toxicities, acquired resistance, spatial and temporal HER2 heterogeneity, and off-tumor exposure. Optimisation of a single component in an ADC, such as improving antibody affinity, increasing the drug-to-antibody ratio (DAR), enhancing linker stability or boosting payload potency, may not expand the therapeutic window because these traits are interdependent, and trade-offs among efficacy, pharmacokinetics, and safety often need to be considered. Therefore, the future development will be a multi-parameter Design strategy of antibody engineering, site-specific conjugation, DAR control, linker optimisation and payload selection. Dynamic assessment of HER2 and reliable biomarkers for resistance need to be developed further to support biomarker-guided patient selection and mechanism-based sequential or combination therapy. Finally, prospectively randomised and tumor-agnostic or cross-tumor studies will need to be conducted to evaluate the long-term efficacy and safety of next-generation HER2-targeted ADCs, optimise patient selection criteria, and determine whether they can overcome therapeutic resistance.

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