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Molecular Framework of T-DXd Acquired Resistance in HER2-Positive Breast Cancer

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Abstract. T-DXd has transformed the world of directed treatment of HER2 positive breast cancer but its clinical benefit over an extended period of time is severely constrained by unavoidable acquired resistance. Although recent studies have examined specific resistance pathways, a comprehensive overview of this very heterogeneous molecular network is still relatively absent. The present study systematically examines the stratified biological processes that form the basis of the acquired T-DXd resistance as informed by the pharmacokinetic profiles of antibody drug conjugates (ADCs) and describes in great detail all the components of a full resistance model, including the loss of antigen binding and intracellular metabolic escape mechanisms. Besides, it also shows what leads to severe resistance by the nuclear DNA damage repair compensation mechanism, illustrated by SLX4-deficiency, which clearly defines the essential evolutionary mechanisms used by the tumor cells to avoid ADC-mediated killing. These main insights offer important theoretical direction to clinicians when developing personalized, evidence-based combination therapies in patients who are progressing post-T-DXd resistance. Nevertheless, the absence of empirical verification in the form of massive clinical cohorts in real life has been an unsolved weakness of this discipline. Next-generation research should be aimed at using non-invasive liquid biopsy techniques to observe the clonal evolution in real-time and fast-track the generation of new combination approaches to eliminate the resistance obstacles conclusively.

Keywords: antibody drug conjugates (ADCs), T-DXd, HER2-positive breast cancer

1. Introduction

Over the past few years, there has been a quick evolution of antibody-drug conjugates (ADCs) that have transformed the context of tumor-targeted treatments. Trastuzumab Deruxtecan (T-DXd) as an example drug of the new generation against the HER2 is successful in various clinical trials and got its first approval in 2019 as a treatment of patients with metastatic HER2-positive breast cancer [1]. For instance, by improving the connection design and using a more potent topoisomerase I inhibitor, it kills adjacent HER2-negative cells through the "bystander effect", demonstrating broader applicability, especially in HER2 low blood type breast cancer patients [2]. However, as T-DXd becomes more widely used in clinical settings and the treatment lines continue to advance, almost

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all patients will face the problem of drug resistance after continuous medication [3]. This has become the core clinical challenge currently restricting the long-term benefits of T-DXd.

Regarding the resistance issue of T-DXd, scholars have conducted extensive basic and preclinical explorations in recent years. For instance, the observed reduction in HER2 expression in some patients at the advanced stage of T-DXd treatment might be sufficient to lower the internalization of T-DXd to the level of clinical treatment resistance [4]. However, current literature evidence indicates that the resistance mechanism of T-DXd is highly heterogeneous and complex. On one hand, the mechanisms related to the "antibody-antigen" level include the downregulation or complete loss of HER2 expression and the loss of HER2 binding ability [4]. On the other hand, the mechanisms related to the target-dependent level (reduced HER2 (ERBB2) abundance and acquired mutations - affecting drug uptake and binding) and the drug-carrying efflux mechanism (KEAP1/NRF2–ABCC1 axis driving multidrug resistance - shortening intracellular drug retention time) [3].

In the face of the complex resistance mechanism, subsequent treatment strategies are also in a diversified exploration stage, including improving the delivery of ADC to tumor tissues and regulating the expression of antigen-targeted proteins, ADC combined with chemotherapy (such as combining with taxanes or anthracyclines), and ADC combined with immunotherapy (its potential mechanisms include inducing immunogenic cell death, promoting dendritic cell maturation, enhancing T lymphocyte infiltration, and enhancing immune memory) [5]. However, as of now, the literature evidence in this field is relatively fragmented and lacks a macroscopic review of the key nodes of the resistance molecular network. Moreover, precise treatment strategies for different resistance phenotypes have not yet formed a standard clinical consensus.

Therefore, in the current context, it is very important to logically and systematically integrate and analyze the existing research results on the resistance of T-DXd. This article aims to systematically summarize the structural characteristics and anti-tumor mechanism basis of T-DXd, the layered biological mechanisms of T-DXd's acquired resistance, the clinical and frontier treatment strategies for overcoming T-DXd resistance, and the challenges of clinical translation. This article aims to build a multi-dimensional resistance mechanism framework to help basic researchers clarify the deep biological laws of cancer cells evading ADC killing, and also facilitate clinical doctors to formulate individualized, evidence-based post-treatment combination plans after the progression of T-DXd, which has significant guiding significance.

2. Structural characteristics and anti-tumor mechanism of T-DXd

2.1. Structure and mechanism of T-DXd

T-DXd is an ADC composed of a humanized anti-HER2 IgG1 monoclonal antibody (with the same amino acid sequence as trastuzumab) covalently linked to the topoisomerase I inhibitor payload (DXd, a derivative of the camptothecin-like drug Exatecan) through a cleavable tetrapeptide linker, as shown in Figure 1.

In comparison to the previous generation of ADCs (e.g., T-DM1, where the drug-to-antibody ratio (DAR) is merely 3.5), the most important structural improvement of T-DXd is its very homogeneous and remarkably high drug-to-antibody ratio (DAR 8) [6] (refer to Table 1). The high-density coupling technology allows a single antibody molecule to provide over two-fold of cytotoxic payload to tumor cells. Also, the GGFG tetrapeptide linker is highly chemically stable in blood circulation system and this makes the premature release of the payload in off-target tissues very low; and upon entering the given intracellular microenvironment, this linker may be rapidly cleaved.

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The cytotoxic properties of the T-DXd payload are not similar to those of other trastuzumab-dependent anti-drugs; the T-DXd payload (DXd) induces ADCC by two ways: the inhibition of topoisomerase I (inhibition of topoisomerase I-DNA complex) and antibody interaction with the FcgRIII domain on the immune effector cell surface. The inhibition of the topoisomerase I-DNA complex will cause inhibition of DNA replication, cell cycle arrest, and apoptosis of the tumor cells. The phosphorylation of Akt (which is a signal transduction pathway that promotes cell survival and growth) is downregulated through antibody binding and phosphorylation of the cell cycle protein kinase inhibitor p27 is upregulated, which finally results in the inhibition of cell proliferation.DXd shows stronger anti-tumor activity in various tumor xenograft models, superior to irinotecan (another topoisomerase I inhibitor that has shown activity in breast cancer, including the tumor with resistant irinotecan) [6]. In contrast, the payload of T-DM1 can prevent the mitosis of dividing cells, while the trastuzumab decalin payload can inhibit DNA alkylation. Although their mechanisms of action are different, all payloads ultimately lead to cell death [6].

Table 1. The DAR of three ADC [7]

Trastuzumab duocarmazine	Trastuzumab emtansine (T-DM1)	Trastuzumab deruxtecan(T-DXd)	Trastuzumab duocarmazine
Structure			
DAR	3.5:1	8:1	2.8:1

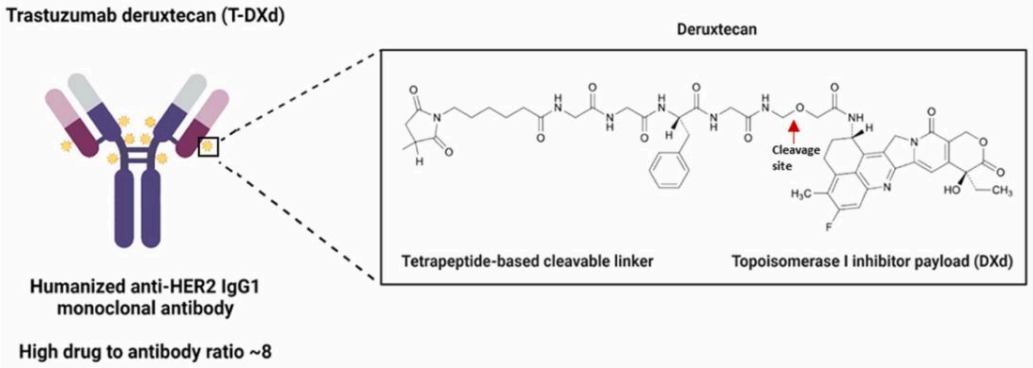

Figure 1. The structure of trastuzumab deruxtecan [7]

2.2. Bystander killing effect (bystander effect) and its microenvironment

Apart from precisely targeting and killing target cells, T-DXd possesses core pharmacological characteristics that distinguish it from many early ADCs - a potent "bystander killing effect"; the DXd payload molecule not only has extremely high cytotoxicity but also has excellent lipid solubility and transmembrane penetration ability. When the target cells (HER2-positive cells) are killed and rupture by DXd, or when DXd is released in lysosomes, the excess DXd can easily penetrate the cell membrane of the target cells and spread into adjacent tumor cells (even if these adjacent cells do not express HER2) and induce their death [8]. This unique bystander effect has significant clinical value: it not only overcomes the common "antigen spatial heterogeneity"

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problem in solid tumors (that is, only some cells in the tumor mass express HER2), but is also the fundamental molecular basis for the success of T-DXd in tumors [1].

3. Hierarchical biological mechanisms of T-DXd

3.1. Resistance acquisition

Unlike traditional small molecule targeted drugs or single-agent chemotherapy, the anti-tumor process of T-DXd is a precise assembly line involving "transmembrane binding, endocytosis transport, enzymatic metabolism, and nucleic acid killing". Therefore, cancer cells will undergo dynamic evolution at different spaces and nodes of this assembly line to survive, resulting in the acquired resistance mechanism of T-DXd showing extremely high spatiotemporal heterogeneity. Based on the pharmacokinetics of ADC drugs, this paper classifies the currently known resistance mechanisms into four levels.

3.2. Obstacles at the antibody and target antigen level

HER2 serves as the anchor point for the precise guidance of T-DXd. Altering the characteristics of the anchor point to evade antibody binding is the most direct method for cancer cells to achieve immune escape. Firstly, the dynamic reduction of HER2 expression levels is one of the most confirmed mechanisms in clinical practice; in the DAISY phase II clinical trial exploring the efficacy of T-DXd for different target expression levels, the subjects were divided into three groups: the first group (HER2 overexpression, n=68), the second group (HER2 low expression, n=72), and the third group (HER2 non-expression, n=37). This trial demonstrated the high anti-tumor activity of T-DXd: the confirmed objective response rate (ORR) in the first group, the second group, and the third group reached 70.6%, 37.5%, and 29.7%, respectively. Moreover, the survival analysis showed that the median progression-free survival (PFS) of the first, second, and third groups reached 11.1 months, 6.7 months, and 4.2 months, respectively. However, despite the significant initial efficacy, in the subsequent translational medical research of this study, approximately 65% of the patients showed a significant decrease or complete loss of HER2 expression after drug resistance progression [9].

Secondly, even if the expression level of HER2 protein does not show a significant decrease, the microscopic variations in the binding surface of the target can also lead to drug resistance. A study in 2026 confirmed through deep sequencing that some patients had new mutations in the ERBB2 gene (especially V597M and P593R mutations) [4]. These mutations are located on the binding surface of the extracellular domain of the receptor, resulting in a severe steric hindrance effect, which not only makes T-DXd unable to firmly bind to the target even when encountering it, but also leads to the failure of a series of anti-tumor mechanisms. Firstly, the endocytosis and payload release process are blocked. Target binding is the prerequisite for ADC drugs to exert cytotoxicity; failure of binding makes T-DXd unable to be smoothly internalized into lysosomes and the linker cannot be cleaved by cathepsins, resulting in the inability of DXd toxin to be released, directly blocking the core DNA damage effect [9]. Secondly, the bystander effect is significantly weakened. Due to the lack of free toxin release and transmembrane diffusion, the tumor local cannot form an effective drug concentration gradient, allowing HER2-negative or low-expression cells around to escape and survive [10]. Moreover, antibody binding failure not only weakens the ADCC immune killing effect but also causes the downstream PI3K/Akt pro-survival signal to lose inhibition. In clinical

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applications, such changes in the binding site often lead to cross-resistance of tumors to other drugs based on trastuzumab (such as T-DM1) [8].

3.3. Toxin load and nuclear-level defense mechanisms

After T-DXd breaks through the intracellular transport barrier, its free load (DXd) needs to enter the nucleus and specifically anchor to topoisomerase I (Topo-I), thereby inducing lethal double-strand DNA breaks. At this point, the change in the abundance of nuclear substrates and the compensatory DNA damage repair (DDR) pathway become the core driving factors of acquired resistance. On one hand, tumor cells can directly downregulate the transcription or protein expression level of Topo-I, reducing the molecular targets of DXd, thereby reducing the cytotoxicity of the drug at the source. On the other hand, the absence of specific DDR key factors plays a crucial role in mediating the extremely resistant phenotype. The in-depth translational medical analysis based on the DAISY phase II clinical trial confirmed that the expression deficiency of the structural-specific endonuclease SLX4 is an important mechanism driving the secondary resistance of T-DXd. In vitro functional experiments precisely revealed this effect: after silencing the SLX4 gene in SK-BR-3 breast cancer cells using siRNA technology, the half-maximal inhibitory concentration (IC50) of DXd by the cells decreased from the baseline of 8.18 nM to 167.27 nM, and the resistance multiple increased by more than 20 times; similarly, in the MCF-7 cell line after knocking down this gene, its IC50 (from 95.10 nM to 502.40 nM) also showed a significant upregulation of over 5 times (Mosele et al., 2023). The above conclusive data indicate that the abnormal repair network represented by SLX4 deficiency endows tumor cells with a powerful resistance potential to escape apoptosis under severe genomic damage stress [9].

4. Conclusion

In conclusion, this article systematically reviews the pharmacological advantages of T-DXd and its complex acquired resistance mechanism; it constructs a hierarchical resistance biological network framework from antigen binding obstruction (such as HER2 downregulation and binding surface mutations) to nucleic acid metabolism defense (such as the DNA repair compensation caused by the absence of SLX4). By using this multi-dimensional approach, this article is able to answer the fragmented literature evidence raised in the introduction, with a deep insight into the high spatiotemporal heterogeneity of ADC resistance. Not only does it assist the fundamental researchers in clarifying the underlying escape mechanism of cancer cells at every pharmacokinetic stage, but it also offers an important theoretical foundation to oncologists in creating individualized, evidence-based targeted sequential or combination drug therapies given particular molecular phenotypes following the emergence of T-DXd resistance. Nevertheless, the present research in the area and the weakness of the presented article can be defined as the fact that most of the resistance mechanisms are still very much reliant on in vitro models of cells or small-sample retrospective translational studies and have no real-world validation of large-scale prospective clinical groups. Consequently, the clinical application of non-invasive liquid biopsy technologies to enable real-time and accurate detection of HER2 dynamic expression and evolution of resistance clones should become the topic of future research. At the same time, accelerating the exploration of combined treatment strategies based on different resistance targets (such as combined immunotherapy or the development of dual-targeted ADC drugs) may be the core breakthrough direction for ultimately overcoming the resistance bottleneck and extending patients' long-term clinical benefits.

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References

[1] Modi, S., Jacot, W., Yamashita, T., Sohn, J., Vidal, M., Tokunaga, E., Tsurutani, J., Ueno, N. T., Prat, A., Chae, Y. S., Lee, K. S., Niikura, N., Park, Y. H., Xu, B., Wang, X., Gil-Gil, M., Li, W., Pierga, J.-Y., Im, S.-A., … Cameron, D. A. (2022). Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. *New England Journal of Medicine, 387*(1). https://doi.org/10.1056/nejmoa2203690

[2] Zhou, M., Huang, Z., Ma, Z., Chen, J., Lin, S., Yang, X., Gong, Q., Braunstein, Z., Wei, Y., Rao, X., & Zhong, J. (2025). The next frontier in antibody-drug conjugates: Challenges and opportunities in cancer and autoimmune therapy. *Cancer Drug Resistance*. https://doi.org/10.20517/cdr.2025.49

[3] Sledge, G. W., Xiu, J., Mahtani, R. L., Sandoval, A. C., Meric-Bernstam, F., Ribeiro, J. R., Kulkarni, N. N., Rampa, D. R., Lee, J., Ueno, N. T., Oberley, M. J., Radovich, M., & Spetzler, D. B. (2025). Mechanisms of resistance to trastuzumab deruxtecan in breast cancer elucidated by multi-omic molecular profiling. *Npj Breast Cancer, 12*(1), 1. https://doi.org/10.1038/s41523-025-00868-y

[4] Chen, W., Gupta, A., Mai, N., Nag, S., Lau, J. S., Singh, S., Chodera, J. D., Liu, B., de Stanchina, E., Pareja, F., Hashmi, A. A., Lieberman, M. M., Modi, S., Bromberg, J., Razavi, P., Drago, J. Z., & Chandarlapaty, S. (2025). Trastuzumab deruxtecan (T-DXd) resistance via loss of HER2 expression and binding. *Cancer Discovery*. https://doi.org/10.1158/2159-8290.cd-25-0647

[5] Xing, P., Yang, C., Hu, H., Qian, T., Xie, B., Huang, J., & Wang, Z. (2025). Antibody-drug conjugates in breast cancer: Current resistance mechanisms and future combination strategies. *Cancer Drug Resistance*. https://doi.org/10.20517/cdr.2025.26

[6] Tsao, L.-C., Wang, J. S., Ma, X., Sodhi, S., Ragusa, J. V., Liu, B., McBane, J., Wang, T., Wei, J., Liu, C.-X., Yang, X., Lei, G., Spasojevic, I., Fan, P., Trotter, T. N., Morse, M., Lyerly, H. K., & Hartman, Z. C. (2025). Effective extracellular payload release and immunomodulatory interactions govern the therapeutic effect of trastuzumab deruxtecan (T-DXd). *Nature Communications, 16*(1). https://doi.org/10.1038/s41467-025-58266-8

[7] Martín, M., Pandiella, A., Vargas-Castrillón, E., Díaz-Rodríguez, E., Iglesias-Hernangómez, T., Martínez Cano, C., Fernández-Cuesta, I., Winkow, E., & Perelló, M. F. (2024). Trastuzumab deruxtecan in breast cancer. *Critical Reviews in Oncology/Hematology, 198*, 104355. https://doi.org/10.1016/j.critrevonc.2024.104355

[8] Ogitani, Y., Hagihara, K., Oitate, M., Naito, H., & Agatsuma, T. (2016). Bystander killing effect of DS-8201a, a novel anti-human epidermal growth factor receptor 2 antibody-drug conjugate, in tumors with human epidermal growth factor receptor 2 heterogeneity. *Cancer Science, 107*(7), 1039–1046. https://doi.org/10.1111/cas.12966

[9] Mosele, F., Deluche, E., Lusque, A., Le Bescond, L., Filleron, T., Pradat, Y., Ducoulombier, A., Pistilli, B., Bachelot, T., Viret, F., Levy, C., Signolle, N., Alfaro, A., Tran, D. T. N., Garberis, I. J., Talbot, H., Christodoulidis, S., Vakalopoulou, M., Droin, N., … André, F. (2023). Trastuzumab deruxtecan in metastatic breast cancer with variable HER2 expression: The phase 2 DAISY trial. *Nature Medicine*, 1–11. https://doi.org/10.1038/s41591-023-02478-2

[10] Fu, Z., Li, S., Han, S., Shi, C., & Zhang, Y. (2022). Antibody drug conjugate: The "biological missile" for targeted cancer therapy. *Signal Transduction and Targeted Therapy, 7*(1). https://doi.org/10.1038/s41392-022-00947-7