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Research

Antibody-Drug Conjugate in Cancer Therapy: Mechanism, Resistance, and Emerging Advances

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	Abstract
Published on: 02 Nov 2025	Antibody-Drug Conjugates (ADCs) are a rapidly evolving type of targeted cancer treatment that is intended to reduce systemic toxicity and improve therapeutic efficacy by delivering cytotoxic chemicals only to cancer cells. Through stable chemical linkers, ADCs combine the efficacy of cytotoxic medicines with the specificity of monoclonal antibodies. The article provides a thorough analysis of the structural and functional importance of the antibodies, linkers, and payloads that make up ADCs. Key mechanisms are covered in detail, such as internalization, trafficking, antigen recognition, and bystander effects. Along with new approaches to combat them, resistance mechanisms such as drug efflux, altered trafficking, and antigen heterogeneity are critically assessed. ADCs that are already FDA-approved are also discussed, along with their clinical implications, limitations, and advancements in next-generation ADC designs. Notwithstanding obstacles, continuous advancements in ADC engineering and therapeutic approaches highlight their bright future in oncology.
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	Keywords: Antibody-drug conjugates (ADCs), Targeted cancer therapy, Monoclonal antibodies, Cytotoxic payload, Linkers, HER2, Trop-2, Bystander effect, Internalization, Drug resistance.

1. INTRODUCTION

Cancer is the world's second greatest cause of death, accounting for an estimated 10 million deaths in 2020 [1]. For decades, chemotherapy has been the major treatment choice however, its lack of selectivity and significant toxic side effects frequently impair efficacy and restrict patient tolerance [2]. To address these constraints, combination therapies based on medications with distinct mechanisms and non-overlapping toxicities were developed, to achieve additive or synergistic anti-tumor actions. Tyrosine kinase inhibitors, monoclonal antibodies, and antibody-drug conjugates (ADCs) have emerged as popular targeted therapies due to their tumor-specific effects [3].

ADCs are a unique class of therapies that combine monoclonal antibodies with strong cytotoxic drugs via stable linkers, allowing chemotherapy to be delivered directly to cancer cells while sparing healthy tissue [1]. Rituximab, the first FDA-approved therapeutic antibody, was released in 1997 to treat B-cell non-Hodgkin's lymphoma [4]. Early hurdles included immunological responses to murine antibodies, which prevented recurrent dosing due to the development of human anti-mouse antibodies (HAMA) [5]. Modern ADCs have overcome many of these challenges by carefully selecting high-affinity antibodies, stable linkers, and powerful cytotoxic payloads, making them attractive agents for future cancer treatment [3].

2. Cancer

Cancer is characterized by uncontrolled cell development that can occur in any organ. It is a major global health challenge, with over 14 million new cases reported each year, including over 1.1 million in India [6]. Men had a slightly higher lifetime risk of invasive cancer (41.6%) than women. Cancer incidence rates in the United States have largely declined in recent decades, although the COVID-19 pandemic resulted in a short dip in new diagnoses for 2020. The National Cancer Institute tracks age-standardized incidence and mortality rates, which vary by cancer type [7].

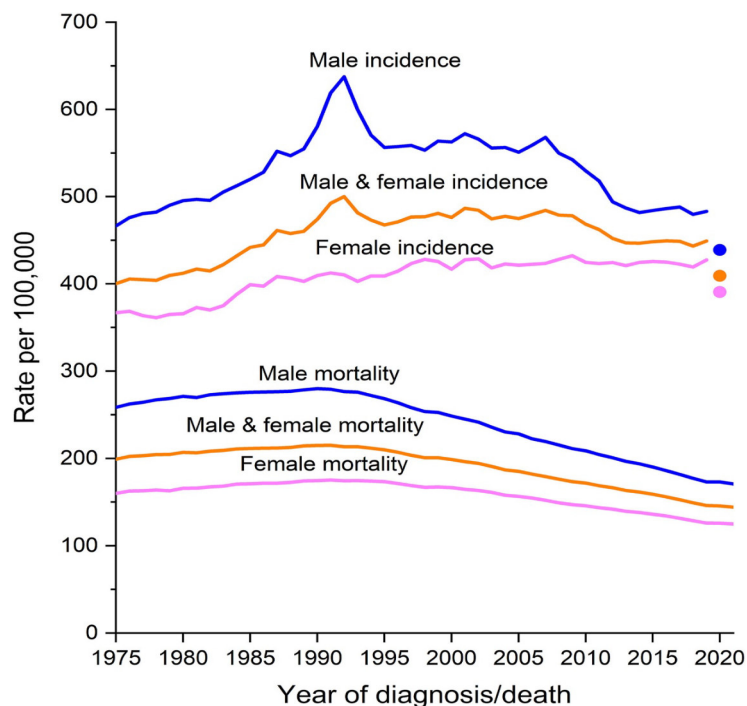


Fig 1: Cancer incidence (1975-2020) and death (1975-2021) trends by gender in the United States.

Rates are age-adjusted for the 2000 US standard population. The incidence rates are also modified to account for reporting delays. Incidence statistics for 2020 are shown separately from trend lines. [8]

3. Antibody Drug Conjugate

ADCs are a new type of targeted anticancer drug delivery agents that deliver cytotoxic drugs to tumors in a sustained and selective manner. The three primary structural components of an ADC are the linker, the cytotoxic agent, and the Antibody. The innovative creation of secure and effective ADCs is made possible by the selection of a strong cytotoxic payload, stable linker, and high-affinity Ab. Even though ADCs are

developed to target antigens unique to tumors, issues with Ab immunogenicity, antigen expression, early drug release, and low chemotherapeutic drug potency still exist [9]. The purpose of ADCs is to increase the therapeutic window of these drugs by delivering them selectively to tumor cells that express a particular antigen that the ADC's monoclonal antibody (mAb) targets. The process of creating new ADCs is ongoing and depends on developments in several technologies, including the biosynthesis of novel linkers, the synthesis and production of mAb, and the introduction of new payloads that are more effective against tumor cells while causing less systemic side effects. [2]

The tripartite structure of ADC consists of an antibody that is engineered to bind precisely to the antigen, a powerful and cytotoxic chemical payload that can cause cell death, and a linker that connects the antibody and payload (Figure 2). Three essential mechanisms of action must be present for ADC to be a therapeutic option. The antibody must first demonstrate a particular and selective affinity for a receptor or antigen found on the surface of the target cell. To start programmed cell death, the payload must, successfully integrate into the target cell and then interact with the chosen target. Last but not least, the linker must effectively separate the payload at the right time throughout the antibody and payload binding and internalization processes within the target cell to maintain a stable relationship between the two. [10]

3.1 Antibody

Glycoproteins known as antibodies have the amazing capacity to attach to particular antigens with selectivity, triggering a strong immune response. [10] To deliver the medication into the cell, the mAb component of an ADC-which binds to an antigen, such as a tumor-associated antigen expressed on the surface of cancer cells-must be effectively internalized into the target cell. Nonetheless, the majority of ADC targets are typically tumor-associated, and tumor-specific antigens are very distinctive. Healthy cells have very little expression of these antigens on their surface [12]. The antibody is made up of one constant fragment (Fc) and two antigen-binding fragments (often referred to as Fabs). Fabs mediate the identification of the antigen, while Fc mediates the antibody's contact with effector immune cells. The target antigen of the tumor should be highly specific for the integrated antibody [13].

For ADCs, the best mAbs must have low immunogenicity, a long blood half-life, and good tumor specificity to minimize off-target damage. The rate of internalization and tumor penetration must be balanced by the antibody's affinity. High antibody affinity reduces the ability of solid tumors to penetrate and promotes receptor internalization. After attaching to a cytotoxic payload, it is also preferable for mAbs to maintain their inherent anticancer activity. mAbs can generate antibody-dependent antitumor immune responses via the Fc part in addition to binding to the target antigen via the Fab portion [14]. Immunoglobulin G (IgG) is the most often utilized kind of immunoglobulin among the five types (M, A, D, E, and G) in ADCs. IgG1 is widely used because of its immunogenic properties, including complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cell-mediated phagocytosis (ADCP). IgG3 is typically not used due to its short half-life [15].

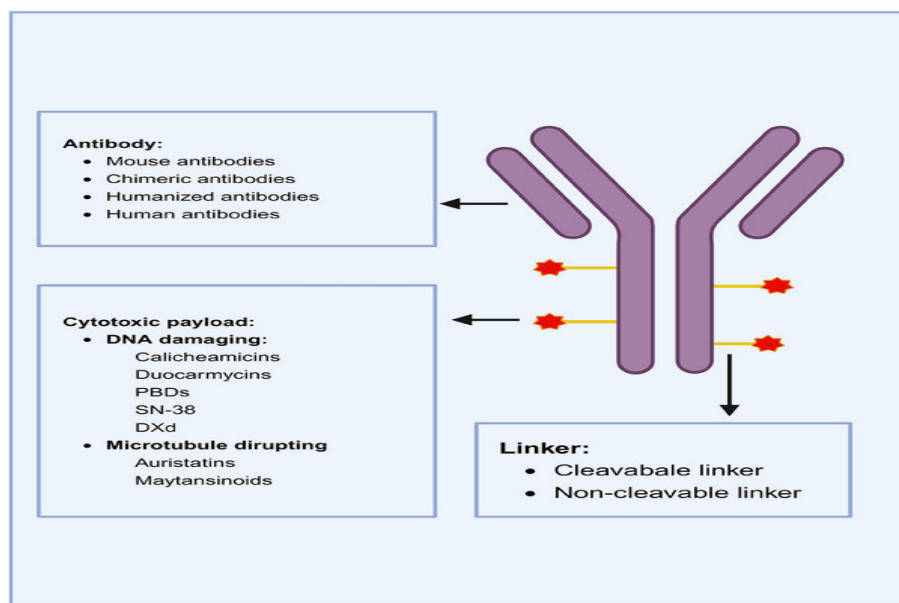


Fig 2: Antibody-drug conjugate structure.

An ADC's three main components are antibodies, cytotoxic payloads, and linkers. There are four main forms of antibodies: mouse antibodies, chimeric antibodies, humanized antibodies, and human antibodies. Cytotoxic payloads are classified into two types based on their mode of action: those that operate on DNA (e.g., calicheamicins, duocarmycins, PBDs, SN-38, and DXd) and those that act on tubulin (e.g., auristatins and maytansinoids). Linkers are divided into two categories: cleavable and noncleavable. PBD stands for pyrrole benzodiazepines, SN38 for 7-ethyl-10-hydroxycamptothecin, and DXd for deruxtecan. [11]

3.2 Linkers

The linker that connects the cytotoxic drug with the antibody in ADCs is crucial for the therapeutic index because it affects payload release and stability. An optimal linker reduces ADC aggregation, limits premature drug release in plasma, and guarantees that the active substance is released only within tumor cells. The metabolic fate of linkers determines whether they are cleavable or non-cleavable. Chemical cleavage types (for example, hydrazone and disulfide bonds) and enzyme-sensitive types (for example, glucuronide and peptide bonds) are examples of cleavable linkers. Hydrazone linkers are stable in circulation but degrade in the acidic environments of lysosomes (pH 4.8) and endosomes (pH 5.5-6.2) to release the payload. Lysosomal proteases break down peptide-based linkages. Non-cleavable linkers, such as thioether and maleimidocaproyl groups, are resistant to enzymatic and chemical degradation, increasing plasma stability and lowering off-target toxicity. Payload release from non-cleavable linkers is dependent on the antibody's proteolytic breakdown, which results in a drug-amino acid combination. Thioether linkers are appropriate for tiny, chemically stable medicines located away from the conjugation site [1].

Upon cellular uptake, linkers must be stable enough to keep the medication connected during circulation and tissue distribution, including tumor penetration, while also allowing for efficient lethal agent release within cancer cells [16]. Unstable linkers limit ADC efficacy by causing early drug loss. The target antigen also influences linker selection, for example, ADCs containing cleavable linkers are active *in-vivo* against multiple B-cell antigens, including CD19, CD20, and CD22 [17].

3.3 Cytotoxic Payloads

Once ADCs have been internalized by cancer cells, the cytotoxic payload is released, causing cell death. Because only around 2% of ADCs reach the tumor site following intravenous administration, payloads must be extremely powerful, with IC₅₀ values in the nanomolar to picomolar range. Furthermore, they must be stable under physiological settings and have functional groups that can be conjugated with antibodies. Currently, the most popular payloads are potent tubulin inhibitors, DNA-damaging compounds, and immune stimulants. Microtubules, which are critical components of the cytoskeleton, are required for cell division, especially in rapidly proliferating tumor cells [1].

ADC payloads are commonly classified as Topoisomerase I inhibitors (e.g., deruxtecan, SN-38), tubulin-binding compounds (auristatins, maytansinoids), and DNA-targeting agents (calicheamicins, duocarmycin). Auristatins are the largest class due to their excellent biochemical characteristics. Newer payloads in development include tyrosine kinase inhibitors, radionucleotides, immunomodulators, and dual payloads [15]. Early ADCs relied on less potent medicines, such as vinblastine and doxorubicin, which limited their effectiveness. Current payloads, on the other hand, are highly cytotoxic and can hurt healthy cells nearby. Thus, even at extremely low concentrations, these payloads must effectively kill target cells while remaining stable in circulation and minimizing premature release [10].

The drug-antibody ratio (DAR), or the amount of payload molecules bound to each antibody, affects ADC potency, therapeutic index, pharmacokinetics, and clearance. DAR ratings for approved ADCs typically range from 2 to 8. Lower DARs lower toxicity and improve the therapeutic index, but they may impair efficacy, whereas larger DARs boost potency while increasing toxicity and clearance rates [14,18].

Mechanism

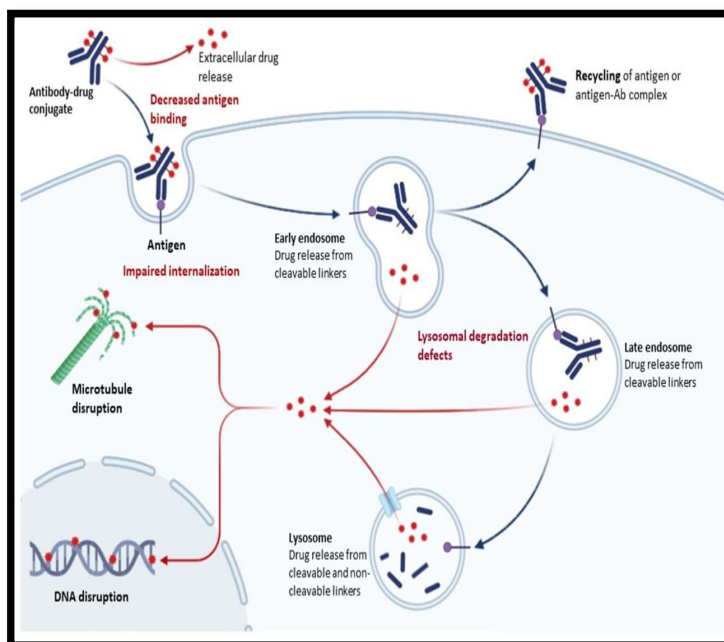


Fig 3: ADC internalization and destruction of both the antibody and the linker

Intravenous administration of ADCs prevents their breakdown by stomach enzymes [2]. When the ADC binds to cancer cell target antigens, it is absorbed and transported via endosomal-lysosomal pathways. Either chemical or enzymatic breakage releases the cytotoxic payload into lysosomes, where it causes microtubule disruption or DNA damage that triggers apoptosis [1].

Cleavable linkers depend on the tumor microenvironment, but non-cleavable linkers need the mAb to be broken down by lysosomes. The efficacy of T-DXd in HER2-low breast malignancies may be explained by the "bystander effect," which is particularly pertinent in heterogeneous tumors and is caused by released payloads diffusing into nearby cells. ADCs not only directly cause cytotoxicity but also trigger immunological responses through mechanisms such as ADCC, ADCP, and CDC, which are primarily made possible by the IgG1 isotype [15]. For example, T-DM1 improves ADCC while maintaining the immunological effects of trastuzumab. Certain ADCs also increase immunogenicity; for example, DS-8201a raises the expression of MHC-I/PD-L1 and CD8⁺ T cells [14].

4.1 Target Antigen Recognition

4.1.1 Role of monoclonal antibodies in ADC Function

The mAb component of ADCs provides specificity by binding to tumor-associated antigens with high affinity, allowing for selective drug administration while limiting off-target damage. High-affinity antibodies ($K_d < 1 \text{ nM}$) can boost tumor localization, although moderate affinity can also improve tumor penetration, especially in solid tumors. Internalization of the antibody-antigen complex is required for payload distribution, which occurs predominantly via receptor-mediated endocytosis. Humanized or completely human antibodies are recommended in ADCs to reduce immunogenicity and increase circulation half-life. Despite their large size ($\sim 150 \text{ kDa}$), mAbs may impede deep tissue penetration, yet they improve pharmacokinetics and can initiate immune responses when attached to antigenic sites. Antibodies remain the primary targeting moiety in ADCs, while other ligands such as peptides or vitamins are being investigated. [12]

4.1.2 Target antigen in ADC mechanism HER2 and Trop-2

The therapeutic efficacy of ADCs begins with the recognition and binding of the antibody component to specific antigens overexpressed on cancer cell surfaces. In ADC therapy, two well-characterized targets are HER2 (Human Epidermal Growth Factor Receptor 2) and Trop-2 (Trophoblast Cell Surface Antigen 2). HER2 is overexpressed in several malignancies, most notably breast and gastric cancers. ADCs such as Trastuzumab emtansine (T-DM1) and Trastuzumab deruxtecan (T-DXd) attach to HER2, internalize, and release their cytotoxic payload intracellularly, resulting in targeted cell death with low systemic toxicity. [14]

TROP2, encoded by the TACSTD2 gene, is a transmembrane glycoprotein that is overexpressed in a variety of malignancies and has been linked to cancer cell proliferation, invasion, and poor prognosis. TROP2 is a promising target for ADC development because of its low expression in normal tissues and strong tumor selectivity. “Sacituzumab govitecan (SG), a third-generation ADC, combines a humanized anti-Trop-2 IgG1 monoclonal antibody (hRS7) with a topoisomerase I inhibitor payload”. SG has shown promise anticancer effectiveness and tolerability in a variety of malignancies, including metastatic triple-negative breast cancer (mTNBC), urothelial carcinoma, and lung cancer, according to phase I/II clinical trials. Notably, the ASCENT trial demonstrated a substantial improvement in progression-free and overall survival in mTNBC patients treated with SG over chemotherapy. [11]

4.2 Internalization of ADCs and Intracellular Trafficking

4.2.1 Internalization ADCs

Since the antibody aids in the internalization of the target antigen-receptor complex, internalization is a crucial step for the effectiveness of the majority of ADCs. For example, tumor growth inhibition and receptor internalization can be induced by the anti-HER3 antibody EV20 alone. ADCs employ a number of endocytic pathways, such as Caveolae-mediated endocytosis, Clathrin/Caveolin-independent processes, and Clathrin-mediated endocytosis (CME). The most popular pathway, CME, is target antigen-dependent and involves proteins like dynamin, epsin, adaptor protein 2 (AP2), and phosphatidylinositol (4,5) bis-phosphate (PIP2) that help ADC internalize and accumulate in endo-lysosomal vesicles [5].

ADC trafficking depends on important proteins including Endophilin A2 (Endo II), which is involved in Clathrin-independent endocytosis. In HER2+ breast cancer models, decreased Endo II expression is associated with resistance to T-DM1 and reduced HER2 internalization. As demonstrated in the n87 gastric cancer cell line, aberrant Caveolae-mediated endocytosis has also been linked to T-DM1 resistance, possibly as a result of inefficient lysosomal trafficking. Acidic pH and active lysosomal enzymes are necessary for the breakdown of ADCs in lysosomes. Lysosomal alkalization and reduced protease activity are two resistance mechanisms that have been observed in T-DM1-resistant breast cancer mice. For drug release, non-cleavable ADCs rely on lysosomal enzymes, which in turn need a very acidic environment that is preserved by V-ATPase. Resistant N87 gastric cancer cells have been shown to exhibit altered V-ATPase activity [14]. Impaired movement of cytotoxic payloads from lysosomes to the cytoplasm is another mechanism of resistance. Certain transporters are needed for non-cleavable linkers. Hamblett *et al.*'s RNA screening revealed that SLC46A3 is a transporter for maytansine-based catabolites; lysosomal buildup and treatment failure result from its silencing [13]. Through its unique method, the payload diffuses into the cytoplasm after being released into endolysosomal vesicles, causing cell death [5].

4.2.2 Non-Internalizing ADCs

ADCs with non-internalizing antibodies mostly use a bystander mechanism to achieve their therapeutic goal. Drug entry into tumor cells through diffusion, pinocytosis, or other pathways is made possible by the release of the cytotoxic payload into the extracellular environment by proteolytic enzymes or lowering circumstances once it reaches the tumor site. Further release of reducing agents or proteases is triggered by the initial wave of cancer cell death, which amplifies payload release and improves tumor cell killing. Notably, this tactic prolongs the lethal effect by allowing drug diffusion into nearby non-target tumor cells. An ADC that targets fibronectin's alternatively spliced extracellular domain A, for instance, showed strong anticancer effect after tumor cell death and extracellular payload release [5].

4.2.3 Failure in Internalization and Trafficking Pathways

Changes in internalization and trafficking pathways might potentially result in resistance to ADCs. Several *in vitro* models of T-DM1 resistance were created; some of these models showed decreased HER2 expression, but the N87-TM model internalized trastuzumab-ADCs into caveolin-1 (CAV-1)-coated vesicles, suggesting changed trafficking and a possible preference for cleavable over non-cleavable linkers. Additionally, Endophilin A2 (encoded by SH3GL1) increases ADC sensitivity and HER2 internalization in HER2-positive breast cancer. Reduced internalization and T-DM1 cytotoxicity were the results of SH3GL1 knockdown [13].

4.3 Bystanders Effects

The bystander effect is an increasingly recognized ADC mechanism in which nearby tumor cells that do not express the target antigen are killed as a result of cytotoxic payload diffusion. This effect is mostly determined by the linker and payload employed in the ADC build. ADCs with cleavable linkers and hydrophobic payloads can diffuse across membranes, resulting in a bystander effect. For example, a comparison between T-DM1 and T-DXd clearly demonstrates this: T-DM1 uses a non-cleavable linker, resulting in a charged payload that is maintained within antigen-positive cells, reducing bystander death. T-DXd, on the other

hand, employs a cleavable linker to release the hydrophobic payload deruxtecan, which can diffuse into adjacent antigen-negative cells. Similarly, trastuzumab duocarmazine, which contains a cleavable linker and a duocarmycin payload, exhibits a strong bystander impact. Other authorized ADCs, including enfortumab vedotin (EV), tisotumab vedotin (TV), and sacituzumab govitecan (SG), have demonstrated this behavior in preclinical research. Furthermore, MMAE based ADCs, such as brentuximab vedotin and polatuzumab vedotin, have cleavable linkers and membrane-permeable characteristics, allowing them to cause bystander effects. In contrast, because of its inability to cross membranes, MMAF does not induce this action and is hence less effective. [18]

4.4 Mechanisms of Resistance to ADCs

ADCs' multi-step process allows resistance to develop at several phases, including lysosomal degradation, payload release, apoptosis induction, and antigen identification and internalization [13]. Even with ADC payloads' great potency, resistance can nevertheless arise, frequently due to ATP-binding cassette (ABC) transporters. Because these drug efflux pumps aggressively export cytotoxic drugs from cancer cells, they have long been linked to decreased chemotherapy efficacy [18].

4.4.1 Dysregulated downstream signaling pathways of HER2

In addition to antigen binding, HER2-targeted mAbs in ADCs have inherent cytotoxic effects. A crucial downstream route of HER2, the PI3K/AKT/mTOR signaling pathway, is essential to the development of cancer [14]. ADC sensitivity can be decreased and the effectiveness of the cytotoxic payload compromised by its activation, which is frequently caused by PIK3CA mutations or PTEN loss. Through PI3K/AKT activation, trastuzumab resistance has been demonstrated to be induced by PTEN knockdown [13]. Fascinatingly, the EMILIA trial showed that T-DM1 was more beneficial than capecitabine and lapatinib for patients with reduced PTEN expression [14]. In the presence of these mutations, T-DM1 may be more effective than other HER2-directed treatments, as evidenced by its persistent efficacy independent of PTEN or PIK3CA status [13].

4.4.2 Drug-Efflux Pumps

ATP-binding cassette (ABC) transporter overexpression promotes drug efflux, which results in multidrug-resistant (MDR) tumor phenotypes and greatly increases treatment resistance. Similar to the payload in T-DM1, maytansinoids are recognized MDR1 substrates. Expression of MDR1 has been associated with resistance to T-DM1. This was addressed by conjugating DM1 with a hydrophilic linker (PEG4Mal), which produced better results in tumors expressing MDR1 than conjugates with a nonpolar SMCC linker [13].

4.4.3 HER2 heterogeneity

HER2 intratumoral heterogeneity, which is frequently seen in breast tumors with low-grade amplification or ambiguous expression, is the term used to describe different HER2 expression or amplification within the same tumor. It is identified by fluorescence in situ hybridization (FISH) showing HER2-negative regions or HER2 amplification in >5% but <50% of tumor cells. Compared to homogeneous HER2-expressing tumors, T-DM1 has demonstrated reduced efficacy in these heterogeneous cancers, as seen by decreased pathological complete response (pCR) rates. T-DM1 was shown to have no effect on HER2⁺ cells in in vitro models that mimicked HER2 heterogeneity, but DS-8201a had a strong bystander effect that efficiently killed nearby HER2⁺ cells. The benefits of DS-8201a in individuals with heterogeneous, decreased, or deleted HER2 expression were further validated by clinical investigations [14].

4.4.4 Apoptotic Dysregulation

ADC sensitivity may be impacted by changes in apoptotic pathways. The overexpression of anti-apoptotic proteins BCL-2 and BCL-XL is linked to resistance to ADCs such as brentuximab vedotin and gemtuzumab ozogamicin, according to evidence from hematological malignancies [13].

4.5 Overcoming resistance to Antibody drug conjugate

Therapeutic resistance is still a significant clinical concern even with the tremendous advancements in ADC technology. Increased drug efflux, modified intracellular trafficking, target antigen heterogeneity or downregulation, and compromised payload release are some examples of resistance mechanisms. Several approaches are being researched in order to address these:

1. Bispecific and Biparatopic ADCs: By binding multiple epitopes or receptors at once, dual-targeted ADCs, such as HER2×HER3 and HER2×PRLR, enhance tumor targeting, internalization, and degradation. This increases their effectiveness in tumors that are resistant to or low in antigen.
2. Intracellular Trafficking Optimization: Payload delivery can be enhanced by shifting endocytic trafficking from recycling routes to lysosomal degradation. Biparatopic antibodies promote internalization and receptor clustering.

3. Improved Linker Design and Hydrophilicity: By avoiding drug-efflux pumps (like MDR1), hydrophilic and cleavable linkers (like sulfo-SPDB-DM4 and PEG4Mal) improve intracellular retention of payloads.
4. Payload Innovation: Using new cytotoxins, like as topoisomerase I inhibitors (found in T-DXd and SHR-A1811, for example), may help overcome resistance to previous payloads such DM1.
5. Enhancement of Bystander Effects: Tumor heterogeneity can be addressed by killing nearby antigen-negative cells with enhanced membrane-permeable payloads and tailored release kinetics.
6. Tumor Microenvironment-Responsive Release: The effectiveness of ADCs is increased by hypoxia- or pH-sensitive linkers, which enable payload release in acidic tumor settings without the need for internalization.
7. Combination Therapies: To improve immune response and get past signaling pathway-mediated resistance, ADCs are being studied in combination with immune checkpoint inhibitors (ICIs) or tyrosine kinase inhibitors (TKIs) (e.g., KATE2, KATE3 trials).
8. Focusing on Resistance Routes: Resistant tumor cells can be re-sensitized by blocking compensatory survival pathways, such as HER3/NRG1 signaling, with PAN-HER treatments or HER3-neutralizing antibodies. [11,13]

5. Approved ADCs and clinical application

After decades of work to refine the main components, approximately 100 ADCs are now in clinical development, and as of December 2021, 14 ADC medicines had achieved marketing approval in various countries globally. Coincidentally, half of the approved ADCs are primarily employed against hematological malignancies, while the remainder are primarily prescribed for solid tumors [1]. Two antibody-drug conjugates are now licensed for usage in the United States and Europe: brentuximab vedotin and ado-trastuzumab emtansine. In general, these antibody-drug conjugates are well tolerated, with deleterious effects commensurate with the established mode of action of the cytotoxic payloads—for example, neutropenia and neuropathy with brentuximab vedotin and increases in hepatic aminotransferase levels with ado-trastuzumab emtansine. However, the exact processes of harmful effects owing to antibody-drug conjugates are complex, including contributions from all conjugate components—i.e., the monoclonal antibody, the linker, and the cytotoxic payload. Non-specific systemic release of the cytotoxic drug as a result of premature linker lysis, as well as internalization of the antibody-drug combination by cells that do not express the target, are two mechanisms. [19]

6. Advances in the development of ADCs

ADC drug development is often separated into three generations based on drug composition and technological aspects. [1]

6.1 The first generation of ADCs

ADCs in the early stages, like BR96-doxorubicin, were made of mouse-derived antibodies connected to traditional chemotherapeutics via non-cleavable linkers. They were very immunogenic and provided little benefit over free medicines. The first approved ADCs, gemtuzumab ozogamicin and inotuzumab ozogamicin, both of which use IgG4 antibodies conjugated to calicheamicin via acid-labile linkers, were the result of later use of humanized mAbs with stronger cytotoxins. However, variable drug-to-antibody ratios (DARs) caused by stochastic conjugation at lysine and cysteine residues led to limited therapeutic windows and less than ideal PK/PD characteristics, underscoring the need for more improvement. [1]

6.2 The Second-generation ADCs

The second-generation ADCs, brentuximab vedotin and ado-trastuzumab emtansine, were released following the optimization of mAb isotypes, cytotoxic payloads, and linkers. Both of these ADCs are based on the IgG1 isotype mAbs, which are more appropriate for bioconjugation with small-molecule payloads and have high cancer cell targeting capabilities compared to IgG4. Another significant advancement in second-generation ADC is the use of more potent cytotoxic medicines, such as auristatins and mytansinoids, which have better water solubility and coupling efficiency. More payload molecules can be loaded onto each mAb without causing antibody aggregation. In addition to the enhancements in terms of the antibody carrier and cytotoxic payload, the linkers in the second-generation ADCs are also upgraded to achieve higher plasma stability. When DAR exceeds 6, the ADC exhibits significant hydrophobicity and tends to reduce ADC efficacy due to quicker distribution and clearance in vivo. In this context, optimization of DAR via site-specific conjugation, as well as continual tuning of mAbs, linkers, and payloads, appears to be critical for the successful development of third-generation ADCs. [1]

6.3 The Third-generation ADCs

Third-generation ADCs have addressed the previously described issues with the heterogeneous DARs of second-generation ADCs. In order to create homogenous ADCs with well-characterized DARs and desired cytotoxicities, site-specific conjugation has been introduced. A single-isomer ADC with a consistent DAR value is produced by the drug's site-specific conjugation to Ab. Bioengineered Abs with site-specific amino acids like cysteine, glycan, or peptide tags can be used to create these ADCs. For instance, by substituting cysteine for the Ala114 amino acid of the IgG Ab's CH1 domain, MMAE was precisely site-specifically conjugated to human IgG, resulting in a selectively designed Ab known as THIOMAB. [9]

7. Limitations of ADC

- ADCs are intended to be target-specific treatments that deliver cytotoxic payloads straight to cancerous cells.
- An important issue with ADCs is off-target toxicity, which results from the early release of cytotoxic payloads into the bloodstream.
- Usually, off-target effects result in toxicity in the respiratory, ophthalmic, hepatic, neurological, or hematologic organs.
- Nectin-2 expression on cardiomyocytes causes cardiotoxicity, while HER2 expression on the skin causes skin toxicity.
- The toxicity increased due to early payload release caused by linker instability in early ADC development; the ADC half-life should be ten times longer than the payload half-life.
- To minimize immunogenicity, maintain appropriate payload delivery, and control early drug release and toxicity, the linker's polarity is essential. Drug tolerance and efficacy may be impacted by hydrophobic cytotoxic chemicals that change the characteristics of antibodies, causing conjugation or aggregation.
- When valine-citrulline linkers in MMAE-based ADCs rupture the linker, serine proteases release MMAE prematurely, which results in neutrophil death.
- Linkers, which can be either cleavable or non-cleavable, are crucial for preserving ADC stability in circulation.
- The bystander effect, which can impact neighboring healthy cells but is beneficial for tumors with low and variable target antigen expression, is frequently seen in cleavable linkers.
- The ADC with MMAE payload, benxumab vedotin, exhibits a bystander effect and employs a cleavable linker.
- While thioether linkers (non-cleavable) do not show bystander cytotoxicity, disulfide linkers, a type of cleavable linker, do.
- The bystander effect is improbable with non-cleavable linkers since the antibody must be completely broken down inside the cell for the payload to be released. The stability of ADC may be impacted by the systemic degradation of the PEG component caused by anti-PEG antibodies. [8]

8. Challenges

- Optimizing the antibody, linker, and payload to maximize efficacy while limiting non-target toxicity is the main issue in the creation of ADCs
- Long-term efficacy can also be diminished by resistance mechanisms, such as target antigen mutations or the activation of alternate signaling pathways.
- ADC performance is further hampered by low antigen specificity, restricted tumor penetration because of the high antibody size, and insufficient internalization.
- ADCs' low therapeutic index, payload-related toxicity, inability to assess reliable biomarkers for patient selection, and ineffectiveness against solid tumors because of obstacles such the tumor stroma all impede their clinical success.
- ADCs with hydrophobic linkers or particular payload-linker combinations, such as MMAE with valine-citrulline, are particularly susceptible to toxicity from premature payload release, heterogeneous antigen expression, and dense tumor stroma that restricts ADC penetration. [4,18]

9. CONCLUSION

ADCs have emerged as a promising precision oncology technique, allowing for the targeted administration of highly potent cytotoxins. Even with clinical triumphs, issues including resistance, off-target effects, and manufacturing complexity still exist. Improving payload design, linker stability, and antigen specificity while combining ADCs with other treatment modalities will be essential for future advancement.

ADCs have the potential to become a vital component of contemporary cancer treatment with further development.

Abbreviations

Abbreviation	Full forms
ADC	Antibody-Drug conjugate
mAb	Monoclonal Antibody
HER2	Human Epidermal Growth Factor Receptor2
Trop-2	Trophoblast cell surface Antigen 2
DAR	Drug-Antibody Ratio
ADCC	Antibody-Dependent Cytotoxicity
CDC	Complement-Dependent Cellular Cytotoxicity
MMAE	Monomethyl Auristatin E
MMAF	Monomethyl Auristatin F
T-DM1	Trastuzumab emtansine
T-DXd	Trastuzumab deruxtecan
SG	Sacituzumab govitecan
HL	Hodgkin Lymphoma
AML	Acute Myeloid Leukemia
PBD	Pyrrolo[2,1-c][1,4] benzodiazepine
CME	Clathrin-Mediated Endocytosis
V-ATPase	Vacuolar-type ATPase
MDR1	Multidrug Resistance Protein 1
CSA	Cyclosporin A
IgG	Immunoglobulin G
Fc	Fragment crystallizable
Fab	Fragment antigen-binding
PEG	Polyethylene Glycol
SLC46A3	Solute Carrier Family 46 Member 3
ADCP	Antibody-dependent cell-mediated phagocytosis
CD19	Cluster of Differentiation 19
CD20	Cluster of Differentiation 20
CD22	Cluster of Differentiation 22
CD8+	Cluster of Differentiation 8
DS-8201a	Trastuzumab deruxtecan
MHC-1/PD-L1	Major Histocompatibility Complex Class I /Programmed Death-Ligand 1.
TACSTD2	Tumor-Associated Calcium Signal Transducer 2
mTNBC	Metastatic Triple-Negative Breast Cancer
EV	enfortumab vedotin
TV	tisotumab vedotin

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