Cancer Biol Med 2026. doi: 10.20892/j.issn.2095-3941.2025.0707

REVIEW

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Drug resistance to antibody-drug conjugates: mechanisms, challenges, and perspectives

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ABSTRACT Antibody-drug conjugates (ADCs) have emerged as a transformative class of cancer therapeutics by combining the target precision of antibodies with the potent cytotoxic effects of chemotherapeutic agents. This unique approach aims to enhance the effectiveness of cancer treatment while minimizing systemic toxicity. This review provides a comprehensive overview of ADC development, starting with explorations of the structural and mechanistic foundations, and advancing to in-depth analyses of ADCs that have achieved clinical success. Despite remarkable progress, the development of drug resistance remains a significant barrier to broader clinical application. We discuss the mechanisms underlying resistance, including alterations in target antigens, disruptions in ADC internalization, and dysfunctions of intracellular trafficking. To address these challenges, we propose several strategies, such as designing next-generation ADCs equipped with improved linkers and novel payloads, implementation of combination therapies, and simultaneous targeting of multiple pathways to circumvent resistance. In conclusion, this review highlights the critical need for innovative approaches in the evolving ADC landscape, aiming to overcome resistance mechanisms and fully harness the therapeutic promise of ADCs in cancer treatment.

KEYWORDS Antibody-drug conjugates; drug resistance; combination strategies; cancer therapy

Introduction

Over the past few decades, cancer therapy has undergone a remarkable transformation, shifting away from the traditional paradigm of cytotoxic chemotherapies towards more targeted and personalized approaches[1-3]. The advent of targeted therapies, such as small-molecule inhibitors and monoclonal antibodies, has revolutionized cancer treatment by selectively targeting specific molecular pathways or antigens overexpressed on tumor cells[4-6]. Furthermore, the emergence of immunotherapies, which harness the power of the patient's immune system to combat cancer, has opened up new frontiers in cancer care[7,8]. Despite these advances, treating cancers remains challenging and there is a constant need for novel therapeutic modalities. Antibody-drug conjugates (ADCs) represent a promising class of targeted therapies that combine the specificity of monoclonal antibodies with the high potency of cytotoxic payloads, offering a unique opportunity to enhance the therapeutic index and reduce off-target toxicity observed in conventional therapies[9,10]. Conceptually proposed more than a century ago by Paul Ehrlich as "magic bullets," early ADC development was limited by insufficient linker stability, suboptimal target selection, and unacceptable toxicity[11]. The modern era of ADCs began in the late 1990s and early 2000s, and was driven by advances in antibody engineering, linker chemistry, and payload design, which collectively improved plasma stability, tumor selectivity, and therapeutic indices[12]. The first marketed ADC, gemtuzumab ozogamicin, was granted accelerated approval in 2000 for acute myeloid leukemia (AML), marking a pivotal milestone despite initial safety challenges[13]. Subsequent technological refinements led to the successful approval of second- and third-generation ADCs, including brentuximab vedotin for CD30-positive lymphomas and trastuzumab emtansine for HER2-positive breast cancer, which demonstrated clear survival benefits and manageable toxicity profiles[14]. Twenty ADC drugs have gained regulatory approval by the Food and Drug Administration (FDA), European Medicines Agency (EMA), and National Medical Products Administration (NMPA) as of December 2025 with

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Received November 12, 2025; accepted February 23, 2026; published online April 6, 2026.
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numerous additional candidates progressing through various clinical development stages (**Table 1**).

The advent of these ADCs underpins a significant shift in oncology, from non-selective cytotoxic agents to targeted therapies. These innovative designs and sophisticated therapeutics represent a new era of precision medicine, offering the ability to deliver potent cytotoxic drugs directly into cancer cells[15,16]. ADCs specifically target cancer cells, unlike traditional chemotherapy that indiscriminately targets all rapidly dividing cells. This targeted delivery system vastly improves the therapeutic index by enhancing efficacy and minimizing toxicity, a feature that holds enormous value in the fight against cancer[17,18]. ADCs also represent a significant stride toward personalized medicine in oncology[19,20]. With the growing understanding of tumor biology and identification of unique tumor markers, ADCs can be custom-designed to target specific patient subpopulations, potentially leading to more effective, individualized treatment strategies[21]. The tumor-specific nature of ADCs translates into improved patient outcomes and quality of life[22,23]. Patients experience fewer side effects compared to traditional chemotherapy. There is also the potential for ADC therapy to be better tolerated, allowing for prolonged treatment and improved disease management[24,25].

While ADCs have achieved significant milestones, the emergence of drug resistance represents a formidable obstacle to widespread clinical application. Resistance can arise at multiple stages of the ADC mechanism of action, from target antigen recognition to payload delivery and execution of cytotoxic effects. Tumor heterogeneity and adaptive mechanisms often enable cancer cells to evade ADC-mediated destruction, which diminishes the therapeutic efficacy over time. Given the growing importance of ADCs in oncology and the challenges posed by resistance, this review aims to provide a comprehensive exploration of the mechanisms underlying ADC resistance and the strategies being developed to address ADC resistance. By synthesizing current knowledge and emerging research, this article seeks to identify key insights that can guide the design of next-generation ADCs and inform clinical practices to maximize their therapeutic potential. An in-depth analysis of the mechanisms contributing to ADC resistance was performed in this review, including antigen-related, internalization deficiency, trafficking dysfunction, impaired lysosomal function, payload-related, tumor microenvironment (TME)-mediated, and cancer stem cell-related mechanisms. The challenges associated with overcoming resistance were also discussed and innovative strategies to circumvent resistance were explored. This review aims to contribute to the ongoing efforts to enhance the efficacy and durability of ADC therapies by focusing on resistance mechanisms and solutions, ultimately improving outcomes for patients with cancer. It should be noted that although ADC-associated toxicities can influence treatment continuity and clinical outcomes, a comprehensive evaluation of safety profiles and toxicity mechanisms is beyond the scope of the present review, which focuses on biological resistance pathways affecting ADC efficacy.

This review was based on a comprehensive and focused literature survey aimed at summarizing current knowledge on mechanisms underlying ADC resistance and strategies to overcome these challenges. Peer-reviewed preclinical and clinical studies published between January 2000 and September 2025 were considered eligible for inclusion. The literature search primarily focused on studies investigating mechanisms underlying ADC resistance, biological determinants of responses, and therapeutic strategies to circumvent resistance with particular emphasis on data derived from FDA-approved ADCs to ensure clinical relevance. Eligible studies included original research articles, translational studies, and clinical trial reports that provided mechanistic insights or therapeutic implications related to ADC resistance. Studies not published in English, not peer-reviewed, lacking relevance to ADC resistance mechanisms, or lacking resistance-overcoming strategies were excluded.

ADC mechanism of action and structure

The mechanisms of action for ADC involve a series of intricate processes that culminate in the selective delivery of cytotoxic payloads to cancer cells, leading to cell death while sparing normal tissues. The canonical process typically involves four steps: binding of the antibody to the target antigen; internalization; linker cleavage; and release of payloads[9,24]. The chronologic events illustrating the mechanisms of action of ADC are detailed in **Figure 1**. The monoclonal antibody component of the ADC selectively binds specific antigens expressed on the surface of cancer cells upon administration and is internalized *via* endocytosis. The process of ADC internalization into cancer cells often involves clathrin-mediated endocytosis, in which the ADC-antigen complex is engulfed by the cell membrane into clathrin-coated pits[26]. This pit then buds off into the cytoplasm, forming a clathrin-coated vesicle that subsequently sheds the clathrin coat and fuses with early endosomes[27]. Payloads connected through acid-labile linkers tend to be released in early endosomes, while payloads linked *via* enzymes or through proteolytic degradation are typically liberated in late endosomes or lysosomes.

ADCs consist of three critical components: the monoclonal antibody; the linker; and the cytotoxic payload (**Figure 2** and **Table 1**)[28]. Target selection is critical because it determines the specificity and efficacy of the resulting ADC[29]. To achieve a sufficient therapeutic window, tumor-associated antigens (TAAs) selectively expressed or overexpressed in tumors compared to normal tissues are usually considered as suitable targets for ADC. These antigens may include cell surface receptors, membrane proteins, or other molecules involved in

Table 1 Summary of ADC drugs approved by the FDA, EMA, and NMPA as of December 2025

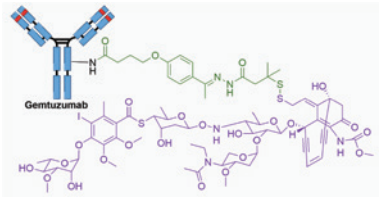
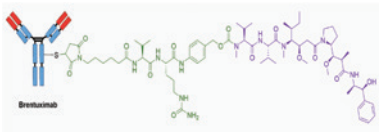
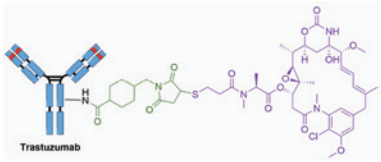
Drug name (Brand name)	Target	Linker	Payload	Approved country (Year)	Indication(s)	Structure
Gemtuzumab ozogamicin (Mylotarg)	CD33	Hydrazone	Calicheamicin	FDA (2000/2017) EMA (2020)	Newly diagnosed CD33-positive AML in adults and pediatric patients ≥ 1 month of age	
					Relapsed or refractory CD33-positive AML in adults and pediatric patients ≥ 2 years of age	
Brentuximab vedotin (Adcetris)	CD30	mc-VC-PABC	MMAE	FDA (2011) EMA (2011) NMPA (2020)	Previously untreated stage III or IV classical HL, in combination with chemotherapy	
					Previously untreated high-risk classical HL in combination with chemotherapy	
					Relapsed classical HL	
					Relapsed primary cutaneous ALCL or CD30-expressing MF	
					Previously untreated systemic ALCL or other CD30-expressing PTCL in combination with chemotherapy	
					Relapsed systemic ALCL	
					Classical HL consolidation	
Ado-trastuzumab emtansine (Kadcyla)	HER2	SMCC	DM1	FDA (2013) EMA (2013) NMPA (2020)	HER2-positive, metastatic BC who previously received trastuzumab and a taxane	
					HER2-positive early BC who have residual invasive disease after neoadjuvant taxane and trastuzumab-based treatment, adjuvant treatment	

Table 1 Continued

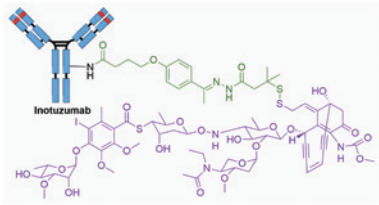
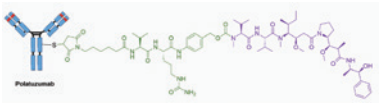
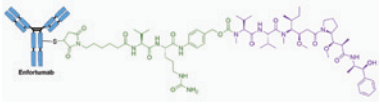
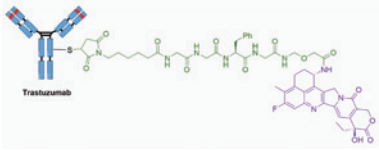
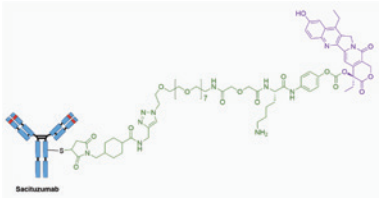
Drug name (Brand name)	Target	Linker	Payload	Approved country (Year)	Indication(s)	Structure
Inotuzumab ozogamicin (Besponsa)	CD22	Hydrazone	Calicheamicin	FDA (2017) EMA (2017) NMPA (2021)	Relapsed or refractory CD22-positive B-cell precursor ALL in adult and pediatric patients ≥ 1 year of age	
Polatuzumab vedotin (Polivy)	CD79b	mc-VC-PABC	MMAE	FDA (2019) EMA (2020) NMPA (2023)	Previously untreated DLBCL, not otherwise specified or HGBL in combination with chemotherapy Relapsed or refractory DLBCL, after at least two prior therapies, in combination with chemotherapy	
Enfortumab vedotin (Padcev)	Nectin-4	mc-VC-PABC	MMAE	FDA (2019) EMA (2020) NMPA (2024)	Advanced/metastatic UC	
Fam-trastuzumab deruxtecan (Enhertu)	HER2	Tetrapeptide	DXd	FDA (2019) EMA (2019) NMPA (2023)	Unresectable or metastatic HER2-positive BC who have received a prior anti-HER2-based regimen Unresectable or metastatic HER2-low BC who have received a prior chemotherapy in the metastatic setting Unresectable or metastatic HER2-positive NSCLC who have received a prior systemic therapy Locally advanced or metastatic HER2-positive gastric or GEJ adenocarcinoma who have received prior trastuzumab-based regimen Unresectable or metastatic HER2-positive solid tumors who have received prior systemic treatment and have no satisfactory alternative treatment options	
Sacituzumab govitecan (Trodelvy)	Trop-2	CL2A	SN-38	FDA (2020) EMA (2020) NMPA (2022)	Locally advanced or metastatic TNBC who have received two or more prior systemic therapies	

Table 1 Continued

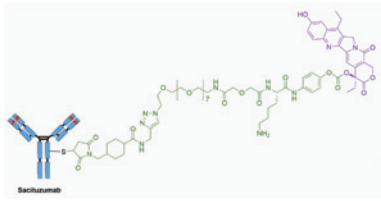
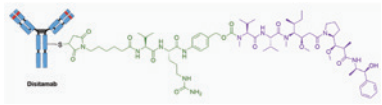
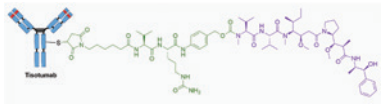
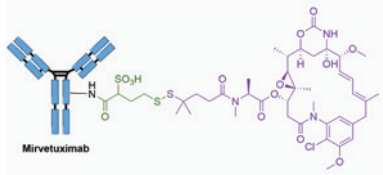
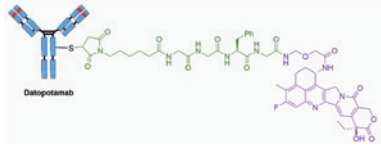
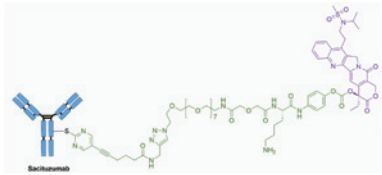
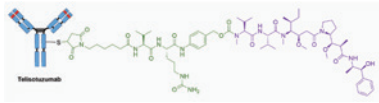
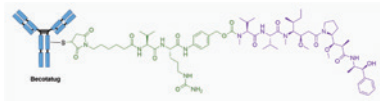
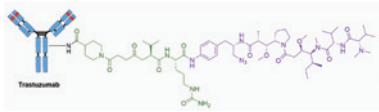
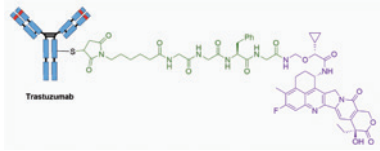
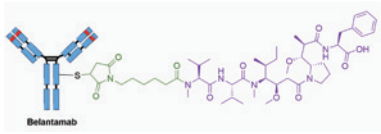
Drug name (Brand name)	Target	Linker	Payload	Approved country (Year)	Indication(s)	Structure
					Locally advanced or metastatic UC who have previously received platinum-containing chemotherapy and either PD-1 or PD-L1 inhibitor	
Loncastuximab tesirine (Zynlonta)	CD19	Dipeptide	PBD dimer	FDA (2021) EMA (2021) NMPA (2024)	Relapsed/refractory DLBCL after two or more lines of systemic therapy	
Disitamab vedotin (Aidixi)	HER2	mc-vc-PABC	MMAE	NMPA (2021)	HER2-overexpressing locally advanced or metastatic gastric cancer (previously treated) and UC	
Tisotumab vedotin (Tivdak)	TF	mc-vc-PABC	MMAE	FDA (2021) EMA (2021)	Recurrent/metastatic cervical cancer with disease progression on or after chemotherapy	
Mirvetuximab soravtansin (Elahere)	FRα	sulfo-SPDB	DM4	FDA (2022) EMA (2022) NMPA (2024)	FRα-positive, platinum-resistant epithelial ovarian, fallopian tube, or primary peritoneal cancer who have received one-to-three prior systemic treatment regimens	
Datopotamab deruxtecan (Datroway)	Trop-2	Tetrapeptide (MC-Gly-Gly-Phe-Gly)	DXd	FDA (2025) EMA (2025)	Unresectable or metastatic HR-positive, HER2-negative breast cancer who have received prior endocrine-based therapy and chemotherapy	
Sacituzumab tirumotecan (Sac-TMT)	Trop-2	Sulfonyl pyrimidine-CL2A-carbonate	KL610023	FDA (2024) EMA (2024) NMPA (2024)	Locally advanced or metastatic EGFR-mutant NSCLC that has progressed after EGFR-TKI and platinum-based chemotherapy Adult patients with unresectable locally advanced or metastatic TNBC who have received at least 2 prior systemic therapies, including at least 1 in the advanced or metastatic setting	

Table 1 Continued

Drug name (Brand name)	Target	Linker	Payload	Approved country (Year)	Indication(s)	Structure
Telisotuzumab vedotin (Emrelis)	c-Met	mc-vc-PABC	MMAE	FDA (2025) EMA (2025)	Locally advanced or metastatic NSCLC with high c-Met protein overexpression who have received a prior systemic therapy	
Becotatug vedotin (MRG003)	EGFR	Valine-citrulline	MMAE	NMPA (2025)	Recurrent or metastatic nasopharyngeal cancer	
Trastuzumab botidotin (A166)	HER2	Valine-citrulline	Duostatin-5	NMPA (2025)	Unresectable or metastatic HER2-positive BC who have received one or more prior anti-HER2 therapy	
Trastuzumab rezetecan (Avida)	HER2	Tetrapeptide	Rezetecan	NMPA (2025)	Unresectable locally advanced or metastatic NSCLC who have HER2-activating mutations and have received ≥ 1 previous systemic treatments	
Belantamab mafodotin (Blenrep)	BCMA	Maleimidocaproyl	MMAF	FDA (2020/2025)	Relapsed or refractory multiple myeloma who have received at least two prior lines of therapy	

ADC, antibody-drug conjugate; ALCL, anaplastic large cell lymphoma; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BC, breast cancer; BCMA, B-cell maturation antigen; CD19, cluster of differentiation 19; CD22, cluster of differentiation 22; CD30, cluster of differentiation 30; CD33, cluster of differentiation 33; CD79b, cluster of differentiation 79b; CL2A, a cleavable complicated ADC linker; c-Met, a receptor tyrosine kinase that binds to hepatocyte growth factor; DLBCL, diffuse large B-cell lymphoma; DM1 and DM4, derivatives of maytansine; DXd, exatecan derivative; EGFR, epidermal growth factor receptor; EGFR-TKI, EGFR tyrosine kinase inhibitor; EMA, European Medicines Agency; FDA, US Food and Drug Administration; FRα, folate receptor-alpha; GEJ, gastric or gastroesophageal junction; HER2, human epidermal growth factor receptor 2; HGBL, high-grade B-cell lymphoma; HL, Hodgkin lymphoma; KL610023, camptothecin derivative; mc-VC-PABC, maleimidocaproyl-valine-citrulline-p-aminobenzoyloxycarbonyl; MF, mycosis fungoides; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin-F; NMPA, National Medical Products Administration, China; NSCLC, non-small cell lung cancer; PBD, pyrrolobenzodiazepine; PD-L1, programmed cell death-ligand 1; PD-1, programmed cell death protein-1; PTCL, peripheral T-cell lymphomas; SMCC, succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate; SN38, active metabolite of irinotecan; Sulfo-SPDB, N-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate linker; TF, tissue factor; TNBC, triple-negative breast cancer; Trop-2, trophoblast cell surface antigen-2; UC, urothelial cancer.

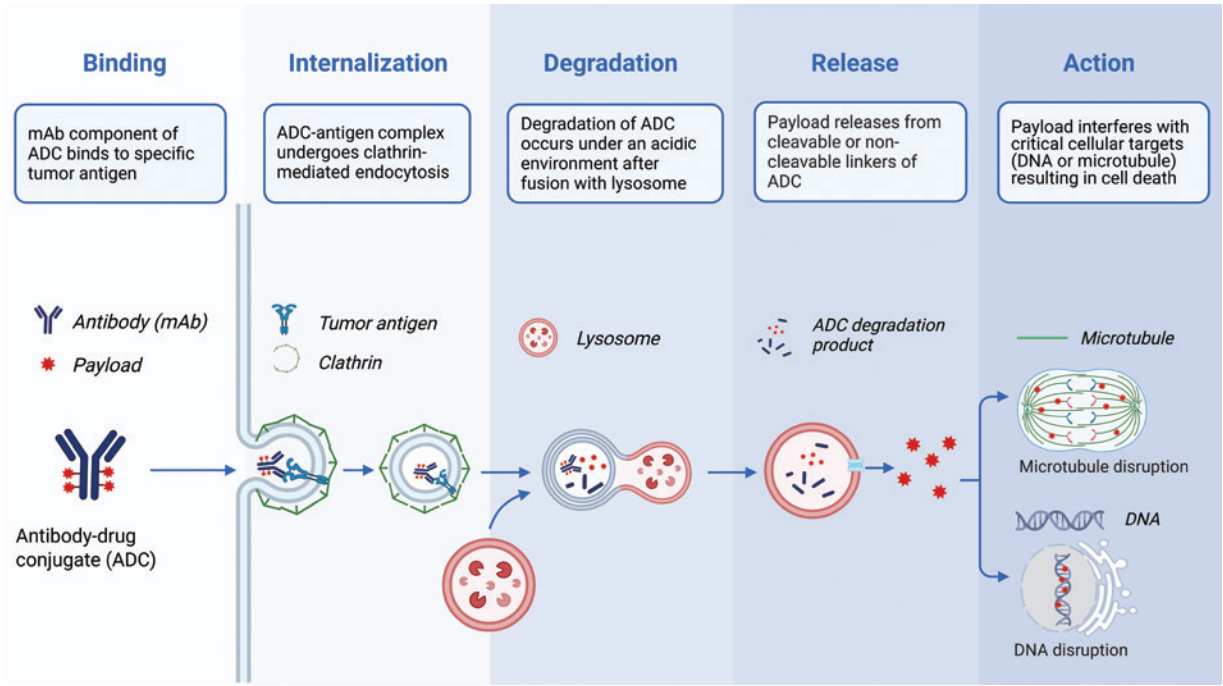


Figure 1 Mechanism of action of antibody-drug conjugates (ADCs). The process involves five main steps: **Binding**, in which the monoclonal antibody (mAb) component of the ADC specifically binds to a tumor antigen on the cancer cell surface; **Internalization**, in which the ADC-antigen complex undergoes clathrin-mediated endocytosis, allowing entry into the cancer cell; **Degradation**, in which the ADC is transported to the lysosome and the acidic environment facilitates degradation; **Release**, in which the payload is released from the cleavable or non-cleavable linkers of the ADC within the lysosome; and **Action**, in which the released payload interferes with critical cellular targets, such as DNA or microtubules, ultimately leading to cell death. Created with BioRender.com.

oncogenic signaling pathways[26,30]. To date, the target antigens of FDA-approved ADCs for solid tumors include human epidermal growth factor receptor 2 (EGFR2/HER2)[31], trophoblast cell surface antigen 2 (TROP2)[32], nectin cell adhesion molecule 4 (Nectin-4)[33], folate receptor alpha (FRα)[34], and tissue factor (TF)[35], which are representative examples of tumor-associated membrane proteins or receptors often implicated in cancer-promoting pathways. Cell lineage markers, like CD19, CD20, CD22, CD33, B cell maturation antigen (BCMA), and CD79b, are consistently expressed on malignant hematologic cells at high levels, making them prime candidates for ADC targets[36,37]. Notably, all antigens targeted by approved ADCs have the capacity to internalize upon binding, a crucial characteristic that enhances ADC effectiveness.

A suitable antibody moiety of the ADC is also crucial because it significantly impacts efficacy, pharmacokinetic/pharmacodynamic properties, and the therapeutic index[38]. The antibody acts as the precise guidance component of the ADC for the specific delivery of the payload into tumor cells through tumor antigen-mediated internalization[17]. The ideal antibody for ADCs should have strong target binding affinity, high stability, low immunogenicity, efficient internalization, and a long plasma half-life. Antibodies used for ADC development are mainly IgG isotypes with IgG1 subclasses being the most common choice due to the long half-life, convenient conjugation, structural stability, Fc-mediated immune functions, and scalability in large scale manufacturing[39,40]. Furthermore, antibody internalization is primarily determined by the target, while its efficiency is closely related to antigen density, antibody affinity, and the epitope involved in antibody-antigen interaction[41]. Different antibodies targeting the same antigen can exhibit varying internalization efficiencies[42]. Therefore, the selection of antibodies with high affinity and high internalization efficiency is crucial for ensuring the targeting specificity and safety of ADCs.

The linker, which connects the antibody to the payload through covalent binding, is an essential component of an ADC. A key requirement for the linker is to ensure the chemical stability of the ADC in the circulation, while allowing rapid payload release at the target site after internalization[43]. There are two main types of linkers used in ADCs: cleavable; and non-cleavable linkers[44]. These linkers have a major role in determining the pharmacokinetic (PK) properties, selectivity, and therapeutic index of ADCs. Cleavable linkers are relatively stable in the circulation but are vulnerable in the acidic or protease-rich TME[45]. Cleavable linkers include acid-labile

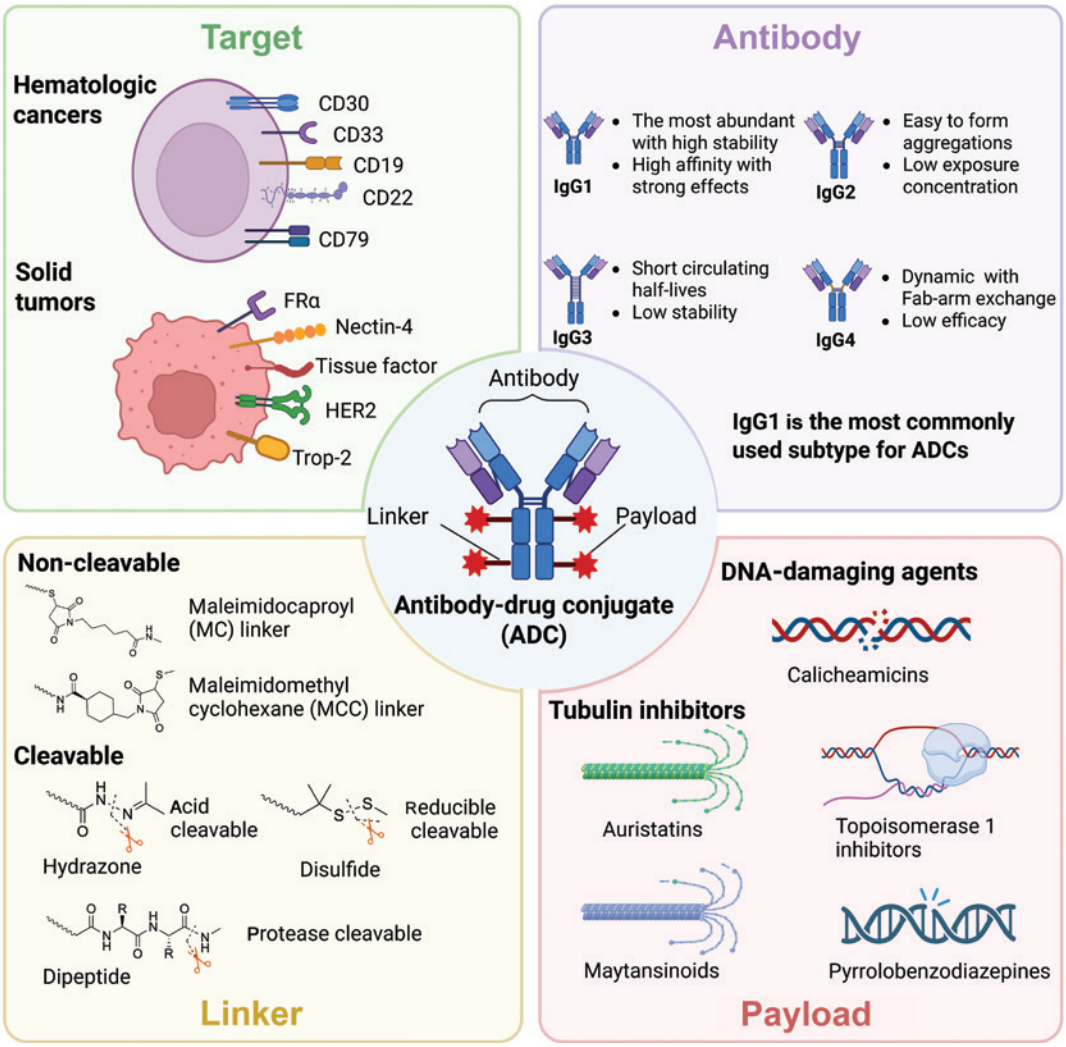


Figure 2 Components and targets of antibody-drug conjugates (ADCs). This figure highlights the key elements of ADCs, including the target antigens found in hematologic cancers (e.g., CD30, CD33, CD19, CD22, and CD79) and solid tumors (e.g., FRα, nectin-4, tissue factor, HER2, and Trop-2). The antibody section describes different IgG subtypes (IgG1, IgG2, IgG3, and IgG4) and notes that IgG1 is the most frequently used subtype for ADCs due to high stability and strong effector functions, like ADCC, ADCP, and CDC. The linker section differentiates between non-cleavable [e.g., maleimidocaproyl (MC) and maleimidomethyl cyclohexane (MCC) linkers] and cleavable linkers (e.g., acid cleavable hydrazone, reducible cleavable disulfide, and protease cleavable dipeptide). The payload section outlines the types of agents used, including DNA-damaging agents (e.g., calicheamicins, topoisomerase I inhibitors, and pyrrolobenzodiazepines) and tubulin inhibitors (e.g., auristatins and maytansinoids). ADC, antibody-drug conjugate; ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CDC, complement-dependent cytotoxicity; CD19, cluster of differentiation 19; CD22, cluster of differentiation 22; CD30, cluster of differentiation 30; CD33, cluster of differentiation 33; CD79b, cluster of differentiation 79b; EGFR, epidermal growth factor receptor; Fab, fragment antigen-binding of an antibody; Fc, the fragment crystallizable region of an antibody; FRα, folate receptor-alpha; HER2, human epidermal growth factor receptor 2; TF, tissue factor; Trop-2, trophoblast cell surface antigen-2. Created with BioRender.com.

hydrazone linkers, which are used in gemtuzumab ozogamicin and inotuzumab ozogamicin[46], reduction-sensitive or glutathione-labile disulfide linkers[47], and various enzyme-cleavable peptide-based linkers[48]. Among the marketed ADCs, eight utilize enzyme-cleavable linkers, such as brentuximab vedotin, polatuzumab vedotin, enfortumab vedotin, trastuzumab deruxtecan, and tisotumab vedotin[49]. In contrast, non-cleavable linkers contain stable chemical bonds that are not susceptible to proteolytic cleavage, which provides greater stability compared to cleavable linkers. ADCs with these linkers rely on lysosomal proteolytic degradation of the internalized antibody to release the payload, resulting in concomitant linker

cleavage[50]. Two approved ADCs (trastuzumab emtansine and belantamab mafodotin) utilize non-cleavable linkers[51]. Of note, the "bystander effect" exhibited by ADCs, in which neighboring cancer cells are destroyed, generally requires a cleavable linker because the charged amino acid drug products released by non-cleavable linkers typically lack cell permeability[52].

Payload is also an important component of ADCs and the activity and physicochemical properties of the payload can greatly impact the efficacy of ADCs. Early-stage payloads of ADCs used traditional chemotherapeutic agents (e.g., methotrexate, vincristine, and doxorubicin) but the suboptimal cytotoxicity and low uptake rate by tumor cells led to unsatisfactory efficacy and clinical failures[10]. Therefore, the payload should possess relatively high potency with IC_{50} values generally in the sub-nanomolar range. Payloads currently used in ADCs mainly fall into three classes: microtubule inhibitors; DNA damaging agents and the topoisomerase 1 (TOPO1) inhibitor[53]. Microtubule inhibitors destroy microtubules, thereby inhibiting chromosome separation during mitosis to eventually cause cell death[54]. Payloads acting via these mechanisms include maytansinoids and auristatins[55]. With IC_{50} values in the picomolar range, the exceptional potencyof maytansine and auristatin derivatives meets the requirements for ADC payloads, making them among the most important and clinically successful payload classes[56]. Specifically, maytansine derivative 1 (DM1) and monomethyl auristatin E (MMAE) are the most frequently used microtubule inhibitor-derived ADC payloads, accounting for nearly one-half of the payloads among marketed ADCs. DNA damaging agents kill tumor cells by causing double-strand breaks, alkylation, intercalation, and crosslinking of DNA[57]. Representative DNA-damaging payloads include calicheamicins and pyrrolobenzodiazepines (PBDs). TOPO1 inhibitors have recently emerged as promising payloads for ADCs. Prominent examples of TOPO1 inhibitors include trastuzumab deruxtecan (Enhertu, which was approved in 2019)[58] and sacituzumab govitecan (Trodelvy, which was approved in 2020)[59]. In addition, novel ADC payloads, such as RNA inhibitors, Bcl-xL inhibitors, proteasome inhibitors, immunoagonists [toll-like receptor (TLR) agonists], and even proteolysis targeting chimeras (PROTACs), are also under development[60].

Mechanisms underlying drug resistance to ADC therapy

Despite transformative potential, the clinical efficacy of ADCs is often hindered by the development of drug resistance. Resistance to ADCs can emerge through diverse mechanisms and these mechanisms can theoretically impact each stage of ADC action, including antigen recognition, internalization, intracellular trafficking, payload release, and cytotoxic activity of payloads (**Figure 3**).

Antigen-related resistance

Resistance to ADC therapy can arise at various stages of the ADC mechanism of action, each involving distinct molecular and cellular processes. The first step in the action of an ADC drug is to bind to the target antigen with the antibody component. Alterations in the target antigen can hinder the ability of the ADC to bind effectively, which may result in drug resistance[61]. Hence, one of the principal factors that contribute to ADC resistance is alteration of the target antigen. These modifications are multifaceted and can occur in several ways, each influencing drug resistance differently.

A common form of this mechanism is antigen downregulation, which involves a reduction in the presence of the target antigen on the surface of the cell[62]. Antigen downregulation would effectively diminish the binding ability and subsequent efficacy of the antibody component of ADCs. In a study conducted by Chen et al., two brentuximab vedotin-resistant hodgkin lymphoma (HL) (L428) and anaplastic large cell lymphoma (ALCL) (Karpas-299) cell lines were established through exposure to brentuximab vedotin[63]. Chen et al.[63] observed that the resistant ALCL cell line demonstrated downregulated CD30 expression compared to the parental cell line. The results from the INO-VATE trial suggested that patients with the lowest sCD22 receptor density appeared to benefit the least from inotuzumab ozogamicin[64]. Similarly, resistance to enfortumab vedotin was assessed by Aggen et al. Low Nectin-4 expression in metastatic biopsies was potentially associated with resistance to enfortumab vedotin[65]. Moreover, studies on the efficacy of T-DM1 in treating HER2-positive breast cancer showed that patients with lower levels of HER2 expression exhibited a poorer response to T-DM1 treatment, highlighting the important impact of HER2 expression levels on the therapeutic action of T-DM1[66]. A study involving early-stage HER2-positive breast cancer patients receiving neoadjuvant treatment with T-DM1 and pertuzumab showed that pre-treatment HER2 heterogeneity was negatively correlated with treatment response. Among patients with heterogeneous pre-treatment biopsy results, none achieved a pathologic complete response (pCR), whereas 55% of non-heterogeneous patients attained a pCR following combined T-DM1 and pertuzumab therapy[67]. Tumors with higher and more homogenous HER2 expression were more likely to respond to therapy[68]. Similarly, HER2 expression and the association with T-DXd was evaluated by Mosele et al. in the DAISY trial[69]. The study enrolled a total of 177 patients with metastatic breast cancer and divided the patients into 3 cohorts based on the level of HER2 expression, as follows: high HER2 expression (68 patients); low HER2 expression (72 patients); and HER2-negative (37 patients). The results showed that the objective

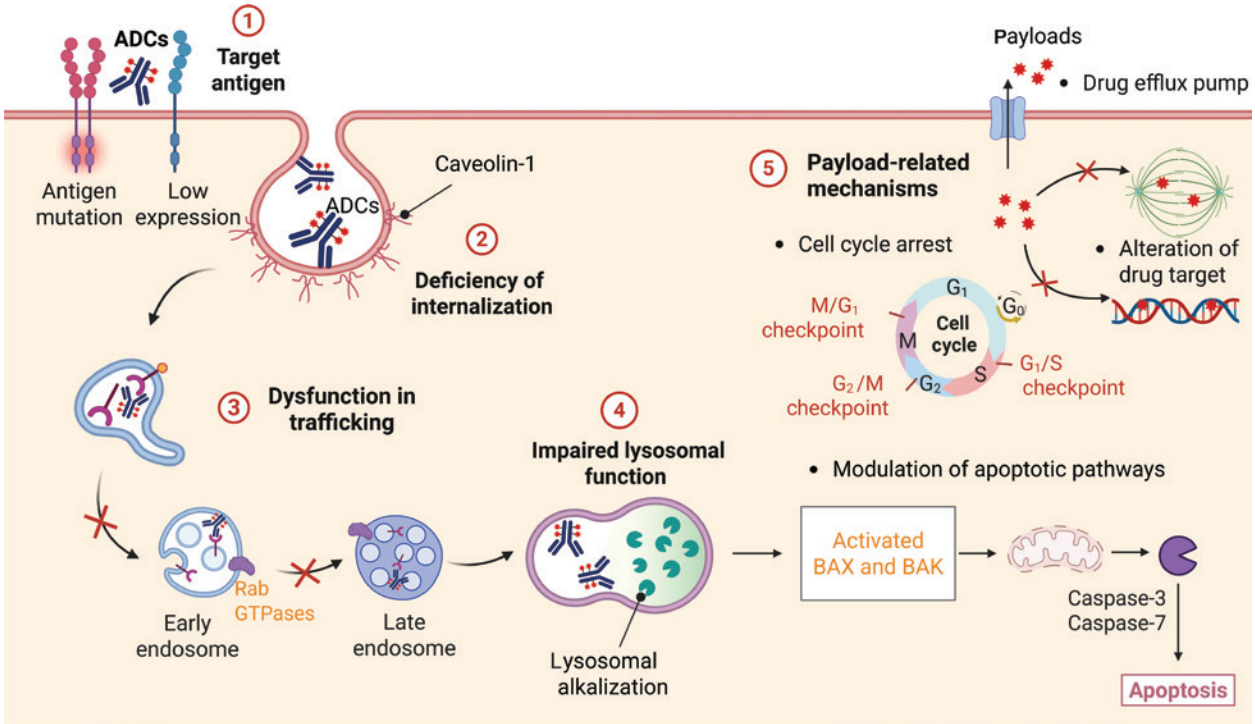


Figure 3 Emerging mechanisms of resistance to antibody-drug conjugates (ADCs). Various potential factors contributing to ADC resistance, including (1) target antigen issues, such as mutations and low expression, (2) deficiency in internalization due to proteins, like caveolin-1, (3) dysfunction in trafficking involving endosomal transport issues with Rab GTPases, (4) impaired lysosomal function resulting in lysosomal alkalization, (5) payload-related resistance through drug efflux pumps and alterations of drug targets affecting cell cycle arrest and apoptotic pathways. ADC, antibody-drug conjugate; BAK, Bcl-2 (B cell lymphoma 2) antagonist/killer; BAX, Bcl-2 associated X protein; GTPase, hydrolase enzymes that bind to nucleotide guanosine triphosphate (GTP); Rab, Ras-related protein. Created with BioRender.com.

response rate (ORR) was 70.6% (48/68), 37.5% (27/72), and 29.7% (11/37), respectively, in the 3 groups. The median progression-free survival (PFS) was 11.1, 6.7, and 4.2 months, respectively. In addition, the biomarker analysis results indicated that the anti-tumor activity of T-DXd was correlated with the level of HER2 expression and the drug distribution in the treated samples also had a moderate correlation with HER2 expression.

Antigen mutation at the antibody binding interface is another mechanism of ADC drug resistance that reduces or prevents binding of the antibody to the target, thereby causing the loss of cytotoxic activity in tumor cells[70]. Mutations in the coding sequence of the target antigen gene can result in structural alterations of the protein, potentially affecting the binding affinity and specificity of the antibody drug[71,72]. For example, Coates et al. identified a novel tumor-associated calcium signal transducer 2 (TACSTD2)/TROP2^{T256R} missense mutation through RNA and whole-exome sequencing of pretreatment and post-progression specimens that confers resistance to sacituzumab govitecan *via* defective plasma membrane localization and reduced cell-surface binding by hRS7[73]. These mutations include point mutations, insertions, deletions, or larger structural rearrangements, all of which can influence the three-dimensional structure and conformation of the target antigen. As a result, the antibody or ADC may no longer be able to recognize and bind effectively to the mutated antigen, rendering ADC ineffective. This phenomenon is particularly relevant in the context of cancer, in which tumor cells exhibit a high degree of genetic instability and accumulate mutations over time, facilitating the emergence of resistant clones[74,75]. Another possible mutation of the antigen involves the accumulation of shortened versions of the antigen ectodomain[16,76]. This accumulation of shortened versions of the antigen ectodomain was observed with trastuzumab, in which tumors with the complete HER2 receptor had a stronger response to the treatment in contrast to the truncated P95HER2 forms, which conferred resistance[77]. Therefore, trastuzumab-based ADCs, like T-DM1 and T-DXd, may cause drug resistance for this reason.

In addition, tumor heterogeneity in antigen expression might affect the efficacy of ADCs[19,78]. Tumor heterogeneity is a well-recognized phenomenon in cancer, in which subpopulations of cells within a tumor exhibit distinct genetic,

epigenetic, and phenotypic characteristics[79]. ADCs rely on the specific binding of the antibody component to the target antigen expressed on cancer cells. However, within a heterogeneous tumor, there may exist subpopulations of cells that express low or undetectable levels of the target antigen. These antigen-negative or -low cells may not be effectively targeted by the ADC, allowing the cells to survive and potentially repopulate the tumor, leading to treatment resistance and disease relapse[80]. Moreover, the emergence of antigen-negative or -low subpopulations can arise through various mechanisms, including genetic alterations (e.g., mutations, gene deletions, or epigenetic silencing of the target antigen gene), phenotypic plasticity (e.g., epithelial-to-mesenchymal transition), or the selection of pre-existing resistant clones under the selective pressure of ADC therapy[81,82]. This heterogeneity in antigen expression can be further exacerbated by the dynamic and constantly evolving nature of tumors, driven by genetic instability and adaptive responses to therapeutic interventions[83,84]. For example, in the KRISTINE trial, a phase II study focusing on early breast cancer patients, the combination of T-DM1 plus pertuzumab was examined in a pre-surgical context[85]. However, T-DM1 plus pertuzumab was shown to be less effective against tumors displaying a mixed pattern of HER2 expression. Notably, patients with tumors exhibiting HER2 intratumoral heterogeneity did not respond to T-DM1 treatment and a decrease in PFS and overall survival (OS) was more frequently recorded compared to patients with uniform HER2 expression, according to the exploratory biomarker analysis[86]. A similar phase II trial indicated that HER2 variability within a tumor might contribute to resistance against T-DM1, identifying the proportion of cells with unamplified HER2 as a crucial determinant[87].

It is noteworthy that these mechanisms of target antigen modulation often occur in concert, compounding the challenges associated with ADC resistance[70]. For example, a tumor cell may initially downregulate the expression of the target antigen to evade the effects of ADCs. Selective pressure imposed by the drug may subsequently lead to the emergence of clones harboring mutations in the antigen gene, further diminishing the binding affinity of the antibody. In addition, these resistant clones may use antigen masking mechanisms, such as overexpressing protective glycoproteins, to further enhance evasion of the therapeutic antibody[88]. A dense extracellular matrix (ECM), enriched in collagen, hyaluronan, and proteoglycans, can physically hinder ADC penetration and restrict antibody-antigen interactions[89]. Cancer-associated fibroblasts (CAFs), a major stromal component of many solid tumors, actively remodel the ECM and promote stromal fibrosis, thereby creating a diffusion barrier that limits uniform ADC distribution within tumors[90]. Preclinical studies have demonstrated that increased stromal density reduces intratumoral accumulation of HER2-targeted ADCs, even in tumors with high HER2 expression, leading to heterogeneous drug delivery and residual viable tumor regions[91]. This phenomenon may explain why some patients with antigen-positive tumors exhibit limited or no clinical response despite adequate target expression by immunohistochemistry. Finally, sensitivity to ADCs can be modulated by the presence of tumor heterogeneity in antigen expression[92]. Cancer cells with low or no antigen expression might not be efficiently lysed by the ADCs and this enables the cancer cells to endure and possibly replenish the tumor, which could result in treatment resistance and cancer relapse.

Internalization deficiency

Efficient internalization of the ADC-antigen complex is a crucial step for ADCs efficacy, as it enables the delivery of the cytotoxic payload to the intracellular compartments where the payload can exert cytotoxic effects[24]. One of the primary mechanisms by which deficiency in internalization can contribute to ADC resistance is through the reduced expression or activity of endocytic receptors and associated proteins involved in the internalization process[26]. ADCs typically leverage the natural endocytic pathways of cells to enter. However, if the expression or activity of key receptors involved in these pathways is diminished, the internalization process can be significantly impaired[93,94]. For example, some cancer cells may exhibit downregulation or mutations in receptors, such as the slow-density lipoprotein receptor (LDLR) or the transferrin receptor (TfR), which are commonly exploited by ADCs for internalization[95,96]. In addition, alterations in the expression or function of associated proteins involved in the endocytic machinery, such as clathrin, adaptor proteins, or dynamin, can also contribute to deficient internalization of the ADC-antigen complex[26,27]. One way ADCs achieve specificity is through clathrin-mediated endocytosis in cells that express the target antigen[97]. However, in a T-DM1-resistant N89-TM cell line, alternative uptake involving caveolin-1 (CAV1)-coated vesicles occurred, which might be less efficient[98]. In support of this finding, recent research in gastric cancer revealed a negative correlation between tumor CAV1 levels and T-DM1 uptake. In various xenograft models, genetic or pharmacologic suppression of CAV1 enhanced T-DM1 uptake and synergized with T-DM1 treatment[99]. In addition, glycosaminoglycan modification can hinder internalization of the tumor antigen, CAIX, and anti-CAIX ADCs, by facilitating the association of CAIX with CAV1 in lipid rafts[100,101].

Another mechanism by which deficiency in internalization can contribute to ADC resistance is through modulation of endocytic pathways and intracellular trafficking routes. Cells possess multiple endocytic pathways, including clathrin-mediated endocytosis, caveolin-mediated endocytosis, and various clathrin- and caveolin-independent pathways[102]. The endocytic pathway choice can influence

subsequent intracellular trafficking and the fate of the internalized cargo, including ADCs. Cancer cells may exhibit alterations in the regulatory mechanisms governing these endocytic pathways, favoring routes that do not lead to efficient delivery of the ADC-antigen complex to the appropriate intracellular compartments, such as lysosomes or endosomes[103]. These alterations can result in the mislocalization or sequestration of the ADC, preventing the release of the cytotoxic payload and rendering therapy ineffectiveness. In addition to limiting ADC access, the TME can directly impair ADC internalization and intracellular processing. Hypoxia, an acidic extracellular pH, and nutrient deprivation have been shown to alter endocytic trafficking and lysosomal function[104]. These conditions may reduce clathrin-mediated endocytosis and compromise lysosomal acidification, thereby attenuating payload release from internalized ADCs. Experimental studies have reported that hypoxic tumor regions exhibit reduced internalization of ADCs and diminished cytotoxic payload activation[105]. Moreover, tumor-associated macrophages (TAMs) expressing Fcγ receptors can sequester ADCs *via* Fc-mediated uptake, diverting TAMs away from tumor cells and further reducing effective internalization. This macrophage-mediated "ADC sink" effect has been observed in preclinical models and may contribute to reduced drug availability in antigen-expressing tumor cells[106].

Dysfunction in trafficking pathways

Efficient intracellular trafficking is crucial for the successful delivery of the cytotoxic payload to the intended subcellular target. Any disruptions in this process can significantly impact the efficacy of ADCs. Cancer cells can acquire resistance by interfering with the proper sorting and delivery of the ADC-antigen complex to the correct intracellular locations. Upon internalization, the ADC-antigen complex must navigate a complex network of endocytic vesicles, endosomes, and lysosomes to reach the appropriate location for payload release[42]. This process is tightly regulated by various trafficking proteins, such as guanosine triphosphatase (GTPase), sorting nexins, and retromer complexes[107,108]. Dysregulation or mutations in these trafficking proteins can lead to missorting or misdirection of the ADC-antigen complex, preventing it from reaching the lysosomal compartment or other intended subcellular destinations[109,110]. For example, alterations in Rab GTPases, which act as molecular switches controlling vesicle trafficking, can disrupt the proper sorting and trafficking of the ADC-antigen complex, leading to accumulation in non-productive compartments or premature degradation[111].

Endophilin A2 (Endo II), a crucial scaffolding protein implicated in non-clathrin endocytosis, has a significant role in internalization of HER2. Diminished expression of Endo II correlates with a drop in HER2 absorption and a reduced reaction to T-DM1 in HER2-positive breast cancer models[112]. Similarly, anomalies in caveolae-dependent endocytosis have been observed in T-DM1-resistant cell lines. For example, a gastric cancer cell line (N87) that developed resistance to T-DM1 exhibited increased caveolae-mediated endocytosis, which was likely due to caveolae inefficiency in drug delivery to lysosomes[98]. In agreement with these findings, the JIMT1-TM cell line, which is resistant to T-DM1, has heightened levels of proteins, such as Ras-related protein Rab-5B (Rab5B), autophagy-related protein 9A (ATG9a), and Huntingtin (HTT), that are responsible for lysosomal processing and vesicle transport. The proteomic analysis specifically pointed out elevated quantities of Rab6, associated with microtubule-dependent transport, and p21-activated kinase 4 (PAK4), linked to cytoskeletal functions[113-115].

An alteration of endosomal maturation and acidification processes also contributes to ADC resistance, except for the abovementioned trafficking pathways. Acidification of endosomes and lysosomes is crucial for the efficient release of the cytotoxic payload from the antibody-drug linker because many linkers are designed to be cleaved in the low pH environment[116,117]. However, cancer cells may exhibit alterations in the mechanisms that regulate endosomal acidification, such as deficiencies in vacuolar ATPases (V-ATPases) or alterations in the expression or activity of ion channels and transporters involved in pH regulation[118]. These disruptions can lead to impaired acidification of endosomes and lysosomes, hindering the efficient release of the payload and reducing the cytotoxic effects. In addition, even when ADCs are correctly internalized, a portion of these endosomes may be quickly recycled back to the cell membrane before the payload is released, resulting in the clearance of ADC from the cell[119,120]. Thus, this excessive recycling in cancer cells could also have a role in the development of resistance.

Impaired lysosomal function

The lysosomal compartment has a crucial role in intracellular trafficking and release of the cytotoxic payload from ADCs. Any disruptions in this process can significantly impact therapeutic efficacy[121]. One of the primary mechanisms by which impaired lysosomal function contribute to ADC resistance is impaired release of the cytotoxic payload from the antibody-drug linker[122]. ADCs are designed to undergo lysosomal degradation upon internalization, in which the low pH and proteolytic enzymes within the lysosome cleave the linker, releasing the potent cytotoxic payload into the cytosol[123]. Lysosomal acidification is critical for efficient linker cleavage and payload activation in many ADCs. Lysosomal pH is typically maintained within a narrow acidic range (pH 4.5–5.0) in ADC-sensitive tumor cells, which supports optimal protease activity. However, multiple studies have reported that ADC-resistant cells exhibit partial lysosomal alkalinization with

pH values increasing to approximately 5.5–6.0[91]. Although modest, this shift in pH is sufficient to impair lysosomal protease function and reduce payload release, thereby attenuating ADC efficacy without affecting antibody binding or internalization[124]. Ríos-Luci and colleagues identified mechanisms of resistance to T-DM1 by studying three distinct HER2-positive resistant cell lines. These cell lines, despite being resistant, had comparable levels of HER2 expression and normal processes for internalization and trafficking compared to non-resistant counterparts. However, these resistant cells exhibited an elevated lysosomal pH, which led to reduced proteolytic activity and accumulation of T-DM1[125]. This decline in lysosomal functionality hinders T-DM1 processing, thereby curtailing the ability to combat tumors. Notably, Trudeau and associates reported on the utilization of UV photoactivation to engineer acidifying nanoparticles, which purposefully alter intralysosomal pH levels, potentially restoring or enhancing the anti-cancer efficacy of ADCs[126].

Several factors can contribute to impaired lysosomal function in cancer cells, including alterations in lysosomal pH, deficiencies in lysosomal enzymes, or dysregulation of lysosomal membrane proteins[127]. Some cancer cells may exhibit increased lysosomal pH due to overexpression of proton pumps or defective vacuolar V-ATPases, which can disrupt the optimal acidic environment required for efficient lysosomal degradation and payload release[128]. For example, compromised V-ATPase activity was noted in the T-DM1-resistant N87 gastric cancer cell line[129]. Applying bafilomycin A1, which inhibits V-ATPase, resulted in reduced production of T-DM1 active metabolites, lowering toxicity in N87 gastric cancer cells, although this effect was absent in T-DM1-resistant N87 cells[129]. A similar scenario occurred in a T-DM1-resistant breast cancer cell line, in which an increase in lysosomal pH and a reduction in lysosomal proteolytic enzyme activity were noted in BT474 cells[125]. In addition, cancer cells may exhibit deficiencies or reduced activity of specific lysosomal proteases, such as cathepsins, which are responsible for cleaving the linker and releasing the payload. These deficiencies can arise from genetic mutations, epigenetic silencing, or post-translational modifications of these enzymes, ultimately leading to impaired payload release and reduced cytotoxicity[130,131].

Another way that defective lysosomal functioning contributes to ADC resistance involves the early breakdown or neutralization of the cytotoxic component. After the payload is released from its bond with the antibody, it needs to remain intact and functional within the cytoplasm to enact toxic effects. However, when the lysosomes are dysfunctional, the cytotoxic payload is not adequately released and the cancer cells do not receive a lethal dose of the drug, resulting in survival and proliferation of cancer cells, even in the presence of ADC therapy[125]. This premature degradation occur due to various factors, such as altered lysosomal pH, deficient or malfunctioning lysosomal enzymes, impaired lysosomal trafficking[132]. Moreover, the role of specific transporters in the efficacy of ADCs with non-cleavable linkers is particularly notable, as these linkers may necessitate a specialized transporter for cytoplasmic delivery. Hamblett and colleagues performed phenotypic RNA screening and identified solute carrier family 46 member 3 (SLC46A3) as a transporter that directly moves maytansine-derived catabolites into the cytoplasm. Inhibiting this transporter leads to the buildup of these catabolites within the lysosomes, culminating in the ineffectiveness of the drug[133]. The effectiveness of brentuximab vedotin in treating lymphoma was shown to be affected by a mechanism like other therapies. Transport of monomethyl auristatin E (MMAE) through the lysosomal membrane is regulated by lysosomal multidrug-resistance protein 1 (MDR1). Studies have suggested that blocking lysosomal MDR1 can increase the lethal effect of brentuximab vedotin in the HL cell line[134].

Impaired lysosomal function can also contribute to ADC resistance by modulating intracellular trafficking and localization of the ADC-antigen complex[135]. Successful delivery of the cytotoxic payload to the intended subcellular target is crucial for efficacy and this process relies on the proper sorting and trafficking of the internalized ADC-antigen complex through the endolysosomal pathway. Several ADCs rely on the lysosomal compartment to release the cytotoxic payloads. These payloads often require enzymatic cleavage or processing within the lysosome to become activated or to reach the full cytotoxic potential. The primary classes of payloads among the FDA-approved ADCs that depend on lysosomal processing include microtubule-disrupting agents (e.g., maytansinoids, auristatins), and DNA-damaging agents (e.g., calicheamicins)[94]. When the payload is released from the ADC in the lysosome, the cytotoxic compound must exit the lysosomal compartment to exert toxic effects. In some cases, the activated drug or metabolites may be expelled from the lysosome into the cytoplasm through a process involving vesicular trafficking[136]. After being processed in the lysosome, the payload may be packaged into vesicles that fuse with the plasma membrane, releasing the drug into the extracellular space or into specific target sites within the cell. Disruptions in this trafficking process can lead to mislocalization or sequestration of the ADC-antigen complex, preventing the ADC-antigen complex from reaching the lysosomal compartment and ultimately hindering payload release[9]. Such disruptions can result from in the endocytic machinery, dysregulation of Rab GTPases or other trafficking regulators, or changes in the composition and organization of endosomal and lysosomal membranes[137,138].

Payload-related resistance

Activation of drug efflux pumps

A key mechanism by which these components may prompt resistance is through activation of drug efflux pumps. Some cancer cells, particularly cancer cells in multidrug-resistant

tumors, can express elevated levels of P-glycoprotein (P-gp) or other members of the ATP-binding cassette (ABC) transporter family[139,140]. These efflux pumps act as molecular "gatekeepers", actively pumping out a wide range of cytotoxic payloads, like maytansinoids or auristatins, before efflux pumps exert cytotoxic effects in the cytoplasm and thereby causing drug resistance in cancer cells. This mechanism is particularly relevant for payloads that are substrates for these efflux pumps, such as payloads derived from natural product sources or some synthetic compounds[53].

Preclinical models have demonstrated that higher MDR1 expression was associated with drug resistance to gemtuzumab ozogamicin in AML cells[141]. Moreover, clinical findings indicate a significant correlation between MDR1 activity and patient outcomes. The AML cells obtained from patients who were sensitive to gemtuzumab ozogamicin were shown to have markedly lower MDR1 activity compared to patients who were not sensitive to gemtuzumab ozogamicin[142]. Similar outcomes were observed with inotuzumab ozogamicin, which also uses calicheamicin as payload[143]. Chronic treatment with ADCs that utilize auristatin derivatives, such as brentuximab vedotin, favors the selection of cell populations expressing MDR1. This is reported in HL cell lines and patient samples who have relapsed or shown resistance to brentuximab vedotin[63]. Resistance to anti-Nectin-4 ADC, like enfortumab vedotin, is also linked to increased MDR1 expression in resistant tumors[144]. Furthermore, Yu et al. developed cell lines from xenograft tumors of non-Hodgkin lymphoma (NHL) that were resistant to anti-CD22-valine-citrulline-MMAE and anti-CD79b-valine-citrulline-MMAE, identifying MDR1 as the primary resistance mechanism to these MMAE-based ADCs[145].

Maytansinoids, which are used in ADCs, like T-DM1, are the substrates of drug transporters, such as MDR1. Previous studies imply that resistance to such ADCs could be associated with MDR1 activity[146]. For example, HER2-positive gastric cancer cells resistant to T-DM1 demonstrated an increase in the expression of the ABC transporters (ABCC2 and ABCG2), while suppression of these transporters was shown to reinstate sensitivity to T-DM1[147]. Moreover, overexpression of multidrug resistance-associated proteins, such as multidrug resistance protein 1 (MRP1), multidrug resistance protein 2 (MRP2), and MDR1, has been identified in various T-DM1-resistant cell lines with sensitivity being potentially recoverable through the use of inhibitors targeting these proteins[113,148,149]. This resistance mechanism is similarly noted in cases involving sacituzumab govitecan, in which overexpression of the breast cancer resistance protein (BCRP) has been confirmed in breast cancer cell lines that are insensitive to sacituzumab govitecan[150].

Effects on the cell cycle

Anti-mitotic agents are frequently used as potent payloads in the composition of ADCs. Anti-mitotic agents interrupt the cell cycle of cancer cells by disrupting the dynamics of the microtubule during mitosis, leading to inhibition of cell division and death[151,152]. The cyclin B/Cyclin-dependent kinase 1 (CDK1) complex is pivotal for cell division and failure, which is termed mitotic catastrophe. Some recent studies have suggested that the mechanism of resistance to ADCs involves the impact of the ADC on cyclin B, a protein that is critical for the G2-M phase progression[153,154]. It has been reported that HER2-positive breast cancer cell lines with reduced cyclin B levels are resistant to T-DM1, whereas the increase in expression partly re-sensitized resistant cells[155]. Interestingly, the therapeutic effect of T-DM1 was aligned with the accumulation of cyclin B in a group of 18 HER2-positive breast cancer fresh specimens. Therefore, this finding suggested that upregulation of cyclin B could serve as a biomarker for T-DM1 sensitivity. In addition, the B-cell lymphoma 2 (BCL-2)/B-cell lymphoma-extra large (BCL-XL) proteins, which regulate cell apoptosis, have been linked with resistance to gemtuzumab ozogamicin in AML and anti-CD79b-vc-MMAE in NHL cell lines[156,157]. Similarly, T-DM1 resistance can be counteracted by inhibiting Polo-like kinase 1 (PLK1) through CDK1-dependent phosphorylation of Bcl-2[158] and the suppression of Bcl-2/Bcl-xl significantly amplifies T-DM1 anti-tumor effectiveness in patient-derived xenograft (PDX) models, independent of the initial sensitivity to T-DM1[159]. Moreover, cell-cycle dynamics can influence gemtuzumab ozogamicin drug activity. Specifically, non-dividing leukemic cells show less uptake and reduced sensitivity to the cytotoxicity of calicheamicin compared to cells that are actively dividing[160].

Payload target alterations

Another potential cause of ADC resistance is mutations in the targets of cytotoxic payloads. Several studies have reported changes in the payload target (TOPO1) after treatment with sacituzumab govitecan. Interestingly, a particular point mutation in TOPO1 (TOPO1^{E418K}) was pinpointed in triple-negative breast cancer (TNBC) patients who are resistant to sacituzumab govitecan. It has been hypothesized that this mutation may alter TOPO1 binding to DNA, thereby obstructing payload interaction at the enzyme-DNA junction[73]. This mechanism has previously been reported in resistance to chemotherapy targeting TOPO1[161]. Drawing parallels with chemotherapies using TOPO1 inhibitors, resistance to ADCs targeting TOPO1, like T-DXd and sacituzumab govitecan, might arise due to mutations that impact the structure or expression of TOPO1. TOPO1 is comprised of four domains. The linker domain that joins the core to the COOH-terminal is highly malleable and not crucial for the catalytic function of the enzyme[162,163]. Moreover, enzyme dysfunction is linked to reduced sensitivity to TOPO1 inhibitors[162,164]. Thus, it has been suggested that increased flexibility in the linker domain could alter enzyme shape upon drug binding, creating a version of the enzyme that resists TOPO1 inhibitors. In the context of ADCs deploying anti-mitotic payloads, such as T-DM1, changes in the

microtubule/tubulin complex were detected in T-DM1-resistant MDA-MB-361 HER2-positive breast cancer cells. These cells were characterized by a lower amount of polymerized tubulin and reduced βIII tubulin levels[165].

Signaling pathway activation

Like various cancer chemotherapies, sustained exposure to ADCs can exert selective pressure that results in the emergence of resistance-causing mutations, particularly through activation of some cellular signaling pathways. For example, resistance to gemtuzumab ozogamicin is associated with activation of the phosphoinositide 3-kinase (PI3K)/Protein kinase B (PKB or AKT) signaling pathway in primary AML cells studied *in vitro*. Application of an AKT inhibitor, MK-2206, demonstrated the potential to restore sensitivity to gemtuzumab ozogamicin or the isolated cytotoxic agent, calicheamicin, in these resistant cells[166]. Loss of the tumor suppressor, phosphatase and tensin homolog (PTEN), is known to trigger PI3K/AKT pathway activation in breast cancer. This pathway is implicated in the development of resistance to T-DM1, as evidenced by lower PTEN levels in resistant cells and indicating active PI3K/AKT signaling as a contributor to resistance. Incorporating the PI3K inhibitor, CDC-0941, had a synergistic anticancer effect, indicating that combining this type of inhibitor could potentially overcome resistance to ADCs[149]. Furthermore, overexpression of the leukemia inhibitory factor receptor (LIFR) in T-DM1-resistant cells triggered the signal transducer and activator of transcription 3 (STAT3) pathway, resulting in increased levels of anti-apoptotic proteins, like Bcl-xL, Bcl-2, survivin, and Mcl-1, thereby contributing to the resistance against T-DM1[167]. It is important to note that Wnt/β-catenin pathway activation may also have a role in ADC resistance. Wu and colleagues detailed the role of Wnt3 in trastuzumab resistance, in which overexpression caused increased β-catenin levels, enhanced growth rates and invasiveness, and resistance to trastuzumab in the affected cells[168]. Although these mechanisms suggest potential pathways of resistance to trastuzumab-based ADCs, the mechanisms have not been definitively linked to T-DM1 or T-DXd resistance.

Apoptotic pathway modulation

Apoptotic pathway modulation is another mechanism by which cancer cells may develop resistance to the lethal effects of ADCs. Typically, the cytotoxic components of ADCs are intended to induce programmed cell death in cancer cells. However, cancer cells might become resistant by altering or disabling parts of the apoptotic system, either as an intrinsic pathway associated with mitochondria or an extrinsic pathway linked to death receptors[169,170]. Resistance can develop due to mutations in crucial regulators of apoptosis, such as the Bcl-2 family or caspases, or by boosting the levels of anti-apoptotic proteins, like inhibitors of apoptosis proteins (IAPs)[171,172]. This phenomenon is particularly relevant in the context of cancer, in which tumor cells exhibit a high degree of heterogeneity and plasticity, enabling tumor cells to rapidly adapt and develop resistance mechanisms.

The importance of pro-apoptotic proteins [Bcl-2-associated X protein (BAX) and Bcl-2 homologous antagonist/killer (BAK)] in determining sensitivity to gemtuzumab ozogamicin in AML has been recognized[173]. Resistance to this ADC has also been associated with the heightened expression of anti-apoptotic proteins (BCL-2 and BCL-XL)[157]. A therapeutic strategy involving BCL-2 antisense (oblimersen sodium) combined with gemtuzumab ozogamicin has been explored in AML patients during the first relapse[174]. Similarly, reduced sensitivity to anti-CD79b-valine-citrulline-MMAE in NHL has been correlated with the BCL-XL level and utilization of a BCL-2 family inhibitor (ABT-263) has been shown to amplify ADC activity. This finding could have implications for resistance to ADCs, like brentuximab vedotin, due to the structural similarities. In cells resistant to T-DM1, an increase in PLK1, a kinase crucial for mitosis, has been noted[158]. Inhibition of PLK1 using volasertib was shown to overturn resistance by inducing mitotic arrest through the spindle assembly checkpoint, followed by phosphorylation and subsequent deactivation of the anti-apoptotic protein, Bcl-2[158].

Drug resistance can also stem from activation of DNA repair pathways[175]. Cancer cells may counter ADC payloads that inflict DNA damage by upregulating DNA repair processes, such as nucleotide excision repair (NER), allowing cancer cells to fix the damage caused by these agents, thus negating the toxic effects[176]. Moreover, cancer cells may mutate enzymes or proteins involved in these repair mechanisms, further enhancing resistance[177]. In addition to TOPO1 mutations that disrupt the target of SN-38, the efficacy of the homologous recombinational repair (HRR) pathway has also been linked to resistance against sacituzumab govitecan by compensating for the DNA damage inflicted by SN-38[178].

Innovations and strategies to overcome ADC resistance

A multifaceted approach is essential to effectively address the challenge of drug resistance to ADCs in the new ADC era. This section will delve into two critical aspects that are shaping the future of ADCs in overcoming resistance. First, molecular design innovations are at the forefront, including strategies, like targeting multiple antigens to enhance selectivity, improving linker and conjugation chemistry for better stability and release profiles, and introducing novel payloads to bypass traditional resistance mechanisms. Second, the strategic use of combination therapies with various agents is gaining traction, offering a synergistic approach to overcoming resistance by simultaneously targeting multiple pathways. These advances collectively represent a comprehensive effort to enhance the

efficacy and durability of ADCs in the evolving landscape of cancer treatment.

Molecular design innovations

Targeting multiple antigens

In the era of new ADC treatment, actively exploring new ADC drugs is a courageous but unknown path[179]. A forward-looking approach to addressing the clinical challenge of ADC resistance is to conjugate bispecific antibodies (BsAbs) with linker-payload complexes, resulting in bispecific antibody-drug conjugates (BsADCs)[180]. The design of BsADCs aims to target two different tumor antigens simultaneously to enhance activity in overcoming drug resistance due to antigen escape and/or tumor heterogeneity[181]. The innovative bi-paratopic or dual-targeting approach of BsADCs not only enhances selectivity by allowing binding to co-expressed antigens in solid tumors but also significantly improves internalization compared to conventional ADCs[182]. These distinctive benefits position BsADCs as a significant component in the next wave of ADC development with more than 10 BsADCs currently in clinical trials[183]. Importantly, recent discussions at the 2025 San Antonio Breast Cancer Symposium (SABCS) further underscored antigen heterogeneity as a dominant driver of acquired resistance to ADCs in solid tumors, particularly in breast cancer. Clinical sequencing data have shown that loss or spatial heterogeneity of HER2 expression following HER2-directed ADC exposure frequently limits the efficacy of subsequent HER2-ADC therapies, thereby providing a strong biological rationale for multi-antigen or bispecific targeting strategies[184]. The design of BsADCs is not intended for linear additive improvement alone. In fact, changes in binding modes affect overall efficacy, requiring comprehensive coordination and optimization of the BsAbs, linkers, and payloads[185,186].

Zanidatamab zovodotin (ZW49) is an innovative ADC based on zanidatamab that utilizes an interchain disulfide cysteine and protease-cleavable linker to conjugate the N-acyl sulfonamide auristatin[187]. The bispecific antibody structure of ZW49 enhances internalization and the Fc region contributes to antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC) effects. This design tackles numerous unaddressed clinical needs in patients with HER2 expression. Preclinical studies have demonstrated that ZW49 has strong internalization and potent tumor-killing effects, and favorable tolerability in animals despite high HER2 affinity[187]. ZW49 is currently being evaluated in a phase I clinical trial (NCT03821233). As of 10 March 2022, among 76 patients receiving ZW49, the confirmed ORR and disease control rate (DCR) was 28% and 72%, respectively, across multiple cancer types in this clinical trial, although the ocular toxicity profile (42% keratitis) warrants attention[188].

MEDI4276 is a tetravalent HER2-targeted ADC that combines the single-chain variable fragment (scFv) of trastuzumab with the N-terminus (39S) of another anti-HER2 IgG1 antibody[189]. While MEDI4276 had significant efficacy in treating resistant HER2-positive cancer xenograft models, it failed to achieve a favorable balance between efficacy and safety in clinical trials[190]. Among 43 breast cancer or gastric cancer patients, the overall ORR was reported to be 9.4% in 32 evaluable patients with a maximum tolerated dose (MTD) at 0.75 mg/kg[190,191], which is lower than many other HER2-ADCs. MEDI4276 had poor tolerability and unfavorable pharmacokinetics (PK) at the MTD due to high toxicity of the payload, suggesting the necessity for further optimization[192].

CD63 is part of the tetraspanin superfamily. CD63 is broadly expressed, primarily on the cell surface, as well as in late endosomes and lysosomes[193]. The unique subcellular localizations of CD63 make it a promising target for BsADCs, which facilitate antigen internalization upon drug binding on the cell membrane and subsequent lysosomal trafficking for more efficient drug delivery and payload release. BsAb combining low-affinity CD63 arm with high-affinity anti-HER2 Fab (designated as HER2 × CD63) was developed[194]. This design leverages antibody-dependent receptor cross-linking to boost the effective internalization of HER2 and facilitate lysosomal co-localization (from 30–40% to 60–80%). The HER2 × CD63 BsAb is linked to the anti-mitotic payload, duostatin-3, *via* a valine-citrulline (vc) linker[194]. However, the suboptimal efficacy in tumors with low HER2 expression suggests the need for further optimization, possibly by increasing the drug-to-antibody ratio (DAR) to improve cytotoxic activity and/or using a different payload with stronger bystander effect.

The prolactin receptor (PRLR), which is frequently overexpressed in malignant breast epithelium, effectively facilitates clathrin-dependent initial internalization and lysosomal transport *via* self-ubiquitination and stimulation of adaptor protein complex 2 (AP2) complex recruitment[195]. A BsAb featuring HER2 and PRLR arms was developed using the "Knobs-into-Holes" (KIH) approach[196]. The BsADC uses a non-cleavable linker to attach Mertansine (DM1) to surface lysine residues, achieving an average DAR of 3.3[186]. Despite relatively low cell surface expression, surface antigens with high turnover rates, like PRLR, can sufficiently induce fast ADC internalization and lysosomal degradation processes, thereby enabling adequate cytotoxic activity of ADCs. Moreover, the phase I global clinical data of EGFR × HER3 dual-targeted ADC IzaBren (BL-B01D1) showed a confirmed objective response rate (cORR) of 33.3% with an acceptable safety profile in patients with metastatic or unresectable NSCLC and other solid tumors, including breast cancer and esophageal adenocarcinoma, which is the first clinical validation of the therapeutic value of dual-targeted ADCs in EGFR-TKI resistant NSCLC[197].

Linker and conjugation chemistry improvement

The linker and conjugation chemistry are also pivotal aspects of ADC design that significantly impact the pharmacokinetics, stability, specificity, and efficacy[38,198]. ADCs can achieve better-targeted delivery, improved stability, and enhanced efficacy against resistant tumors through the rational design of optimized linkers. For example, the traditional non-specific conjugation methods using lysine residues or cysteine thiols lead to significant heterogeneity in the DAR and hydrophobicity[199]. The ADC drug load ranges from unloaded (DAR = 0) to highly loaded (DAR ≥ 6). Highly-loaded ADCs are unstable in plasma and exhibit increased non-specific hepatic uptake, resulting in off-target toxicities[200]. ADCs with homogenous DAR distributions have been developed with technological advances. This uniformity can enhance the therapeutic window of ADCs and reduce resistance by ensuring consistent delivery of the cytotoxic payload to the tumor cells. The thiomab site-specific conjugation, which was developed by Panowski et al.[201], introduces a cysteine residue at the ALA114 position in the antibody CH1 domain, providing an exposed free thiol (–SH) group for site-specific conjugation.

In addition to site-specific conjugation, modified and engineered linkers, such as polyethylene glycol (PEG)ylated linkers, can also improve the PK, tolerability, and efficacy of ADCs by tuning the hydrophilicity[202,203]. The development of self-immolative linkers has also enhanced the stability and tolerability of ADCs[204]. In the early 1980s, a paraaminobenzyloxycarbonyl (PABC)-based self-immolative linker was developed for prodrug design[205]. Since the early 1980s, PABC linkers have been widely applied in ADCs, especially in MMAE-conjugated ADCs[206]. After ADC internalization into the target cells, the linker is cleaved by lysosomal enzymes and the PABC spacer in the vc-PAB-MMAE linker/payload undergoes a cascade of cleavage reactions to facilitate the intracellular release of MMAE. The vc-PABC linker exhibits superior plasma stability and better toxicity profiles compared to earlier cleavable linkers[48,207]. This linker technology has been successfully utilized in the four FDA-approved MMAE-based ADCs.

Recently, Pillow et al. developed more sophisticated self-immolative linkers that allow direct conjugation of the cytotoxic payloads to engineered cysteine residues[208]. This engineered cysteine conjugation approach enables a more uniform distribution of the cytotoxic payload across the entire ADC. IgGs generally contain 8 cysteine residues, allowing for the generation of ADCs with a DAR of 2–4 through this site-specific engineering[209]. These engineered ADCs have demonstrated significant potential and tolerability in preclinical studies compared to traditional ADCs[24,210]. Moreover, this approach has improved the tolerability of pyrrolobenzodiazepine (PBD)-based ADCs. The novel self-immolative disulfide linker ADC containing the same PBD payload exhibited similar *in vivo* potency in xenograft tumors compared to conjugation through a peptide linker, while largely improved tolerability in rats, suggesting the unique advantage of this disulfide linker in balancing efficacy and toxicity in preclinical studies[208].

Payload innovations

Innovative ADC payloads with different mechanisms of action are also crucial to overcome drug resistance to current-generation ADCs. Observed resistance mechanisms involve the payload itself affecting efflux proteins because many frequently used ADC payloads, like MMAE, MMAF, and SN38, are substrates of ABC transporters, which inevitably leads to ADC resistance[51]. Therefore, using novel payloads with different mechanisms of action can effectively address payload-related resistance. For example, ADC resistance driven by changes in topoisomerase expression or downstream signaling mechanisms can be overcome by using topoisomerase inhibitor payloads[211]. Similarly, it has been noted in NHL tumor models that substituting an auristatin-based payload with an anthracycline-based payload in the ADC enhances patient responses to the treatment[145]. Similarly, the cells remained sensitive to T-DXd in HER2-positive gastric cancer cells resistant to T-DM1[147]. This finding was further expanded *in vivo* and xenograft tumors derived from T-DM1-resistant cells showed volume reduction when treated with T-DXd[147]. The application of this principle includes developing other HER2- or TROP-2-targeted ADCs, which may provide alternative payloads to overcome payload-related resistance. However, emerging clinical observations presented at the American Society of Clinical Oncology (ASCO) 2025 annual conference raised concerns regarding cross-resistance among ADCs sharing similar payload classes, particularly TOPO-I inhibitors. Sequential use of multiple TOPO-I-based ADCs was associated with attenuated responses in heavily pretreated populations, suggesting that payload class, rather than antibody target alone, may critically shape resistance trajectories[212]. These findings highlight the necessity of incorporating payload diversification or rotation strategies into ADC development and sequencing paradigms to mitigate cumulative payload-driven resistance.

In addition, to improve the immunomodulatory capabilities of ADCs, immunostimulatory antibody conjugates (ISACs) were developed. In contrast to ADCs, which depend exclusively on payload internalization and direct cytotoxic effects for anti-tumor activity, ISACs leverage payloads to mediate reprogramming of the TME. Mechanistically, ISACs promote targeted recruitment and activation of innate immune cells, including macrophages and dendritic cells, leading to enhanced antigen presentation and subsequent priming of adaptive immunity. Preclinical studies have shown that ISAC treatment can result in a 2–5-fold increase in intratumoral $CD8^+$ T-cell infiltration, accompanied by significant upregulation of antigen presentation markers, such as MHC class I and II on tumor-associated antigen-presenting cells[213]. This immune activation cascade has been associated with the induction of systemic anti-tumor immune responses, and

in some models, the establishment of long-lasting immunologic memory, as evidenced by protection against tumor rechallenge[214]. ISACs achieve these effects by replacing conventional cytotoxic payloads with immunostimulatory molecules, most frequently pattern recognition receptor (PRR) agonists, including Toll like receptor 7/8 (TLR7/8) or stimulator of interferon genes (STING) agonists[215]. While PRR agonists have demonstrated potent anti-tumor activity, systemic administration as free agents is often limited by widespread immune activation and dose-limiting toxicities. Indeed, free PRR agonists typically induce systemic cytokine release at doses one-to-two orders of magnitude lower than required to achieve robust anti-tumor efficacy[216]. ADC enables selective tumor delivery of PRR agonists, resulting in markedly enhanced intratumoral immune activation with substantially reduced systemic cytokine exposure[81,217]. ISACs have been shown to induce robust macrophage reprogramming toward a pro-inflammatory phenotype in preclinical models, increase antibody-dependent cellular phagocytosis by approximately 2- to 3-fold, and promote dendritic cell maturation and cross-presentation. ISACs targeting a range of antigens, including cancer embryonic antigens, HER2, tumor-associated antigens, and PD-L1, are currently under clinical evaluation.[218,219]. Notably, combination strategies further amplify ISAC efficacy. When combined with PD-1 blockade, ISACs or PRR agonist-based conjugates have demonstrated significantly greater tumor growth inhibition and survival benefit compared to monotherapy, even in tumor models resistant to PD-1 antibodies alone[220,221]. This synergistic effect has been attributed, at least in part, to enhanced macrophage polarization toward anti-tumor phenotypes and increased T-cell infiltration, highlighting the potential of ISACs to overcome immunologically "cold" tumor states.

In addition to using immunostimulatory agents, a variety of innovative cytotoxic ADC payloads can also be used. For example, there are new ADCs that use the same antibody to conjugate two different payloads, which allow for the accumulation of multiple cytotoxic mechanisms to enhance anti-tumor activity and reduce tumor cell resistance[222-224]. Current chemotherapy regimens often use combination drug approaches. Considering the benefits of combination therapy, using ADCs with two or more payloads may help avoid the development of ADC resistance. Dual-payload ADCs aim to address tumor resistance and heterogeneity by integrating the mechanisms of multiple cytotoxic drugs. Levengood et al. reported the first dual-payload ADC in 2017, which conjugated MMAE and MMAF to a short peptide linker containing orthogonally protected cysteine residues, with a DAR of 16 (8 + 8)[225]. MMAE and MMAF have complementary physiochemical properties. MMAE has good cell permeability and a bystander effect but reduced potency against MDR-high cells, while MMAF has activity against MDR+ cells but lower cell permeability. Further activity analysis showed that the generated dual-payload ADC exhibited potent anti-tumor activity and overcame tumor cell resistance by incorporating complementary payloads. Kumar et al. reported a second dual-payload ADC in 2018 that was composed of MMAE and a PBD dimer[226]. The first payload (MMAE) was attached *via* a hydrazone linker, while the second payload (PBD-SG3557) was installed *via* CuAAC. The evaluation of activity *in vitro* showed that the dual-payload ADC had equal potent anti-tumor activity compared to the PBD single-payload ADC. A new dual-payload ADC was developed in 2021 using click chemistry, enabling flexible DAR combinations of 2 + 2, 4 + 2, and 2 + 4[222]. The design incorporated an azido-dibenzocyclooctyne (DBCO) click pair and a methyl-tetrazine-trans-cyclooctene (TCO) click pair, in which the me-tetrazine-TCO cycloaddition did not interfere with the azide-DBCO coupling. The click pairs were conjugated to PEG spacers, a cleavable Glu-Val-Cit linker, PABC, and the payloads (MMAE/MMAF). Cytotoxicity studies showed the dual-payload ADC maintained potent anti-tumor activity in the HER2-low, hydrophobic chemotherapy-resistant JIMT-1 breast cancer cell line. Furthermore, in a breast tumor xenograft model with HER2 heterogeneity/resistance, the dual-payload ADC exhibited better anti-tumor activity compared to single-payload ADCs or the co-administration of single-drug variants[222].

Combination therapies

In addition to the innovative improvement of ADC structure, drug combination is one of the frequently used strategies to solve drug resistance caused by signal pathway changes, especially in the treatment of cancer, in which it is often difficult to achieve the expected efficacy with a single drug. Therefore, the combination of ADCs with other anti-tumor agents is being actively explored in preclinical models and clinical trials. Notably, major oncology meetings in 2025, including the ASCO and the European Society For Medical Oncology (ESMO), reflected a paradigm shift in which ADCs are increasingly positioned as components of rational combination regimens rather than stand-alone cytotoxic agents[227-229]. Drug combinations not only overcome single-drug resistance but enhance the overall efficacy. The combination regimens can be roughly divided into four categories: combined with chemotherapy drugs; combined with targeted drugs; combined with anti-angiogenesis drugs; and combined with immunotherapy drugs (**Figure 4**). **Table S1** summarizes the representative ongoing clinical trials which evaluate ADC combination therapies for different cancer indications.

Combination with chemotherapy

Optimizing ADC combination therapy requires understanding how ADCs and chemotherapeutic agents interact to regulate the cell cycle and modulate target antigen expression. Growing preclinical and clinical evidence demonstrates varying levels

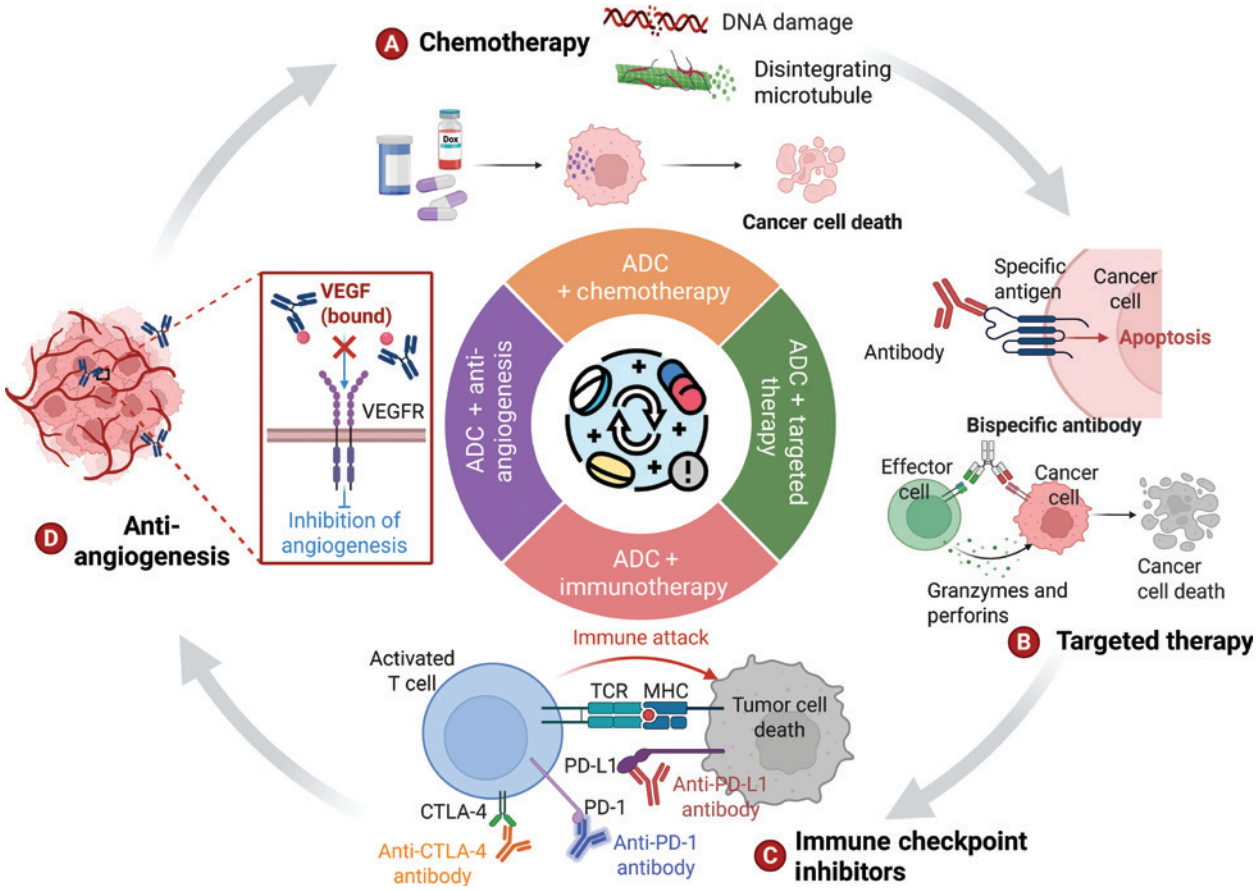


Figure 4 Combination strategies of antibody-drug conjugates (ADCs) in cancer therapy and mechanisms of action. (A) Chemotherapy: the cytotoxic agents for chemotherapy could target cancer cells, causing DNA damage and microtubule disintegration, leading to cancer cell death. (B) Targeted therapy: the monoclonal antibody can target specific antigens on cancer cells, inducing apoptosis while bispecific antibodies can engage effector cells to release granzymes and perforins, causing cancer cell death. (C) Immune checkpoint inhibitors: combination with immune checkpoint inhibitors, such as anti-CTLA-4 and anti-PD-1 antibodies, can enhance the immune attack on tumor cells, leading to tumor cell death. (D) Anti-angiogenesis: anti-angiogenesis agents can inhibit angiogenesis by blocking VEGF from binding to the VEGF receptor (VEGFR), thereby preventing the formation of new blood vessels that supply the tumor. ADC, antibody-drug conjugate; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; MHC, major histocompatibility complex; PD-L1, programmed cell death-ligand 1; PD-1, programmed cell death protein-1; TCR, T-cell receptor; VEGF, vascular endothelial growth factor; VEFGR, vascular endothelial growth factor receptor. Created with BioRender.com.

of effectiveness and lays the groundwork for advancing drug development. For example, DNA-damaging agents, such as platinum agents and topoisomerase inhibitors, which induce G2/M cell cycle arrest, can be combined with microtubule-disrupting agents, such as paclitaxel and docetaxel, to maximize the destruction of cells arrested in G2/M[230,231]. This strategy of combining drugs has yielded encouraging outcomes in ovarian cancer models using carboplatin with mirvetuximab soravtansine [FRα-DM4], anetumab ravtansine (mesothelin-DM4), or luveltamab tazevibulin (FRα-SC239)[232-235].

When combining ADCs with chemotherapy, the timing of administration is critical. Microtubule polymerization is essential for the endocytosis and internalization of ADCs. Additionally, G2/M arrest induced by DNA damage requires sufficient time to sensitize cells to microtubule-disrupting agents. Researches involving colorectal, lung, and breast cancer models have shown that administering SGN-15 (Lewis Y antigen-adriamycin) and paclitaxel sequentially leads to more DNA damage than concurrent administration, suggesting that delayed administration of the anti-microtubule agent after DNA-damaging therapy may enhance the therapeutic effect[236]. However, this approach has not been tested in clinical trials and requires further exploration based on the tumor-specific ADC internalization rate and cell cycle dynamics.

Chemotherapies may also modulate the expression of the targeted surface antigen on tumor cells[237]. For example, studies

have indicated that gemcitabine increases HER2 expression in pancreatic cancer cells, which can improve the effectiveness of T-DM1 when used in combination with gemcitabine[238,239]. Consequently, some chemotherapies might be better suited for use with ADCs based on the capacity to boost the target antigen, a discovery that calls for additional research into other ADC-chemotherapy pairings.

Combination with targeted therapy

ADCs have a higher therapeutic index and targeted tumor cell cytotoxicity compared to standard chemotherapy, which may allow for better combination with targeted therapies. Many studies have attempted to replace standard chemotherapy with ADCs and combine ADCs with targeted agents but the results have been disappointing. Clinical trials, like KAITLIN, KRISTINE, and MARIANNE, that combined T-DM1 with pertuzumab did not show improved efficacy in the neoadjuvant and metastatic settings compared to paclitaxel + trastuzumab + pertuzumab, especially in patients with low HER2 expression or high HER2 heterogeneity[240-242]. While these results were not ideal, it does not preclude the possibility that updated ADCs combined with targeted therapies may show improved efficacy in select clinical settings. Increasing evidence supports the combination of ADCs with drugs targeting the resistance mechanisms. For example, HER2-driven malignant transformation of breast epithelial cells depends on cyclin D1. Therefore, combining the CDK4/6 inhibitor with T-DM1 in HER2-resistant patients showed moderate tumor regression with manageable toxicity[243,244]. However, CDK4/6 inhibitors block cell cycle progression into the S or M phase, which may reduce the efficacy of T-DM1. As a result, optimizing the treatment sequencing to enhance efficacy and minimize toxicity needs to be considered. A notable advance involves the anti-HER2 ADC, trastuzumab deruxtecan, combined with the monoclonal antibody, pertuzumab. The FDA approved this combination in December 2025 for first-line treatment of adults with unresectable or metastatic HER2-positive breast cancer based on results from the phase III DESTINY-Breast09 trial, which showed a significant improvement in PFS compared to the standard taxane plus trastuzumab and pertuzumab regimen[245]. Specifically, T-DXd plus pertuzumab reduced the risk of disease progression or death by 44% (hazard ratio = 0.56) and achieved a median PFS > 3 years *vs.* 26.9 months with the standard regimen. This approval represents a new standard of care in this setting and highlights the potential of combining ADCs with other monoclonal antibodies to improve outcomes.

Furthermore, various tyrosine kinase inhibitors (TKIs) have been shown to modulate cell surface antigen expression, which may further potentiate ADC activity and sensitize tumors with low antigen expression[246,247]. In the TEAL study, the regimen of T-DM1 combined with the pan-HER inhibitor, lapatinib, and albumin-bound paclitaxel for preoperative therapy of HER2-positive breast cancer demonstrated superior effectiveness relative to the conventional treatment combining paclitaxel, trastuzumab, and pertuzumab, with a more notable advantage observed in patients with hormone receptor-positive (HR+) tumors[248]. Similarly, exposure of melanoma cell lines to B-Raf proto-oncogene, serine/threonine kinase (BRAF)/Mitogen-activated protein kinase kinase (MEK) inhibitors upregulated surface Tyrosine-protein kinase receptor UFO (AXL), which in turn increased the sensitivity to enapotamab vedotin (AXL-MMAE)[249]. In the combination anti-HER2 therapy setting, the phase I study involving T-DM1 plus the HER2-TKI, tucatinib, in HER2-positive metastatic breast cancer achieved a median PFS of 8.2 months[250]. Based on this finding, the HER2CLIMB-02 study involving tucatinib plus T-DM1 and the HER2CLIMB-04 study involving tucatinib plus T-DXd are ongoing[251,252]. The combination of T-DXd and pertuzumab achieved an ORR of 16% in the DESTINY-Breast 07 study, which is comparable to single-agent T-DXd[253]. Emerging evidence presented at the 2025 European Lung Cancer Congress (ELCC) further supported the role of ADCs as effective partners for overcoming acquired resistance to targeted therapies. Early clinical data demonstrated that combining the TROP2-directed ADC, datopotamab deruxtecan, with osimertinib produced encouraging activity in EGFR-mutant non-small cell lung cancer patients with post-TKI resistance, highlighting a novel strategy whereby ADCs eradicate heterogeneous resistant clones, while TKIs suppress residual oncogenic signaling[254].

Combination with anti-angiogenesis therapy

Anti-angiogenic agents can promote tumor vessel normalization, which can enhance the penetration and exposure of ADC drugs in the tumor tissue[255,256]. The combination of anetumab ravtansine or mirvetuximab soravtansine with bevacizumab achieved complete responses in preclinical ovarian cancer models. Anetumab ravtansine combined with bevacizumab showed improved *in vivo* efficacy in the OVCAR-3 ovarian cancer model with high mesothelin expression[257]. Similarly, mirvetuximab soravtansine combined with bevacizumab improved the *in vivo* efficacy in platinum-resistant ovarian cancer in the FRα-high epithelial ovarian cancer model with most tumor-bearing mice showing tumor regression or complete elimination[233].

Based on these preclinical studies, a phase Ib study using mirvetuximab soravtansine in combination with bevacizumab to treat heavily pre-treated, platinum-resistant, FRα-high ovarian cancer patients achieved an ORR of 39%, which was superior to the benchmark from the AURELIA trial (ORR 27%) with bevacizumab plus chemotherapy[258]. In addition, studies are exploring the direct design of ADCs targeting the VEGF pathway. An innovative approach involved conjugating bevacizumab and MMAE *via* a protease-cleavable linker (bevacizumab vedotin), which can target the VEGF/VEGFR pathway and rapidly release MMAE in the presence of exogenous protease B[259]. Bevacizumab vedotin exhibited good anti-proliferative, pro-apoptotic, and cell cycle arrest effects

on glioblastoma, hepatocellular carcinoma, and breast cancer *in vitro*[259]. Fundamental research has shown that HER2 is an inducer of VEGF expression and overexpression of the HER2 signaling in breast cancer models led to increased VEGF mRNA expression[260,261]. *In vitro* studies have also confirmed that dual-targeting therapy using a VEGF inhibitor combined with trastuzumab was more effective in suppressing the growth of HER2-overexpressing breast cancer than either agent alone, suggesting potential synergistic effects between T-DXd and anti-angiogenic agents[262].

Combination with immunotherapy

Increasing evidence suggests that ADCs may be able to enhance the efficacy of immune-oncology (IO) therapies[263]. The proposed mechanisms include inducing immunogenic cell death, dendritic cell maturation, increased T-cell infiltration, and enhanced immune memory, as well as the expression of immune regulatory proteins (e.g., PD-L1 and MHC)[264]. Using low-dose ADCs as immune stimulants may help potentiate the activity of IO drugs while minimizing the risk of toxicities[264]. Immunotherapy and ADC co-treatment strategies have progressed to clinical evaluation. Although preclinical studies and initial clinical findings indicate an enhancement in anti-tumor effects, randomized trials are still needed to demonstrate superior benefits over standard-of-care.

Several HER2-targeted ADCs, including T-DM1, T-DXd, and RC48, have undergone evaluation in combination with immune checkpoint inhibitors (ICIs) *in vitro* and *in vivo*. These combinations have demonstrated synergistic benefits, notably in promoting the movement and activation of immune cells[265-267]. The KATE2 trial (NCT02924883), which is a randomized controlled study, assessed the efficacy of combining T-DM1 with atezolizumab *versus* T-DM1 with a placebo in patients with previously treated HER2-positive breast cancer. Although this combination did not significantly enhance the median PFS (8.2 *vs.* 6.8 months; $P = 0.33$)[268], there was an observable trend towards improved PFS in patients whose tumors were PD-L1 positive (8.5 *vs.* 4.1 months; $P = 0.099$), suggesting that HER2-targeted ADC plus ICI may only benefit the PD-L1-positive population. Despite this disappointing result, preliminary studies suggested that a combination of ICIs with ADCs that target antigens other than HER2 might be effective in a range of cancers, such as urothelial, small cell lung, ovarian, cervical cancer, TNBC, and HL. Combination therapy has the potential to yield higher response rates compared to historical data on ICI monotherapy[269-271].

The KATE2 study showed a trend towards improved survival in the PD-L1-positive subgroup of breast cancer patients who progressed on trastuzumab plus taxane, with 1-year OS rates of 94.3% for T-DM1 plus atezolizumab *versus* 87.9% for T-DM1 alone. Building on this finding, the KATE3 study (NCT04740918) is further exploring the efficacy of T-DM1 plus atezolizumab in HER2-positive, PD-L1-positive advanced breast cancer[272]. In both HER2-positive and -low breast cancer populations, dose-escalation and expansion studies of T-DXd in combination with nivolumab are ongoing, showing ORRs of 65.5% in HER2-positive and 50% in HER2-low patients. The anticipated analyses in the PD-L1-positive subgroups may yield more promising results.

EV-302 was a randomized, controlled, phase III clinical trial designed to evaluate the efficacy of enfortumab vedotin in combination with pembrolizumab *versus* platinum-based chemotherapy in patients with previously untreated locally advanced or metastatic urothelial carcinoma. The EV plus pembrolizumab combination regimen nearly doubled the PFS and OS outcomes compared to platinum-based chemotherapy (PFS: 12.5 months *vs.* 6.3 months, $P < 0.001$; OS: 31.5 months *vs.* 16.1 months; $P < 0.001$). The incidence of treatment-related adverse events ≥ grade 3 was reduced in the combination group (55.9% *vs.* 69.5%)[273]. At present, the EV plus pembrolizumab combination has been established as the preferred first-line treatment option for patients with locally advanced or metastatic urothelial carcinoma (la/mUC) who are cisplatin-eligible or -ineligible. This regimen has also received regulatory approval in multiple countries and regions, including the United States, Canada, the European Union, Brazil, and Japan. In addition to efficacy improvement, ADC-induced immunogenic cell death and myeloid cell reprogramming were highlighted as key mechanisms by which immunologically "cold" tumors may be sensitized to PD-1/PD-L1 inhibition, thereby expanding the therapeutic relevance of ADC-ICI combinations beyond traditionally responsive tumor subsets[274].

Limitations and challenges

While the innovations in molecular design and combination therapies have shown promise in preclinical and early clinical settings, the translation to broad, durable clinical benefit is hindered by underappreciated limitations that are rooted in biological complexity, therapeutic trade-offs, and clinical implementation gaps. A critical reassessment of these strategies is imperative to avoid overoptimism and guide rational development of next-generation approaches.

BsADCs are designed to address antigen heterogeneity and escape but the efficacy is constrained by strict biological prerequisites and practical trade-offs. BsADCs require simultaneous expression of two targets on tumor cells, which is often heterogeneous across patients and even within tumors. For example, HER2 × CD63 BsADC shows suboptimal efficacy in HER2-low tumors because the low-affinity CD63 arm cannot compensate for insufficient HER2 binding[275]. Moreover, off-target toxicity arises from cross-reactivity with normal tissues expressing either target at low levels. ZW49 (an HER2 bispecific ADC) exhibited a 42% incidence of keratitis that was likely due to low-level HER2 expression in corneal epithelial cells, which is an issue exacerbated by the high affinity of biparatopic antibody'[276]. In addition, bispecific antibody

scaffolds often have a reduced serum half-life and stability compared to conventional IgG1, necessitating more frequent dosing or higher concentrations, which in turn increase the risk of toxicity. MEDI4276, a tetravalent HER2 BsADC, failed to meet efficacy expectations in clinical trials due to poor tolerability and unfavorable PK, which was attributed to payload toxicity and scaffold instability[190].

Similarly, site-specific conjugation and advanced linkers improve DAR uniformity and stability but introduce new challenges. Site-specific conjugation requires specialized protein engineering and purification, increasing production costs 2- to 3- fold compared to traditional lysine conjugation[277]. Increased production costs translate to higher patient burden. For example, site-specific ADCs, like loncastuximab tesirine, cost approximately 20,000 USD per cycle, which restricts access in low-resource settings. In addition, linker stability-to-release trade-offs remain unresolved. The cleavable linkers enable bystander effects but carry the risk of premature cleavage in the circulation, as occurs with T-DXd interstitial lung disease toxicity, which is linked to off-target payload release in lung tissue[278]. Non-cleavable linkers avoid this but rely on efficient lysosomal degradation, which is impaired in ~30% of resistant tumors with lysosomal dysfunction[279].

Novel payloads aim to bypass traditional resistance but face intrinsic limitations. The balance between synergy and toxicity is difficult to achieve for dual-payload ADCs. Combining two potent cytotoxins (e.g., MMAE + PBD) increases the risk of off-target toxicity, while combining complementary mechanisms (e.g., microtubule inhibitor + STAT3 inhibitor) may require precise DARs that are challenging to manufacture uniformly[200]. Preclinical studies have shown that dual-payload ADCs often exhibit narrow therapeutic windows with efficacy gains offset by increased myelosuppression[280]. Second, ISACs suffer from TME-mediated immune suppression. TLR7/8 agonists conjugated to antibodies can activate dendritic cells but this is abrogated in tumors with high myeloid-derived suppressor cells (MDSCs) or TAMs[281]. Moreover, systemic leakage of immunostimulatory payloads induces a cytokine storm and limits dose escalation, which were observed in early-phase ISAC trials[282].

Combining ADCs with chemotherapy leverages synergistic cell-cycle effects but is undermined by toxicity overlap and compromised ADC efficacy. For example, myelosuppression and mucosal toxicity are exacerbated when the MMAE-based ADCs (e.g., brentuximab vedotin) and taxanes combined, leading to severe neutropenia (grade 3/4 incidence > 50%) in combination trials[283]. Similarly, anti-angiogenic agents (e.g., bevacizumab) improve ADC penetration by normalizing tumor vasculature but this strategy faces narrow therapeutic windows and hypoxia-induced resistance. The window during which blood vessels are normalized (reducing hypoxia without inducing ischemia) is narrow and patient-specific, requiring precise dosing and timing that are difficult to standardize clinically. Only 39% of patients achieved objective responses in a phase Ib trial of mirvetuximab soravtansine + bevacizumab with 27% experiencing grade 3/4 hypertension attributed to off-target vascular disruption[284].

Conclusions

Current strategies to overcome ADC resistance are hampered by inherent biological trade-offs, translational gaps, and failure to account for dynamic resistance evolution. While molecular design innovations address specific resistance mechanisms, molecular design innovations are constrained by target dependency, toxicity, and manufacturing complexity. Combination therapies suffer from toxicity overlap, compensatory signaling, and poor patient selection. To move forward, the following must be prioritized in future efforts: (1) developing biomarkers to stratify patients based on resistance profiles and TME features; (2) designing "multi-mechanism" ADCs (e.g., dual payloads + immunostimulatory moieties) that target cancer cells and the TME; (3) optimizing combination dosing and sequencing to maximize synergy while minimizing toxicity; and (4) advancing preclinical models that recapitulate human tumor heterogeneity and dynamic resistance. Only through a critical, evidence-based reassessment of current strategies can we avoid translational failures and unlock the full potential of ADCs for patients with refractory cancer.

Grant support

This work was supported by the Joint Innovation Funding of the Natural Science Foundation of Hubei Province-Jiangsu-Hengrui Pharmaceuticals Company Ltd. (Grant No. 2024AFD450) and the National Natural Science Foundation of China (Grant Nos. 82073402 and 82204758).

Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

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Data availability statement

The data generated in this study are available upon request from the corresponding author.

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Cite this article as: Liu Y, Liu Y, Wu T, Bai X, Wang S, Zhang L, et al. Drug resistance to antibody-drug conjugates: mechanisms, challenges, and perspectives. Cancer Biol Med. 2026; 23: 833-864. doi: 10.20892/j.issn.2095-3941.2025.0707