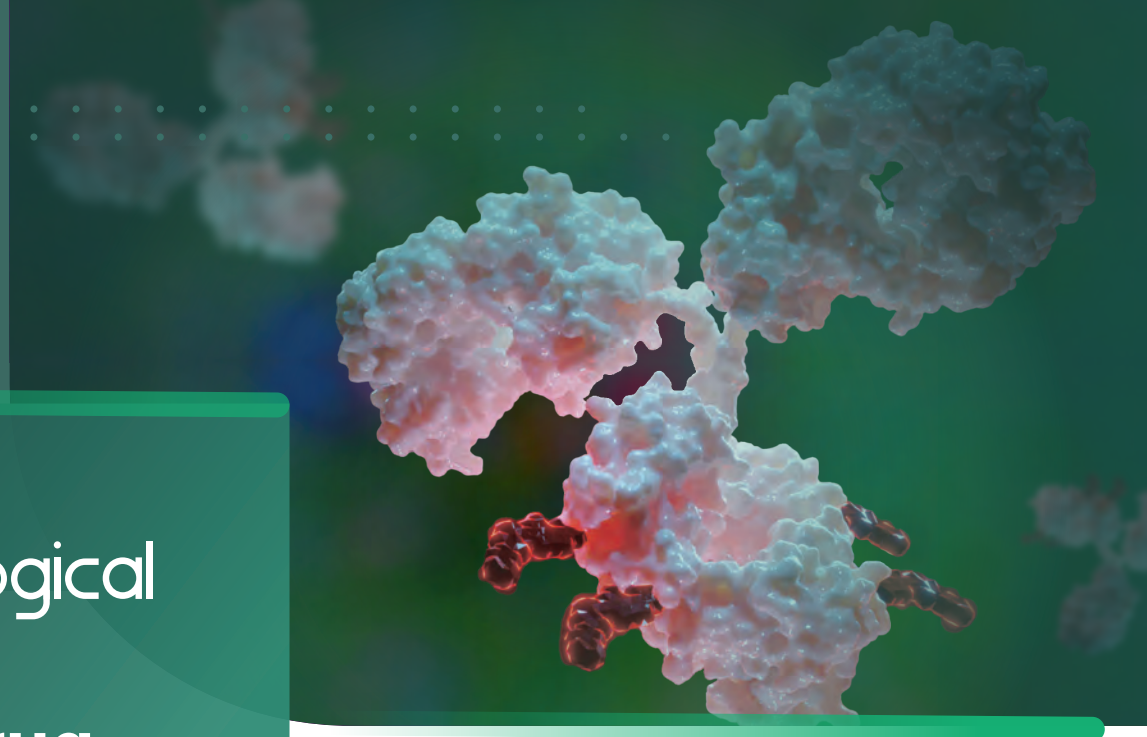


Antibody-drug Conjugates: Smart Chemotherapeutics

Antibody-drug conjugates (ADCs) have emerged as a promising class of anticancer therapeutics, demonstrating improved precision and reduced off-target toxicity in targeting cancer cells.[1] The fundamental mechanism involves monoclonal antibodies binding to overexpressed target antigens on cancer cells, leading to internalization, lysosomal fusion, and subsequent release of cytotoxic payloads, inducing tumor cell death.[2] Additional mechanisms like antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis (ADCP) also contribute to the overall anticancer effect.[3] The development of ADCs involves careful consideration of various components, including target-specific antibodies, cytotoxic payloads, and linkers.[4] This article explores the structure, mechanism of action, evolution of three generations, clinical success stories, challenges faced, and future directions of ADCs.

Mechanism of Action

ADCs target cancer cells with improved precision and reduced off-target toxicity compared to conventional chemotherapy agents. The mechanism involves the monoclonal antibody binding to an overexpressed target antigen on cancer cells, leading to internalization and formation of endosomes. Lysosomal fusion triggers detachment of cytotoxic payloads which specifically target subcellular structures such as DNA or microtubules leading to cell death. Cytotoxic payloads can also be released at the cell membrane and induce a bystander effect, targeting neighboring cells and contributing to overall tumor cell destruction.[4] Additionally, mechanisms like ADCC, CDC, and ADCP may enhance the ADCs anticancer effect. The Fab segment binds to the antigen, while the Fc segment engages immune cells and components of complement system for direct cell death.[5] Moreover, monoclonal antibodies can directly inhibit downstream signal transduction, exemplified by trastuzumab blocking HER2-related pathways for apoptosis.[4,5]

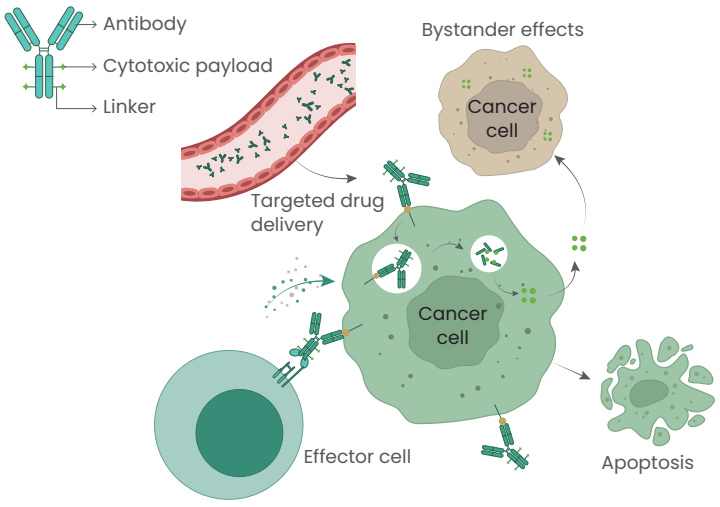


Figure 1. Mechanisms of Antibody-Drug Conjugates (ADCs) to selectively kill cancer cells. DOI: 10.1038/s41392-022-00947-7

ADC Building Blocks

An ADC consists of a target-specific mAb, a cytotoxic payload, and a chemically synthesized linker that covalently links the toxin and the antibody.[4,6,7] The mAb binds to specific antigens on the surface of tumor cells, and ADCs are internalized into tumor cells during the formation of antibody-antigen complexes.[1,3] ADCs are typically transported from the endosome to the lysosome, where the linker is cleaved and the small molecule cytotoxins are released, leading to tumor cell death.[1,4,7] Overall, ADC development requires consideration of the target antigens, antibodies, cytotoxic payload, and linker (Figure 2).

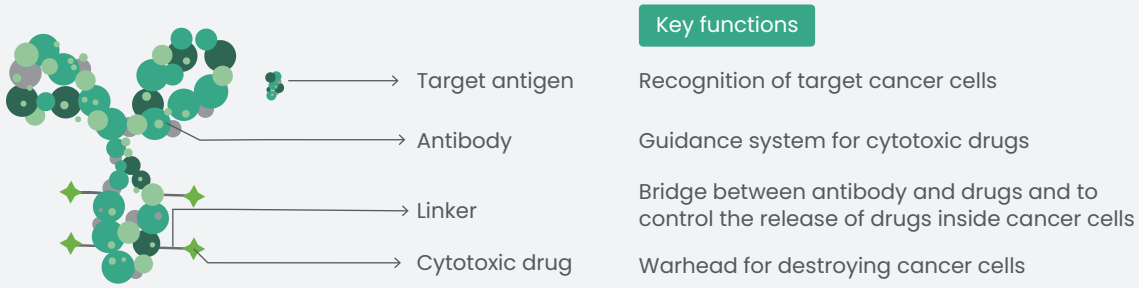


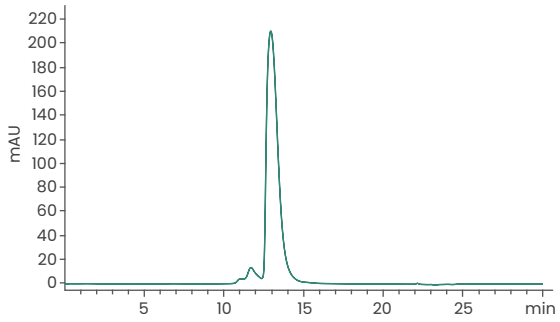
Figure 2. Basic components of The ADC include antibody, linker, and cytotoxic drug. Antibody targets binding, payload destroys cancer cells, and linker controls drug release.
DOI:10.1016/j.biopha.2023.114408

Antibodies

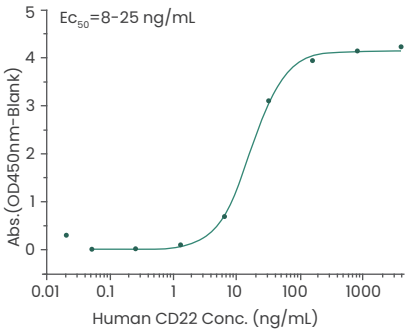
Target antigens for ADCs should be non-secretory, mainly on tumor cell surfaces, and internalize post-binding for effective cytotoxic payload release into tumor cells.[8] Popular ADC targets include HER2, Trop2, Nectin4, EGFR, CD19, CD22, CD33, CD30, BCMA, and CD79b.[1,3,4,7,9] The evolving landscape includes emerging targets like EpCAM, CD70, CD25, CD166, showing promising results.[10] Immunoglobulins (IgGs), particularly IgG1, are commonly employed in clinical studies for ADCs.[1] IgG1, abundant in serum, induces strong effector functions, including ADCC, ADCP, and CDC, due to high Fc receptor binding affinity.[1,3] Ideal antibodies for ADCs require high affinity, efficient internalization, low immunogenicity, and a long plasma half-life.[3,7] Current ADCs predominantly use fully humanized antibodies, minimizing immunogenicity compared to earlier mouse antibodies.[1,3,9]

Featured Hematological Malignancy Target Protein From Sino Biological

Human CD22/Siglec-2 Protein | Cat#: 11958-H08H HPLC-verified



≥ 90 % as determined by SEC-HPLC.



Binding assay: Immobilized anti-CD22 antibody can bind human Siglec-2/CD22 protein.

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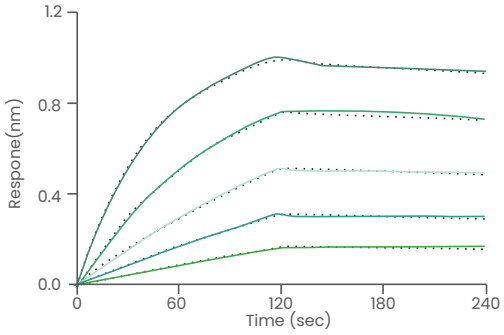
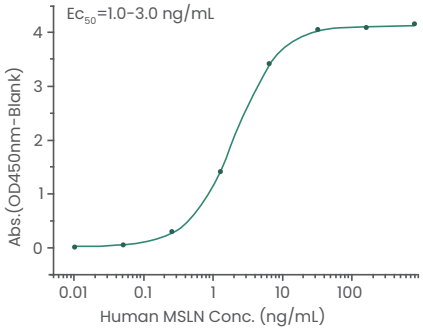
Featured Solid Tumor Target Protein From Sino Biological

Human Mesothelin/MSLN Protein | Cat#: 13128-H08H1 HPLC-verified



BLI assay: Loaded anti-MSLN antibody on ProA Biosensor can bind human Mesothelin protein.



Binding assay: Immobilized anti-MSLN antibody can bind human MSLN/Mesothelin protein.

Cytotoxic Payload

Less than 2% of administered ADC reaches targeted tumors.[5] Therefore, high potency (nano and picomolar IC50) is essential for ADC payload compounds.[5] Current cytotoxic payloads include tubulin inhibitors and DNA damaging agents.[1,7,11] Ideal cytotoxins need high toxicity, low immunogenicity, and stability, with modifiable functional groups for mAb linkage.[1] The drug-antibody ratio (DAR) is crucial. Low DAR may reduce efficacy, while high DAR may cause instability and off-target toxicity in ADC development.[1,4]

Novel approaches in payload design for ADCs include the exploration of non-toxic payloads like RNA inhibitors, immuno-agonists (such as Toll-like receptors and STING agonists)[1,4], and apoptosis-promoting Bcl-xL inhibitors,[1] aiming to enhance anti-tumor immune responses, broaden treatment windows, and improve safety and efficacy. Recent developments include PROTAC (Proteolysis-targeting chimeras) molecules that aims to combine catalytic properties to target undruggable proteins,[5] and the development of hybrid payloads that combine different mechanisms to achieve synergistic effects, targeting multiple refractory cancers with heterogeneity and drug resistance.[5]

Linker

Cleavable and non-cleavable linkers are used to control cytotoxic payload release.[3,4] Cleavable linkers leverage environmental differences between circulation and tumor cells, utilizing chemical cleavage (hydrazone and disulfide bonds)[4,9] or enzyme cleavage (glucuronide and peptide bonds).[1,9] Hydrazone-linked ADCs remain stable in circulation and release cytotoxic payloads in lysosomes (pH 4.8) and endosomes (pH 5.5–6.2) upon internalization into cancer cells.[1] Non-cleavable linkers depend on lysosomal degradation of the entire structure, retaining charged amino acids in the payload.[3,4,9] The linker's role is critical in ensuring effective release within target tumor cells, preventing premature release in the plasma that could harm normal tissues.

Conjugation Methods

Two types of conjugation techniques are commonly used; stochastic and site-specific.[1] Stochastic conjugations by amide coupling in ADCs like gemtuzumab ozogamicin, T-DM1, and inotuzumab ozogamicin, leads to variable drug-antibody ratios (DAR 0–8) due to randomness among numerous lysine residues.[1] This may cause instability, premature payload release, off-target toxicity, and challenges in achieving consistency.[3,7] In contrast, site-specific conjugation relies on cysteine-based coupling, exposing disulfide bonds for a controlled approach, reducing heterogeneity.[1,5] However, it may compromise antibody integrity.[1]

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Evolution of ADCs

ADC drug development has evolved through three generations. The initial generation, exemplified by BR96-doxorubicin, utilized non-cleavable linkers with mouse-derived antibodies, facing potency and immunogenicity concerns. Further improvements, seen in gemtuzumab ozogamicin, incorporated humanized antibodies and potent cytotoxic agents but retained challenges like uncontrollable payload release.[1,3,5] The second generation, featuring brentuximab vedotin and ado-trastuzumab emtansine, optimized mAb isotypes, cytotoxic payloads, and linkers, enhancing clinical efficacy and safety.[1] Despite progress, issues like off-target toxicity and high drug-antibody ratios persisted.[1,7] The third generation, represented by polatuzumab vedotin, enfortumab vedotin, and fam-trastuzumab deruxtecan, focuses on overcoming limitations, employing specific conjugation technologies for homogenous ADCs with consistent drug-antibody ratios, resulting in improved anticancer efficacy, lower toxicity, and increased stability.[1]

Clinical Application of ADCs

As of February 2024, the FDA has approved 12 ADCs for various cancers, encompassing lymphomas, leukemia, myeloma, and solid tumors like HER2+ breast cancer, urothelial cancer, metastatic triple-negative breast cancer, and gastric cancer. Examples include trastuzumab emtansine (T-DM1) for HER2-positive breast cancer and inotuzumab ozogamicin for acute lymphoblastic leukemia. Additionally, there are over hundred ADC candidates in clinical trials, with many of them already demonstrating remarkable success in advanced phases.[12] These success stories underscore the potential of ADCs to transform the landscape of cancer treatment.[12]

Table 1. FDA Approved ADCs[1,9]

Approved ADC	Target	Indication	Approved Year/ Company	Payload	Linker
Gemtuzumab Ozogamicin (Mylotarg®)	CD33	Acute myeloid leukemia	2000, 2017; Pfizer	N-acetyl-γ-calicheamicin	Hydrazone
Brentuximab Vedotin (Adcetris®)	CD30	Hodgkin lymphoma, systemic anaplastic large-cell lymphoma-cell lymphoma	2011; Seagen	MMAE	mc-VC-PABC
Trastuzumab Emtansine (Kadcyla®)	HER-2	Breast cancer	2013; Roche	DM1	SMCC
Inotuzumab Ozogamicin (Besponsa®)	CD22	B-cell precursor acute lymphoblastic leukemia	2017; Pfizer	N-acetyl-γ-calicheamicin	Hydrazone
Moxetumomab pasudotox (Lumoxiti®)	CD22	Hairy cell leukemia	2018; AstraZeneca	mc-VC-PABC	PE38
Polatuzumab Vedotin (Polivy®)	CD79b	Diffuse large B-cell lymphoma	2019; Roche	MMAE	mc-VC-PABC
Enfortumab Vedotin (Padcev®)	Nectin-4	Urothelial cancer	2019; Seagen	MMAE	mc-VC-PABC
Trastuzumab Deruxtecan (Enhertu®)	HER-2	Breast cancer	2019; Daiichi Sankyo	DXd	tetrapeptide
Sacituzumab Govitecan (Trodelvy®)	Trop-2	Breast cancer, urothelial cancer	2020; Immunomedics	SN38	CL2A
Belantamab Mafodotin (Blenrep®)	BCMA	Multiple myeloma	2020, Withdrawal in 2022; GSK	MMAF	mc
Loncastuximab Tesirine (Zynlonta®)	CD-19	Large B-cell lymphoma	2021; ADC Therapeutics	PBD dimer	dipeptide
Tisotumab Vedotin (Tivdak®)	Tissue factor	Cervical Cancer	2021; Genmab/ Seagen	MMAE	mc-VC-PABC
Mirvetuximab Soravtansine (Elahere®)	FRα	Ovarian cancer	2022; ImmunoGen	DM4	sulfo-SPDB

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Challenges and Future Directions

ADCs encounter several challenges like complex pharmacokinetics, side effects, limited tumor targeting, and drug resistance.[4,7,9] In addition, tumor penetration is hindered by ADCs' large molecular weight.[1,4,5] To overcome these challenges, next-generation ADCs focus on optimizing monoclonal antibodies, linkers, and payloads, utilizing bispecific antibody technology, and employing dual-payload ADCs.[1,4,5] Smaller molecular weight ADCs, like PEN-221, aim to enhance penetration efficiency, particularly in challenging tumors.[1] Finally, the use of two cytotoxic agents as payloads to reduce ADC resistance is also promising.[8]

Sino Biological are at the forefront of advancing ADC research with a comprehensive suite of products and services. Our portfolio boasts a diverse range of ADC target proteins for precise cancer cell drug delivery, complemented by MMPs, Cathepsins, and uPA enzymes for optimal linker cleavage. High-quality FACS antibodies and reliable Fc receptor proteins aid immune cell analysis and therapeutic development. Specialized anti-payload antibodies assess efficacy. Beyond products, our services encompass rigorous in vitro bioactivity validation, internalization assay studies, binding activity assays, neutralizing/blocking assays, anti-idiotypic antibodies development for PK/ADA assays, and control antibody production, ensuring a holistic approach to ADC research.

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