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Antibody-Drug Conjugates: Understanding Associated Drug Design and Pharmacology

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ABSTRACT

Antibody-drug conjugates (ADCs) are currently among the fastest growing drug classes in oncology, combining the specificity and targeting capabilities of monoclonal antibodies (mAbs) with the potent cytotoxicity of small molecule drugs. Considered the "biological missiles" of cancer therapy, ADCs are composed of 3 key elements: (1) a mAb framework that selectively binds to an antigen on the tumor cell surface, (2) a cytotoxic drug payload, and (3) a chemical linker attaching the 2 entities. Because each of these components can vary widely among ADCs, the associated drug design is relatively complex, with subtle differences leading to immense diversity in the overall drug structure and associated pharmacological and clinical properties. As medical communication experts, it is essential to have a basic understanding of the various components of ADC design and their potential impact on drug efficacy, safety, and capability in targeting certain degrees of antigen expression and tumor types. This review aims to provide a basic understanding of each component related to ADC design and the role they play in defining the pharmacological properties of a particular ADC.

BACKGROUND

First proposed by Paul Ehrlich in the early 1900s, the foundational concept of a "magic bullet" as a way to selectively transport cytotoxic drugs to a specific target tissue has become an ever-closer reality, passing through key milestones over the last century.[1,2] The development of chemotherapy in the 1940s was a first major step in the transition from concept to reality.[3] However, the lack of high-level specificity and targeting capabilities with cytotoxic agents led to a high degree of systemic toxicities and has remained an ongoing challenge. The advent of hybridoma technology and the development of monoclonal antibodies (mAbs) in the 1970s established a highly effective method for targeting specific antigens expressed on the tumor cell surface.[1,4,5] This led to the development of targeted therapeutics that have become an attractive method for improving tumor selectivity and reducing the systemic toxicity associated with traditional chemotherapy.[6] Combining these 2 technologies enabled the development of the first antibody-drug conjugate (ADC). In recent decades, ADCs have become a rapidly expanding therapeutic drug class specifically designed to overcome the shortfalls associated with chemotherapeutic agents.[6,7] Currently, there are 13 ADCs that have received US Food and Drug Administration approval for various hematological and solid tumor cancers.[1,8-20]

ADC MECHANISM OF ACTION

ADCs are a group of tripartite drugs made up of a tumor-specific mAb conjugated via a stable linker to a potent cytotoxic payload.[21,22] The core concept of an ADC is to use the specific recognition between an antibody and antigen to selectively deliver cytotoxic drugs to the tumor site, after which the payload is released in the tumor via a specific release mechanism.[23] The general mechanism of action for an ADC can vary depending on the inherent design (Figure 1, next page).

ADC DESIGN

The clinical success achieved with a particular ADC is contingent upon several key factors: (1) target antigen, (2) antibody framework, (3) method of conjugation, (4) chemical linker, and (5) cytotoxic payload (Table 1, next page).

Target Antigen

To achieve a favorable therapeutic index and reduce the potential for off-target toxicity, the selected target antigen should be tumor-specific or tumor-associated with a high level of expression in tumor cells and minimal to no expression in healthy tissues.[24,28] Following ADC binding, target antigens should internalize efficiently via endocytosis to enable ADC entry into the cell and subsequent cellular transport and payload release.[1,21,29] The target antigen should also undergo efficient recycling or replenishment on the cell surface with no associated antigen shedding into

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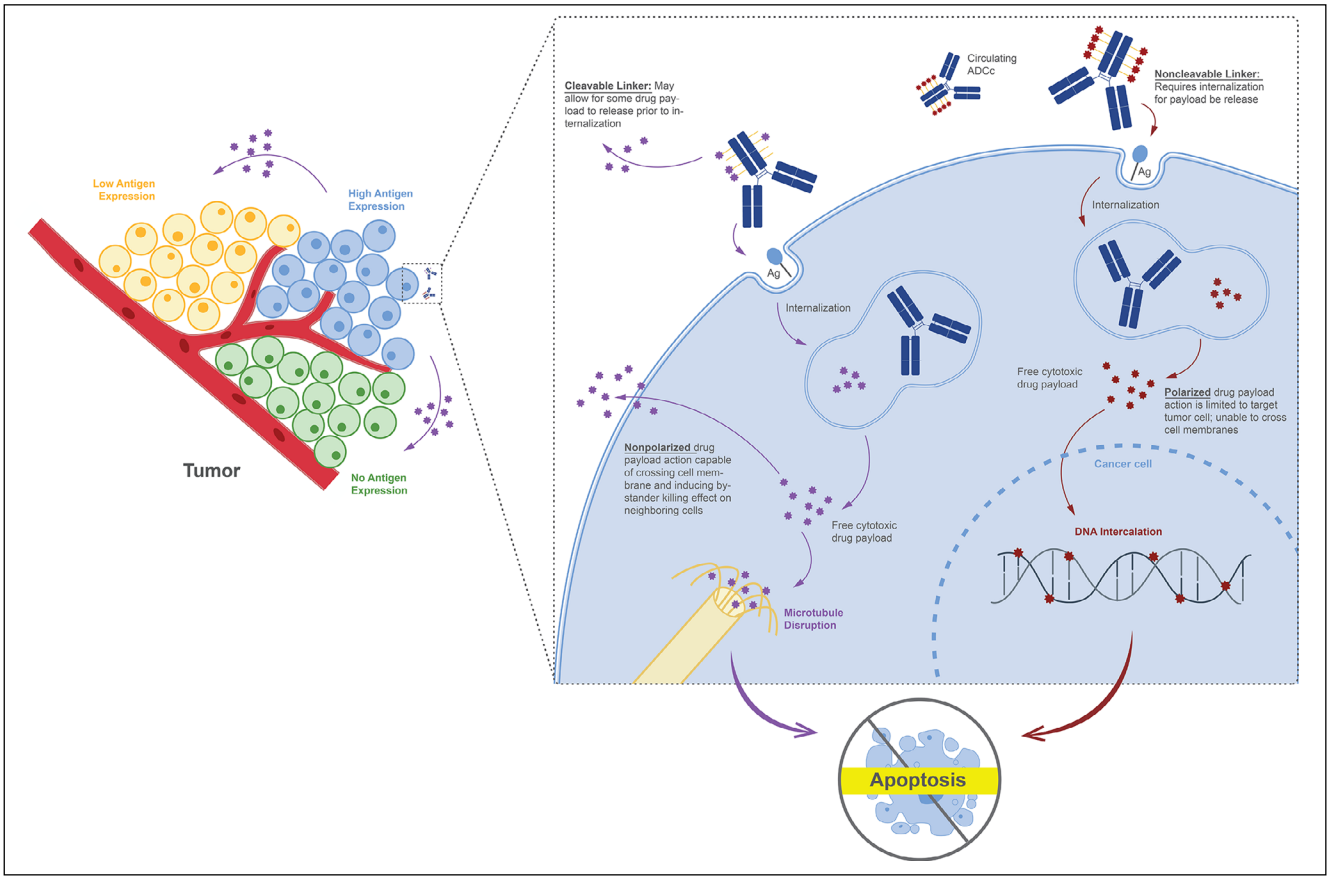
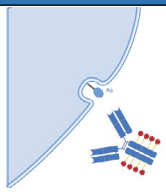
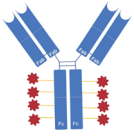
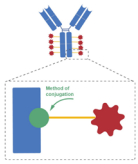
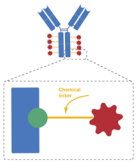
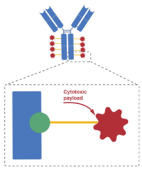


Figure 1. General mechanism of action of ADCs with and without bystander killing effect.[5,21-23] Following introduction of the antibody-drug conjugate (ADC) into the plasma circulation by intravenous injection, the target antigen is recognized on the tumor cell surface, leading to subsequent binding and formation of an antigen–ADC complex (ADCc). The complex is then internalized via receptor-mediated endocytosis. For ADCs with cleavable linker design, some initial release of drug payload may occur prior to internalization. Once inside the cytosol, endosome, or lysosome, the chemical characteristics of these environments (eg, low pH, high glutathione/thiol levels, proteolytic enzymes) allow for further payload release. In the case of ADCs with noncleavable linker design, compete degradation of the ADC is typically required for payload release. The resulting free cytotoxic drug payload then exerts its cellular destruction via a pathway-specific mechanism (eg, microtubule disruption, DNA intercalation). Hydrophobic or nonpolar payloads are capable of crossing cell membranes, thereby exerting a so-called bystander effect. The bystander killing effect involves the diffusion of free drug across cell membranes from the target tumor cell and into neighboring tumor cells, thereby expanding antitumor activity to tumor cells with low or no target antigen expression, or those that are less accessible directly from the circulatory system, eg, solid tumor cells. Ag, antigen.

Table 1. Components of ADC Drug Design[1,7,25,26]

Target antigen	Antibody framework	Method of conjugation	Chemical linker	Cytotoxic payload
				
Ideal characteristics • Highly expressed in tumor cells • Homogeneous expression in tumor cells • Minimal presence in circulation and healthy cells	**Ideal Characteristics** • Minimal cross reactivity with healthy tissues • Low immunogenicity • Strong binding affinity for the target antigen • Long PK half-life	**Conventional/stochastic** • Lysine sites • Reduced cysteine sites **Site-specific** • Engineered reactive cysteine residues • Disulfide re-bridging • Unnatural amino acids • Enzyme assisted ligation • Glycan remodeling and glycoconjugation • pClick technology	**Cleavable linkers** *Chemically cleavable* • Acid sensitive • Glutathione sensitive *Enzymatically cleavable* • Peptide based (protease sensitive) • B-glucuronide based • Phosphate based **Noncleavable linkers** • Thioether (SMCC) • Maleimido propionyl • Maleimido caproyl	**Tubulin Inhibitors** • Auristatins (eg, MMAE, MMAF) • Maytansinoids (eg, DM1, DM4) • Tubulysins (eg, tubulysin A) **DNA damaging agents** • Calicheamicins (eg, ozogamicin) • Duocarmycins (eg, duocarmazine) • Pyrrolbenzodiazepines • Camptothecin analogues (eg, govitecan, DXd) **Immunomodulators** • TLR agonists • STING agonists

Abbreviations: Ag, antigen; DM1, mertansine; DM4, ravtansine; DXd, deruxtecan; Fab, fragment antigen binding; Fc, fragment crystallized; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin F; PK, pharmacokinetic; SMCC, sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carbocylate; STING, stimulator of interferon genes; TLR, toll-like receptor.

circulation to improve tumor cell targeting and reduce the risk of toxicity.[30,31] Solid tumors present a challenge in terms of both the drug accessibility and intratumoral heterogeneity of the target antigen. Therefore, certain characteristics of ADC design, such as cleavable linkers or nonpolarized payloads, which permit the release or distribution of drug to neighboring tumor cells with low or no antigen expression (the so-called bystander effect), may be advantageous in ADCs targeting these tumor types.[31]

Antibody Framework

The antibody framework used in the ADC structure is essential for facilitating selective binding of target antigens and can significantly influence the overall efficacy, therapeutic index, and pharmacokinetic and pharmacodynamic characteristics of the end drug product.[1,21] Ideally, the selected mAb should possess a strong binding affinity with high target specificity and minimal cross-reactivity with healthy tissues.[24] The selected mAb should also facilitate efficient internalization, demonstrate low immunogenicity, and have a long half-life. Among the 5 major subtypes of human antibodies (eg, immunoglobulin [Ig]A, IgD, IgE, IgG, and IgM), IgG antibodies are most often used for ADC development because of their plasma stability and strong binding affinity for the fragment crystallized (Fc) receptor.[1,21,32] Moreover, because of their decreased potential for immunogenicity, humanized or fully human mAbs are typically favored in ADC design as opposed to murine or chimeric mAbs.

Method of Conjugation: Conventional

The method of conjugation in ADC design refers to the approach used to connect the linker and payload to the mAb framework.[1] Historically, conventional conjugation methods have been the most widely used in ADC design. Conventional conjugation entails either the alkylation or acylation of lysine side chains or the reduction of disulfide bonds to liberate cysteine residues for conjugation sites.[21,33] Notably, mAbs such as IgG contain a natural abundance of lysine (80-100) and cysteine (32-40) residues, which offer ideal sites for linker attachment via conventional conjugation involving coupling reactions.[1,27,33-36]

Conventional conjugation using lysine and cysteine residues is stochastic. This can lead to heterogeneous mixtures of ADC species with varying sites of conjugation and drug-to-antibody ratios (DARs), defined as the number of drug payloads conjugated to each mAb.[37] DARs associated with ADCs produced by conventional conjugation methods often vary, ranging from 0 to 8 or more.[38] DAR-associated variability can potentially impact certain ADC characteristics, such as hydrophobicity, charge, polarity, pharmacokinetics, and thermostability of mAbs.[38-40] In some cases this may result in insufficient stability, causing premature payload release and increased potential for off-target toxicities.[1] Elevated DARs can also lead to aggregation, increased metabolism, or disruptive coupling within the antigen binding region of the mAb.[21,41,42] Because lysine residues are distributed throughout both heavy and light chain regions of the antibody, coupling reactions resulting in payload conjugation near antibody–antigen recognition sites could interfere with ADC target binding.[1,39] Moreover, ADCs with cytotoxic payloads conjugated to heavy chain regions of the mAb have lower in vivo efficacy compared with light chain conjugates.[24,44] Consequently, novel site-specific conjugation strategies have been developed that produce more homogeneous ADC products with favorable pharmacokinetic and antigen binding properties.[1,37,41]

Method of Conjugation: Site-Specific

Site-specific conjugation strategies are categorized according to their associated methodology and generally include the following: (1) engineered cysteine residues, (2) disulfide rebridging, (3) engineered unnatural amino acids, (4) enzymatic assisted ligation (5) glycan remodeling/glycoconjugation, and (6) proximity-induced antibody conjugation method (pClick) technology.[1,21,45,46] Site-specific cysteine residues are commonly engineered using THIOMAB technology, which allows specific positioning of conjugation sites within both heavy chain and light chain regions of the antibody.[47] Drug conjugates created using THIOMAB technology are typically referred to as THIOMAB-drug conjugates and are shown to have improved safety and therapeutic indexes.[46]

Disulfide rebridging is a process in which 4 interchain disulfide bonds in an IgG antibody are reduced and subsequently treated with a cysteine-selective cross-linking reagent.[48] This rebridging process enables simultaneous reattachment of polypeptide chain and installation of drug molecules or function groups that may be further modified. Genetically encoded unnatural amino acids, such as *p*-acetylphenylalanine and *p*-azidophenylalanine, are other site-specific conjugation methods that have demonstrated specific advantages in terms of optimizing physical properties of the ADC, and improved associated efficacy, pharmacokinetic, and safety profiles.[41,49] Compared with conventional cysteine conjugated ADCs, those using unnatural amino acids for site-specific conjugation demonstrate superior *in vitro* selectivity and efficacy, particularly in low antigen expressing tumor cells.[50] Enzyme-assisted ligation techniques use enzymes such as transglutaminase to conjugate specific amino acid sequences or tags that are genetically engineered and artificially induced to express in the antibody.[1,21,51] Glycan remodeling or glycoconjuga-

tion methods exploit naturally-occurring glycosylation sites at the N297 residue in the CH2 domain of IgG antibodies.[52] However, relative to other site-specific conjugation approaches, glycan remodeling has limited control over site placement. A site-selective conjugation method using pClick has more recently emerged and uses a proximity activated crosslinker to covalently attach to a specific antibody site, which eliminates the need for additional antibody engineering or post-synthesis treatments.[53]

Chemical Linker

The primary function of chemical linkers in ADC design is to effectively bridge the mAb to the cytotoxic drug payload.[1] ADC specificity, potency, safety, and overall activity are greatly influenced by the associated linker chemistry.[24] Linkers are generally designed to remain stable in circulation and release the drug payload once the ADC has reached the target tumor site.[2,24] Chemical linkers commonly employed in ADC design are classified into 2 main categories: cleavable and noncleavable linkers.[1,24]

Cleavable Linkers

Cleavable linkers are designed to exploit cancer-specific cellular conditions and are generally divided into 3 primary categories: acid or pH sensitive, glutathione or redox sensitive, and enzymatically cleavable (eg, protease sensitive).[21,24,28] Acid-sensitive linkers, such as hydrazone, are designed to release the drug payload within acidic environments, such as lysosomal or endosomal cellular compartments, while remaining stable within the blood's neutral pH environment.[1,31] Glutathione-sensitive or disulfide linkers exploit the high levels of glutathione and other thiols present in the cytosol of cancer cells, thereby enabling selective cleavage of the cytotoxic payload at the tumor site.[24,28,54] Protease sensitive or peptide-based linkers most often consist of dipeptide linkers (eg, valine-citrulline), which require enzymatic cleavage of peptide bonds for payload release.[28,31,55] Compared with chemically liable linkers (eg, hydrazone and disulfide), peptide-based linker technologies allow for greater control of drug delivery, with improved systemic stability and rapid enzyme-mediated release of the drug payload within the target cell.[31]

Noncleavable Linkers

In contrast with cleavable linkers that rely on tumor-specific cellular conditions for payload release, noncleavable linkers require internalization via antigen-mediated endocytosis and lysosome-mediated proteolytic degradation for payload release.[24,31,56] Generally, noncleavable linkers are associated with greater plasma stability, longer half-lives, and pose a reduced risk for off-target side effects. However, the actions of ADCs with noncleavable links are typically restricted to the target tumor cell. Moreover, amino acid-drug metabolites resulting from the drug payload release tend to be more hydrophilic and have greater intrinsic polarity, which reduces bystander effect. Commonly used noncleavable linkers in ADC design include the thioether linker succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate or maleimide moieties, such as maleimdopropionyl and maleimdocaproyl.[25]

Cytotoxic Drug Payload

The drug payloads employed in the design of ADCs are typically 100 to 1,000 times more potent than the cytotoxic agents used in traditional chemotherapy.[24,57] Cytotoxic payloads are generally subdivided into 2 main categories: (1) DNA-damaging agents, including calicheamicins, duocarmycins, pyrrolobenzodiazepines and camptothecin analogues; and (2) anti-tubulin agents, including auristatins, maytansinoids, and tubulysins.[1,24,28]

Calicheamicins (eg, ozogamicin) were first isolated from the actinomycete *Micromonospora echinospora* in the mid-1980s, and act by binding the minor groove of DNA, causing site-specific, double-stranded DNA breaks.[31,58-60] Notably, calicheamicins are highly hydrophobic, which limits the DAR or number of payload molecules able to be attached per mAb. Duocarmycins are potent alkylating compounds derived from the bacteria species *Streptomyces zelensis*. Duocarmycin and its derivatives, such as duocarmazine, act by binding to and alkylating adenine within the minor groove of DNA, leading to subsequent DNA strand cleavage and cellular apoptosis.[61-64] Pyrrolobenzodiazepine (PBD) dimers, such as tesirine, are DNA-damaging agents derived from anthramycin, an antitumor antibiotic isolated from Streptomyces.[65-68] PBD dimers bind the DNA minor groove, forming covalent interstrand DNA crosslinks within tumor cells that lead to cytotoxicity and cell death.[28,69,70] The formation of crosslinks resulting from PBD activity, which occurs rapidly and with minimal DNA distortion, is thought to contribute to their persistence and evasion of DNA repair mechanisms. Campothecin is a pentacyclic alkaloid isolated from the stem wood of *Camototheca acuminata*, a tree indigenous to China.[71-73] Campothecin and its derivatives (eg, govitecan, deruxtecan) act by inhibiting topoisomerase I enzyme activity, which results in double-stranded DNA breaks and subsequent cell death.[72-75]

Auristatins commonly used as ADC payloads include monomethyl auristatin E and monomethyl auristatin F, which are both synthetic analogues of dolastatin 10, a naturally-occurring antimitotic drug isolated from the sea hare *Dolabella auricularia*.[28,76,77] Auristatins inhibit tubulin polymerization by attaching to the same binding site as vinca alka-

loids (eg, vincristine, vinblastine), which are frequently used as traditional chemotherapy agents.[78-80] Maytansinoids are benzoansamacrolides derived from the bark of an African shrub, *Maytenus ovatus*.[81] Similar to auristatins, maytansinoids bind at or near the vinca-binding site, thereby inhibiting microtubule assembly and inducing mitotic arrest.[31,82] Maytansinoids commonly used in ADC design include 2 thiomethyl derivatives of maytansine: mertansine and ravtansine.[1,31,83] Tubulysins comprise a family of more recently discovered cytostatic peptides isolated from the myxobacteria species, *Archangium gephyra* and *Angiococcus disciformis*, that act by inhibiting microtubule polymerization during mitosis, thereby inducing apoptosis.[28,84,85]

DISCUSSION

Rapid advances in molecular and genomic technologies over the last several decades have ushered in a new era of precision medicine and led to the increased use of these technologies as important clinical tools for the diagnosis, classification, and treatment of disease.[86] As the use of precision-based treatment strategies has expanded, biomarker testing has become an increasingly important prognostic and predictive tool to improve disease management and enabled the development of numerous targeted therapies.[88,89] The effect of precision-based approaches on the treatment landscape has been especially profound in the oncology space, as reflected the growing number of biomarker-driven clinical trials, which increased from 15% in 2000 to 55% in 2018.[89] Although targeted therapies have led to major improvements in progression-free and overall survival, acquired drug resistance has led to associated therapeutic limitations and the need for other treatment options.[86] ADCs in particular represent a unique treatment approach that combines precision-based technology used in targeted therapy approaches with chemotherapy-based strategies using cytotoxic agents with greater potency.

Since the approval of the first ADC in 2000, continued technological advancements have led to an explosion in the number of ADCs under clinical development over the last few decades. As of 2022, a total of 12 ADCs with 9 associated biomarker targets have been approved for use in the treatment of both solid tumor and hematological malignancies.[7,90] In addition, over 80 ADCs are currently being evaluated in clinical trials, suggesting that utilization of these novel agents will continue to increase.

Most recently, the clinical development of novel ADCs has had a profound impact on the treatment landscape for solid tumors. The DESTINY series of clinical trials, investigated the human epidermal growth factor receptor 2 (HER2)-directed ADC, trastuzumab deruxtecan (T-DXd) in a number of solid tumor types.[91] Resulting data from these trials, led to approved indications in HER2-positive metastatic breast cancer (mBC), HER2-low mBC, and gastric or gastroesophageal cancer, as well as accelerated approval in non-small cell lung cancer.[16,92-95] Notably, DESTINY trial data surrounding T-DXd in mBC were not only unprecedented, but also transformative, and have played a major in reshaping how HER2 expression in tumors is assessed and managed. Historically, HER2 expression assessed via immunohistochemistry methods has been associated with a binary classification, namely HER2-positive or HER2-negative.[96] Early targeted therapies directed toward the HER2 receptor only demonstrated efficacy in HER2-positive mBC, with no effect in those with HER2-negative status.[96] However, the unique ADC design associated with T-DXd and the resulting bystander effect have expanded observed therapeutic responses to patients with lower levels of HER2 expression.[97]

CONCLUSION

As the incidence of cancer increases worldwide, growing demand for safer, more personalized cancer therapies with fewer side effects will undoubtedly propel further advances in ADC technology. Over the last 5 years alone, 39 clinical trials have investigated over 19 ADCs.[98,99] Given the pace at which ADC therapeutics are being introduced, the need for clear and accurate communication of information regarding these sophisticated treatments will also continue to grow. We as medical communicators must make it our mission to educate ourselves and our readers so that they can approach the literature critically and make informed decisions about their use.

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