

Monoclonal Antibodies in Targeted Cancer Therapy Advances, Resistance Mechanisms, and Next-Gen Formats (Bispesifics, ADCs)

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Abstract

Breast cancer is one of the leading causes of cancer-related morbidity and death worldwide, which emphasizes the need for more effective and focused treatment strategies and antibody-mediated drug delivery techniques. Specifically, antibody–drug conjugates (ADCs) have emerged as a potential approach that combines the specificity of monoclonal antibodies with the potent cytotoxicity of anticancer drugs. By selectively targeting tumor-associated antigens, ADCs enhance drug accumulation within cancer cells while minimizing systemic toxicity associated with conventional chemotherapy. This review discusses the fundamental principles of antibody-mediated drug delivery, including the roles of monoclonal antibodies, linkers, and cytotoxic payloads in ADC design. Various classes of cleavable and non-cleavable linkers, mechanisms of drug release, and advancements in conjugation technologies are examined. The article further explores the mechanisms of action, clinical applications, and evolutionary development of ADCs from first- to third-generation platforms, with particular emphasis on breast cancer treatment. Additionally featured are new developments like immune-stimulating conjugates, dual-payload ADCs, and bispecific ADCs. Despite significant clinical success, challenges including complex pharmacokinetics, limited tumor penetration, payload release efficiency, and drug resistance continue to hinder optimal therapeutic outcomes. Future advancements in antibody engineering, linker chemistry, payload development, and personalized medicine are expected to further improve the safety, efficacy, and clinical utility of ADCs. Overall, antibody-mediated drug delivery represents a transformative strategy in precision oncology, offering substantial potential for improving cancer treatment and patient outcomes.

Keywords: Antibody–drug conjugates (ADCs), monoclonal antibodies, breast cancer, targeted drug delivery, linkers, cytotoxic payloads, precision oncology, HER2, antibody-mediated therapy, cancer treatment

INTRODUCTION

By combining the cytotoxicity of chemotherapeutic medicines with the specificity of monoclonal antibodies, antibody–drug conjugates (ADCs) offer a paradigm change in targeted cancer therapy [1]. Because of their sensitivity and specificity, therapeutic antibodies have been an important part of cancer treatment for the past thirty years [2]. Breast cancer (BC) is the most common type of cancer diagnosed worldwide and a major cause of cancer-related mortality among women [3]. With 10.0 million cancer-related deaths in 2020, cancer has emerged as the second biggest danger to

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world health [4]. For many years, the primary method of treating a variety of tumors has been chemotherapy based on cytotoxic drugs [5]. Paul Ehrlich first proposed the concept of “magic bullets” at the beginning of the 20th century, hypothesizing that certain drugs might directly target particular cell targets to treat diseases [6]. As a major source of death and morbidity, cancer continues to be one of the world’s most important public health issues. Nearly 10 million people died from cancer in 2020, according to the World Health Organization (WHO), and estimates indicate that this number will rise over the coming decades [7]. BC is a biologically varied disease that is frequently described using human epidermal growth factor receptor 2 (HER2) and hormone receptor expression (estrogen receptor [ER] and progesterone receptor [PR]) [8]. Every subtype has unique clinical outcomes, therapeutic responses, and prognostic traits. For instance, HER2+ and TNBC are frequently linked to aggressive disease progression, a poor prognosis, and therapy resistance [9]. One of the mainstays of BC treatment is still conventional chemotherapy. However, because of systemic toxicity, immunological suppression, gastrointestinal side effects, and hair loss, its non-selective mechanism of action severely lowers the patients’ quality of life [10, 11]. With the goal of increasing therapeutic precision and lowering systemic toxicity, targeted therapy has emerged as a revolution in cancer. Due to their excellent specificity for tumor-associated antigens (TAAs), antibody-mediated targeted drug delivery systems (DDS) have drawn a lot of attention. It is possible to create monoclonal antibodies (mAbs) that can target particular surface indicators that are primarily expressed on cancer cells [12]. Examples include trastuzumab emtansine (T-DM1), which combines the cytotoxic effects of DM1 with the HER2-targeting characteristics of trastuzumab in the case of HER2+BC [13]. There is an urgent need to compile current data on antibody-mediated DDS development, use, and prospects in BC because of the growing clinical significance of antibody–drug conjugates (ADCs) and the evolving targeted therapies landscape. ADCs are designed with a potent payload and high-affinity targeting; they also provide a positive bystander impact in nonhomogeneous lesions when cleavable linkers are used [14]. Additional advantages of polymerizing antibody-conjugated nanoparticles (ACNPs) include enhanced drug dose, regulated or accelerated release, and active targeting via antibody decorating that increases tumor deposition and lowers systemic exposure [15].

PRINCIPLES OF ANTIBODY-MEDIATED DRUG DELIVERY

To chemically connect mAbs or antibody fragments to cytotoxic substances (payloads), such as cytotoxic drugs, a cleavable or non-cleavable linker is utilized. Consequently, the payload, linker, and antibody come together to create potent ADCs (Figure 1). These incredibly effective anti-tumor reagents can be used to deliver chemotherapeutic drugs to cancer cells in a highly targeted and direct manner against the specific cells under strict control of antibodies with great specificity and affinity [16, 17]. An overview of BC’s antibody-mediated drug delivery methods is shown in Table 1.

We describe the several ADC kinds that are pertinent to BC in the paragraphs that follow, highlighting their parts, workings, and therapeutic uses.

Mabs

One of the most crucial components of ADC design is the antibodies’ high antigen specificity in relation to non-target proteins. Unexpected results can happen when low specificity antibodies cross-react with distinct antigens. Additionally, they may be removed before to the treatment location or react with healthy tissues, leading to off-target toxicities [18]. IgG is the best choice for ADCs out of the five immunoglobulin groups (IgA, IgM, IgE, IgG, and IgD) since it is less immunogenic, abundant in the body, and maintains a stable structure [19]. Its Fc component helps keep it stable so that DDS can be safely introduced into cancer cells and cause cell death. IgG is the ideal substance to use as the foundation for the creation of ADCs to fight cancers because of its ability to easily penetrate cancer cells. Because of these features, IgG is the most effective antibody for delivering extremely large toxic loads in ADCs designed to target cancer cells [20, 21]. Its IgG1 subclass is preferred because it has a longer blood serum half-life than smaller antibody fragments, allowing it to be exposed to target cells

for a longer period. It increases the anti-tumor efficacy of cytotoxic drug delivery by activating immunogenic vectors like ADDC and CDC [22, 23]. On the other hand, IgG4 can form half-bodies, which may play a unique role in producing hybrid molecules that resemble bispecific immunoglobulins [24].

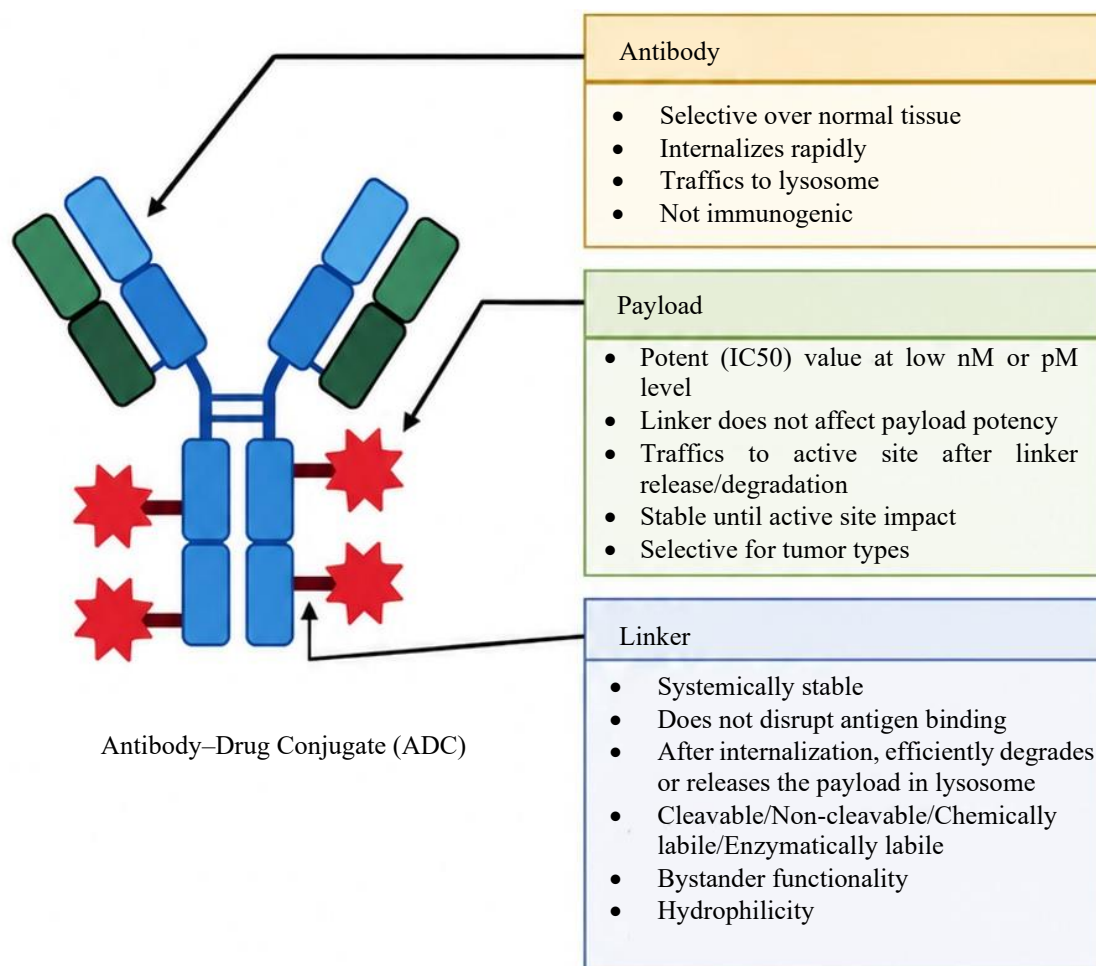


Figure 1. Structure of antibody-drug conjugates (ADCs).

Table 1. Antibody-based medication delivery method for breast cancer.

System/ component	Defining features	Typical target antigens in BC	Representative payload classes or cargo	Release/ activation paradigm	Key advantages	Principal challenges in BC	Representative examples/ status
Monoclonal antibodies	High-affinity antigen recognition; Fc-mediated effector functions	HER2, EGFR, MUC1, GPNMB, TROP-2*	– (no cytotoxic payload)	Receptor blockade; ADCC/CDC	Target specificity; validated biomarkers	Limited efficacy in low- antigen tumors; resistance via signaling bypass	Trastuzumab, pertuzumab (approved)
ADCs	Antibody + linker + cytotoxic payload	HER2, TROP-2, others under evaluation	Microtubule inhibitors (DM1, MMAE), Topo-I inhibitors (DXd, SN-38), DNA-	Cleavable (acid-labile, disulfide, enzyme- cleavable) or	High therapeutic index; delivers potent drugs to cancer cells	Antigen heterogeneity; on- target off- tumor	T-DM1, trastuzumab deruxtecan, sacituzumab

			damaging (PBDs)	non-cleavable (thioether); bystander effect depends on linker/payload		toxicity; interstitial penetration, resistance (efflux, lysosomal processing)	govitecan (approved)
Antibody-conjugated nanoparticles (ACNPs)	Antibody-decorated liposomes/polymeric NPs; active targeting	HER2, EGFR, MUC1, etc.	Chemo payloads (e.g., doxorubicin, paclitaxel), siRNA/miRNA, combinations	Triggered release (pH, enzyme, redox) and EPR + active binding	High drug loading; controlled/triggered release; multi-cargo potential	Scale-up/reproducibility; protein corona; heterogeneity of EPR; translation	Anti-HER2 immunoliposomes; investigational ACNPs (clinical/late preclinical)
Linkers	Connect Ab to payload; govern stability & release	—	—	Cleavable: acid-labile (hydrazone), disulfide, enzyme-cleavable (cathepsin-B peptides); Non-cleavable: thioether (SMCC)	Tuneable stability; enables bystander effect (cleavable)	Premature cleavage/systemic toxicity vs. over-stability/low release	See Supplementary Table S1 (chemistries & examples)
Payloads	Ultra-potent cytotoxins suited for intracellular release	—	MMAE/DM1 (microtubules), DXd/SN-38 (Topo-I), PBDs (DNA)	Endosomally/lysosomal release; diffusion if membrane-permeable	High potency at low DAR	Bystander toxicity (membrane-permeable), efflux, payload-specific AEs	Payloads as used in approved ADCs
Mechanism Targeting → internalization → endolysosomal processing	Antigen-mediated uptake; trafficking dictates release	—	—	Linker cleavage or proteolysis; possible extracellular release (bystander)	Selective intracellular delivery	Requires sufficient antigen density and internalization kinetics	—
Antigen selection	Tumor-associated, high density, internalizing	HER2, TROP-2, EGFR, MUC1, GPNMB	—	—	Improves selectivity; guides patient selection	Heterogeneous expression; normal-tissue expression	HER2 & TROP-2 clinically validated targets

In certain malignancies, ADC's capacity to cause cancer cell death is enhanced by the ability of large immunoglobulin IgM to target low antigen-expressing cancer cells. However, the conjugation and internalization of medicines are complicated by its vast size and complex structure [25]. To treat chronic lymphocytic leukemia, IgMADC was recently created. It is specifically conjugated at the C-terminus, producing specific selenocysteines on each heavy chain [26, 27]. To achieve constant drug: antibody ratios and improved stability, site-specific conjugation technologies (click chemistry, enzymatic ligation) have been developed for drug targeting by antibody. Bispecific and biparatopic antibodies boost tumor selectivity by overcoming antigen escape and tumor selectivity. Innovative Fc engineering and glyco-optimization methods to improve immunogenicity and circulatory half-life, as well as nanobody conjugation and fragments to improve tumor penetration and targeted DDS [28].

Linkers

Linkers are extremely versatile parts of an ADC since they are essential in establishing a link between the mAb and the cytotoxic payload. They have a major impact on the ADC's biophysical and functional properties such as stability, potency, efficacy, and toxicity. Furthermore, linkers play an important role in ensuring that the drug is released effectively at the target location and preventing the early release of the cytotoxic agent in the bloodstream [29]. Based on the payloads' release mechanisms (Figure 2), linkers can be broadly classified into two classes: noncleavable (thioether bond and amide bond) and cleavable (hydrazone bond, disulfide bond, peptide bond, and β -glucuronide linker), which are described below [30]. The active payload is released when a self-immolative spacer collapses; cleavable linkers are activated in endo/lysosomes, whereas non-cleavable linkers rely on the antibody's lysosomal proteolysis; and the antibody-linker-payload circulates undamaged.

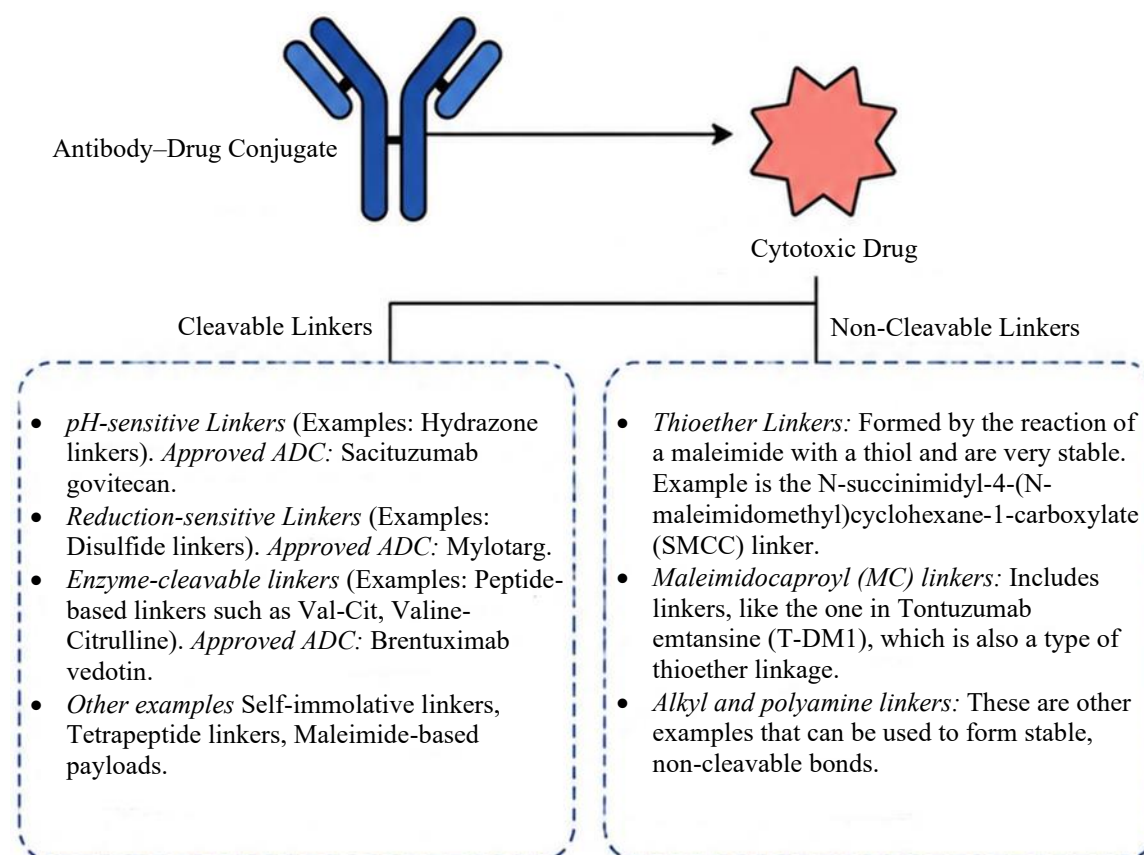


Figure 2. Antibody-Drug Conjugate (ADC) Linker Classification. The two primary classifications of linkers employed in ADCs – cleavable and non-cleavable – are depicted in the diagram. Hydrazone, disulfide, peptide, and β -glucuronide-based linkers are examples of cleavable linkers that release the cytotoxic medication in response to certain intracellular stimuli like acidic pH, reducing agents, or enzymatic activity. Drug release from non-cleavable linkers, like amide and thioether bonds, depends on the antibody's total breakdown inside the lysosome.

Cleavable Linkers

Because they provide greater control over the timing and location of the cytotoxic drug's release, cleavable linkers are favored over non-cleavable linkers in ADCs. Cleavable linkers are made to be stable in the bloodstream but degrade when they meet certain circumstances that are present in cancer cells like acidic environments, proteases, or reducing agents. Whereas the near-neutral extracellular or plasma pH of approximately 7.4 allows for the selective hydrolysis of acid-labile linkers, such as hydrazone or cis-aconityl bonds, the acidic circumstances that lead to cleavage usually correlate to endosomal or lysosomal compartments with pH values ranging from ≈ 4.5 to 6.0. Off-target toxicity is

less likely, and the medicine can be delivered to cancer cells primarily without damaging healthy tissues, enhancing the therapeutic impact and limiting damage to good tissues [31].

Chemically Cleavable

Acid labile linkers: At physiological pH (~7.4), acid-cleavable hydrazone linkers show slow hydrolysis with low-level payload release over 24–48 hours, as opposed to rapid cleavage in acidic endosomal/lysosomal compartments (pH~4.5–6) [32]. This was demonstrated in early research on Pfizer's gemtuzumab ozogamicin (Mylotarg), which has an acid-sensitive N-acyl hydrazone linker. Effective DDS is ensured when the linker hydrolyzes in acidic conditions, releasing the cytotoxic drug [33].

Disulfide linkers: Because disulfide linkers are stable at physiological pH but break down when thiols are present within cells, they are frequently utilized in ADCs. Clinical ADCs – such as Pfizer's Mylotarg and Besponsa – have demonstrated the efficacy of these linkers [34]. Conventional aliphatic disulfide linkers cannot live in human plasma because they are susceptible to thiol-disulfide exchange with plasma thiols, particularly the free Cys34 residue of human serum albumin, which poses a risk of premature payload release. Classifying Linkers in Antibody–Drug Conjugates (ADCs), as demonstrated in clinical Figure 2. The two primary classifications of linkers utilized in ADCs – cleavable and non-cleavable – are seen in the diagram. Examples of cleavable linkers that release the cytotoxic drug in response to specific intracellular stimuli such as acidic pH, reducing agents, or enzymatic activity include hydrazone, disulfide, peptide, and β -glucuronide-based linkers. To boost plasma stability while preserving intracellular reducibility, Rejili Discover Oncology (2026) 17:377 Page 8 of 44 designs rely on chemically modified disulfides such as sterically-hindered tertiary/aryl disulfides, disulfide masking, or self-immolative structures. Drug release from non-cleavable linkers, such amide and thioether linkages, depends on the complete disintegration of the antibody inside the lysosome. On the other hand, these linkers can be selectively broken down by high amounts of glutathione (GSH) in cancer cells, which releases drugs and makes them useful for targeting cancer cells [35].

Exogenous stimuli causing cleaving: Drug release control is improved, and biological variances are accommodated by using external triggers to break ADC linkers. Stenton et al. created a thioether-based linker that releases its drug when exposed to a palladium catalyst, acting similarly to Dox with a HER2-targeting nanobody. However, safer palladium complexes are required for clinical applications [36, 37].

Cleavable enzyme linker Dipeptide-containing linkers: Enzymes, like cathepsin, which are active in tumor cells, may cleave linkers that contain dipeptides. This would reduce the negative effects and ensure that the drugs were primarily delivered within the target cells [38].

β -Glucuronidase-cleavable linkers: By using the enzyme β -glucuronidase, which is released in necrotic tumor regions and active in lysosomes, these linkers enable ADCs to target both intracellular and extracellular environments (Rejili Discover Oncology (2026) 17:377 Page 9 of 44). They were first investigated for prodrugs in 1988, and Seagen Inc. (previously Seattle Genetics) modified them for ADCs in 2006. In preclinical research, these linkers showed remarkable stability and effectiveness when paired with a para-aminobenzylcarbamate (PABC) spacer [39].

Other Enzyme-Responsive Linkers

Phosphatase-triggered phosphate/phosphoramidate cages have been studied preclinically for bioconjugates and prodrugs, but they are still considerably less established than β -glucuronide or peptide (cathepsin-cleavable) motifs and have not been clinically verified for ADCs. They are, therefore, not regarded as a basic class in this context [40, 41].

Non-Cleavable Linkers

Non-cleavable linkers create stable connections that are not chemically or enzymatically broken in the tumor environment; payload release only happens when the antibody is lysosomally broken down,

producing a payload-linker catabolite that is still active. Depending on the activity of the resultant catabolite, non-cleavable designs can enhance plasma stability and lessen bystander impacts. To create a charged, membrane-impermeable catabolite in non-cleavable designs, the ADC needs to be fully proteolyzed in lysosomes. Since this catabolite cannot diffuse through membranes, the cytotoxic reaction is localized on the antigen-positive cell that was infected by the ADC; the surrounding cells are unaffected, indicating that there is no bystander effect [42].

The following are a few non-cleavable linkers:

- *Maleimide Linkers*: Maleimide linkers selectively react with thiol groups (-SH) through a Michael addition to create stable thioether linkages that precisely attach the cytotoxic payload to the antibody. This site-specific conjugation maximizes ADC potency while reducing possible toxicity by enabling efficient management of the DAR [43].
- *Amide and Amino Acid Linkers*: Because amide linkers are stable and non-cleavable, they are essential to ADCs. They ensure that the medication is retained until lysosomal breakdown by forming strong connections between it and the antibody. Even if they stop premature release, the resulting drug-linker conjugate may be less effective due to its attachment to the antibody [44].
- *Hydrophilic Linkers*: Hydrophilic linkers enhance the solubility and pharmacokinetics of ADCs by including substances like sugars or polyethylene glycol (PEG). Particularly in ADCs with high DAR, this design ensures efficient DDS to tumors, reduces off-target toxicity, and improves blood circulation [45].

Payload

A crucial part of an ADC is its payload, which, once inside the tumor cell, effectively kills cancer. For a payload to be effective, its IC₅₀ value must be between 0.1 and 10 nM. Additionally, they must have functional groups that may chemoselectively couple into the antibody using an appropriate linker and be stable throughout the body [46, 47].

TYPES AND CLASSIFICATION OF MONOCLONAL ANTIBODIES IN CANCER

Monoclonal antibodies used in oncology can be classified based on their structure, source, and mechanism of action:

Based on Structure and Origin

- Antibodies derived entirely from mice such as Muromonab-CD3.
- Chimeric antibodies (such as Rituximab; approximately 65% human).
- Humanized antibodies, such as trastuzumab, which are about 90% human.
- Completely human antibodies (100% human; Panitumumab, for example) according to mechanism and function.
- Antibodies that directly target (e.g., cetuximab binds EGFR to block signaling).
- Immune checkpoint inhibitors, such as nivolumab, which increases T-cell activation by blocking PD-1.
- ADCs, or antibody-drug conjugates such as ado-trastuzumab emtansine.
- Bispecific antibodies, such as Blinatumomab, which connects T cells and cancer cells.
- The variety and growing complexity of antibody-based treatments are reflected in this classification [48].

Mechanisms of Action in Cancer Therapy

Monoclonal antibodies can destroy cancer cells through various mechanisms:

Direct Antitumor Activity

Tumor cell binding to target receptors can directly disrupt signaling pathways, stop cell division, or trigger apoptosis. For instance, trastuzumab, which is used to treat HER2-positive breast cancer, stops the HER2 receptor from dimerizing and causes cell cycle arrest [49].

Immune-Mediated Cytotoxicity

Monoclonal antibodies can engage the host immune system through mechanisms such as:

- Cellular cytotoxicity dependent on antibodies (ADCC).
- Cytotoxicity that is dependent on complement (CDC).

Rituximab uses both ADCC and CDC to eradicate cancerous cells by targeting CD20 on B-cells [50].

Immune Checkpoint Blockade

Checkpoint inhibitors, including Nivolumab (anti-PD-1) and Ipilimumab (anti-CTLA-4), activate cytotoxic T-cells by obstructing inhibitory signals that cancer cells utilize to avoid immune monitoring [51].

Drug Delivery Vehicles

Cytotoxic chemicals are delivered to cancer cells only by antibody–drug conjugates (ADCs), protecting healthy tissues. Brentuximab vedotin, which gives CD30+ lymphoma cells a tubulin inhibitor, is one example (Table 2) [52].

Table 2. Clinical applications of monoclonal antibodies [53].

Drug name	Target	Cancer type
Rituximab	CD20	Non-Hodgkin's Lymphoma.
Trastuzumab	HER2	Breast, Gastric.
Cetuximab	EGFR	Colorectal, Head & Neck.
Bevacizumab	VEGF	Colorectal, NSCLC.
Nivolumab	PD-1	Lung, Melanoma, RCC.
Daratumumab	CD38	Multiple Myeloma.

Advantages of Monoclonal Antibody Therapy

- *Specificity:* Focuses on antigens linked to tumors while having little effect on healthy tissues.
- *Decreased Systemic Toxicity:* Particularly in contrast to conventional chemotherapy.
- *Immune Activation:* Able to use the patient's immune system to produce long-lasting reactions.
- *Personalization Potential:* Biomarker-driven treatments such as PD-L1 or HER2.
- *Combination Therapy Compatibility:* mAbs can be utilized in conjunction with radiation, chemotherapy, or other biologics [54].

Limitations and Challenges

Despite their advantages, mAbs have certain drawbacks: Growth of Resistance The antibody may become ineffective if tumor cells undergo antigen mutations or downregulate target expression [55].

Immune-Related Adverse Effects (irAEs)

Because checkpoint inhibitors overactivate the immune system, they can result in autoimmune adverse effects such as pneumonitis, hepatitis, or colitis [56].

MECHANISM OF ACTION OF ADC

When combined, ADCs have a “specific” targeting role and a “efficient” cancer cell-killing impact. These drugs can attack cancer cells precisely, much like a precision-guided “biological missile,” increasing the treatment window and reducing unwanted side effects [57]. One of ADC's primary modes of action is shown in the upper-right panel of Figure 3. Once mAb of ADC is bound to the target antigens that are specifically expressed on the cancer cells, ADC is endocytosed/internalized by cells to form an early endosome, which then develops into late endosomes before fusing with lysosomes. Through chemical or enzyme-mediated release in the lysosomes, the cytotoxic payloads that target DNA or microtubules ultimately result in cell death or apoptosis [58]. The discharge of a permeable or

transmembrane payload may also have a bystander effect to increase ADC's effectiveness. Additionally, these medications' bystander effect may change the tumor microenvironment, which could increase ADCs' ability to kill [59].

ADVANCE IN THE DEVELOPMENT OF ADC

The development of ADC pharmaceuticals can often be categorized into three generations based on chemical composition and technological features (Table 3).

The First-Generation ADCs

Early ADCs, like BR96-doxorubicin, were mostly made up of a traditional chemotherapeutic medication coupled to a mouse-derived antibody via a non-cleavable linker [60, 61]. Those ADCs' immunogenicity was often an issue, and their potency was not better than that of free cytotoxic medicines [62]. The first-generation ADCs, such as gemtuzumab ozogamicin and inotuzumab ozogamicin, were later approved for sale due to the use of significantly stronger cytotoxic chemicals in conjunction with humanized mAbs. In these two compounds, the powerful cytotoxic calicheamicin was coupled to humanized mAbs of the IgG4 isotype using acid-labile linkers [63, 64].

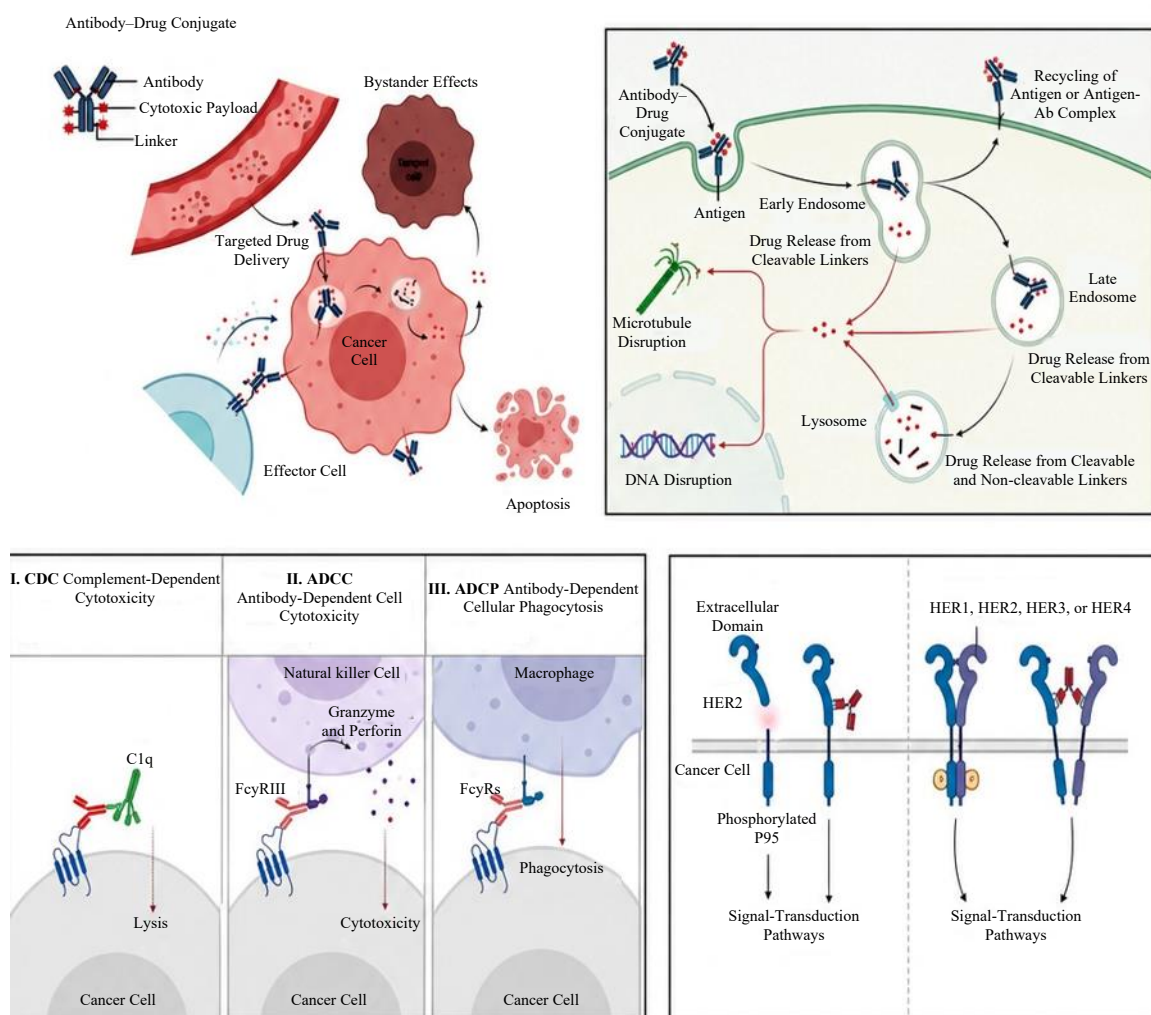


Figure 3. This image summarizes the several ways that ADC kills cancer cells. Upper-Right: ADCs' primary mode of action; Lower-Left: To produce antitumor immunity, including CDC, ADCC, and ADCP effects, the antibody component of ADCs interacts with immune effector cells; Lower-Right: Because the antibody component of ADCs maintains its activity profile, it can disrupt target function and reduce downstream signaling to stop the growth of tumors. BioRender.com was used to create it.

The Second-Generation ADCs

The second-generation ADCs, represented by brentuximab vedotin and ado-trastuzumab emtansine, were introduced after mAb isotypes, cytotoxic payloads, and linkers were optimized. These two ADCs are based on IgG1 isotype mAbs, which are more suitable for bioconjugation with small-molecule payloads than IgG4 and have a greater ability to target cancer cells [65, 66]. The adoption of more potent cytotoxic medications with enhanced water solubility and coupling efficiency, such as auristatins and maytansinoids, is another significant advancement in second-generation ADC [67].

The Third-Generation ADCs

Third-generation ADCs include polatuzumab vedotin, enfortumab vedotin, fam-trastuzumab deruxtecan, and later approved ADCs. The discovery of the site-specific conjugation technique allowed for the creation of homogenous ADCs with well-characterized DARs (2 or 4) and the necessary cytotoxicity [68].

ADCs with stable DARs exhibit improved pharmacokinetic efficiency and reduced off-target toxicity [69]. Furthermore, the third generation uses entirely humanized antibodies rather than chimeric ones to lessen immunogenicity. Moreover, antigen-binding fragments (Fabs) are being developed to replace complete mAbs in some ADC candidates because they are more stable in systemic circulation and may be more readily internalized by cancer cells [70].

Table 3. The evolution of the ADC drug development.

	First-generation ADC	Second-generation ADC	Third-generation ADC
Antibodies	Mouse-original or chimeric humanized antibodies	Humanized antibodies	Fully humanized antibodies or Fabs.
Linkers	Unstable	Improved stability; cleavable and non-cleavable linkers;	Stable in circulation; precise control drugs release into tumor sites.
Payloads	Low potency, including calicheamicin, duocarmycin and doxorubicin	Potency such as auristatins and maytansinoids	High potency, such as PBDs and tubulysin, and novel payloads like immunomodulators.
Conjugation methods	Random lysines	Random lysines and reduced interchain cysteines	Site-specific conjugation.
DAR	Uncontrollable (0–8)	4–8	2–4.
Representative drugs	Gemtuzumab ozogamicin and inotuzumab ozogamicin	Brentuximab vedotin and ado-trastuzumab emtansine	Polatuzumab vedotin, enfortumab vedotin, and fam-trastuzumab deruxtecan.
Advantages	Specific targeting Increase therapeutic window to some extent	Improved targeting ability More potent payloads Lower immunogenicity	Higher efficacy though in cancer cells with low antigen; Improved DAR along with improved stability and PK/PD; More potent payloads Less off-target toxicity.
Disadvantages	Heterogeneity; Lack of efficacy; Narrow therapeutic index; Off-target toxicity as premature drug loss; High immunogenicity	Heterogeneity; Fast clearance for high DARs; Off-target toxicity as premature drug loss; Drug resistance	Possible toxicity due to highly potent payloads; Catabolism may be different across species Drug resistance.

Clinical Applications Across Cancer Types

Monoclonal antibodies have been widely used as monotherapy or in conjunction with other modalities for a variety of cancer types.

Breast Cancer

Breast cancer has benefited considerably from mAb therapy, particularly HER2-positive subtypes.

- When combined with chemotherapy, trastuzumab increases both disease-free survival (DFS) and overall survival (OS) [71].
- Another HER2-targeting mAb that works well for dual HER2 blocking is pertuzumab, which binds to a distinct HER2 domain.
- These drugs are used to downstage tumors prior to surgery in the neoadjuvant situation.

Non-Hodgkin's Lymphoma (NHL)

The first monoclonal antibody (mAb) authorized for use in cancer treatment was rituximab, which targets CD20. It is still the mainstay of treatment for follicular lymphoma and diffuse large Bcell lymphoma (DLBCL).

Rituximab-CHOP (R-CHOP) considerably increased response rates and survival when compared to CHOP alone, according to research by Czuczman et al. (2017) [72].

Cancer of the Colon

EGFR-targeting monoclonal antibodies (mAbs) as cetuximab and panitumumab are used to treat RAS wild-type metastatic colorectal cancer (mCRC). These medications decrease the growth and spread of malignancies by inhibiting EGFR signaling [73].

Bevacizumab, which targets VEGF to decrease angiogenesis, is commonly used in combination with chemotherapy.

Lung Cancer

Immunocheckpoint inhibitors have transformed the treatment of lung cancer. Nivolumab, atezolizumab, and durvalumab are used to treat NSCLC with varying levels of PD-L1 expression.

Nivolumab versus docetaxel enhanced OS in NSCLC patients, according to a seminal trial by Borghaei et al. (2015) [74].

Melanoma

- Immune checkpoint inhibition has made melanoma a model illness.
- The first medication to demonstrate OS benefit was ipilimumab.
- Although there is a higher chance of immune-related adverse events (irAEs), the combination of nivolumab and ipilimumab delivers improved results [75].

Next-Generation ADC Platforms and Combination

Strategies

Bispecific and Dual-Target ADCs: By simultaneously binding two different antigens, bispecific ADCs can combat tumor heterogeneity and antigen-mediated resistance [76–78]. Bispecific ADCs can continue to function even when one antigen is downregulated since they target two antigens that are expressed on tumor cells. Furthermore, by necessitating co-expression of both antigens for the best binding and internalization, bispecific forms can improve tumor specificity [79]. Preclinical and early clinical development is underway for a number of bispecific ADC forms [80, 81].

Dual-Payload ADCs

Potential benefits of ADCs coupled to two distinct cytotoxic payloads include simultaneous targeting of several cellular pathways and possible payload synergy [82–84]. This tactic may delay or eliminate resistance by forcing tumor cells to create defense systems against two distinct payloads. However, there are technological difficulties due to the intricacy of producing dual-payload ADCs and maximizing the ratio of each payload [85].

Immune-Stimulating ADCs

Immune-stimulating antibody conjugates transfer immunomodulatory payloads, such as Toll-like receptor agonists or activator of interferon gene agonists, to the tumor microenvironment to promote

anti-tumor immunity while maintaining tumor-targeted delivery [86–88]. Immune-stimulating antibody conjugates have been shown to increase T cell infiltration and cause immunogenic cell death in preclinical settings. Safety and initial efficacy are being assessed in early clinical trials. Combining ADCs with immune checkpoint inhibitors makes sense because preclinical research indicates that ADCs may boost immunogenicity by inducing immunogenic cell death and releasing tumor antigens [89–91].

Combination with Targeted Agents

Based on preclinical synergy, combinations of ADCs with other targeted therapies, such as tyrosine kinase inhibitors, poly (ADP-ribose) polymerase inhibitors, and cyclin-dependent kinase 4/6 inhibitors, are being studied [92–94]. Controlling the toxicity of mixed regimens and identifying patient populations most likely to benefit are difficult tasks. Additionally, sequential approaches – in which ADCs are given gradually after targeted therapy – have been investigated [95].

CURRENT CHALLENGES AND NEXT GENERATIONS OF ADCS

It is evident from the licensed medications and in-development candidates mentioned in earlier sections that current generation ADCs are outperforming earlier generations in terms of selectivity and cytotoxicity characteristics. However, the development of anti-cancer ADCs still faces numerous obstacles such as drug resistance, inadequate tumor targeting and payload release, and complicated pharmacokinetics [96].

- *Major Challenges:* intricate pharmacokinetic profiles. Three primary forms – the intact ADC, the bare antibody, and the free cytotoxic payload – may be found in the systemic circulation following the administration of an ADC (usually via intravenous infusion) [97]. The ratios of these three forms will fluctuate dynamically due to target binding, elimination, and deconjugation [98]. The concentration of both conjugated ADC and naked antibody decreases when ADC internalization and antibody clearance occur in a normal pharmacokinetic profile of ADC [99]. Neonatal Fc receptor (FcRn)-mediated recycling and the mononuclear phagocyte system are factors that influence antibody clearance [100, 101]. FcRn exports ADC to an extracellular compartment for recycling after interacting with it in an endocytic vacuole [102–103].
- *Unavoidable Side Effect:* intricate pharmacokinetic profiles. Three primary forms – the intact ADC, the bare antibody, and the free cytotoxic payload – may be found in the systemic circulation following the administration of an ADC (usually via intravenous infusion) [97]. The ratios of these three forms will fluctuate dynamically due to target binding, elimination, and deconjugation [98]. When ADC internalization and antibody clearance take place in a typical ADC pharmacokinetic profile, the concentration of both conjugated ADC and bare antibody drops [99]. Neonatal Fc receptor (FcRn)-mediated recycling and the mononuclear phagocyte system are factors that influence antibody clearance [100, 101]. FcRn exports ADC to an extracellular compartment for recycling after interacting with it in an endocytic vacuole [104, 105].

Tumor Targeting and Payload Release

When compared to conventional cytotoxic medications, the molecular weight of ADC is significantly greater than the drug's ability to penetrate complex pharmacokinetic characteristics. After an ADC is administered (typically by intravenous infusion), the systemic circulation may contain three main forms: the complete ADC, the bare antibody, and the free cytotoxic payload [97]. Target binding, elimination, and deconjugation will cause these three forms' ratios to change dynamically [98]. When ADC internalization and antibody clearance take place in a typical ADC pharmacokinetic profile, the concentration of both conjugated ADC and bare antibody drops [99]. Neonatal Fc receptor (FcRn)-mediated recycling and the mononuclear phagocyte system both affect antibody clearance [100, 101]. After connecting with ADC in an endocytic vacuole, FcRn exports it to an extracellular compartment for recycling. is restricted. The potency of payloads must be considered while constructing ADC because current research indicates that only a tiny portion of ADC input into patients can reach tumor cells [106]. The internalization of the generated ADC-antigen complex via antigen-dependent

endocytosis or antigen-independent pinocytosis is necessary for the delivery of payloads for ADC medicines. The ADC antigen complex will be carried to the lysosome or endosome for the release of payloads upon internalization. The payload will be released in late endosomes or lysosomes for ADCs that need specific proteases; otherwise, it is likely to be released in the early endosome when attached by an acid cleavable linker [107].

Drug Resistance

Drug resistance is another obstacle to the development of ADCs. Tyrosine kinase inhibitor (TKI) drug resistance typically involves an escape mutation of the therapeutic target [108]. However, the mechanism underlying ADCs' drug resistance has not been adequately described. Because of the underlying MOA of ADCs, they are probably more varied and complex. According to available data, cancers can evade ADC activity in a variety of ways, including by lowering antigen expression levels, altering intracellular transport pathways, and developing drug resistance to payloads [109].

Future Generation ADCs

Replacing any one of the three components that make up ADC – monoclonal antibody, linker, and payload – may have an impact on its efficacy. Different antibodies that target the same antigen may attach to it differently and affect receptor dimerization and antigen internalization in different ways. According to recent research, ADC internalization and the intracellular transport mechanism significantly influence the cytotoxic efficacy of ADC [110]. Mutant proteins are typically more ubiquitinated than wild-type proteins and are more easily absorbed and broken down [111].

Future Perspectives

Currently, research is concentrated on:

- Tumor genomics-based customized antibody treatment.
- Antibodies have multiple specificities that can target multiple pathways at once.
- Intratumoral administration for reduced systemic toxicity and local effects.
- CAR-T cell therapy, an extension of antibody technology based on cells.
- The use and safety of mAbs in oncology will continue to grow thanks to developments in immune profiling, drug conjugation, and antibody design [112].

CONCLUSION

By fusing the strong cytotoxic activity of anticancer agents with the high specificity of monoclonal antibodies, antibody-mediated drug delivery systems, especially antibody–drug conjugates (ADCs), have become a gamechanger in the treatment of breast cancer and other cancers. The treatment efficacy has been greatly increased by developments in payload design, linker technology, and antibody engineering. Pharmacokinetic properties, and safety profiles of ADCs compared with conventional chemotherapy. The evolution from first-generation to third-generation ADCs has resulted in enhanced tumor selectivity, reduced systemic toxicity, and improved clinical outcomes for patients with difficult-to-treat cancers, especially HER2-positive and triple-negative breast cancer.

Despite these achievements, several challenges remain, including tumor heterogeneity, limited tumor penetration, complex pharmacokinetics, off-target toxicities, and the development of drug resistance. Ongoing research focused on site-specific conjugation technologies, novel linker systems, bispecific and dual-payload ADCs, immune-stimulating conjugates, and combination treatment strategies is expected to further enhance the clinical potential of these agents. Additionally, advances in biomarker identification, precision oncology, and personalized medicine are likely to facilitate better patient selection and treatment optimization.

Overall, antibody-mediated drug delivery represents a promising and rapidly evolving field in cancer therapeutics. Continued innovation in ADC design and a deeper understanding of tumor biology will contribute to the development of next-generation targeted therapies capable of achieving superior

efficacy while minimizing adverse effects, ultimately improving survival and quality of life for patients with breast cancer and other cancers.

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