Fundamental research on Antibody-Drug Conjugates and their applications in solid tumors and hematologic malignancies

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Abstract. Antibody-Drug Conjugates (ADCs) have emerged as a promising targeted therapy for cancer, combining the specificity of monoclonal antibodies with the potent cytotoxic effects of chemotherapy agents. This paper provides a comprehensive review of the fundamental mechanisms, clinical applications, and potential future developments of ADCs in the treatment of both solid tumors and hematologic malignancies. ADCs work by selectively targeting cancer cells through specific antigens, delivering cytotoxic drugs directly into these cells while minimizing damage to healthy tissue. Key components, including the choice of antibodies, cytotoxic payloads, and linkers, are discussed in detail to highlight their roles in optimizing ADC efficacy. The clinical success of various ADCs, such as Gemtuzumab ozogamicin in acute myeloid leukemia and Ado-trastuzumab emtansine in HER2-positive breast cancer, underscores their potential in improving patient outcomes. However, challenges such as toxicity and drug resistance remain, necessitating further research.

Keywords: Antibody-Drug Conjugates, solid tumors, hematologic malignancies.

1. Introduction

Cancer treatment remains a significant challenge, with conventional methods such as chemotherapy and radiation therapy offering only limited success. These traditional approaches, while effective at targeting rapidly dividing cells, often lack specificity, leading to damage to healthy tissues and causing severe side effects. Additionally, their effectiveness is often compromised by the development of drug resistance and the inability to fully eradicate tumors, especially in advanced stages. In response to these limitations, ADCs have emerged as a promising targeted therapy. ADCs combine the specificity of monoclonal antibodies with the potent cell-killing capability of cytotoxic drugs, allowing for targeted delivery directly to cancer cells while minimizing off-target effects on healthy tissue [1]. This novel approach holds significant potential to improve the precision and efficacy of cancer treatment, making it a focal point of ongoing research and clinical development. The main objective of this paper is to provide a comprehensive review of the mechanisms, clinical applications, and prospects of antibody-drug conjugates. By analyzing current research progress and clinical trial outcomes, this paper aims to highlight the potential of ADCs as an innovative targeted therapy in cancer treatment. In-depth research and optimization of ADCs are expected to further advance the development of cancer therapies.

2. Fundamentals and components of ADCs

2.1. Definition and working principle of ADCs

ADCs are a novel class of cancer therapeutics that chemically link a monoclonal antibody to a cytotoxic drug, enabling highly targeted drug delivery within the body. This technology combines the high specificity and selectivity of antibodies with the cytotoxic effects of the drug, aiming to enhance therapeutic efficacy while minimizing damage to normal tissues [2].
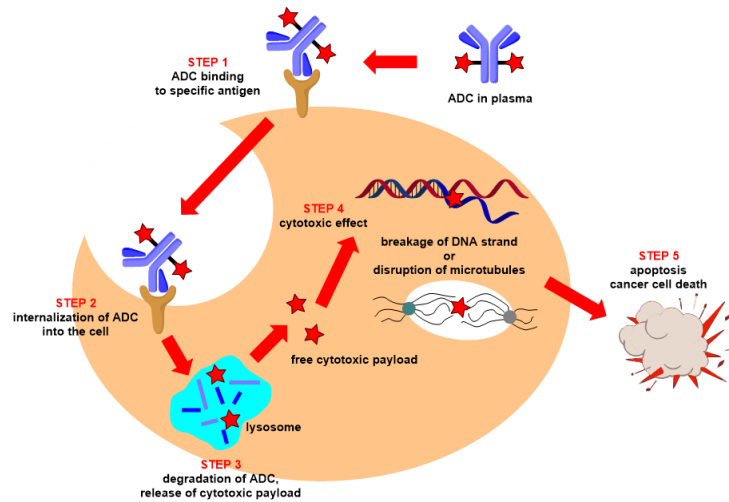

Figure 1. ADC mechanism of action (source: https://www.santiago-lab.com/)

As shown in Figure 1. The mechanism of action of antibody-drug conjugates involves several key steps. First, the antibody component of the ADC specifically recognizes and binds to a particular antigen on the surface of cancer cells, ensuring that the ADC selectively targets cancer cells without affecting normal cells. Next, the cancer cell internalizes the ADC complex through endocytosis, forming an endosome. The endosome then fuses with a lysosome, where, in the acidic environment and in the presence of proteases, the linker is cleaved, releasing the cytotoxic drug carried by the ADC. Once activated, these drugs enter the cytoplasm and exert their toxic effects by disrupting microtubules or causing DNA damage, interfering with the cell's survival processes, ultimately leading to cancer cell death. This entire process demonstrates the high specificity and precision of ADCs in targeting and killing cancer cells, effectively eliminating them while minimizing damage to normal cells [3].

2.2. Antibody and antigen in ADCs

The antibody is the primary component of an Antibody-Drug Conjugate (ADC) responsible for recognizing and binding to tumor cells. It must possess high affinity for the target antigen and low immunogenicity, meaning the antibody can bind tightly and specifically to the antigen, even at low concentrations, effectively attaching to cancer cells [4]. Low immunogenicity also reduces the likelihood of the host immune system attacking the antibody, allowing the ADC to maintain prolonged activity in the body. Additionally, the antibody should have a long plasma half-life and the ability to facilitate rapid internalization, ensuring that the ADC circulates in the body for a longer period and increasing its chances of reaching the tumor site. There is a high degree of correlation between the antibody and antigen, and the antibody should not interfere with antigen internalization, ensuring the drug is effectively delivered into the cancer cells [5]. Moreover, selecting an appropriate target antigen is crucial; the antigen should be highly expressed on tumor cells while being minimally expressed on healthy cells to minimize adverse effects on normal tissues. The antigen should be present on the exterior surface of cancer cells, enabling the antibody to accurately and quickly recognize and bind to it, enhancing specificity. At the same time, the antigen should have a strong capacity for internalization, allowing the ADC molecule to enter the cell and release its payload at the intracellular target [6].
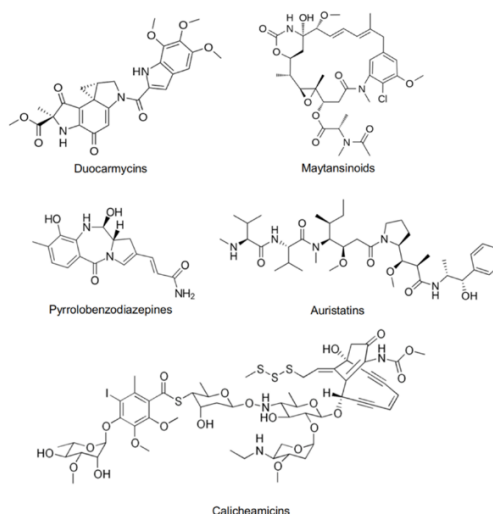
2.3. Cytotoxic Payloads in ADCs

The cytotoxic payload is the active component of an ADC, consisting of highly toxic compounds that exert therapeutic effects on target cells [7]. These compounds bind to intracellular targets, disrupt cellular processes, and promote cell death. Ideally, the payload should efficiently reach the target site without releasing any off-target effects and should be able to kill cancer cells without harming normal healthy cells. Notably, each payload must have very high toxicity, as each ADC molecule can only attach a limited number of payloads (typically 3-4 per antibody molecule). This high toxicity ensures that even with the limited amount of payload released after the ADC enters the tumor cell, it can effectively kill cancer cells at low concentrations. These payloads are generally categorized into two types: microtubule disruptors and DNA-damaging agents [8,9].

Microtubule disruptors prevent cell division by disturbing the dynamic stability of microtubules. Microtubules are crucial components of the cytoskeleton, composed of α and β-tubulin dimers, and play a key role during cell division process. The processes of microtubule polymerization and depolymerization are essential for forming the mitotic spindle, separating chromosomes, and completing cell division. The main function of microtubule disruptors is to inhibit microtubule assembly or disassembly, thereby blocking cell cycle progression and ultimately leading to apoptosis. As shown in the Figure 2a, common microtubule disruptors include Auristatins and Maytansinoids. DNA-damaging agents induce cell death by directly damaging the DNA structure or interfering with DNA repair mechanisms. These drugs primarily cause DNA double-strand breaks or interstrand cross-links, which obstruct DNA replication and transcription, ultimately resulting in apoptosis. Common DNA-damaging agents include Calicheamicins, Pyrrolobenzodiazepines, and Duocarmycins [10,11].

2.4. The linkers in ADCs

In ADCs, linkers play a crucial role by attaching cytotoxic drugs to antibodies and ensuring that the drug is effectively released within specific tumor cells. Linkers need to be sufficiently stable to prevent degradation or premature release of the drug before it reaches its target, thereby avoiding off-target effects and unnecessary toxicity [12]. Additionally, linkers must be able to be activated after entering the tumor cells to release the drug in the intracellular environment. Based on their characteristics, linkers are categorized into two main types: non-cleavable linkers and cleavable linkers. Cleavable linkers are designed to break under specific conditions within tumor cells, ensuring precise drug release with high specificity and potent drug activity, but they may be unstable in systemic circulation, leading to off-target effects. Non-cleavable linkers, on the other hand, are more stable and do not break prematurely in the body, reducing non-specific toxicity [13,14]. However, their drug release efficiency is lower because release depends on the complete degradation of the ADC within the tumor cell.
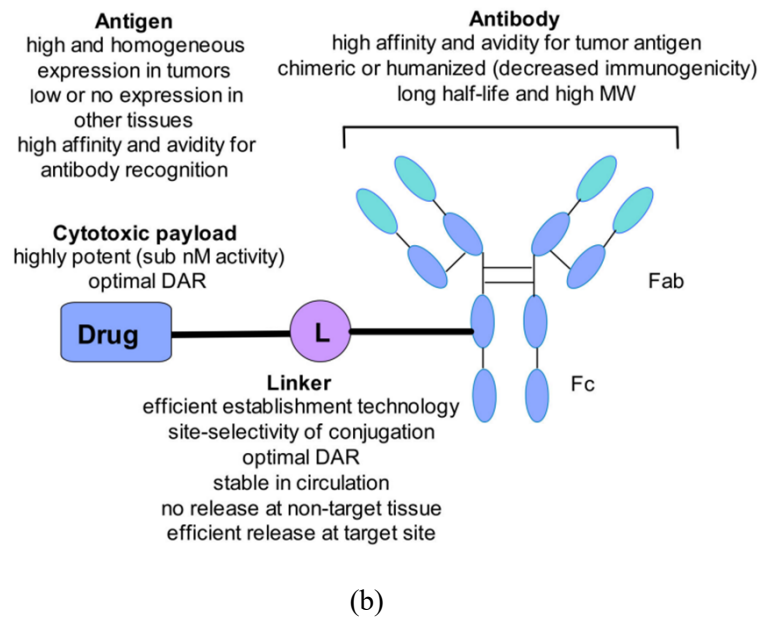

(a)

(b)

Figure 2. (a) Molecular structure of several cytotoxic payloads including microtubule disruptors and DNA-damaging agents. (b) General structure of ADC with its key components and the pivotal considerations when combining the different components [3].

3. Development and clinical use of ADCs

3.1. Treatment for hematologic malignancies

ADCs have shown varying efficacy in treating hematologic malignancies, which encompass a variety of diseases, including acute myeloid leukemia (AML), Hodgkin lymphoma (HL), anaplastic large cell lymphoma (ALCL), and acute lymphoblastic leukemia (ALL), among others. Gemtuzumab ozogamicin is a representative ADC used in the treatment of hematologic malignancies. It consists of a monoclonal antibody targeting the CD33 antigen conjugated to the potent cytotoxic agent calicheamicin via an acid-sensitive linker. The CD33 antigen is highly expressed on most AML cells, and calicheamicin, a potent DNA-damaging agent, induces DNA double-strand breaks, leading to apoptosis. Therefore, Gemtuzumab ozogamicin might be expected to have significant effects in treating AML. However, clinical trials have shown that this ADC can cause serious side effects, such as delayed hematopoietic recovery and hepatic veno-occlusive disease (VOD), indicating that further research and optimization of this ADC are needed [15, 16].

Inotuzumab ozogamicin is another potent drug for treating hematologic malignancies. It consists of a monoclonal antibody targeting the B-cell antigen CD22 conjugated to calicheamicin via an acid-sensitive linker. The CD22 antigen is highly expressed on the surface of B cells, making this ADC particularly effective in treating ALL. Like Gemtuzumab ozogamicin, the cytotoxic drug calicheamicin induces DNA double-strand breaks, leading to cell death. Clinical trial data have shown that Inotuzumab ozogamicin achieves better remission rates and patient survival outcomes for ALL compared to other treatment options, indicating its significant efficacy in treating ALL [17].

Lastly, Brentuximab vedotin is composed of a monoclonal antibody targeting the CD30 antigen conjugated to the cytotoxic agent MMAE (Monomethyl auristatin E) via a cleavable valine-citrulline dipeptide linker [18]. CD30 is highly expressed on malignant cells in Hodgkin lymphoma (HL) and anaplastic large cell lymphoma (ALCL) [19], suggesting potential therapeutic effects of this ADC for these diseases. MMAE is a potent microtubule disruptor that destroys microtubule structures, preventing cell division and leading to tumor cell death. Clinical trials have shown that Brentuximab vedotin has

higher remission and cure rates for HL and ALCL compared to other therapies[20]. However, it may cause some neurotoxicity, necessitating further evaluation of its safety and efficacy.

3.2. Treatment for solid tumors

Ado-trastuzumab emtansine is an ADC specifically designed for the treatment of HER2-positive breast cancer. It consists of the anti-HER2 monoclonal antibody trastuzumab conjugated to the cytotoxic drug DM1 (maytansinoid microtubule disruptor) via a non-cleavable linker, SMCC [21]. Ado-trastuzumab emtansine combines the targeting ability of trastuzumab with the cytotoxicity of DM1, enabling it to specifically target HER2-positive cancer cells and release DM1 inside the cells, thereby disrupting microtubule structures, inhibiting cell division, and inducing apoptosis [22,23]. In clinical trials, it has demonstrated significant antitumor activity, with improved response rates and patient survival.

Enfortumab vedotin is an ADC used for the treatment of urothelial carcinoma. It consists of a monoclonal antibody targeting the Nectin-4 antigen conjugated to the cytotoxic drug MMAE via a valine-citrulline dipeptide linker [24]. Enfortumab vedotin combines the targeting ability of the anti-Nectin-4 antibody with the cytotoxicity of MMAE, enabling it to specifically recognize and bind to the Nectin-4 antigen, which is highly expressed on the surface of urothelial carcinoma cells [25]. Once inside the cell, the linker is cleaved, releasing MMAE, which disrupts microtubule structures, inhibits cell division, and induces tumor cell death. In clinical trials, Enfortumab vedotin has demonstrated significant antitumor activity, especially in patients who have previously received platinum-based chemotherapy and PD-1/PD-L1 inhibitors, showing a high response rate [26].

Sacituzumab govitecan is used for the treatment of metastatic triple-negative breast cancer (TNBC). It consists of a monoclonal antibody targeting the Trop-2 antigen conjugated to the cytotoxic drug SN-38 via an acid-sensitive, cleavable linker (CL2A) [27]. Trop-2 is an antigen highly expressed in various epithelial cancers, and Sacituzumab govitecan combines the targeting ability of the anti-Trop-2 antibody with the cytotoxicity of SN-38. It specifically recognizes and binds to the Trop-2 antigen, which is highly expressed on the surface of TNBC cells. Once inside the cell, the linker is cleaved, releasing SN-38, a potent DNA inhibitor that suppresses DNA topoisomerase I activity, thereby preventing cell proliferation and inducing tumor cell death. In clinical trials, Sacituzumab govitecan has demonstrated a high response rate and prolonged progression-free survival in patients with metastatic TNBC who were previously unresponsive to other treatments [28,29].

Table 1. ADC types and their key features and corresponding cancers treated

ADC name	Target antigen	Cytotoxic payload	Linker	Tumor types
Gemtuzumab ozogamicin	CD33	Calicheamicin	Acid-labile hydrazone linker	Acute myeloid leukemia
Inotuzumab ozogamicin	CD22	Calicheamicin	Acid-labile hydrazone linker	Acute lymphoblastic leukemia
Brentuximab vedotin	CD30	MMAE	Valine-citrulline dipeptide linker	Anaplastic large cell lymphoma
Ado-trastuzumab emtansine	HER2	DM1	SMCC	HER2-positive breast cancer
Enfortumab vedotin	Nectin-4	MMAE	Valine-citrulline dipeptide linker	Urothelial carcinoma
Sacituzumab govitecan	Trop-2	SN-38	CL2A	Metastatic triple-negative breast cancer

3.3. Challenges and advancements of ADCs

The preceding text detailed several ADCs used in the treatment of hematologic malignancies and solid tumors, demonstrating the significant potential of this technology in cancer therapy. However, despite the attention attracted by successful ADCs, the technique still faces several challenges in clinical application. ADCs suffer from a relatively poor balance between therapeutic efficacy and toxicity, as their potent payloads can lead to severe toxic reactions at slightly higher doses, limiting the range of usable doses. Additionally, tumor cells may develop resistance to ADCs over time by downregulating target antigens, increasing drug efflux capabilities, or altering intracellular drug processing mechanisms, thereby reducing efficacy [30]. Furthermore, the development of ADCs involves complex integration of antibodies, linkers, and payload drugs, with significant manufacturing challenges and high costs, making it difficult to ensure consistency and quality control across batches [31]. Future technological advancements should focus on enhancing antibody selectivity for tumors, developing more stable linker technologies capable of precise drug release in the tumor microenvironment, exploring new efficient payloads to enhance anticancer effects, and minimizing impact on healthy tissues. Moreover, further research into biomarkers to predict patient response to ADCs for personalized treatment could improve efficacy and safety. These improvements will help expand the application of ADCs and enhance their clinical effectiveness.

4. Future improvement of ADCs

4.1. Enhance selectivity and reducing toxicity

Optimization of antibody design can enhance the binding affinity of antibodies to tumor-specific antigens on the surface of cancer cells, thereby reducing attacks on normal cells. This can be achieved through structural biology techniques combined with antibody engineering to design antibodies with higher affinity, improving recognition of cancer-specific antigens. Simultaneously, liquid biopsy techniques can be integrated to detect circulating tumor DNA or exosomes containing tumor biomarkers, allowing real-time monitoring of the patient's response to ADC therapy, and enabling treatment adjustments [32,33]. Additionally, to minimize off-target effects, future research can explore the use of humanized or chimeric antibodies to reduce the immunogenicity of ADCs against healthy cells. Modifying the Fc region of the antibody can further reduce interactions with non-target cells, thereby lowering systemic toxicity. Nanotechnology also shows promise in reducing ADC toxicity, as combining antibodies with drug-loaded nanoparticles can enhance the precision of drug release, minimizing release in non-target areas [34].

4.2. Design more stable and controllable linker

First, it is essential to ensure that the linker remains highly stable during systemic circulation to prevent the premature release of cytotoxic drugs, thereby reducing damage to healthy tissues. Non-cleavable linkers, such as the maleimidocaproyl linker used for MMAF, can significantly enhance systemic stability. Additionally, introducing hydrophilic spacers like PEG can reduce the risk of ADC aggregation and extend circulation time. Moreover, effective release in the tumor environment is crucial. Cleavable linkers can be designed to take advantage of tumor-specific characteristics, such as the cleavage of specific peptide sequences by cathepsin B to release the drug, or acid-sensitive linkers that trigger drug release in the acidic environment of the tumor lysosome [12]. Furthermore, by incorporating site-specific conjugation techniques, such as engineered cysteines or non-natural amino acids, the linker can be precisely conjugated to the antibody, reducing heterogeneity and improving drug distribution [35]. By combining these techniques, more stable linkers with efficient release characteristics in the tumor microenvironment can be designed.

4.3. Overcome drug resistance mechanism

Over time, cancer cells can develop resistance to ADCs through several pathways, including the downregulation of the target antigens that ADCs recognize or by increasing the activity of efflux pumps,

which actively expel the cytotoxic drug from the cancer cells. To address these challenges, research should focus on thoroughly understanding the underlying mechanisms of resistance. One potential strategy is to employ combination therapies that target multiple pathways simultaneously, reducing the likelihood of resistance development [36]. For example, combining ADCs with small-molecule inhibitors, immune checkpoint inhibitors, or other chemotherapeutic agents could enhance the overall therapeutic effect and prevent or delay the emergence of resistance. Additionally, modifying ADC design, such as using novel payloads or more potent linkers, can help to bypass or mitigate these resistance mechanisms, ensuring sustained efficacy in the long term [37].

4.4. Other aspects
In the future, the success of Antibody-Drug Conjugates (ADCs) in cancer treatment will increasingly depend on the principles of personalized medicine. Identifying biomarkers that can predict a patient's response to specific ADC therapies is a crucial step in improving efficacy and safety. By studying biomarkers such as tumor antigen expression levels, drug resistance mechanisms, or the genetic characteristics of tumors, companion diagnostic tools can be developed to help clinicians tailor the most suitable ADC treatment plan for individual patients. Furthermore, the complex production process of ADCs, which involves the conjugation of antibodies, cytotoxic drugs, and linkers, presents challenges in manufacturing and quality control, affecting the accessibility and cost of these therapies. Therefore, future research should focus on optimizing production processes, improving batch consistency, and reducing overall costs, making ADCs available to a broader range of patients. Although ADCs are primarily used in cancer treatment, their unique mechanism of precisely targeting specific cells holds promise for the treatment of other diseases. Future exploration of ADCs in autoimmune and infectious diseases could help reduce systemic side effects, further expanding the scope of this technology's applications.

5. Conclusion
ADC as an innovative cancer therapy, demonstrate the unique advantage of combining the targeting specificity of monoclonal antibodies with the potent cytotoxic effects of chemotherapy drugs. By selectively targeting cancer cells and releasing the drug within the cells, ADCs have made significant progress in treating various cancers, including solid tumors and hematologic malignancies. However, despite the notable therapeutic potential of ADCs, their clinical application still faces several challenges, such as toxicity management, the development of drug resistance, and the complexity and cost of manufacturing. In the future, the key lies in further optimizing the selectivity of antibodies, developing more stable and controllable linker technologies, and finding new, highly effective cytotoxic payloads. Additionally, the development of personalized treatment strategies and biomarkers for different patients will help enhance the efficacy and safety of ADCs. Overall, with continuous technological advancements and in-depth research, ADCs are expected to become an important tool in the field of cancer treatment, significantly improving patient outcomes and quality of life.

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