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The ABC of ADCs (Antibody-Drug Conjugates): A Comprehensive Review of Technical, Regulatory, and Clinical Challenges

Kishore Kumar Hotha

Veranova, Devens, MA, USA
Email: drhotha@gmail.com

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Abstract

Over the past several decades, there has been a significant surge in the development of Antibody-Drug Conjugates (ADCs). Designing an ideal ADC presents a multifaceted challenge, requiring the precise orchestration of various elements such as antigens, antibodies, linkers, and payloads. While ADCs aim to target tumor cells specifically, several antigens can also be found in regular tissues, potentially compromising the specificity of ADCs in therapeutic applications. The complexity extends to antibody selection, necessitating effective targeting of the desired antigen and ensuring compatibility with linkers for effective payload delivery. Additionally, the linker and payload combination are critical for the ADC's therapeutic efficiency, balancing stability in circulation and timely payload release upon target binding. ADC doses must be safe for normal tissues while ensuring the released payloads are effective. The success of ADCs is attributed to their unmatched efficacy compared to traditional chemotherapy agents. The current research article aims to provide a technical review of Antibody-Drug Conjugates (ADCs) for cancer therapies. A brief discussion on the basics of ADCs, regulatory approach, overview, and technical complexities for quantification is presented. This review also summarizes recently approved ADCs and introduces the concepts of antibodies, linkers, and payloads. The article also outlines cancer-specific ADCs currently in late-stage clinical trials for cancer treatment.

Keywords

Antibody-Drug Conjugates, Cancer Therapy, Payload, Linker, Conjugation Chemistry, Antibody, Analytical Development, Manufacturing of ADCs

1. Introduction

Cancer therapy has undergone a remarkable transformation since the 20th cen-

tury, particularly by introducing biological products that offer excellent selectivity for different types of antitumor drugs [1]. Traditional chemotherapy, while effective, has significant limitations, most notably its non-specific nature, which inadvertently damages healthy cells, resulting in severe side effects. On the other hand, while monoclonal antibodies have emerged as a promising solution due to their precise targeting abilities, their efficacy against solid tumors is compromised due to poor penetrability owing to their large molecular size [1].

In the quest to overcome these challenges, recent innovations in tumor-targeted therapy have garnered significant attention thanks to their potent anti-tumor activities and excellent targeting properties. ADCs stand out as a groundbreaking advancement. Traditional drug substance manufacturing involves creating the active pharmaceutical ingredient (API) that renders the desired therapeutic effect. It can encompass organic synthesis, fermentation, and crystallization [1]. Conversely, ADC manufacturing is a multi-faceted endeavor. It involves the meticulous production of a monoclonal antibody, a cytotoxic drug, and a linker molecule, which are subsequently conjugated to form the ADC (**Figure 1**). The complexity doesn't end at synthesis; the purification of ADCs demands the removal of unconjugated components, ensuring a consistent drug-to-antibody ratio crucial for efficacy and safety [2]. This inherent intricacy in ADC production necessitates more rigorous quality control and regulatory oversight, reflecting the diverse nature of the molecule and its potential therapeutic implications [2].

The clinical properties of ADCs depend on the characteristics of all three of these components. The mechanism of action of ADCs is complex, releasing its payload upon drug internalization followed by intracellular processing [2]. Unlike many standard oncological therapies, ADCs must act upon cancer cells for optimal effectiveness. An improved understanding of the interactions between ADCs and tumors is essential to realize this drug class's true potential for cancer treatment [2]. The most common linkers are statistical cysteine, statistical lysine, and engineered cysteine (site-specific). Typical payloads include auristatin, PBDs, and irinotecan, with a trend towards increased diversity. Combining these elements creates various ADC molecules currently in pre-clinical and clinical development [3].

The critical component in the function of ADCs is the linker that connects the antibody to the cytotoxic payload (**Figure 2**). The real challenge in developing linkers is ensuring high circulation stability and the payload's specific release in the target tissue. The linker can be either cleavable or non-cleavable, with the linkage achieved through various conjugation technologies. A cleavable linker possesses a chemical trigger that can be efficiently cleaved to release the cytotoxic payload within the tumor. Most of the clinically approved ADCs utilize cleavable linkers. In contrast, non-cleavable linkers lack chemical triggers, and the linker remains part of the payload. This type of linker has only been used in ado-trastuzumab emtansine (Kadcyla, T-DM1) among the approved ADCs [1].

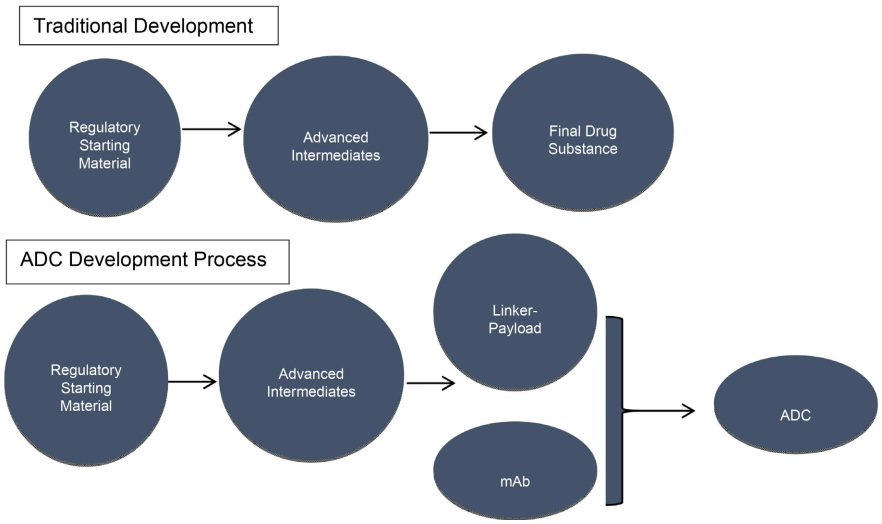

Figure 1. Overview of traditional drug development & ADC development.

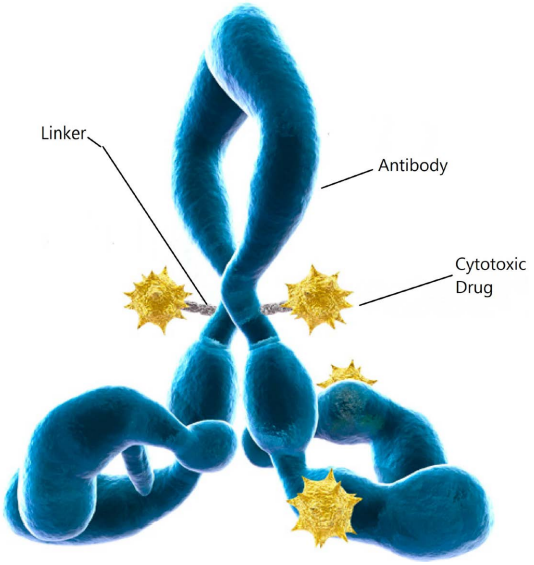

Figure 2. Antibody drug conjugate (Figure reference adapted from http://www.biooncology.com/).

2. Overview and Current State of ADCs

The FDA approved the first ADC drug in 2000. However, the subsequent decade saw no new ADC approvals. Since 2011, there's been a significant uptick in the number of FDA-approved ADC drugs. By the end of 2020, nine ADCs had received approval, highlighting an accelerating trend in ADC clinical development. As of the latest data, the FDA has approved 14 ADCs for cancer treatment, as detailed in the table below [3]. These ADCs address a range of cancer types, encompassing breast, lymphoma, leukemia, and multiple myeloma. The development of ADCs is ongoing, with a plethora of these drugs currently in clinical trials. The FDA is also in the process of reviewing several new ADCs. As of March 8, 2023, 18 ADCs are under the FDA's evaluation. These potential new entries

target various cancers, including but not limited to breast, lung, colorectal, and pancreatic cancer. In reviewing ADCs, the FDA adopts a rigorous stance [4]. The agency meticulously assesses the safety and efficacy of these drugs, paying heed to potential off-target effects. The FDA's ultimate commitment is to ensure that ADCs are safe and effective for cancer patients (**Table 1**). Over nearly three decades since 1997, the landscape of ADC clinical trials has significantly evolved. Beginning with the pioneering ADC clinical trial, 266 ADCs have been brought under the investigative lens across more than 1200 clinical studies. Distinctly, 54 ADC initiatives reached an endpoint and were officially terminated, while another 38 faded from corporate development pipelines. For clarity in this analysis, the ADCs are stratified into three groups: Endorsed (those approved by the FDA), Operational (those in active clinical trials but without FDA approval) and Retired (those no longer present in a company's development pathway, irrespective of a formal discontinuation declaration). As illustrated in **Figure 3**, it's pertinent to note that every ADC that has gained FDA endorsement also enjoys approval in several countries outside the United States [4] [5]. Yet, to avoid redundancy, these globally approved ADCs have been excluded from the "Operational" count, emphasizing the rigorous standards and wide-reaching impact of ADC research (**Figure 3**).

3. Regulatory Considerations

As ADCs enter clinical development and reach the market, it is important to understand the regulatory requirements for these products in different countries. Globally, there is a growing trend towards regulating ADCs as biologics. However, there is still some variation in the regulatory requirements for ADCs in different countries.

In the United States, ADCs are regulated as combination products by the Food and Drug Administration (FDA). This means that both the small molecule drug and the monoclonal antibody must be approved by the FDA before the ADC can be marketed. The FDA has issued draft guidance for the clinical pharmacology of ADCs, but there is no specific guidance for the CMC aspects of ADCs [6] [7] [8].

In the European Union, ADCs are regulated as biologics by the European Medicines Agency (EMA). The EMA has issued some limited guidance on the CMC aspects of ADCs, with more promised [9]. In Japan, ADCs are regulated as new active substances by the Pharmaceuticals and Medical Devices Agency (PMDA). This means that ADCs must undergo a full clinical and non-clinical development program before they can be marketed. In Canada, ADCs are regulated as biologics by Health Canada. Health Canada has issued draft guidance on the CMC aspects of ADCs, as well as clinical and non-clinical guidance. In Brazil, ADCs are regulated as biologics by the National Health Surveillance Agency (ANVISA). ANVISA has not issued any specific guidance on the CMC aspects of

Table 1. Overview of Recently approved ADC's.

Year	ADC	Target	Cancer Type	Remarks
2000	Gemtuzumab ozogamicin (Mylotarg)	CD33	Acute myeloid leukemia (AML)	Discontinued in 2010, reapproved in 2017.
2011	Brentuximab vedotin (Adcetris)	CD30	Anaplastic large cell lymphoma (ALCL), Hodgkin lymphoma, systemic anaplastic large cell lymphoma (sALCL), cutaneous T-cell lymphoma (CTCL)	
2013	Ado-trastuzumab emtansine (Kadcyla)	HER2	Breast cancer, gastric cancer, metastatic urothelial carcinoma	
2017	Inotuzumab ozogamicin (Besponsa)	CD22	B-cell acute lymphoblastic leukemia (ALL)	
2017	Gemtuzumab ozogamicin (Mylotarg)	CD33	Acute myeloid leukemia (AML)	
2018	Moxetumomab pasudotox (Lumoxiti)	CD22	Hairy cell leukemia (HCL)	
2019	Polatuzumab vedotin-piiq (Polivy)	CD79b	Diffuse large B-cell lymphoma (DLBCL)	
2019	Enfortumab vedotin (Padcev)	Nectin-4	Urothelial carcinoma	
2019	Trastuzumab deruxtecan (Enhertu)	HER2	Breast cancer, gastric cancer	
2020	Sacituzumab govitecan (Trodelvy)	Trop-2	Metastatic triple-negative breast cancer (mTNBC), metastatic urothelial carcinoma	
2021	Belantamab mafodotin-blmf (Blenrep)	BCMA	Multiple myeloma	
2021	Loncastuximab tesirine-lpyl (ZYNLONTA)	CD19	Diffuse large B-cell lymphoma (DLBCL), high-grade B-cell lymphoma (HGBL), primary mediastinal large B-cell lymphoma (PMBCL)	
2021	Tisotumab vedotin-tftv (Tivdak)	Trop-2	Recurrent or metastatic cervical cancer	
2022	Mirvetuximab soravtansine (ELAHERE)	Folate receptor alpha (FRα)	Ovaian cancer, fallopian tube cancer, primary peritoneal cancer	Discontinued in August 2023 due to commercial considerations.
2023	Tesetaxel (Padcev T)	Trop-2	Metastatic urothelial carcinoma (mUC)	
2023	Tucatinib-trastuzumab vedotin (T-DXd)	HER2	Metastatic breast cancer	

ADCs. In China, ADCs are regulated as biologics by the National Medical Products Administration (NMPA). The NMPA has issued draft guidance on the CMC aspects of ADCs, as well as clinical and non-clinical guidance.

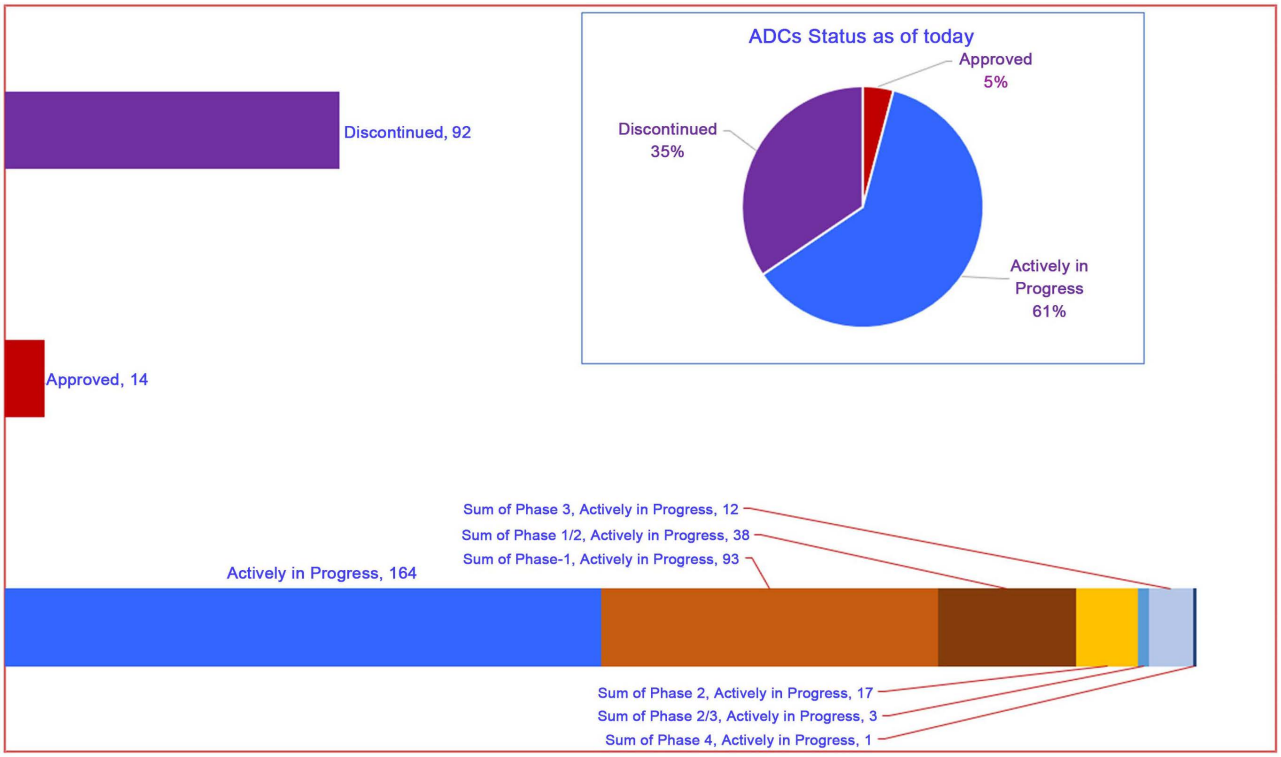

Figure 3. Current Status of ADCs.

In Korea, ADCs are regulated as biologics by the Ministry of Food and Drug Safety (MFDS). The MFDS has issued draft guidance on the CMC aspects of ADCs, as well as clinical and non-clinical guidance [9] [10] [11] [12].

Notably, the FDA classifies ADCs as biologics rather than chemically synthesized entities. For generic small molecules, bioequivalence to an FDA-approved reference-listed drug is required. In contrast, the approval process for ADCs is more intricate. Applicants for generic ADCs must showcase similarity to the reference product, ensuring clinical significance in safety, purity, and potency. While these rigorous regulatory stipulations provide robust market protection against subsequent competition, exceptions can be made. If ADC generic manufacturers employ a well-established antibody, linker, or payload, which are perceived as safer and more cost-effective, they could gain a competitive and clinical edge [9]. However, if the conjugation in ADCs affects the safety, purity, or potency of an already licensed antibody, licensing and exclusivity provisions will be adjusted accordingly (**Table 2**).

4. Linkers and Payloads

The antibody provides specificity to cancer cells, while the linker connects the antibody to the payload, ensuring the cytotoxic drug reaches its target. With over 100 ADCs currently in clinical trials and an expanding developmental landscape, the search for the perfect linker and payload is gaining momentum (**Table 3**).

Linkers, crucial for ensuring stability and the timely release of the payload, have

Table 2. Regulatory considerations of ADC's.

Country	Health authority	Availability of the ADC Guidance	ADC CMC Regulated as a biologic?	Source
United States	FDA	No	No	Draft guidance available on Clinical Pharmacology Considerations for Antibody-Drug Conjugates. Regulated as combinational products through small molecules and biologics.
European Union	EMA	No	Yes	Same as FDA guidance regulated as combinational, new substance through complete application and mAb and drug-linker are considered as drug substance intermediates
Japan	PMDA	No	Yes	JNDA Procedure to be followed for submission. Both small molecule and biological regulations are applied.
Canada	Health Canada	No	Yes	No added Perceptions
Brazil	ANVISA	No	Yes	No added perceptions
China	NMPA	Yes, in Draft	Yes	Draft CMC for clinical and nonclinical drafts available
Korea	MFDS	No	Yes	Both small and large molecules are applicable. High-level requirements for ADC CMC Information

Table 3. Linkers and Payloads approved by FDA.

Linker	Payload	Payload Class	Cyclic	Approval Status
Maleimidocaproyl (MC)	Monomethyl auristatin E (MMAE)	Auristatin	Yes	Approved
Maleimidocaproyl (MC)	Monomethyl auristatin F (MMAF)	Auristatin	Yes	Approved
Valine-citrulline (VC)	Monomethyl auristatin E (MMAE)	Auristatin	Yes	Approved
Valine-citrulline (VC)	Monomethyl auristatin F (MMAF)	Auristatin	Yes	Approved
Glucuronide	Monomethyl auristatins (MMAs)	Auristatin	Yes	Approved
Glucuronide	Maytansines (MTs)	Maytansine	Yes	Approved
Glucuronide	Duocarmycins (DCs)	Duocarmycin	Yes	Approved
Hydrazone	Monomethyl auristatins (MMAs)	Auristatin	Yes	Approved
Hydrazone	Maytansines (MTs)	Maytansine	Yes	Approved
Hydrazone	Duocarmycins (DCs)	Duocarmycin	Yes	Approved
Disulfide	Monomethyl auristatins (MMAs)	Auristatin	Yes	Approved
Disulfide	Maytansines (MTs)	Maytansine	Yes	Approved
Disulfide	Duocarmycins (DCs)	Duocarmycin	Yes	Approved
Click chemistry	Monomethyl auristatins (MMAs)	Auristatin	Yes	Approved
Click chemistry	Maytansines (MTs)	Maytansine	Yes	Approved
Click chemistry	Duocarmycins (DCs)	Duocarmycin	Yes	Approved
Click chemistry	Pyrrolobenzodiazepines (PBDs)	PBD	No	Approved

undergone refinements to enhance therapeutic indices. Meanwhile, payloads dictate the ADC's potency in eradicating cancer cells with minimal adverse effects [4] [10]. As the arsenal of available linkers and payloads grows, the ADC community relentlessly pursues combinations that maximize efficacy and minimize toxicity, signaling that the ADC era is just starting.

5. Cleavable vs. Non-Cleavable Linkers in Antibody-Drug Conjugates (ADCs)

5.1. Cleavable Linkers

Cleavable linkers utilize the differences between tumor microenvironments and standard physiological conditions to release their drug payload. This release might cause a 'bystander effect,' where the freed drug affects nearby cells not directly targeted by the ADC. While this can amplify the therapeutic impact, it also poses risks: cleavable linkers are more vulnerable to unintended, off-target toxicities. Many clinical trials lean towards dipeptide, disulfide, and enzyme-cleavable linkers. The challenge lies in enhancing these linkers' stability during circulation, spurring continued research into their modification and optimization [1] [11] [12].

Enzyme-cleavable linkers, such as those targeting cathepsins, are crafted to be cleaved by enzymes overexpressed in tumor tissues. This specificity adds another targeting layer, further ensuring drug release exclusively at the intended site.

5.2. Non-Cleavable Linkers

When an ADC binds to its target via the antigen-antibody interaction, non-cleavable linkers demand the antibody component's degradation before the drug is released. This mechanism could diminish the bystander effect. However, the advantages of non-cleavable linkers encompass enhanced stability and a reduced risk of unintended side effects. These linkers might also be effective against multi-drug resistant (MDR) tumors. The challenge hinges on the ADC's design: the internalization and lysosomal degradation of the antibody are crucial for activating the payload. Moreover, payloads connected to polar amino linkers necessitate specialized transport mechanisms to transition from the lysosome to the cytoplasm [1] [11] [12].

5.3. Choosing between Cleavable and Non-Cleavable Linkers

The decision between the two types of linkers hinges on the anticipated therapeutic outcome and the specifics of the targeted tumor. About two-thirds of ADCs in clinical trials use cleavable linkers, especially dipeptide, disulfide, and enzyme-cleavable variants. While cleavable linkers offer a more direct mechanism of action, their vulnerability to off-target toxicities is challenging. Conversely, their superior stability makes non-cleavable linkers require a more nuanced ADC design due to their drug release mechanism [13] [14] [15].

The type of linker used in an ADC can affect the efficacy and safety of the drug. For example, maleimide linkers are often used because they are relatively

stable in the bloodstream but can also be toxic to healthy cells. Glutathione-cleavable linkers are less harmful to healthy cells but can be less effective at killing cancer cells. Anthracyclines are very effective at killing cancer cells but can also be toxic to healthy cells. Topoisomerase I inhibitors are less harmful to healthy cells but can be less effective at killing cancer cells. Researchers are constantly looking for new ways to improve the efficacy and safety of ADCs (**Table 4**). One of the most promising research areas is the development of new linkers and payloads. By developing new linkers and payloads, researchers hope to create ADCs that are more effective at killing cancer cells and less toxic to healthy cells [13] [14] [15].

An interesting comparison is between Kadcyla™ and Enhertu™. The latter uses a cleavable linker and has shown promising results, especially in tumors with varied levels of the HER2 antigen. Recent trials showed Enhertu™ outperforming Kadcyla™ in efficacy, although both presented with similar safety profiles. However, it's essential to consider other factors like payload type and tumor properties, which might also influence these outcomes [4].

Lastly, the linker's physical properties can make a difference too. Some studies suggest that linkers with higher water affinity can enhance the solubility and pharmacokinetic traits of ADCs, especially those paired with less water-friendly drug compounds [4].

In antibody-drug conjugates (ADCs), linkers are typically categorized into two primary classes: cleavable and non-cleavable (**Figure 4**). A significant portion, about 53% of clinical ADCs, employs cleavable linkers, making them the more prevalent choice. Notably, among approved ADCs, ten out of eleven harness the power of protease-cleavable linkers. On the other hand, non-cleavable linkers are incorporated in 16% of clinically examined ADCs, with Blenrep™ being one of the ADCs showcasing their utility. Only Kadcyla™, among approved ADCs, opts for a non-cleavable linker. The type of linker they employ remains unspecified for a sizable 31% of ADCs under clinical examination.

6. Payloads in Antibody-Drug Conjugates (ADCs)

The cytotoxic agent often called the payload, is a crucial component of ADCs. An ideal payload should exhibit high cytotoxicity at low concentrations to effectively target tumor cells. The choice of payloads depends on several parameters, including solubility, hydrophilicity, permeability, and modifiability. The design also needs to account for potential challenges such as aggregation, the bystander effect, and stability in circulation [4] [11] [15] [16]. In ADCs, selecting payloads, which are the active drug components, plays a pivotal role in their efficacy. Broadly, four main classes of payloads have garnered significant attention in research and development. First, the microtubule inhibitors, which stand out as the most popular choice, comprise 61% of all payloads undergoing clinical trials. Their prominence is further solidified by the fact that seven of the eleven FDA-approved ADCs use them. Second, DNA-damaging agents comprise 18% of ADCs in clinical studies. The targeted small molecules, which encompass approximately

Table 4. Types of linker features and its details and features.

Type of Linker	Details/Features
Enzyme Cleavable Linkers	
Enzyme Activable Linkers	- Specifically cleaved by tumor-associated enzymes. - The Glu-Val-Cit linker demonstrates outstanding activity and stability.
Sulfatase-Cleavable Linkers	- Cleaved by enzymes in the lysosome, particularly sulfatases. - Noted stability in both human and mouse plasma, and efficient payload release.
Galactosidase Cleavable Linker	- Targeted cleavage by β-Galactosidase present in the lysosome. - Exhibits increased potency in certain ADC designs.
Lysosomal Protease-Sensitive Linkers/	- Specifically cleaved by lysosomal proteases, such as cathepsin B. - Val-Cit stands as a frequently utilized peptide-based linker in ADC construction.
Peptide-Based Linkers	
Glucuronide Linker	- Cleaved by the β-glucuronidase enzyme. - Its hydrophilic nature makes it suitable for hydrophobic payloads.
Chemically Cleavable Linkers	
Acid Sensitive Cleavable Linker	- Undergoes cleavage in acidic milieus. - Generally not recommended for highly cytotoxic drugs.
Glutathione-Sensitive Disulfide	- Designed to be cleaved in environments with elevated glutathione, commonly found in cancer cells.
Non-Cleavable Linkers	- Require complete degradation of the antibody to release the payload. - Superior stability in plasma.

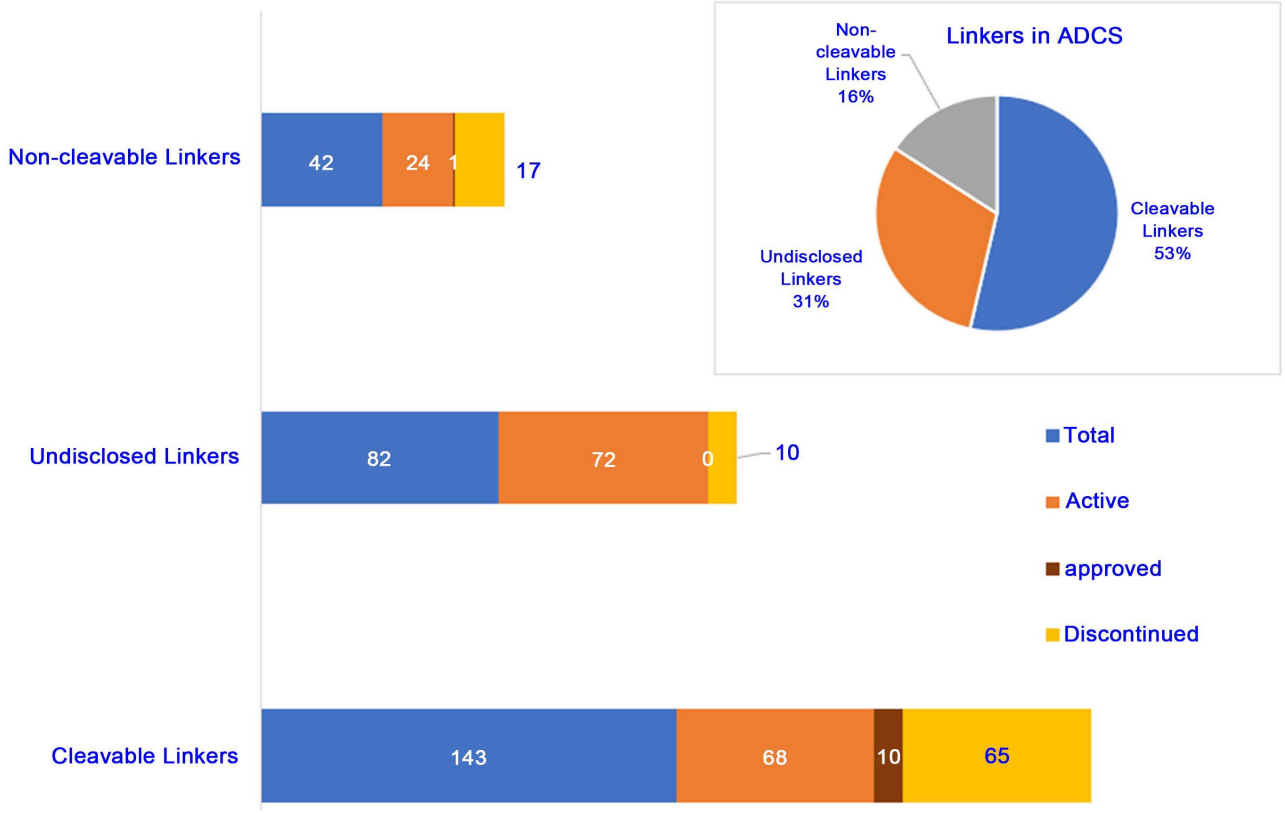

Figure 4. Current status of linkers.

5% of ADCs, are carving a niche for themselves, even though they haven't secured FDA approval yet (**Figure 5**). It's also worth noting that for 16% of the ADCs under clinical evaluation, the specific nature of the payload remains undisclosed [4].

6.1. DNA Damaging Payloads

6.1.1. Double Strand Break Agents

Calicheamicin induces DNA cleavage by binding to the minor groove. ADCs such as gemtuzumab ozogamicin and inotuzumab ozogamicin utilize calicheamicin as a payload, targeting CD33 and CD22 antigens, respectively.

6.1.2. Topoisomerases

Topoisomerase I Enzymes: Compounds like camptothecins (topotecan, irinotecan, and belotecan) inhibit DNA re-ligation, leading to DNA strand breaks and subsequent cell death. SN-38, the active metabolite of irinotecan, is used in the ADC sacituzumab govitecan.

Topoisomerase II Inhibitors: Agents such as anthracyclines (doxorubicin, daunorubicin, epirubicin, idarubicin) induce double-strand breaks in DNA. Among these, epirubicin boasts a prolonged half-life, while idarubicin's lipophilic nature ensures enhanced cellular uptake compared to daunorubicin.

6.1.3. Alkylating Agents

Compounds like duocarmycin and the indolinobenzodiazepine dimer-IGN irreversibly alkylate DNA, triggering cell death. Duocarmycin is featured in anti-HER2 ADCs like SYD983 and remains potent, even against multi-drug resistant (MDR) tumors.

6.1.4. Crosslinkers

Pyrrolobenzodiazepines (PBD) covalently bind to the minor groove of DNA, forming crosslinks that prevent cell division. This method mitigates the potential for drug resistance. The ADC Vadastuximab taurine, which targets CD33, utilizes PBD as its payload.

6.2. Payloads Inhibiting Tubulin Polymerization

1) Maytansinoids: DM1 and DM4 target microtubules, inhibiting cell proliferation during mitosis. ADCs that incorporate DM1 include Trastuzumab-MCC-DM1 (T-DM1) and lorvotuzumab mertansine. These ADCs differ in linker compositions: T-DM1 utilizes a non-cleavable linker, while lorvotuzumab mertansine opts for a cleavable one.

2) Auristatins: MMAE and MMAF obstruct tubulin polymerization, leading to cell cycle arrest in the G2/M phase. Brentuximab vedotin, an ADC targeting CD30, features MMAE as its payload. Likewise, polatuzumab vedotin, which targets CD79b, employs MMAE in treating specific lymphomas.

These payloads form the foundation for effective ADCs, ensuring targeted tumor cell destruction. However, the therapeutic index of the ADC and potential

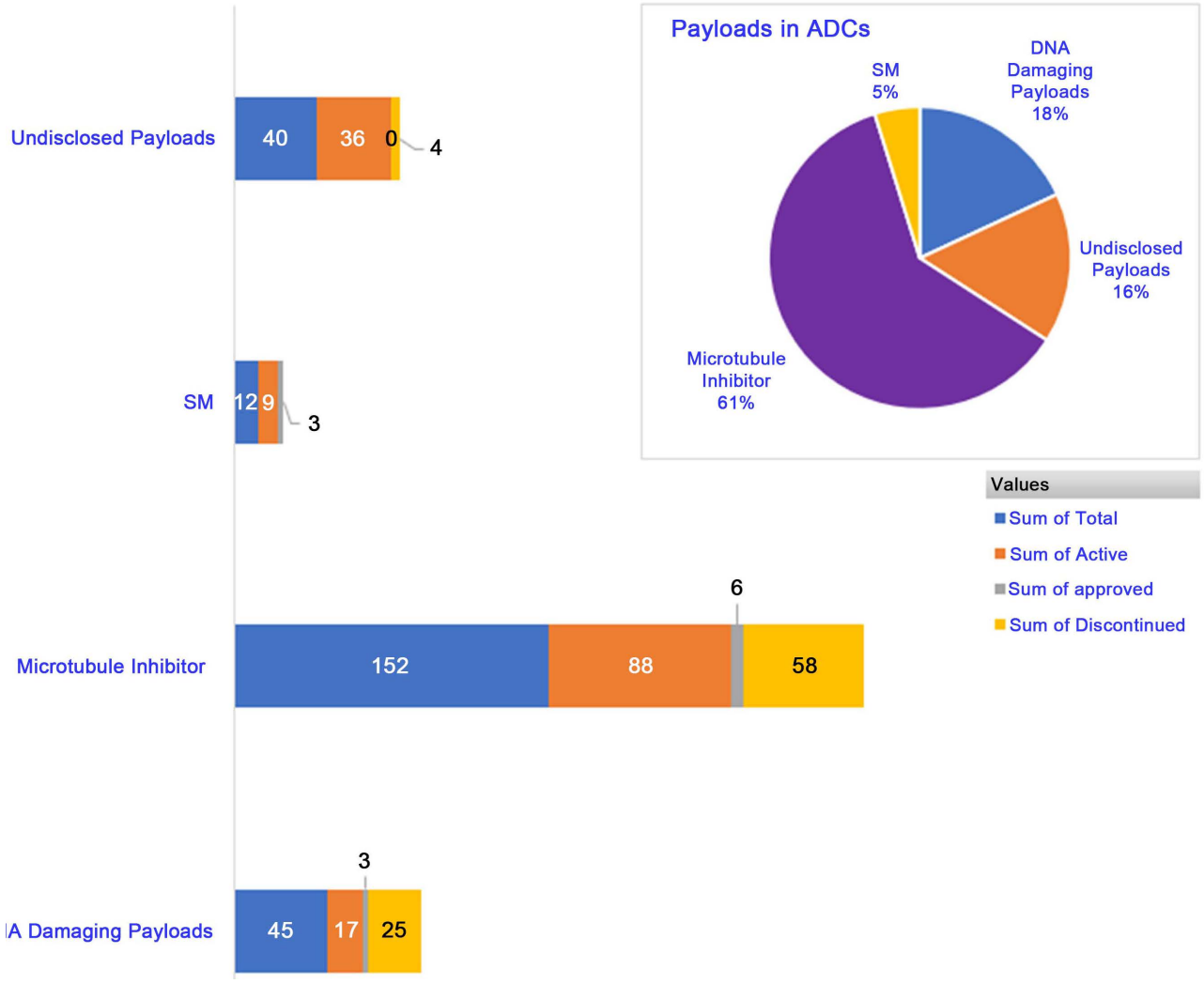

Figure 5. Current status of payloads.

side effects are dictated not solely by the payload but also by the antibody's specificity and the stability of the linker. As a result, the design of ADCs remains a collaborative endeavor, interweaving insights from molecular biology, pharmacology, and medicinal chemistry to provide targeted and potent therapies (Table 5).

7. Synthesis of ADC Linker and Payloads

ADC linkers are vital components of ADCs, integral to their stability, payload release, and efficacy. Synthesizing these linkers is challenging due to their chemical complexity [16] [17]. These linkers consist of multiple chemical entities that necessitate sequential assembly. Their chemical and biophysical attributes significantly impact the stability and release of the payload within target tissues.

The synthesis process demands numerous steps and meticulous purification to achieve the desired purity [16]. Advanced synthetic methods, such as protective group chemistry and reaction optimization, are essential for obtaining the

Table 5. Types of payload examples.

Type	Payload/Examples
DNA Damaging Payloads	
Double Strand Break Agents	Calicheamicin (gemtuzumab ozogamicin, inotuzumab ozogamicin)
Topoisomerase I Enzymes	Sacituzumab govitecan
Topoisomerase II Inhibitors	Doxorubicin, epirubicin, daunorubicin, idarubicin
Alkylating Agents	Duocarmycin, SYD983, SYD985
Crosslinkers	Pyrrolobenzodiazepines (PBD), Vadastuximab talirine
Payloads Inhibiting Tubulin Polymerization	
Maytansinoids	Trastuzumab-MCC-DM1 (T-DM1), lorvotuzumab mertansine
Auristatins	Brentuximab vedotin, Polatuzumab vedotin

specific chemical structure. Once synthesized, the end product requires rigorous characterization using tools like HPLC, mass spectrometry, and NMR spectroscopy to confirm its purity and efficacy [16] [17].

Linker design is crucial for ADCs' systemic circulation stability. It ensures the ADCs retain their potency in circulation while enabling the specific release of the payload in the target tissues. Their design influences the *in vivo* stability of the conjugate, which subsequently affects the ADCs' pharmacokinetic, efficacy, and toxicity profiles [18]. The structural integrity of the conjugate heavily depends on the linker's stability, which can be tailored by adjusting the linker's chemical structure. Notably, cleavable linkers release their payload through enzymatic cleavage in target tissues, while non-cleavable ones require the complete degradation of the ADC [18].

Translating the synthesis of ADC linkers from a small laboratory scale to a large-scale manufacturing setting presents challenges, such as ensuring reproducibility, purification, and yield consistency [16] [18]. As more ADCs transition into clinical trials, the scalability of these synthesis processes becomes paramount. An exciting advancement in this area is the scalable synthesis approach for uncommon chemotypes like methyl adamantane, spotlighting the potential of leveraging such techniques for broader research.

Safety is another concern, given the potential toxicity of ADC linkers and payloads [16] [17]. They mandate cautious handling, protective gear usage, and stringent testing to ascertain the safety of the resultant product. Creating ADCs entails rigorous R&D to refine design and mitigate toxicity threats.

In summary, the synthesis and development of ADC linkers necessitate expertise spanning synthetic chemistry, molecular biology, and toxicology [16] [18]. Their attributes play a pivotal role in the overall performance of ADCs. Continued research is imperative to unearth novel linkers with enhanced characteristics

and streamline ADC synthesis and manufacturing processes.

8. Analytical Development of ADCs

Ensuring the paramount quality, safety, and efficacy of Antibody-Drug Conjugates (ADCs) necessitates the establishment of meticulous analytical methodologies. These methods are imperative to corroborate the ADC's identity, purity, potency, conjugation chemistry, and retention of stability during storage. The synergy of monoclonal antibodies' precision targeting with potent therapeutic agents in ADCs enhances the therapeutic assault on malignant cells, while simultaneously minimizing collateral damage to healthy tissues. Comprehensive analytical scrutiny of ADCs demands a multifaceted approach, ensuring the integrity and performance of both the linker and the payload components [12] [13] (Table 6).

1) Characterization and Stability of the Linker: The linker in ADCs connects the antibody to the cytotoxic payload. It's paramount to employ analytical methodologies to evaluate the resilience and integrity of this linker, especially under varied conditions *in vivo* and *in vitro* settings. Leading-edge techniques like high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy are the frontrunners for this intricate characterization [13] [14] [15].

2) Drug-to-Antibody Ratio (DAR) Ascertainment: A typical ADC involves conjugating multiple therapeutic drug entities to a single antibody. The DAR is a pivotal metric denoting the average number of drug molecules attached to each antibody unit. Precision-driven analytical tools, such as liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis (CE), are vital for deciphering the DAR, a cornerstone in evaluating the ADC's therapeutic strength and efficacy [16] [17].

Table 6. Analytical challenges and overview of ADCs.

Analytical Aspect	Techniques/Tools Used	Current Trends
Characterization and stability of the linker	HPLC, MS, NMR	Advanced techniques such as NMR are becoming increasingly common.
Drug-to-antibody ratio (DAR) ascertainment	LC-MS, CE, ELISA	The development of more sensitive and accurate DAR assays is a current trend.
Evaluating the potency of the payload	Cell viability assays, ELISA, flow cytometry	Developing more sensitive and specific assays for evaluating payload potency is a current trend.
Assessment of purity and detection of impurities	HPLC, MS, gel electrophoresis	The use of high-resolution mass spectrometry (HRMS) is becoming increasingly common.
Stability profiling	Varies depending on the aspect of stability studied (e.g., HPLC for degradation pathways)	The development of more predictive stability models is a current trend.
Bioanalytical assay deployment	ELISA, LC-MS, immunoassays	The development of more sensitive and specific bioanalytical assays is a current trend.

3) Evaluating the Potency of the Payload: Rigorous assessment of the cytotoxic payload's potency within ADCs is crucial, ensuring their formidable efficacy against cancerous targets. Cutting-edge analytical techniques, like cell viability assays, enzyme-linked immunosorbent assays (ELISA), and flow cytometry, are pivotal for assessing the drug's potential to induce cellular apoptosis.

4) Assessment of Purity and Detection of Impurities: ADCs demand an analytical regimen that attests to the impeccable purity of the linker and payload components and remains vigilant against potential impurities, quantifying them if they emerge. Forefront modalities like HPLC, mass spectrometry, and gel electrophoresis ensure purity and ward off impurities.

5) Stability Profiling: In the realm of ADCs, stability is paramount, ensuring these bio-conjugates maintain their functional integrity and therapeutic efficacy from research to clinical application. Comprehensive analytical techniques delve into evaluating their physical and chemical robustness, tracing potential degradation pathways, and identifying any resultant degradation byproducts.

6) Bioanalytical Assay Deployment: For a thorough ADC assessment, bioanalytical assays are fundamental. These assays scrupulously quantify the ADC, its linker, and payload concentrations across different biological matrices such as blood, plasma, and tissue specimens. Tools like ELISA, LC-MS, and specialized immunoassays provide intricate insights into the pharmacokinetics (PK) and pharmacodynamics (PD) profiles of ADCs [18].

9. Challenges in ADC Application

1) Dynamic Pharmacokinetics: The fluctuating nature of ADC pharmacokinetics complicates predicting the concentration of the different components at varied timeframes post-administration. It poses hurdles in constructing precise PK/PD models, which are pivotal for deciphering the safety and effectiveness of ADCs. Furthermore, the dynamics of ADC pharmacokinetics can complicate personalized treatments. The most beneficial dose and regimen of an ADC might shift based on patient-specific factors like tumor classification, tumor magnitude, and immunological status. Nevertheless, despite these hurdles, ADC development remains a beacon of hope in oncological treatment. By melding the targeting precision of antibodies with the cytotoxic prowess of drugs, ADCs hold the potential to deliver targeted treatments to malignant cells, sparing normal cells from undue harm. Active research is underway to surmount the challenges presented by dynamic pharmacokinetics in ADC applications. These endeavors encompass the innovation of novel linker mechanisms, the introduction of cutting-edge bioanalytical assays, and pioneering strategies for tailoring ADC treatments [19] [20].

2) Inherent Adverse Effects: A majority of ADCs have been associated with serious hematotoxicity. The unintended consequences of ADCs frequently yield side effects similar to conventional chemotherapy. Some anti-HER2 ADCs have demonstrated potential pulmonary toxicity, underscoring the urgency for vigi-

lant patient monitoring [21].

3) Tumor Penetration and Drug Release: The sizable molecular weight of ADCs impedes their efficient penetration into tumors. Post ADC-tumor antigen binding, the intracellular delivery of cytotoxic drugs hinges on effective endocytosis. The variance in antigen expression and subsequent drug dispensation further muddies this dynamic.

4) Developing Drug Resistance: Tumors often exhibit resilience against ADC interventions, evident through diminished antigen expression, modulated intracellular processes, or direct payload resistance. This composite resistance considerably undermines ADC's effectiveness [22].

5) Visions for Upcoming ADC Generations: Though ADCs have emerged as an encouraging novel class of oncological interventions, they have limitations, including restricted tumor penetration and the emergence of drug resistance. The upcoming generation of ADCs aims to surmount these challenges by several factors as follows

6) Modified Monoclonal Antibodies: Directing their focus on oncogenic mutant proteins can heighten therapeutic specificity. Furthermore, deploying bispecific antibodies that target multiple antigens concurrently has showcased potential in optimizing ADC internalization and drug transport [23].

7) ADC Structural Innovations: Leveraging CDMO services and streamlining tech transfers, the integration of smaller molecular elements, like polypeptides or single-chain variable fragments, can amplify tumor infiltration. This strategy is specifically designed to boost ADC efficiency, especially for elusive tumor locations [24] [25].

8) Payload Innovations: The new wave of payloads isn't restricted to conventional cytotoxic agents but encompasses targeted drugs and immunotherapies. This diversification is envisioned to counteract drug resistance and amplify therapeutic potency.

Next-generation ADCs also promise heightened specificity, potency, and stability. They're being crafted to target novel tumor variants and harmonize with other cancer treatments. The rapid advancements in ADC development echo these therapies' transformative potential for cancer management [26].

10. Conclusions

ADCs herald a new epoch in precision-based cancer interventions. The synthesis of antibody precision and chemotherapeutic power holds the promise of strategically targeting malignant cells, sparing healthy tissue. Yet, their intricate design presents daunting challenges.

Systemic toxicity remains a prime concern despite ADCs' transformative targeting prowess. The reality is that only a fraction of the administered ADCs reach tumors, which stokes apprehensions about indiscriminate payload releases. Such unintentional discharges, given the payloads' acute toxicity, can give rise to severe side effects, imposing constraints on the Maximum Tolerable Dose (MTD).

Yet, the ADC evolutionary trajectory over the past years radiates hope. Technological strides and a nuanced comprehension of ADC dynamics have heralded potent agents making discernible differences in oncological treatment. Yet, the road ahead demands a deeper grasp of ADC functionality post antibody-antigen binding.

The Tumor Microenvironment (TME) is another crucial realm warranting deeper exploration concerning ADCs. Also, the intricacies behind ADC resistance in patients remain to be fully deciphered.

Recent advancements in biological engineering have demonstrated the potential to elevate the therapeutic index, as evidenced by preclinical studies. Embracing a comprehensive strategy that merges meticulous target selection with the concurrent optimization of the antibody, linker, and payload, all tailored to the specific indications under consideration could pave the way for the forthcoming generation of ADC approvals.

ADCs represent a beacon of hope in transforming cancer treatment paradigms. Yet, their multifaceted nature demands a nuanced approach to their development and deployment. We can harness the full spectrum of ADC potential to redefine cancer care only through rigorous research, comprehensive understanding, strategic CDMO partnerships, and an openness to challenge conventional wisdom.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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