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Antibody-drug Conjugates in Cancer Treatment: An Overview

Nikita Gupta [a++], L. Sai Geethika [b#*] and Payavula Sneha [b#]

[a] Department of Pharmacy Practice, St Pauls College of Pharmacy, Turkyamjal, India.
[b] St Pauls College of Pharmacy, Turkyamjal, India.

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Review Article

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ABSTRACT

The development of antibody-drug conjugates (ADCs) has significantly impacted cancer therapy, progressing from foundational discoveries in the late 19th century to contemporary clinical applications. With the approval of the first ADC in 2000 and subsequent advancements, including over 30 ADCs in advanced clinical development, the therapeutic landscape for cancer patients has undergone a notable transformation. From initial cleavable linker technologies to the latest third-generation ADCs, continuous innovation in ADC design has been evident. Novel conjugation and linker technologies, alongside the identification of specific target antigens in solid cancers, have reinvigorated prospects for treating challenging malignancies. However, challenges such as off-target toxicity and heterogeneous antigen expression persist. The prevailing empirical approach to systemic cancer therapy administration presents challenges, including potential under-treatment of aggressive disease and over-treatment of indolent conditions, along with frequent adverse effects. Robust prognostic markers are essential to differentiate disease aggressiveness levels, guide

[++] Assistant Professor;
[#] Pharm D III Year;
**Corresponding author: Email: geethika123laddika@gmail.com;*

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treatment decisions, and anticipate adverse effects. Companion diagnostics for targeted therapies, such as HER-2 status for trastuzumab in breast cancer and BCR–ABL mutations for imatinib resistance in CML, enable personalized treatment strategies. Similarly, BRCA mutations predict response to PARP inhibitors in breast and ovarian cancers, while BRAF mutations guide treatment with BRAF inhibitors in melanoma. Patient selection strategies for clinical trials involving ADCs rely on prospective selection or retrospective analysis, each with its merits and challenges.

Keywords: Target therapy; antibody-drug conjugate; cancer treatment; off-target toxicity; personalized treatment.

1. INTRODUCTION

Cancer treatment has significantly shifted towards targeted interventions, moving away from traditional systemic chemotherapy. This change is driven by a deeper understanding of the molecular mechanisms behind cancer progression, inspiring a variety of innovative strategies and treatment approaches. As targeted therapies become more prominent, they bring inherent challenges like on-target side effects, necessitating a thorough examination of their impact [1]. Inquire about endeavors, exemplified by considers in focused on treatment in oncology [2] and the comprehensive audit in the EPRA Universal Diary of Investigate and Advancement (IJRD) centering on novel instruments for the conveyance of cancer therapeutics, reflect the continuous commitment to unraveling the complexities of focused on approaches [3].

The future direction of cancer treatment unfurls with the rise of focused on cancer treatment, challenging the ordinary dependence on systemic chemotherapy. Inside this scene, antibody-drug conjugates (ADCs) stand out as pioneers in the interest of exactness and effectiveness [4]. These conjugates hold guarantee in overcoming the restrictions of conventional medications, introducing transformative conceivable outcomes, especially in breast cancer [5]. Regulating the cytotoxic sedate payload to the tumor location with specificity is the primary reason of the counter acting agent moiety. There are various tumor-related antigens that have as of late been proposed as potential for immunotherapy-based cancer treatments, but there are significantly less reasonable cellular targets that are suitable for ADC-directed intercession [6-8]. The ADC methodology, in differentiate to unconjugated counter acting agent treatment alternatives, does not require the counter acting agent to have any utilitarian action (such as antibody-dependent cellular cytotoxicity, or ADCC), in any case these highlights may have extra restorative benefits [9-11]. Moreover, building an ADC having particular effector capabilities or the capacity to communicate with the resistant framework may be suitable depending on the planning movement profile. This can be carried out by designing the Fc locale or selecting a fitting IgG subclass [4]. Such nanomedicine-based approaches speak to an early but promising region in cancer treatment, advertising potential headways past the surface of current strategies [12,13-14].

This article explores the history and present status of antibody-drug conjugates (ADCs) as targeted therapies in cancer treatment. We compiled publications ranging from Paul Ehrlich's collected works (1956) to current progress until 2024. Also reviewed is the evolution of ADC technology from early concepts to contemporary clinical applications, with an emphasis on advances in monoclonal antibody engineering, linker chemistry, and cytotoxic payloads. The paper outlines the main challenges and opportunities facing the development of ADCs, such as on-target toxicity, improved conjugation strategies, individualized care, and improved patient strategies to enhance treatment outcomes in clinical settings.

2. ANTIBODY–DRUG CONJUGATE TECHNOLOGY

2.1 Historical Development of Antibody Drug Conjugates

Antibody–drug conjugate technology was not quite simple; rather, it resembled a century-long adventure from the end of the nineteenth century to the first clinical uses. Consequently, in 1890, Emil von Behring and Shibasaburō Kitasato noticed that the infected cohort might be cured via the administration of serum from animals impervious to diphtheria to animals who were ill. Ehrlich first proposed the "sidechain theory" in 1890, which postulated that "toxins" attach to side chains (receptors) on the cell surface. Paul

Ehrlich to begin with presented the term "antibodies" in 1891, stamping a noteworthy turning point in immunology. Ehrlich was the pioneer in putting forth a hypothesis in which the antibody was branching and comprised several sites for binding to an antigen and triggering the complement system (immune system) [15].

The influential German scientist Paul Ehrlich first suggested the idea of combining a harmful action for a diseased cell or organism with particular binding to that cell or organism in a single molecule more than a century ago [16]. Antibodies' unique binding characteristics and protein structure led to early attempts to combine cytotoxic medicines with serum immunoglobulins for specificity [17]. But the idea that antibodies may provide a cell-killing agent the selective binding Ehrlich had envisioned did not receive much attention until the discovery of monoclonal antibodies in 1975 [18].

In 1957, Mathé revealed that methotrexate could be conjugated to antileukemia 1210 antigen immunoglobulins by a diazo coupling process, but not to normal gamma globulin, to provide cell-specific antiproliferation activity against L1210 leukemia cells. Several additional organizations conducted more in-depth studies on ADC in the late 1960s and early 1970s following Mathé's publication. How to convert the research using animal immunoglobulins into therapeutic applications was the biggest obstacle at the time [19]. A covalent conjugation between the immunoglobulin and the drug is necessary to achieve the tumor targeting effect when using an alkylating chemotherapy agent, as was shown in the early 1970s by Ghose and collaborators at Dalhousie University in Canada and Rowland and colleagues at Searle Research Laboratory in the UK [20]. In 1975, Sela and colleagues at the Weismann Institute of Science in Israel discovered that daunomycin and Adriamycin could be covalently linked to anti-bovine serum albumin (BSA) immunoglobulins through various reactions. However, only the periodate oxidation method was found to retain both drug and antibody activity [21].

The 1980s saw an expansion in the ADC industry for a number of reasons. Beginning with Milstein and Koch's successful development of a monoclonal antibody [22]. The problem of antibody manufacturing and purification has been resolved by monoclonal antibody technology. FDA authorized muromonab-CD3 (OKT3 ®), the first monoclonal antibody medication, in 1986 as an immunosuppressive treatment for patients receiving kidney transplants [23]. Consequently, concerns over immunogenicity of immunoglobulins as the conventional murine monoclonal antibodies experienced has been significantly diminished by the application of recombinant technology to make humanized antibody, initially as chimeric antibody and then as humanized antibodies [24]. Furthermore, the discovery of several novel biomarkers, including vascular endothelial growth factor and HER2, has enabled immunologists to concentrate on the function as well as the structure of antigens as targets for developing anticancer monoclonal antibodies [25-26]. According to several successful feasibility studies, the field of ADC immunotherapy research matured in the 1990s. The concept of employing monoclonal antibodies, specifically ADC, in treatments has been further backed by new technologies in the development and production of human monoclonal antibodies, such as the phage-display approach and single-chain Fv polypeptides, or scFv [27-28].

The first antibody-drug conjugate (ADC) was approved in 2000, and the market for these targeted medications has changed significantly, significantly altering the course of treatment for several advanced-stage malignancies, including solid tumors [29]. Over the past ten years, antibody-drug conjugates (ADCs) have advanced significantly. An ADC is a vector-based chemotherapy that enables the targeted delivery of a strong cytotoxic agent inside a tumor. An ADC is created when a cytotoxic agent is generally randomly grafted onto a monoclonal antibody (mAb) through a carefully designed spacer arm. This is a complex mixture of immunoconjugates with varying drug loading and distribution (DLD) and drug-to-antibody ratio (DAR), which represent the number of [30]. Novel conjugation and linker technologies, in addition to target and payload diversification, are at the forefront of next-generation ADC development, reviving hopes that these targeted drugs will be able to treat cancers that are difficult to treat and other diseases [31]

2.1.1 Mylotarg, besponsa: the first-generation cleavable linker

In 2000, the FDA (Food and Drug Administration) approved Besponsa, Mylotarg (Gemtuzumab ozogamicin), and the first-generation Cleavable Linker, for use in patients with acute myeloid leukemia. However, the drug's clinical success

was limited because it failed to demonstrate a survival rate and caused fatal toxicity in patients [30]. In 2010, gemtuzumab ozogamicin was voluntarily removed from the market after preliminary results from a phase III study by the Southwest Oncology Group (SWOG) were released. These results led to the drug being approved in the U.S. for older patients in their first relapse when standard therapy was not suitable. This approval set the stage for evaluating the drug in patients with newly diagnosed high-risk acute myeloid leukemia (AML) or myelodysplastic syndrome [32,33-36].

Besponsa® (inotuzumab ozogamicin), approved by the FDA in 2017 against acute lymphoblastic leukemia (ALL), was created by grafting calicheamycin onto inotuzumab, a mutated anti-CD22 IgG4. Similar linkers were developed and used to reapprove Mylotarg® in 2017, using lower doses, a modified administration schedule, and for a different patient population [37].

In phase 3 clinical trials, inotuzumab ozogamicin was associated with veno-occlusive liver disease as a significant adverse event. Nevertheless, the treatment resulted in prolonged rogression-free and overall survival rates, with a higher remission rate compared to standard therapy. Furthermore, a substantial proportion of patients in the trials achieved results below the threshold for minimal residual disease [16].

2.1.2 Polivy®, Adcetris®: The second-generation cleavable linker

A targeted antibody-drug conjugate (ADC) active against CD30-positive cancer cells, such as those linked to classical Hodgkin lymphoma, is intravenous brentuximab vedotin (ADCETRIS®) Monomethyl auristatin E (MMAE), a powerful microtubule-disrupting agent, is covalently linked to a human chimeric immunoglobulin G1 antibody directed against CD30 by a protease-cleavable linker to form benuximab vedotin. A series of events culminate in the apoptotic death of the CD30-expressing tumor cell when brentuximab vedotin binds to CD30 on the tumor cell membrane. With a half-maximal inhibitory concentration of 3–50 pmol/L, brentuximab vedotin demonstrated strong, highly selective activity against CD30-positive HL and ALCL cells in vitro; CD30-negative cells were approximately 1000 times less sensitive to brentuximab vedotin than CD30-positive cells. When used as a retreatment in patients who had previously experienced an objective response to brentuximab vedotin therapy but later experienced a relapse, brentuximab vedotin produced high objective response statistics observed in individuals with recurrent or resistant CD30-positive Hodgkin lymphoma (HL). With few treatment options available for many patients with relapsed or refractory HL, benuximab vedotin demonstrated an acceptable tolerability and safety profile in both the clinical trial and real-world context, where most adverse events can be controlled through dose adjustments [17].

Similarly to benuximab vedotin, The ADC PolivyTM (polatuzumab vedotin-piiq) consists of a monoclonal antibody against CD79b. On June 10, 2019, the USA granted polatuzumab vedotin its first worldwide approval for use as a combination with bendamustine and rituximab as a treatment for adults with relapsed/refractory Diffuse large B-cell lymphoma (DLBCL), unspecified subtype, after receiving at least two prior treatments [18]. Hoffmann-La Roche launched a phase 3 trial in November 2017 to compare polatuzumab vedotin plus rituximab-CHP with rituximab-CHOP in patients with previously untreated DLBCL. The study, referred to as POLARIX, aims to recruit 875 participants and is projected to conclude in 2025 [18].

2.1.3 TRODELVY®, ENHERTU®: The third generation cleavable linker

The advent of third-generation Antibody Drug Conjugates (ADCs) has addressed concerns with second-generation ADCs, emphasizing site-specific conjugation and expanding the cytotoxic payload arsenal. The focus is on enhancing the therapeutic index by selecting target antigens elevated in solid cancers, employing a humanized monoclonal antibody with high tumor selectivity and internalization. The cytotoxic payloads with known toxicity profiles, moderate potency, and established pharmacological activity, alongside a moderately stable linker [38].

Evaluation of TROP-2 expression in tumor response to Sacituzumab (Trodelvy®) therapy showed enhanced responsiveness in transfected cells, attributed to increased exposure to SN-38. Sacituzumab govitecan demonstrates internalization and potential "bystander" effects in the tumor microenvironment. Clinical trials commenced in 2012, showing promising results in diverse metastatic epithelial cancers, although the correlation between TROP-2 levels in archived tumor specimens and responsiveness remains inconclusive [39].

Trastuzumab deruxtecan (ENHERTU®), approved in the USA for unresectable or metastatic HER2-positive breast cancer after two or more prior anti-HER2-based regimens, is under regulatory review in Japan for HER2-positive metastatic breast cancer. The first ADC approved for solid tumors was ado-trastuzumab emtansine (TDM1; Kadcyla) in 2013, based on overall survival data in HER2-positive metastatic breast cancer patients. Unlike T-DM1, which released a positively charged, membrane-impermeable payload, trastuzumab deruxtecan, upon internalization, releases DXd, a highly membrane-permeable agent that inhibits topoisomerase I-DNA complexes, inducing tumor cell apoptosis [40].

Trastuzumab deruxtecan exhibits a higher drug-to-antibody ratio and demonstrated durable antitumor activity in phase 2 trials, with promising median progression-free survival and overall survival rates. However, it is associated with serious adverse reactions, including interstitial lung disease, pneumonia, and vomiting, occurring in a notable percentage of patients [41].

2.2 Structure and Mechanism of ADCs

Antibody-drug conjugates (ADCs) consist of monoclonal antibodies chemically linked to cytotoxic agents via specialized linkers. In modern ADC research, the preferred delivery platform involves humanized or fully human monoclonal antibodies (hmAb) to ensure precise cell targeting, extended circulating half-life (up to three weeks for immunoglobulin G (IgG)), and minimal immunogenicity [42,43]. This conjugation enhances the pharmacokinetic profile by reducing the distribution volume and prolonging both the distribution and elimination phases. This method allows for a gradual release of the active drug from the carrier, leading to sustained high levels of intratumoral drug and lower plasma concentrations. Monoclonal antibodies (mAbs) are particularly suited for their unmatched selectivity and adaptability, as evident in recent successful developments targeting crucial components of biological pathways. This progress significantly expands treatment options for patients dealing with various cancers [43,44]

ADC molecules, comprising antibodies, linkers, and cytotoxic drug payloads, are administered into the bloodstream to recognize and bind to highly expressed antigens on cancer cells [45]. The released payload rapidly reaches the systemic circulation after an ADC administration. Much of the initial payload deconjugation in the systemic circulation is related to plasma exposure to free payload (e.g., due to poor linker stability) [46]. Immediate and efficient internalization of the ADC-antigen complex occurs after an ADC binds to a tumor-associated target. Several parameters, notably the epitope on the selected target antigen bound by the ADC, the affinity of the ADC–antigen interaction, and the intracellular trafficking pattern of the ADC complex, are considered to influence the rate of internalization, despite their lack of clarity. The association between an antibody's Fc component and cells expressing Fc receptors (FcRs) may determine the antibody's biological action [47-49].

As a result, choosing the right antibody format for an ADC is crucial. In order to ascertain the impact of format on ADC function, McDonagh et al. coupled anti-CD70 antibody immunoglobin G (IgG) variants (IgG1, IgG2, and IgG4) to an auristatin (ADC toxin monomethyl auristatin F; MMAF) [50].

Upon internalization, lysosomal processing releases the cytotoxic payload (typically antimitotic agents), inducing cell death through various mechanisms. Challenges in ADC development include solubility issues, instability, aggregation, and unwanted toxicity. The efficiency of antibody-antigen complex internalization depends on the binding affinity, with higher affinity promoting rapid internalization. However, elevated antigen affinity in antibodies may hinder penetration into solid tumors due to the "binding site barrier (BSB)." To address this, researchers have explored miniaturizing antibodies by removing the FC segment. This approach retains high affinity and specificity, facilitating easier penetration into solid tumors. Yet, such modifications may reduce in vivo half-life, necessitating careful consideration in ADC design [45].

Therapeutic antibodies eliminate target cells through two main mechanisms: one involves receptor signaling, leading to apoptosis or disrupting essential growth signal transduction (e.g., bevacizumab), while the other utilizes antibody effector functions like ADCC (Antibody-Dependent Cell-Mediated Cytotoxicity) and CDC (Complement-Dependent Cytotoxicity) (mediated by IgG1 and IgG3). However, these mechanisms may not suffice, prompting the conjugation of specific antibodies to potent cytotoxic drugs for

enhanced efficacy. For a monoclonal antibody (mAb) to diffuse effectively into a tumor, a substantial concentration of unbound molecules is essential. The binding of antigens with slow dissociation rates (high affinity) can diminish the concentration of unbound mAb or single-chain variable fragments (scFv), thereby restricting penetration. Further reduction in free mAb concentration occurs if the antigen undergoes internalization before mAb dissociation. Although the internalized mAb may undergo degradation in endosomes and lysosomes, the antigen can be recycled or replaced by newly synthesized proteins. In such scenarios, especially in large tumors with dense antigen expression, the tumor can act as a notable reservoir for free mAb, impeding homogeneous distribution [45,51].

Advances in target identification, selecting specific mAbs, optimizing linker technology, and improved conjugation methods have led to FDA-approved ADCs, with over 30 in advanced clinical development. Enhancing target molecule density aids drug introduction into cells, and alongside selective expression in cancer cells, internalization through the early endosome-lysosome pathway is crucial. While humanized or fully human antibodies are prevalent, alternative proteins like soluble forms of the HIV-1 receptor CD4 (sCD4) show promise in targeting cytotoxic drugs to HIV-1-infected cells expressing viral envelope glycoproteins (Envs) [45].

2.3 Antibody Selection and Linker Chemistries

The initial step in the development of an optimal Antibody Drug Conjugate (ADC) involves the careful selection of a target antigen. This selection is paramount for mitigating off-target toxicity. The ideal antigen should exhibit overexpression on the surface of cancer cells relative to healthy cells, thereby distinguishing cancerous cells from their normal counterparts. This criterion ensures a decreased risk of off-target toxicity and forms a foundational aspect of ADC development. In the field of antibody-drug conjugate (ADC) development, the choice of an appropriate target antigen is crucial. An ideal target, exemplified by HER2 in triple-positive breast cancer, exhibits markedly elevated expression levels in cancer cells relative to normal counterparts. Notably, the antigen's binding site should be outward facing, facilitating ADC binding before internalization. Moreover, the target antigen must remain confined within the tumor microenvironment to prevent unintended ADC binding in the systemic circulation. Furthermore, the selected antigen must possess the capability to internalize bound ADCs efficiently. Commonly employed targets for both hematological and solid tumors in ADC research encompass CD33, CD30, CD22, BCMA, CD19, CD79B, HER2, Nectin-4, Trop-2, EGFR, and Tissue Factor (TF). Moreover, extracellular matrix constituents, angiogenesis-promoting factors, and elements of the tumor microenvironment have emerged as potential targets for cancer cell eradication, demonstrating efficacy in both preclinical and clinical investigations [52].

In an optimal scenario, once an ADC engages with a tumor-associated target, the subsequent internalization of the ADC-antigen complex should occur swiftly and effectively. While not fully elucidated, numerous factors are likely to impact the rate of internalization, including the specific epitope on the target antigen bound by the ADC, the strength of the ADC-antigen interaction, and the intracellular trafficking pathway followed by the ADC complex. For instance, studies have demonstrated that anti-Her2 antibodies targeting distinct epitopes on Her2 can induce varying patterns of downstream trafficking and lysosomal accumulation, despite their binding to the same cell surface receptor. Sufficient presence of the target antigen on the cell surface is essential for effective binding by circulating ADCs. For instance, melanoma cell lines exhibiting heightened expression levels of the p97 receptor, ranging from 80,000 to 280,000 receptors per cell, displayed susceptibility to the ADC L49-vcMMAF. On the other hand, cancer cell lines with reduced p97 expression exhibited resistance to L49-vcMMAF. Gemtuzumab ozogamicin has demonstrated effectiveness even at relatively low CD33 expression levels (ranging from 5000 to 10,000 receptors per cell), unlike trastuzumab emtansine (T-DM1), which typically requires elevated ErbB2 expression levels (>2 million receptors per cell) [53].

Some antigens facilitate the swift accumulation of ADCs within cells. For example, when bound to ligand-activated EGFR, the Her2 monomer is internalized at a rate up to 100 times faster than carcinoembryonic antigen (CEA). Similarly, antibodies targeting CD74 exhibit a catabolic rate approximately 100 times faster than those targeting antigens like CD19 and CD22, which are known for their rapid internalization. Preclinical data for milatuzumab-DOX (Immu-110), an anti-CD74 doxorubicin conjugated in

early clinical trials, suggests its potency is comparable to ADCs with more potent drug payloads targeting slower internalizing antigens [54].

In order to alleviate off-target effects, the target antigen should primarily or exclusively be expressed on cancer cells, with minimal expression on healthy tissue. Four of the approved ADCs specifically target CD22, CD33, CD30, and CD79, showing consistent expression across cancer cells. Moreover, the target antigen should have minimal secretion into the bloodstream to avoid non-specific antibody binding [53].

The predominant focus of advanced antibody-drug conjugates (ADCs) in clinical trials lies within hematological malignancies, largely attributed to the relatively uniform expression of antigens in liquid tumors, despite often having low receptor densities. Conversely, the treatment of solid tumors with heterogeneous antigen expression poses significant challenges, as the potential for bystander killing may inadvertently harm normal cells, contributing to systemic toxicity [54].

An essential criterion for the antibody component in ADCs is its capacity to selectively bind to antigens on tumor cells, facilitating the concentration of the cytotoxic agent at the tumor site while mitigating binding to healthy cells. Among the five antibodies present in human serum, immunoglobulin G (IgG) emerges as the most abundant, constituting approximately 70–85% of total antibodies and boasting a half-life of approximately 21 days (about 3 weeks). Given its prevalence and potent immune effector capabilities, IgG serves as the predominant antibody class utilized in ADC development. Specifically, IgG1, among the four subclasses of IgG antibodies, stands as the most employed in ADC development due to its established efficacy as an immune effector. Notably, antitumor antibody drugs primarily operate through Fc-mediated effector mechanisms, including antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis (ADCP) [52].

Linkers are pivotal components in controlling the "bystander effect," a phenomenon wherein neighboring antigen-bearing tumor cells release payloads that lead to the destruction of antigen-negative cells. They facilitate regulated permeability of released payloads, allowing for a purposeful bystander effect within ADCs. Investigation into huC242-maytansinoid conjugates containing disulfide or thioether linkers underscored the necessity of lysosomal processing for cell-autonomous killing activity. While disulfide-linked ADCs demonstrated bystander killing of adjacent cells, thioether-linked ADCs did not exhibit this effect [55].

Cleavable linkers, such as hydrazone, peptide (significantly more stable than hydrazone), and disulfide linkers, undergo reactions influenced by various intratumoral microenvironments, including acidic pH, intracellular protease presence (e.g., cathepsin B), and high glutathione concentration. However, a notable challenge with cleavable linkers is the bystander effect observed in adjacent cells. ADCs often employ the dipeptide VC linker, known for its stability. For example, brentuximab vedotin (ADCETRIS®) utilizes a dipeptide VC linker that is cleavable by cathepsins [56].

Non-cleavable linkers, such as thioether or maleimidocaproyl (MC), undergo lysosomal enzymatic degradation to release payloads. Unlike cleavable linkers, they do not elicit bystander effects due to drug accumulation in tumor cells facilitated by charged lysine or cysteine amino acids. These linkers demonstrate enhanced stability in circulation compared to cleavable counterparts, thereby prolonging ADC half-life [56].

2.4 Cytotoxic Payloads

The efficacy of antibody-drug conjugates (ADCs) relies significantly on their ability to undergo internalization into tumor cells, subsequently facilitating the liberation of active cytotoxic compounds within the cytoplasmic milieu. In instances where tumors exhibit suboptimal expression levels of target antigens, it becomes imperative for the payload to demonstrate sufficient potency, ensuring the eradication of cancerous cells even when administered at reduced doses [55].

2.4.1 High cytotoxicity

The efficacy of Antibody-Drug Conjugates (ADCs) is contingent upon the potency of their payloads, especially in the context of tumors with limited expression of targeting antigens and constrained internalization of monoclonal antibodies. This necessitates payloads with

robust cytotoxicity to ensure cancer cell death even at low doses [55].

2.4.2 Low immunogenicity

The ADC may accumulate as a consequence of its hydrophobicity, which may result in immunogenicity. The creation of ATAs (Anti Therapeutic Antibodies) against the cytotoxic drug is the ADC structure. Due to the possibility of cell death resulting from non-target immune cells absorbing massive ADC-ATA immune complexes, these ATAs may be toxic [57]. Immunogenicity poses a significant concern for ADCs, as protein drugs may induce immune responses that compromise efficacy or endanger patient safety. To mitigate this risk, payloads are often sourced from non-human organisms or designed as smaller molecular entities to minimize immunogenic potential [55].

2.4.3 High stability

Stability is crucial for ADC payloads to maintain efficacy both in circulation and within the tumor microenvironment. Payloads must resist degradation under acidic conditions and retain potency post-conjugation, particularly when employing non-cleavable linkers [55].

2.4.4 Functional group modifiability

ADC payloads should possess modifiable functional groups conducive to conjugation with monoclonal antibodies while preserving potency. Careful selection of modification sites is essential to ensure payload efficacy even after linker cleavage [55].

2.4.5 Bystander killing effects

Payloads that induce bystander killing effects offer advantages in tumors with heterogeneous antigen expression. However, achieving a balance between bystander effects and systemic toxicity is critical for clinical utility [55].

2.4.6 Proper water solubility

Payloads must exhibit appropriate water solubility to facilitate conjugation and maintain stability under physiological conditions. Excessive hydrophobicity can lead to aggregation and instability of ADCs, necessitating careful consideration of payload hydrophilicity [55].

2.4.7 Intracellular targeting

ADC payloads should target intracellular components to ensure effective payload delivery into tumor cells. Payloads with extracellular targets are unsuitable for ADC use due to their inability to penetrate cell membranes for intracellular release [58].

The discourse delineates a comprehensive examination of various classes of payloads employed in the construction of Antibody-Drug Conjugates (ADCs), along with the strategies employed in their development:

Auristatins: This class of compounds holds significant prominence in the realm of ADCs, with one of its most prominent members, monomethyl auristatin E (MMAE), being integral to therapeutic agents such as Adcetris© and Polivy©. The narrative underscores the presence of over ten ADCs incorporating auristatins like MMAE or monomethyl auristatin F (MMAF) in clinical trials. Moreover, it highlights the preferred method of attaching auristatins that possess both amine and alcohol functionalities, favoring amine attachment facilitated by a carbamate linkage. Additionally, the discussion underscores the pioneering efforts of Seattle Genetics in devising a novel strategy for the bioconjugation of alcohol-containing payloads to antibodies, specifically through the utilization of the methylene alkoxy carbamate (MAC) self-immolative unit. This approach, characterized by the strategic positioning of both basic and electron-withdrawing groups proximal to the aminal linkage, yields conjugates that exhibit robust stability under physiological conditions, potent pharmacological activity, and discernible immunological specificity both *In vitro* and *In vivo.*

Maytansine: Despite its potent inhibitory effects on microtubule assembly, maytansine presents challenges for conjugation owing to its lack of reactive functional groups. To circumvent this limitation, derivatives incorporating an SMe group were synthesized, serving as prodrugs of SH liberated post cellular uptake via a reduction process mediated by glutathione. Notably, the narrative highlights the preparation of maytansine-based ADCs utilizing the secondary hydroxyl group as the preferred site of attachment, often accompanied by the incorporation of a linker facilitating transglutaminase bioconjugation.

Tubulysins: As potent disruptors of microtubule polymerization, tubulysins engender rapid disintegration of the cytoskeleton in dividing cells, culminating in apoptotic cell death. The discourse underscores the diversity of attachment points that have been devised to harness tubulysins as payloads for ADCs.

Cryptomycins (CR): This cohort of macrocyclic depsi peptides manifests robust anticancer activity through their affinity for microtubules at the vinca binding site. Despite concerted endeavors to advance their clinical utility, outcomes from clinical trials have revealed prohibitive levels of toxicity at therapeutic doses.

Antimitotic EG5 Inhibitors: These compounds target the kinesin spindle protein (KSP, also known as Eg5 or KIF11), thereby disrupting an essential event in mitosis and yielding potent antitumor effects. The narrative delineates the innovative approach adopted by researchers at Novartis, wherein imidazole containing KSP inhibitors were utilized as a foundation for the installation of non-cleavable linkers featuring a maleimide end group. This strategy, when implemented in conjunction with antibodies targeting HER2 and c-KIT, resulted in ADCs exhibiting superior in vivo efficacy compared to established therapies such as ado-trastuzumab emtansine [59].

3. PERSONALIZED MEDICINE AND BIOMARKERS SELECTION

The current approach to systemic cancer therapy administration is predominantly characterized by empirical methodologies. This approach, however, poses several challenges, including the potential for under-treatment of patients with aggressive disease and over-treatment of those with indolent conditions.

Additionally, while some patients may benefit from therapy, adverse effects are frequent, with a subset experiencing severe and occasionally fatal toxicities. To address these shortcomings, there is a critical need for robust prognostic markers capable of effectively distinguishing between patients with differing disease aggressiveness levels. Such markers would facilitate the avoidance of unnecessary chemotherapy in patients with indolent disease while identifying suitable candidates for aggressive therapeutic interventions. Moreover, the development of predictive markers for treatment response or resistance is imperative to ensure that patients receive tailored and effective therapies. Furthermore, the identification of markers to anticipate severe treatment-related toxicities is essential for the implementation of personalized and safer cancer treatment strategies [60].

4. PATIENT SELECTION STRATEGY

Patient selection for clinical trials involving antibody-drug conjugates (ADCs) relies on various strategies, each with distinct merits and drawbacks. These strategies, including prospective selection and retrospective analysis, are influenced by factors such as target prevalence, biology, assay limitations, and operational feasibility. Prospective selection, where target expression is confirmed before enrollment, was employed in developing several ADCs. Conversely, retrospective analysis allows flexibility in assessing target expression levels and their association with clinical activity during trials, particularly in diseases with high target prevalence. However, this approach carries the risk of treating patients with inadequate target expressions. Challenges in patient selection have been observed, with discrepancies in clinical responses based on target expression levels across different studies and scoring methods. These complexities underscore the importance of robust patient selection strategies in ADC development [61].

5. CONCLUSION

In conclusion, the trajectory of antibody-drug conjugates (ADCs) from their foundational discoveries in the late 19th century to their contemporary clinical applications has markedly impacted cancer therapy, ushering in an era of personalized medicine.The development and evolution of ADC technology, which spans over a century from Ehrlich's pioneering insights to current advances in monoclonal antibody engineering and linker chemistry, reveals a journey marked by continuous advancement and innovation. This advancement has resulted in the approval of multiple generations of ADCs, each with enhanced targeting capabilities and lower toxicity profiles than the previous generation.

Moving forward, current work in target identification, innovative linker technologies, and payload diversity promise to improve ADC efficacy and safety. The introduction of third-generation ADCs, which include site-specific conjugation and improved cytotoxic payloads,

reflects the increasing importance toward enhancing treatment outcomes while minimizing side effects.

Moreover, the quest for robust prognostic markers and predictive biomarkers remains crucial for optimizing treatment outcomes and minimizing adverse effects. Serum-based and tissue-based markers have emerged as indispensable tools across various cancer types, offering valuable insights into disease prognosis and treatment response. The integration of companion diagnostics for targeted therapies further enhances personalized treatment strategies, ultimately benefiting patient care.

The incorporation of these biomarkers into clinical practice represents a significant advancement in treatment decision-making, ultimately improving patient outcomes and optimizing healthcare resource allocation. Nevertheless, concerted efforts to address methodological limitations and refine personalized medicine approaches are essential for maximizing the potential of biomarker-guided cancer therapy within academic and clinical realms.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. ChatGPT version 3.5, the input prompts include

   a. Relevant subtopics in “Antibody Drug Conjugates”
   b. ADCs and personalized medicines

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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