Research Advances in Antibody-Drug Conjugates for Cancer Therapy

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Abstract

Antibody-drug conjugates (ADCs), as a key representative of targeted therapies, link specific monoclonal antibodies to potent cytotoxic drugs via linkers. This approach aims to achieve precise tumour treatment while overcoming the systemic toxicity issues associated with conventional chemotherapy. This review systematically elucidates the core components of ADCs: monoclonal antibodies must possess high target specificity, appropriate affinity, and favourable pharmacokinetic properties; cytotoxic payloads (including marine-derived and metal-based drugs) require exceptional potency and conjugatability; while linkers must maintain stability in the circulatory system and facilitate efficient drug release within tumour cells. The article provides a detailed analysis of ADC mechanisms of action, encompassing targeted binding, endocytosis, and intracellular drug release processes. It summarises their significant therapeutic efficacy in haematological malignancies (such as acute myeloid leukaemia and lymphoma) and solid tumours (including breast cancer and urothelial carcinoma). Despite their exceptional efficacy, ADCs face clinical challenges due to toxicity, primarily interstitial lung disease, haematological toxicity, and organ-specific toxicity. These stem from off-target effects and on-target off-tumour effects. Future advancements in linker stability, novel payload development, site-specific conjugation techniques, and combination therapy strategies hold promise for enhancing the therapeutic index of ADCs and expanding their clinical applications.

Keywords: antibody-drug conjugates, cytotoxic drugs, linkers, targeted therapy, tumour treatment

1. Introduction

Cancer treatment remains a significant challenge for medicine in this century. Although numerous studies have introduced anticancer drugs into therapy, Traditional chemotherapy employs chemical agents (such as paclitaxel and cisplatin) to directly kill rapidly proliferating cancer cells. However, the systemic distribution of these drugs and their effects on normal tissues result in non-specific toxicity to the body. Against this backdrop, research into antibody-drug conjugates (ADCs) aims to achieve targeted tumour treatment by precisely delivering payloads to cancer cells via the specific binding of antigens and antibodies. This review systematically outlines the fundamental structure and mechanism of action of ADCs, their developmental history and representative examples, alongside their applications and challenges in cancer therapy.

2. Overview of Antibody-Drug Conjugates

Antibody-drug conjugates (ADCs) represent highly complex targeted therapeutics. By linking monoclonal antibodies (mAbs) to cytotoxic payloads via a linker, they combine the mAb's high-specificity advantage in precisely recognising target cell surface antigens with the potent tumour-cell-killing capacity of conventional chemotherapeutic agents. This dual approach enhances the efficacy and safety of cancer treatment, enabling precise and efficient tumour destruction.

2.1 Monoclonal Antibodies

Hybridoma technology fuses B lymphocytes capable of producing specific antibodies with myeloma cells that can proliferate indefinitely. After screening for hybridoma cells and cloning them for culture, it enables the large-scale production of structurally uniform, highly specific monoclonal antibodies (mAbs).

The fundamental structure of antibodies comprises two identical light chains and two identical heavy chains, forming a characteristic Y-shaped configuration through covalent and non-covalent interactions both between and within chains[i]. Both light and heavy chains comprise variable regions (V-terminus) and constant regions (C-terminus). Within the variable regions, three areas exhibiting higher amino acid sequence variability—VHR1, VHR2, and VHR3—play a pivotal role in antigen recognition and binding . The C-terminal regions CH1 and CH2 of the heavy chain are connected via a flexible hinge region rich in proline residues, which is susceptible to protease degradation and enhances the water solubility of the mAb[ii].

For ADCs to effectively target cells, mAb selection must meet the following criteria: (1) Maintain stability in the circulatory system for a sufficient duration to ensure precise release of cytotoxic drugs and minimise off-target effects. This principle also applies to payload selection. (2) An ideal mAb requires appropriate affinity. However, the exceptionally strong affinity of antibodies can reduce penetration efficiency in solid tumours, meaning high-affinity mAbs do not necessarily exhibit high clinical efficacy[iii].(3) The target antigen exhibits high tumour specificity, being highly expressed on tumour cell surfaces with efficient internalisation, whilst exhibiting minimal expression in normal tissues . Given payload efficacy, the antibody must possess high selectivity for the target receptor and maintain its half-life and biological properties post-conjugation. (4) Selection of antibody subclasses: Different constant regions of antibody subclasses engage IgG-Fc receptors, yielding distinct effector functions that profoundly influence mAb efficacy, pharmacodynamics, and safety[iv].(5) Optimisation of pharmacokinetic (PK) properties: For instance, enhancing FcRn binding through Fc region mutations can prolong antibody

half-life; YTE mutations extend the half-life of IgG1 monoclonal antibodies by 3-4 fold[v]; or analysing the impact of antibody hydrophobicity, glycosylation, and charge on PK[vi].

2.2 Cytotoxic Agents

Cytotoxic payloads encompass traditional chemotherapeutic agents, including novel compounds with unique and potentiating effects whose release exerts lethal toxicity against tumour cells[vii]. Payload diversification constitutes a critical step in ADC development. The killing capacity of the payload significantly influences tumour cell destruction rates; many compounds are discarded due to excessive toxicity, yet their conjugation with mAbs can yield substantial therapeutic efficacy[viii].

Payload design must address the following aspects: (1) High toxicity: Only approximately 0.1% of the administered mAb dose reaches tumour cells, meaning therapeutic efficacy relies heavily on payloads with potent cytotoxicity. Payloads must be capable of killing tumour cells at extremely low concentrations. (2) Beyond potency, the molecular structure, chemical composition, and resistance mechanisms of the payload are critical determinants of mAb safety and efficacy. The payload's molecular structure must possess the capacity for conjugation with linkers. (3) Furthermore, as both mAb preparation and administration occur in aqueous environments (intravenous delivery), the aforementioned requirements for sufficient water solubility and long-term stability in blood are equally vital[ix].

2.2.1 Introduction to Conventional Payloads

Cytotoxic drugs, serving as the "warhead" of ADCs, predominantly originate from natural products and their derivatives. Examples include microtubule inhibitors such as MMAE/MMAF, which inhibit microtubule polymerisation or depolymerisation, thereby disrupting spindle formation during mitosis. This leads to cell division arrest and induces apoptosis, ultimately killing the cells. DNA-damaging agents constitute another prevalent payload category. These drugs directly target DNA, disrupting double-strand structures or alkylating DNA to interfere with gene expression, ultimately inducing cell death. Calicheamicin and DXd represent widely utilised exemplars within this class.

2.2.2 Development of Novel Payloads

Marine organisms thrive in unique environments characterised by high salinity, pressure, and hypoxia, enabling them to produce cytotoxic drugs with potent toxicity (pM-nM range) and distinctive structures (containing active groups), such as sea hare toxin and lichenin. These compounds have become a significant source for payload research and development[x]. Moreover, many marine drugs target microtubulin or DNA while possessing structural differences from their terrestrial counterparts, thereby offering potential to overcome drug resistance. Examples include the microtubule inhibitors sea hare toxin and spongin, and the DNA-damaging agent tunicatin.

Metal-based drugs (e.g., platinum and ruthenium compounds) have garnered significant attention in recent years as novel payloads. Traditional protein-small molecule conjugation techniques exhibit certain limitations, including time-consuming and difficult automation, multiple reaction steps, and incompatibility between chemical reagents and protein formulation buffers. However, through non-traditional synthetic approaches for studying metal-drug-based ADCs, several protein-metal complex ADCs have been developed and flexibly applied in clinical diagnostics[xi].

2.3 Linkers

mAbs and payloads play irreplaceable roles in ADC development, while linker chemistry and conjugation are also critical factors for successful construction. Coupling is the pivotal step connecting mAbs and payloads, directly impacting ADC stability, safety, and efficacy. The linker determines the payload release mechanism, with linker structures categorised into two main types: cleavable and non-cleavable .

Through in vivo and in vitro evaluations of various developed linker types, the criteria for successful ADC construction emerge: (1) Optimal linker stability: The linker must maintain long-term stability in plasma, enabling the ADC to circulate and reach target cells before payload release. Instability leads to premature payload release, causing systemic toxicity through damage to normal tissues and negating the ADC's therapeutic advantage; (2) Rapid cleavage capability: Once internalised, the linker must cleave rapidly to enable timely payload release and exert toxic effects on tumour cells; (3) Hydrophilicity: Linker hydrophobicity impacts pharmacokinetics and efficacy. Early ADCs utilised hydrophobic linkers, which proved disadvantageous during treatment. Enhanced interprotein hydrophobic interactions formed high-molecular-weight aggregates, and these aggregated proteins tended to be rapidly sequestered in the liver and cleared by the reticuloendothelial system, leading to hepatotoxicity. Furthermore, aggregated proteins may act as immunogenic substances, triggering immune responses within the bloodstream[xii]35.

2.4 Drug-Antibody Ratio (DAR) and Conjugation Technology

The drug-to-antibody ratio (DAR) denotes the average number of cytotoxic drugs conjugated per antibody molecule. DAR values and their uniformity fundamentally determine the efficacy and toxicity of ADCs, constituting a core quality attribute: excessively low DAR (e.g., DAR=0–2) indicates insufficient payload on the antibody to achieve lethal cell killing, resulting in poor therapeutic response; Conversely, excessively high DAR values (e.g., DAR > 4) may precipitate several complications. Firstly, over-modified antibodies may exhibit increased aggregation, accelerating plasma clearance and compromising drug stability. Secondly, ADCs with excessively high DAR values are prone to premature payload release

during circulation, amplifying off-target toxicity and damaging healthy human cells.

The homogeneity and controllability of DAR are directly dependent upon the conjugation technology employed for the ADC drug. Traditional conjugation methods primarily fall into two categories: lysine amide conjugation and cysteine conjugation.

Lysine amide conjugation: Lysine residues (Σ-amino group) on the antibody surface react with the active moiety of the payload-linker (containing an activated carboxylate ester) to form an amide bond for conjugation. Amide conjugation is one of the highest-yield, most reliable, and predominant conjugation methods. However, this method exhibits high heterogeneity and may face affinity reduction due to residue modifications. This conjugation pattern typically yields mixtures of multiple ADC variants with variable DARs and conjugation sites. Generally, a broad DAR distribution significantly impacts ADC cytotoxicity, potentially leading to a poor therapeutic index in clinical applications.

Cysteine conjugation: Antibody cysteine residues lack free thiols; reducing the disulphide bonds between chains generates free thiols that react specifically with thiol-reactive functional groups (maleimide linkers) on the payload. This coupling method yields a more uniform DAR and benefits from established processes. However, maleimides readily bind to albumin in plasma, undergoing thiol exchange and potentially causing off-target toxicity[12]36.

Next-generation site-specific conjugation technologies (e.g., engineered cysteine, non-natural amino acids, enzyme-catalysed conjugation) employ chemical and molecular biology techniques to establish precise payload attachment sites on antibodies, achieving site fixation. This fundamentally resolves the inherent DAR variability of traditional conjugation methods by eliminating ineffective components with excessively low DAR values and toxic components with excessively high DAR values. ultimately enhancing the DAR uniformity and safety of the produced ADC drugs.

3. Mechanism of Action and Application of Antibody-Drug Conjugates in Cancer Therapy

Following intravenous administration, ADCs circulate systemically and accumulate in tumour tissues. Here, the antibody's antigen-binding site specifically binds to tumour cell surface antigens. This high-affinity binding concentrates the drug predominantly within tumour tissue, minimising damage to normal cells (though off-target toxicity remains possible). Subsequently, the target antigen-ADC complex undergoes receptor-mediated endocytosis, transferring the ADC into tumour cells to form endosomes (whilst newer non-internalising ADCs may release the drug directly within the tumour microenvironment, simultaneously exerting bystander effects to kill neighbouring cells) for subsequent transport and processing. Subsequently, the endosome fuses with lysosomes and is degraded by lysosomal enzymes or within the acidic environment, causing linker cleavage and release of the cytotoxic payload. If the released payload is a microtubule inhibitor, it disrupts microtubules to arrest mitosis and block tumour cell division. If it is a DNA-damaging agent, it directly damages tumour cell DNA to induce apoptosis or necrosis[xiii].

ADC technology has achieved breakthroughs in haematological malignancies. Gilteritinib-ozemiparib, targeting CD33, became the first ADC approved for relapsed or refractory acute myeloid leukaemia.Vitiliximab[xiv], targeting CD30, has significantly improved outcomes for patients with Hodgkin lymphoma and systemic anaplastic large cell lymphoma. In recent years, polatuzumab vedotin targeting CD79b, combined with chemotherapy and rituximab, has become one of the new standard regimens for diffuse large B-cell lymphoma[xv].In the field of solid tumours, ADC drugs have also yielded remarkable results. HER2-targeted agents are prime examples: Trastuzumab Emtansine (T-DM1) and Trastuzumab deruxtecan (T-DXd) have successively become pivotal therapies for HER2-positive breast cancer, [xvi]with the latter even redefining clinical classification due to its efficacy against HER2-low breast cancer. Furthermore, gosatuzumab, targeting TROP-2, offers a new option for triple-negative breast cancer patients;[xvii]while enfortuzumab exceperdin, targeting Nectin-4, has demonstrated significant efficacy in urothelial carcinoma.[xviii]ADCs successfully combine the precision of targeted therapy with the potency of cytotoxic agents, demonstrating deep and durable responses across multiple haematological malignancies and solid tumours[xix].They have become indispensable therapeutic weapons in modern oncology. With ongoing technological advancements and accumulating clinical experience, ADCs are poised to play an increasingly central role in precision cancer therapy[xx].

4. Clinical Challenges and Future Prospects of Antibody-Drug Conjugates

The toxicity of ADCs constrains their clinical application, making research into and management of these toxicities a prerequisite for safe administration[xxi].ADC toxicity primarily stems from the payload, though other components may also contribute. Its root causes lie in "off-target" effects (premature release of the payload in the circulation, indiscriminately killing proliferating cells) and "on-target off-tumour" effects (arising from low expression of the target antigen in normal tissues)[xxii].Different payloads induce specific toxicities: MMAE causes neuropathy, MMAF correlates with ocular toxicity, and DM1 leads to gastrointestinal reactions. Normally dividing cells, such as those in the gastrointestinal tract and hair follicles, are also susceptible. Interstitial lung disease (ILD) is particularly prevalent with HER2-targeted ADCs (e.g., T-DXd), warranting a black box warning[xxiii].Close imaging surveillance is required; upon suspicion, immediate discontinuation and initiation of glucocorticoids are necessary. Haematological toxicity commonly presents as neutropenia and thrombocytopenia[xxiv].Regular blood counts must be monitored, with growth factor support or dose adjustments administere[xxv].Hepatotoxicity manifests as elevated transaminases, with severe cases potentially leading to acute liver failure. Regular liver function monitoring is essential[xxvi]. Specific toxicities such as ocular toxicity (in TF-targeted ADCs) and peripheral neuropathy (in ADCs with microtubule inhibitors as payloads) require prophylactic medication and symptomatic

management[xxvii].ADC toxicity management is central to their clinical success. Future advancements in developing more stable linkers, novel payloads, and identifying predictive biomarkers hold promise for further enhancing their therapeutic index[xxviii].Future directions include: developing novel targets (e.g., B7-H3, CLDN18.2, ROR1), employing site-specific conjugation technologies to produce more homogeneous ADCs, exploring "smart" controllable-release linkers, and combining them with other therapies such as immunotherapy. (Combination strategies have achieved breakthroughs in multiple tumour treatments: FDA-approved Enfortumab vedotin (targeting Nectin-4) combined with Pembrolizumab (anti-PD-1) has demonstrated efficacy far exceeding chemotherapy in clinical applications for urothelial carcinoma; Sacituzumab Govitecan (targeting TROP-2) combined with Pembrolizumab has also demonstrated significant potential in areas such as triple-negative breast cancer), aiming to further expand its therapeutic scope and benefit more patients[xxix].

5. Conclusion

Antibody-drug conjugates (ADCs) represent a revolutionary breakthrough in precision oncology by combining the targeted delivery capability of monoclonal antibodies with the potent cytotoxic effects of drugs. This review systematically outlines research advances in oncology concerning ADCs, focusing on their core components, conjugation techniques, mechanisms of action, and clinical applications. Studies indicate breakthrough achievements in treating haematological malignancies (such as acute myeloid leukaemia and lymphoma) and solid tumours (including breast cancer and urothelial carcinoma), demonstrating exceptional therapeutic efficacy.

Nevertheless, clinical implementation of ADCs remains fraught with significant challenges. Toxicity issues arising from "off-target" and "on-target off-tumour" effects—such as interstitial lung disease and haematological toxicity—severely constrain their clinical application. Moving forward, the development of novel targets, advancement of site-specific conjugation technologies, optimisation of stable linkers, and exploration of combination strategies with other therapies hold promise for further enhancing ADC efficacy. As these technologies mature, ADCs are poised to assume an increasingly central role in cancer treatment, offering renewed hope to a growing number of patients.

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