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Advances in antibody–drug conjugates: a new era of targeted cancer therapy

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Abstract

Antibody–drug conjugates (ADCs), a potent class of anticancer therapeutics, comprise a high-affinity antibody (Ab) and cytotoxic payload coupled via a suitable linker for selective tumor cell killing. In the initial phase of their development, two ADCs, Mylotarg®, and Adcetris® were approved by the US Food and Drug Administration (FDA) for treating hematological cancer, but the real breakthrough came with the discovery of the breast cancer-targeting ADC, Kadcyla®. With advances in bioengineering, linker chemistry, and potent cytotoxic payload, ADC technology has become a more powerful tool for targeted cancer therapy. In addition, ADCs with improved safety using humanized Abs with a unified ‘drug:antibody ratio’ (DAR) have been achieved. Concomitantly, there has been a significant increase in the number of clinical trials with anticancer ADCs with high translation potential.

Keywords

antibody drug conjugates; targeted cancer therapy; receptor mediated endocytosis; receptor overexpression; antibody bioengineering; advance linker; protein scaffold; Mylotarg; Adcetris; Kadcyla

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Teaser: Discovery of antibody–drug conjugates (ADCs) bridged together by smart linkers created a powerful entity that can deliver highly efficacious anticancer agents targeted to tumor cells and tissues with an improved therapeutic window. Advancement of protein engineering, linker chemistry, and new cytotoxic payloads herald ADCs as safe and effective anticancer therapeutics for personalized medicine.

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Introduction

ADCs are an emerging class of targeted anticancer drug delivery agent that confer selective and sustained cytotoxic drug delivery to tumors [1]. An ADC can be divided into three main structural units (Figure 1a): the Ab; the cytotoxic agent; and the linker. Selecting a high-affinity Ab, stable linker, and potent cytotoxic payload enables the novel development of safe and efficient ADCs [2]. Many monoclonal Ab (mAb), such as avastin, rituximab, and cetuximab, are well known as standard treatments for solid tumors and hematological cancers [3]. By contrast, pristine chemodrugs, such as vinblastine, doxorubicin, and paclitaxel, have limited use for cancer treatment because of their nonspecific toxicity, thus narrowing the therapeutic window and increasing drug resistance [4,5]. Discovery of ADCs bridged the gap between the Ab and cytotoxic drug, creating highly specific anticancer agents with an improved therapeutic window [6]. The first FDA-approved ADC, Mylotarg®, a conjugate of the CD33 Ab and a calicheamicin payload, was developed for acute myelogenous leukemia (AML), although this was withdrawn from the market 10 years after its initial approval. Adcetris®, a conjugate of the CD30 Ab and monomethyl auristatin E, was approved for the treatment of lymphoma [2]. In 2013, Kadcyla® was commercialized for targeting HER-2-positive metastatic breast cancer, with emtansine (DM1) as the cytotoxic agent [1]. Although ADCs are designed against tumor-specific antigens, there are challenges associated with Ab immunogenicity, antigen expression, premature drug release, and low chemotherapeutic drug potency [7]. However, over time, advances in bioengineering have improved the safety profile of ADCs, particularly third-generation ADCs. In this review, we discuss current prospects for, and technical improvements in, ADCs towards developing safer and more efficacious personalized cancer medicine.

Criteria for a successful ADC

Selection of targeting antigen

The selection of the targeting antigen the first and most important determining factor for a successful ADC. The ideal characteristics of a useful antigen are: (i) higher-fold expression in the tumor than in the healthy tissue; for example, Adcetris® targets the CD30 antigen, whose expression on the surface of mature and immature myeloid cells is high (90%–100%) in all patients with AML [8], whereas Kadcyla® targets the HER2 receptor, whose expression is almost 100-fold higher in cancer cells than in healthy cells [1]; (ii) internalization of antigen via endocytosis in the presence of ligand and its recycling back to the plasma membrane [9]; and (iii) homogeneous antigen expression in the tumor microenvironment and low antigen abundance in circulation [10] (Figure 1b). Therefore, given that ADCs target tumor-associated antigens, there should be minimum expression of such antigens on healthy cells to reduce any adverse effects [11]. For example, prostate-specific membrane antigen (PSMA) is expressed on the surface of cancer cells, whereas, in the healthy prostate, it is found in the cytoplasm; therefore, noncancer cells are not affected by PSMA-targeting ADCs. Thus, the indium (In^{111})-labeled PSMA-targeting Ab, ProstaScint® can be clinically used for the early detection of prostate cancer [12]. It is suggested that 10 000 antigens per cell is the minimum number of antigens required to

ensure the selective delivery of lethal cytotoxic drugs to cancer cells [2]. A major challenge to solid tumor therapy arises from antigen expression, which varies with tumor volume, heterogeneity, and treatment [13]. In addition to specific and sufficient expression, an optimal targeting antigen should also stimulate effective ADC internalization [14]. This internalization efficiency depends on the choice of Ab, epitopes of the antigen, and type of target (Figure 2). It has been reported that some targets frequently internalize regardless of ligand binding, whereas others reside permanently on the cell surface [2] (Figure 2). It was thought that the anticancer efficacy of ADCs relies on their internalization by cancer cells. However, recent work on spliced domain fibronectin-conjugated maytansinoid (SIP-F8-SS-DM1) showed that the internalization of antigen is not always necessary to achieve a therapeutic effect [15]. This is because this disulfide-linked ADC is reduced at the subendothelial extracellular matrix of solid tumors and increases the local concentration of cytotoxic payload near the tumor vasculature, enabling the drug to diffuse to the neoplastic mass. Abs of ADCs are occasionally capable of inducing antigen-mediated anticancer activity in addition to the cytotoxicity of the ADC payload [16]. This occurs when the antigen inhibits the downstream signaling of cancer cells when binding with the Ab.

Other challenges to targeting tumor antigens with ADCs include high interstitial tumor pressure, physical and kinetic barriers, and a bystander effect associated with the heterogeneous expression of antigen [7]. The bystander effect is a phenomenon of nonspecific systematic toxicity that arises because of the diffusion of the membrane-internalized cytotoxic payload from antigen-positive tumor cells to neighboring antigen-negative healthy cells [17].

Antibody selection

Important properties of Abs in ADCs include their antigen affinity, target specificity, good retention, minimal immunogenicity, low cross-reactivity, and along circulation in plasma. Typically, the binding affinity of the Ab component of ADCs is in the range 0.1–1 nM. Murine Abs were used in first-generation ADCs, which caused severe immunogenicity by producing human antimouse Abs in patients; however, bioengineering research resulted in the discovery of chimerized, humanized, and fully human Abs [6]. Today, most Abs used in humans are either human derived or humanized. Humanized Abs mainly derive from human sources except for the complementarity-determining regions (CDRs), which have murine origins. In humanized Abs, mouse CDRs are either grafted or resurfaced to the human Fv surface and constant regions (Figure 3a) [18]. Kadcyla® is an example of a CDR resurfacing humanized ADC. Most Abs in ADCs utilized in clinical trials are human immunoglobulin (Ig)-G isotypes, particularly IgG1. Occasionally, the unmodified Fc regions of Abs bind to Fc-gamma receptors (FcγR) of immune cells, such as macrophages and natural killer cells, and induce Ab-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), resulting in induction of antitumor immune responses [7]. The effects of ADCC and CDC are particularly prominent in human IgG1 isotype mAbs rather than in IgG4, and IgG2 mAbs; for example, trastuzumab, which is the drug in Kadcyla®, was found to initiate ADCC and CDC-associated tumor cell killing.

In addition to monospecific Abs, bispecific Abs have been developed to enhance therapeutic functionality. These are used for targeting two antigens of tumor cells and tumor-associated immune cells [19]. In 2014, the FDA approved blinatumomab, a bispecific T cell engager (BiTE) for the treatment of Philadelphia chromosome-negative acute lymphoblastic leukemia (ALL) [20]. Few reports are available regarding bispecific ADC and most are still in preclinical investigations. One such bispecific ADC is bsHER2xCD63his-ADC, which targets two antigens of cancer cells, HER2 and CD63 (a protein that is responsible for communicating between the cell membrane and intracellular compartments) [20].

Linker selection

The linker has a key role in ADC outcomes because its characteristics substantially impact the therapeutic index, efficacy, and pharmacokinetics of the ADC [6,21]. The stable linkers in ADCs can maintain the Ab concentration in the blood circulation and do not release the cytotoxic drug before reaching the target, resulting in minimum off-target effects. However, the linker should be labile enough to rapidly release the cytotoxic drug once the ADC is internalized to the tumor cells [10]. Another critical consideration is how many drug molecules should be loaded onto the Ab: the so-called 'DAR'. Therefore, optimization of DAR is necessary because attaching too few drug molecules will lead to lower efficacy, whereas attaching too many will alter the pharmacokinetics and further increase instability and toxicity [7]. The FDA has approved ADCs with proven activity that are manufactured by nonspecific conjugation to lysine residues of Abs; however, these generated an undesirable heterogeneous mixture of ADCs, with high DAR values. Therefore, research efforts are directed to the design of homogeneous ADCs with a high number of drug molecules stably linked to the Ab. The aim of most common ADCs is to attain a DAR value close to 4 [10,22].

Cleavable linker—The first type of cleavable linker can be classified into three types: hydrazone, disulfide, and peptide linkers. Each corresponds to a different tumor-specific intracellular condition: low pH sensitive, glutathione sensitive, and protease sensitive, respectively [23]. Acid-sensitive hydrazone linkers, which take advantage of the low pH (4~6) within the endosomes and lysosomes of cancer cells, undergo acid hydrolysis and release the cytotoxic payload [24,25].

Disulfide linkers are more stable in the circulation, and exploit higher concentrations of glutathione within cancer cells. Glutathione concentration is higher in cancer cells, because it is associated with cell survival and tumor growth, and is also elevated during cell stress conditions, such as hypoxia [26–28].

Another form of cleavable linker is a (lysosomal protease-sensitive) peptide, which is more stable than the other forms of cleavable linker discussed above. It also enables improved control of drug release by attaching to the cytotoxic drug with mAbs [29]. Peptide-based linkers are optimized to release their toxic payload upon cleavage by distinct intracellular enzymes (proteases). For example, cathepsin B (a tumor-specific protease) recognizes and cleaves a specific dipeptide bond inside the tumor cells [30,31]. FDA-approved Adcetris® contains a cathepsin B-sensitive dipeptide linkage (valine-citrulline).

Another protease-sensitive linker, β -glucuronide, undergoes degradation and hydrolysis by β -glucuronidase, which is a lysosomal enzyme overexpressed in many cancers and utilized for selective payload release [32]. Burke *et al.* showed that the PEGylated self-immolative linker, β -glucuronide-conjugated monomethyl auristatin E (MMAE), had a DAR value of 8, and enhanced pharmacokinetic stability and potency in xenograft models compared with nonPEGylated linkers [33]. However, more research is required for the clinical improvement of glucuronic acid linker-based ADCs.

Noncleavable linkers—The other class of ADC linker comprises noncleavable thioether or maleimidocaproyl (mc), which both depend on the lysosomal enzymatic degradation of ADC to release the cytotoxic payload after internalizing to cancer cells [34,35]. For example, Kadcyla® was successfully designed to utilize a noncleavable mc-linker that links maytansinoid toxin to the HER2 Ab [36,37]. As a result of the stable linker, Kadcyla® is stable in serum for more than 3 days [38].

The advance design has extended the emergence of innovative linkers for toxic payload conjugation at the linker site instead of the Ab site. For example, SpaceLink Technology, developed by Syntarga, is a highly flexible linker-based ADC [39]. This linker can reversibly attach to the toxic payload and Ab. As a result, this allows the selection and optimization of the payload and linker–payload combination to generate ADCs with maximal therapeutic potency [39]. An excellent example of this promising technology is anti-HER2-duocarmycin conjugates, which exhibit *in vivo* antitumor efficacy with reduced off-target toxicity [39].

Thus, researchers are currently investigating several other innovative linkers for the safe and efficacious delivery of ADC to treat chronic diseases.

Cytotoxic payloads for ADCs

The effectiveness of ADCs depends on the internalization of the ADC, followed by the release of active cytotoxic molecules inside the cytoplasm of tumor cells. For tumors with insufficient expression of targeting antigens, the potency of the payload should be sufficient to kill the cancer cells, even at low doses [40]. Given that ADCs currently require intravenous administration, it is crucial that the cytotoxic payload exhibits extended stability in the circulation. Apart from these physicochemical features, the chemical structure of cytotoxin should also allow conjugation to the linker while maintaining the internalization property of the Ab and promoting its antitumor effects [41]. The drug payload in the ADC should demonstrate a high potency therapeutic index because it is projected that only approximately 2% of the injected ADC dose will reach the tumor site, resulting in low intracellular drug delivery [2]. A drug that is suitable for use in an ADC should be potent enough to kill aggressively dividing cancer cells but not lethal to healthy cells. In fact, drugs used in clinical trials are limited to six to eight compounds; the majority of these payloads originate from natural product sources, and are categorized as follows [41]: (i) Microtubule inhibitors (DM1, DM4, MMAF, and MMAE); DNA synthesis inhibitors [calicheamicin, doxorubicin, duocarmycin, and pyrrolobenzodiazepines (PBD)]; and (iii) topoisomerase inhibitors [SN-38 (quinoline alkaloids)].

Consequently, an ideal payload for an ADC should have an *in vitro* subnanomolar IC₅₀ (half maximal inhibitory concentration) value against tumor cell lines and a suitable functional group with adequate solubility in aqueous solutions for chemical conjugation with the Ab and for improving the solubility of the resulting ADC [42,43]. Derivatives of auristatin, maytansinoid, calicheamicin, duocarmycin, PBD, and amanitin are currently being utilized as cytotoxic payloads, and are described below [44].

Auristatin

Auristatin is a dolastatin 10-based auristatin analog [45]. Clinical trials of Dolastatin 10 did not progress because of its nonspecific toxicity. However, its synthetic derivatives MMAE and MMAF are currently used as cytotoxic payloads in ADCs. MMAE and MMAF function as mitotic inhibitors. The first and only FDA-approved auristatin-based ADC is Adcetris®, which is used for lymphoma and systemic anaplastic large cell lymphoma (sALCL) [40]. However, more than ten auristatin-based ADCs are currently in clinical trials [44] (Table 1).

Maytansinoids

Maytansinoids (DMs) are thiol derivatives of maytansines and their function is similar to that of *Vinca* alkaloids. Maytansines bind to the ‘plus’ end of the growing microtubule and block the polymerization of tubulin dimers, thus preventing the formation of mature microtubules [44,46]. However, maytansine derivative (DM1 or DM4)-conjugated ADCs can selectively deliver the payload in cancer cells, thus improving the therapeutic index. Some maytansinoid-based ADCs are currently in clinical trials for solid and hematological cancers [7]. Among these, trastuzumab-DM1 (Kadcyla®), a maytansinoid-linked HER-2-targeting ADC, was approved by the FDA for use against HER-2-positive metastatic breast cancer.

Calicheamicin

Calicheamicin, a potent DNA-targeting agent, is used as a toxic payload for ADCs [47]. Calicheamicin recognizes the minor groove of the TCCTAGGA sequence of DNA and inhibits DNA replication [44]. Mylotarg®, a calicheamicin γ 1- anti-CD33 conjugate, has an acid-cleavable hydrazone linker. Mylotarg® was withdrawn from market 10 years after its initial approval, because of adverse effects such as premature release of calicheamicin, low conjugation efficiency of drug to Ab, exchange of F_{ab} arm with serum Ab, and unwanted binding of ADC to CD33-positive liver cells [1]. CMC-544, a calicheamicin-linked CD22 (IgG4)-targeting ADC has been pursued in multiple clinical trials for different hematological cancers, such as ALL and non-Hodgkin’s lymphoma (NHL) [48].

Duocarmycin

Duocarmycin, a potent antitumor antibiotic, has an IC₅₀ of 40–100 pM [49]. It targets the minor groove of DNA, where it alkylates adenine bases. Synthetic analogs of duocarmycin, such as CC1065, are to be a potent payload for ADCs; currently, BMS-936561 (MDX-1203) is in a Phase I clinical trial [50]. CC1065-conjugated ADCs are efficacious against multidrug resistant (MDR) cell lines (DLD-1 and HCT-15) and showed promising *in vivo* antitumor effect at lower dose [44].

Amatoxin

Amatoxin is a cyclic peptide that binds RNA polymerase II, leading to the inhibition of DNA transcription and ultimately programmed cell death [51]. ChiHEA125-Ama is anti-EpCAM linked to α -amanitin ADC [52].

Other ADC payloads

Some miscellaneous cytotoxic payloads, such as derivatives of PBDs and centanamycin (indole carboxamide), are also considered as potent payloads for ADC development. These molecules bind to double strands of DNA and either alkylate or intercalate the DNA, thereby inhibiting DNA replication. The PBD-containing ADCs (SGN-CD33A and SGN-CD70A) are currently in Phase I clinical trials [44,53] (Table 1). PBDs are naturally produced and intercalate specific regions of DNA within the tumor cell [44]. The blocking of cancer cell division potentially inhibits tumor growth without distorting its DNA helix structure, thereby avoiding emergent drug resistance [54].

Doxorubicin

Doxorubicin binds to DNA by intercalation and inhibits DNA synthesis [55]. For example, milatuzumab-conjugated doxorubicin ADC (IMMU-110) has undergone Phase I/II clinical trials for CD74-positive relapsed multiple myelomas [56].

The primary challenge to developing a new cytotoxic drug is that it should have a high therapeutic index value; therefore, a low dose of drug would be enough to kill the tumor cells, resulting in the reduction of adverse effects.

Advances in ADC technology

Since the breakthrough discovery of the first FDA-approved ADC, Mylotrag®, anticancer therapy research has diversified, resulting in the development of several novel, safer ADCs. Herein, we describe advances in the development of ADCs towards targeted anticancer drug delivery.

First-generation ADCs

The primary focus in the design of first-generation ADCs was faster body clearance to reduce adverse effects. To achieve this, ADCs were made with murine-derived Ab backbones; however, in humans, these generated immunogenic human antimouse Abs, which accelerated the clearance of ADCs by the immune system [44]. The choice of antigen in first-generation ADCs was not specific to tumor cells, resulting in adverse effects. The linkers of ADCs were also not stable enough in the circulation and the toxic payload used was less potent (micromolar IC₅₀ range). For example, BR96-Dox, an anti-Lewis Y-targeting BR96-mAb conjugated with doxorubicin, was terminated in Phase II clinical trials against Lewis Y-expressing epithelial tumors [57]. Similarly, the FDA-approved CD33-targeting Mylotrag® was also withdrawn from market. Both ADCs showed acute adverse effects and morbidity in patients, attributed to acid-labile weak hydrazone linkers and nonspecific antigen expression in healthy cells [58].

Second-generation ADCs

The limitations and failures of first-generation ADCs were eliminated in second-generation ADCs. The premature release of drugs because of the unstable hydrazone linker in Mylotrag® has been avoided in second-generation FDA-approved ADCs, by using different linkers, such as the valine-citrulline (cathepsin cleavable) linker in Adcetris® and a thioester (noncleavable) linker in Kadcyra®. The cytotoxic payloads used in second-generation ADCs are also more potent than in first-generation ADCs. For example, tubulin-targeting agents, such as MMAE used in Adcetris® is approximately 100–1000-fold stronger than DNA-intercalating doxorubicin of BR96-Dox [59]. The IC₅₀ of MMAE is approximately 1 nM in different human cancer cell lines, whereas doxorubicin IC₅₀ is in the 1–6 µM range [60]. Despite the improvement in cytotoxic payloads and the introduction of stable linkers, second-generation ADCs have significant limitations in terms of their heterogeneous DAR, resulting from stochastic coupling strategies between the Ab and drug [61]. Typically, chemical conjugation between the drug and Ab occurs via the lysine or cysteine residue of the mAb, which generates DAR (range 0–8) with an average value of 3–4. Therefore, heterogeneous ADCs can contain a mixture of unconjugated, partially conjugated, and overconjugated Abs and, therefore, there will be competition between unconjugated Abs and drug-conjugated species for antigen binding that can diminish the activity of the ADC. By contrast, overconjugation of the drug to the Ab can result in Ab aggregation, a decrease in stability, incremental increases in nonspecific toxicity, and a reduction in the half-life of ADCs in the circulation [22]. Overall, heterogeneous ADCs have a limited therapeutic index and tumor penetration abilities, which are associated with the induction of drug resistant in the tumor microenvironment.

Third-generation ADCs

The aforementioned concerns regarding the heterogeneous DARs of second-generation ADCs have been addressed in third-generation ADCs. Site-specific conjugation has been introduced to produce homogenous ADCs with well-characterized DARs and desired cytotoxicities [62]. The site-specific conjugation of the drug to Ab provides a single-isomer ADC with a uniform DAR value. Such ADCs can be made using bioengineered Abs containing site-specific amino acids, such as cysteine, glycan, or peptide tags [63]. For example, precise site-specific conjugation of MMAE to human IgG was developed by replacing the Ala₁₁₄ amino acid of the CH1 domain of the IgG Ab with cysteine to create a selectively engineered Ab, called THIOMAB [64]. The site-specific coupling of drug with THIOMAB creates THIOMAB drug conjugates (TDCs) that have homogeneous DARs, low hydrophobicity, and better safety profile for patients [62]. Alternative approaches to site-specific drug conjugation include: (i) a thio-bridge approach that links drugs to the interchain disulfide bond of Abs (four per mAb) [14]; (ii) introduction of unnatural amino acids, such as p-acetylphenylalanine, or noncanonical amino acids, such as phenylselenocysteine, using an encoded gene of the amber stop codon (TAG), along with corresponding orthogonal tRNA/aminoacyl-tRNA synthetase [65]. Recently, a novel chemoenzymatic approach, SMARTag™, was used for the site-selective modification of Abs that generated uniform DAR in ADCs. In this method, the formylglycine-generating enzyme (FGE) recognition sequence was inserted at a specific location in the Ab backbone using molecular biology techniques. In eukaryotic cell FGE, an endogenous enzyme of eukaryotic

cells catalyzes the conversion of the cystine to a formylglycine residue (fGly) [66]. The aldehyde group of fGly is involved in the site-specific reaction of drugs, such as PBDs, auristatin, and duocarmycin. SMARTag™-based ADCs, such as CD22–4AP, are a proprietary technology of Catalent's pharma solution and preclinical studies of CD22–4AP showed it to have a promising therapeutic index with minimum adverse effects and to avoid the development of drug resistance. Similarly, other enzymatic strategies, such as SMAC-TAG™ and TG-ADC™, have been developed for site-specific ADC preparation [67] (Figure 3b). Efforts are continuing to develop more efficient cytotoxic payloads in third-generation ADCs so that a smaller amount of drug is needed to achieve the desired therapeutic effect and to eliminate drug-resistant tumor cells. The development of PBDs, derivatives of tricyclic antibiotics, has attracted interest over other DNA-alkylating agents. PBDs are potent compounds, some of which have subpicomolar IC₅₀ and do not show cross-resistance with other chemotherapeutics, such as cisplatin [68]. Four PBD-containing ADCs are currently in clinical trials: SC16LD6.5 is in Phase II trials for small cell lung carcinoma; SGN-CD33A is in Phase II trials for AML; SGN-CD70A is used to treat patients with CD70-positive cancer, and ADCT-301 is in Phase I trials for CD25 lymphoma [69].

Clinical trials of ADCs

The FDA withdrawal of mylotarg® (10 years after its initial approval) was a lesson learned for redesigning ADC components. Researchers have made significant progress in improving conjugation technology, Ab bioengineering, linker chemistry, and the identification of potent drugs has resulted in more successful clinical outcomes for ADCs (Table 1). This led to the FDA approval of Adcetris® and Kadcyla®, and additional clinical trials, such as 50 active trials for solid tumors and 20 active clinical trials for hematological cancers [44,70]. Pharma Source's trend reports (www.pharmasource.com/trend/adc-market-opportunity-for-cmos) on ADCs reported that more than 15 clinical trials were pursued in 2015, six to eight trials were completed in 2016, and that this trend will continue in 2017. The promising ADC, inotuzumab ozogamicin (CMC 544), was recently withdrawn for the treatment of relapsed NHL. However, CMC-544 is in active Phase III trials for ALL, and was recognized as an orphan drug by the FDA. Some Phase I trials show some success in the use of drugs from the maytansinoid or auristatin classes of molecules for various cancers. Furthermore, the most commonly used Ab is IgG1, although there are trials on going with IgG2 and IgG4. Despite the strong clinical outcome, the cost of ADC development remains a major limitation. For example, the cost of treatment with the mAb trastuzumab is approximately US\$50 000 per year, and the price almost doubles for the trastuzumab-DM1 conjugate Kadcyla®. The development of ADCs is currently directed at decreasing the costs of production by using cheaper nonanimal recombinant mAb technologies and repurposing existing drugs [71]. Therefore, based on the success rate of these clinical trials, it is evident that there is much scope for the further study of ADCs.

Concluding remarks: challenges and hope for ADCs

Extensive research is on going to improve all the components of ADCs that can enhance their targetability and therapeutic efficacy against tumors. A better understanding of ADC-targeting strategies can speed up the FDA approval rate of ADCs and drastically increase the

number of clinical trials, especially in solid tumors. However, the failure of some ADCs, such as Mylotrag®, IMGN242-for gastric cancer in Phase II trials, and SGN15-for ovarian cancer in Phase II trials, is mostly attributed to payload-related toxicity and low therapeutic index [44]. These limitations are being addressed in various ways, such as the development of homogenous ADCs with narrowed DARs, using bispecific Ab with enzyme-activated linkers, and high potent payloads [20,69]. Another challenge to the clinical success of ADCs is the evaluation of viable biomarkers, which will ensure the effectiveness of ADCs for selective cancer targeting. Immunohistology (IHC) is a widely used method of determining antigen expression and requires tissue biopsies, which sometimes limits its use. Recently, other efforts, such as the use of circulating tumor cells and imaging techniques, avoid the limitations of IHC-based methods and are being used to determine the patient population likely to respond to ADC therapy [72]. Another approach to increasing the binding affinity of ADCs is to use protein scaffolds, which have well-defined 3D polypeptide frameworks and their binding affinity to antigens is in the nM to pM range. Clinically utilized protein scaffolds are categorized into two classes: non-Ig scaffolds, such as the Kunitz domain scaffold-based DX88 and affibody scaffold-based ABY-220; and Ab-derived scaffolds, such as the BiTE scaffold-based blinatumomab and nanobody scaffold-based Alx-0141 [73]. These scaffolds appear promising in the development of higher-affinity ADCs. A major hurdle to ADCs targeting solid tumors is the tumor stroma, which is a thick, connective, functionally supportive framework layer comprising tumor and nontumor cells. This tumor stroma has been identified as crucial factor in promoting tumor growth, angiogenesis, metastasis, and immune suppression. Recently, emphasis has shifted to inhibiting fibroblast activation protein (FAP)-expressing tumor stromal cells [74]. FAP-targeting mAbs are still in development and only a few have been utilized in clinical trials. Therefore, it is hoped that, in the near future, tumor stroma-targeting ADC could open a new therapeutic window for the treatment of solid tumors.

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highlights

- ADC represents a new class of targeted anticancer therapeutics
- Elucidates selection criteria for tumor specific antigen and ADC development
- Smart linkers and fixed DAR value for reducing side effects of ADCs are covered
- Current clinical trial trends and market prediction of ADCs are summarized

Figure 1.

(a) Structural characteristics of earlier generation and advanced-generation antibody–drug conjugates (ADCs) [75]. **(b)** List of targetable tumor antigens: epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR); tyrosine-protein kinase met (C-Met); epithelial cell adhesion molecule (EPCAM); carbonic anhydrase- IX (CA-9); tumor-associated calcium signal transducer 2 (TROP-2); vascular endothelial growth factors receptor-2 (VEGFR-2); prostate specific membrane antigen (PSMA); endothelin receptor-B (ET-B); matrix metalloproteinase-9 (MMP-9); and fibroblast activated protein (FAP) [1].

Figure 2. Mechanistic pathways of antibody–drug conjugate (ADC) internalization and cancer cell killing [2].

Figure 3.

(a) Schematic representation of fully human (blue), mouse (green), chimeric (fused mouse-originating antigen-binding domain with human constant domain, green-blue) and humanized [fused mouse CDR domain with a human immunoglobulin (Ig)-G backbone] antibody. Fab and Fc are antibody subdomains and Fv is a variable domain. (b) Advances in ADC technologies and representative images of first-generation (i), second-generation (ii) and third-generation (iii) ADCs. Adapted, with permission, from [7] (bi,ii), [76] (biii), [14] (biv) and [66] (bv).

Table 1.List of ADCs in clinical trials^{a,b}

Clinical trial stage	ADC name	Antibody/Linker	Cytotoxic drugs	Cancer type (s)	Antigen targeted
Phase I	ASG-22ME	Hu IgG1/valine-citrulline (V.C)	MMAE	Urothelial and other malignant solid tumors	Nectin-4
	SAR566658	H ₂ IgG1/DS6 disulfide	Maytansinoid	Malignant neoplasm, triple-negative breast cancer	CA6
	BAY 94-9343	H IgG1/disulfide, HuIgG1/SPDB	Maytansinoid, (DM4)	Mesothelin-positive solid tumors	Mesothelin
	IMGN388	IgG1/disulfide	Maytansinoid	Solid tumors	Integrin α_v
	BIIB015	IgG1/disulfide	Maytansinoid	Anti-Cripto, refractory solid tumors	Cripto-1
	SGN-75	Anti D70/dipeptide	Auristatin	Renal cell carcinoma	CD70
	AGS-22M6E	Anti-Nectin fully human IgG/dipeptide	Auristatin	Malignant solid tumors	Nectin4
	IMGN529	K7153A humanized IgG1 thioether	Maytansinoid	B cell malignancies	CD37
	AMG595	Anti-EGFRvIII fully human IgG1/thioether	Maytansinoid	Glioblastoma	EGFRvIII
	RG7593/DCDT2980S	H ₂ IgG1/valine-citrulline	MMAE	NHL	CD22
	SAR566658	Hu IgG1/SPDB	Maytansine (DM4)	Solid tumors	CA6
	Labestuzumab-SN-38	H ₂ IgG1/phenylalanine-lysine	SN38	Colorectal cancer	CD66e/CEACAM5
	AGS-16C3F	Hu IgG2/maleimidocaproyl	MMAF	Renal cell carcinoma	ENPP3
	SGN-CD33A	H ₂	PDB dimer	AML	CD33
	SGN-CD19A	H ₂ IgG1/maleimidocaproyl	MMAF	B cell lymphoma	CD19
	SGN-LIV1A	H ₂ IgG1/V.C	MMAE	Metastatic breast cancer	LIV-1
	RG7596	H ₂ IgG1/V.C	MMAE	NHL	CD79b
	ASG-5ME	Hu IgG2/V.C	MMAE	Pancreatic, gastric, an prostate cancers	SLC44A4
	BAY 79-4620	Hu IgG1/V.C	MMAE	Advanced solid tumors	CA-IX
	AGS-67E	Hu IgG2/V.C	MMAE	Lymphoid malignancies, leukemia	CD37
	AMG-172	Hu IgG1/MCC	Maytansine (DM1)	Clear cell renal cell carcinoma, renal cell adenocarcinoma and carcinoma	CD27L
	AGS15E	Hu IgG2/[cleavable]	MMAE	Metastatic urothelial cancer	SLITRK6
	GSK2857916	H ₂ IgG1/maleimidocaproyl	MMAF	Multiple myeloma	BCMA
	IMGN289	SMCC	Maytansine (DM1)	EGFR-positive solid tumors	EGFR
	AMG 595	SMCC	Maytansine DM1	Advanced malignant glioma, anaplastic astrocytomas, glioblastoma multiforme	EGFRvIII

Clinical trial stage	ADC name	Antibody/Linker	Cytotoxic drugs	Cancer type (s)	Antigen targeted
	IMGN242 (huC242-DM4)	Humanized IgG1/huC242 disulfide	Maytansinoid	Solid tumors	CanAg
Phase I/II	Immunomedics (IMMU)-110 (hLL1-DOX)	Milatumzumab hydrazone	Doxorubicin	Multiple myeloma	CD74
	Lorvotuzumab mertansine (IMGN901)	Humanized IgG1/huC242 disulfide	Maytansinoid	Multiple myeloma, solid tumors	CD56
	IMMU-132	Hz IgG1/CL2A	CPT-11 SN38	Colorectal cancer, gastric adenocarcinoma, esophageal cancer, hepatocellular carcinoma	TROP-2
	Milatumzumab doxorubicin	Hz IgG1/hydrazone	Doxorubicin	Multiple myeloma	CD74
	HuMax®-TF	Hu IgG1/V.C	MMAE	Solid tumors	Tissue Factor
Phase II	SAR3419 (huB4-DM4)	huB4/humanized IgG1 disulfide, Hz IgG1/SPDB	DM4	B cell NHL	CD19
	BT062	ChIgG4/SPDB, anti-CD138 chimeric IgG4 disulfide	DM4	Multiple myeloma	CD138, Syndecan1
	Glembatumumab vedotin (CDX-011)	Hu IgG2/valine-citrulline, anti-CR011 dipeptide	MMAE	Breast cancer melanoma, squamous cell carcinoma of lung	gpNMB
	Anti-PSMA ADC	Hu IgG1/V.C	MMAE	Prostate cancer	PSMA
	MLN0264	Hu IgG1/V.C	MMAE	Gastrointestinal malignancies	Guanylyl cyclase C
	Lorvotuzumab mertansine	Hz IgG1/SPP	DM1	Solid tumors	CD56
	PSMAADC	Anti-PSMA fully human IgG1/dipeptide	Auristatin	Metastatic, hormone-refractory prostate cancer	PSMA
Phase III	Inotuzumab ozogamicin (CMC 544)	Humanized IgG4/G5/44 hydrazone	Calicheamicin	B- cell lymphomas, NHL	CD22
FDA Approved	Gemtuzumab ozogamicin (mylotarg®), Now terminated	Hu IgG4/hydrazone	calicheamicin	AML	CD33
	Trastuzumab-emtansine (Kadcyla®)	HzIgG1 trastuzumab/thioether	DM1	Metastatic breast cancer	HER2
	Brentuximab vedotin (Adcetris®)	Ch IgG1/V.C	MMAE	Hodgkin's lymphoma	CD30
Terminated	LOP628	HzIgG1/(noncleavable)	Maytansine	AML/C-Kit-positive solid tumors	cKit

^aFrom [clinical trials.gov](https://clinicaltrials.gov).

^b Abbreviations: Ch, chimeric antibody; Hu, fully human; Hz, humanized.