Antibody–Drug Conjugates: A Comprehensive Review

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ABSTRACT

Antibody–drug conjugates (ADC) are one of the fastest growing anticancer drugs. This approach comprises a mAb conjugated to the cytotoxic payload via a chemical linker that directed toward a target antigen expressed on the cancer cell surface, reducing systemic exposure and therefore toxicity. ADCs are complex molecules that require careful attention to various components. Selection of an appropriate target, an mAb, cytotoxic payload, and the manner in which the antibody is linked to the payload are key determinants of the safety and efficacy of ADCs. This review provides an overview of the systemic evaluation of each component of an ADC design, improved understanding of the mechanism of action of ADC, and mechanistic pathways involved in ADC resistance and various strategies to optimize ADC design. Moreover, this review also shed light on the current status of ADCs that have gained regulatory approval from the FDA including a description of biology and chemistry, metabolic profiles, adverse events, drug interactions, and the future perspective on combination strategies with other agents, including immunotherapy.

Introduction

Cancer is the second most common fatal disease, causing approximately 8.2 million deaths worldwide each year (1). The therapeutic interventions used for treating can/tumor include chemotherapy, immunotherapy, radiation, stem cell therapy, laser treatment, hyperthermia, surgery, photodynamic therapy, etc. Among these treatment options, chemotherapy was the principal therapeutic intervention for treating cancer (2, 3). This concept is based on the premise that these agents would not harm normal cells while preferentially kill rapidly dividing tumor cells. On the basis of this concept, nitrogen mustard was tested in humans resulting in the eradication of bone marrow and lymphoid tissues in patients suffering from cancer (4). This chemotherapeutic agent exerts its cellular apoptotic action by DNA alkylation. Thereafter, antifolates such as methotrexate, DNA synthesis inhibitors like thioguanine, 5-fluorouracil, and cytosine arabinoside (ara-C), and DNA interacting agents like cisplatin, actinomycin D, anthracyclines, and Vinca alkaloids entered the foray of drugs used for the treatment of cancer. Despite advances in anticancer chemotherapy, the use of small-molecule anticancer drugs as the most widely used chemotherapeutic drugs (5), has withstood enormous hurdles demonstrating limited selectivity against cancer cells, systemic toxicity, and drug resistance development that results in the narrow therapeutic window, and thus limiting its efficacy (6).

For the anticancer drugs to have improved therapeutic index, it is important to enhance the potency of the cytotoxic agent to reduce the minimum effective dose (MED), or to increase tumor selectivity to escalate the MTD. An ideal solution would be the development of agents that would both decrease the MED and increase the MTD, thus increasing the overall therapeutic index of the cancer drug (7).

Antibody–drug conjugate (ADC) is a new emerging class of highly potent pharmaceutical drugs, which is a great combination of chemotherapy and immunotherapy. The concept of ADC was first presented by the German physician and scientist Paul Ehrlich almost 100 years before. He described the antibody as a "magic bullet" that identifies their target themselves without harming the organism (8). Ehrlich also anticipates attaching toxin to the antibodies to improve their therapeutic specificity. Forty-five years later, in accordance with the concept of Paul Ehrlich, methotrexate was attached to an antibody against leukemia cells (9). In the 1980s, clinical trials of ADCs grounded on mouse IgG molecules were performed. The first ADCs based on chimeric and humanized mAbs were testified in the 1990s (10). This technology consists of highly specific mAbs attached to extremely cytotoxic agents with the help of various linkers. ADCs empower selective delivery of highly potent drugs to tumor cells while sparing healthy cells, attenuating the main clinical obstacle of traditional chemotherapy, thus providing a broad therapeutic window.

Key Requirements of ADCs

Target antigen selection

One of the most important aspects of ADC development for cancer is the identification of the unique antigenic target of the mAb component. There are 328 unique antigens used in antibody-based therapy as a target (11). The selected antigen needs to fulfill several requirements. First, the target antigen needs to have high expression in the tumor and no or low expression in the healthy cell (12). For example, the HER2 receptor, which is almost 100-fold highly expressed in the tumor cell compared with the healthy cell (13). Second, the target antigen should be displayed on the surface of the tumor cell to be available to the circulated mAb (14). Third, the target antigen should possess internalization properties as it will facilitate the ADC to transport into the cell, which will in turn enhance the efficacy of the cytotoxic agent (15), although some studies have demonstrated that noninternalized ADC product directed against components of the tumor microenvironment can efficiently detach their drug in the extracellular space and arbitrate a potent therapeutic activity in some cases and that ADCs often induce a strong "bystander effect" (16).

In ADCs, the most targeted antigens are ERBB2, CD19, CD33, CD22, and MSLN (mesothelin). In addition, over 50 different known antigens have been used in ADC as a target (11). However, previous studies showed that some tumor antigens also show low expression in the normal cell. For example, the antigen MSLN that is overexpressed

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in mesothelioma, ovarian, pancreatic, and lung adenocarcinomas was found to be low expressed in the healthy cell. Golfier and colleagues (17) developed new ADC against mesothelin that showed great efficacy in patient-derived xenograft tumor models, although it also displayed a bystander effect on neighboring mesothelin-negative tumor cells.

Selection of antibody moiety

The antibody is the main component of the ADC design; it should possess the following characteristics: first is target specificity, that is, antibody should deliver the cytotoxic drug to the tumor cell (18). Second is target-binding affinity, that is, antibody should possess a high binding affinity to the tumor cell-surface antigens. In addition, the antibody should also bear good retention, low immunogenicity, low cross-reactivity, and appropriate linkage-binding properties (19).

In the first-generation ADCs, a murine antibody against the target antigen was used. The drawback of this murine antibody was that it showed a strong immune response, and many patients produced anti-human antibodies resulting in reduced efficacy of the treatment (20). However, with the advancement in gene engineering technology, this issue was resolved and resulted in the second-generation ADCs. In the second-generation ADCs, the murine antibody was converted into a mouse/humanized chimeric antibody (21). The chimeric antibody contains the mouse light- and heavy-chain variable regions that are linked to human constant regions (22). The human constant regions aid to reduce the immunogenicity and human anti-mice antibody. This mouse/humanized chimeric antibody showed promising therapeutic efficacy (23). An example of the chimeric antibody used in ADC design is the new generated ADC by Wang and colleagues This ADC consists of a chimeric anti-CD30 mAb linked to lidamycin, a potent cytotoxic agent, via a nonprotease peptide linker (24). This new generated ADC has a specific affinity, strong cytotoxicity, and high efficacy against CD30-overexpressing tumor cells. The chimeric antibody-based ADC showed great efficacy in cancer treatment. However, in some cases, decreased therapeutic efficacy of the chimeric antibody is observed as the chimeric antibody showed predominantly human anti-chimeric antibody response to the murine variable regions of the antibody (25). To tackle this problem, efforts were put forward to design a humanized mAb. Humanized antibody contains only the complementarity determining regions of the rodent variable region that are grafted into the human variable region framework (26). An example of a successfully humanized antibody used in ADC design is the Kadcyla that is the combination of humanized mAb against HER-2–positive cells linked to the DM1 cytotoxic agent. This ADC is used for the treatment of patients with HER-2–positive metastatic breast cancer that do not respond to antibody treatment alone or to chemotherapy (27). Recently, next-generation or third-generation ADCs are replacing the second-generation ADCs as in this generation, a fully human antibody is used instead of a chimeric antibody (28). The advantage of this ADC design over the second generation is that fully human antibody does not produce an immune response and ultimately anti-human antibodies. Gallery and colleagues (29) successfully demonstrated fully human antibody-based ADC. In their study, they used ADC consisting of a fully human anti-Guanylyl Cyclase C (GCC) mAb conjugated to a highly cytotoxic drug monomethyl auristatin E (MMAE) through a protease-cleavable peptide linker. This ADC displayed promising antitumor activity in GCC-expressing cells both *in vitro* and *in vivo*.

Linkers

The linkers play a key role in ADC design as linkers link the cytotoxic drug to the mAb. When the ADC complex circulates in the blood, the linker must be stabilized to avoid the release of the cytotoxic drug in the off-target tissue, and the linker must maintain the conjugate in an inactive, nontoxic state while bound to the antibody (30). At the same time, the linker should possess the property of unleashing the cytotoxic drug upon internalization (31). There are two types of linkers that ensure the abovementioned conditions: noncleavable and cleavable linkers (**Fig. 1**).

Noncleavable linkers

Noncleavable linkers consist of stable bonds that resist proteolytic degradation and provide higher stability than the cleavable linkers (32). The mechanism of action of noncleavable linkers is based on the internalization of the ADC complex followed by degradation of the mAb component in the lysosome, resulting in the release of a cytotoxic drug that kills tumor cells. They do not unleash cytotoxic agents at off-target sites and thus do not harm healthy cells (33). Furthermore, the noncleavable linkers make it possible to modify the chemical properties of small molecules to modulate the affinity of the transporter and to improve the efficiency. Gail and colleagues (34) demonstrated that trastuzumab conjugated via nonreducible thioether linkage (SMCC) to the DM1 cytotoxic drug, exhibited superior activity in comparison with the unconjugated trastuzumab, or trastuzumab conjugated to other maytansinoids via disulfide linkers. Also, the increased serum concentration of trastuzumab-SMCC-DM1 was observed in comparison with different conjugates, and toxicity in rats was insignificant as compared with the free DM1 or trastuzumab conjugated via a reducible linker to the cytotoxic drug DM1. This is one of the successful examples of ADCs using a noncleavable linker. For noncleavable linkers to release active drugs, they need to degrade the mAb in the lysosome after internalization of the ADC. To this end, variances between parent drug and potential ADC metabolites must be considered. For instance, MMAE is a protein-based antimitotic drug that is most effective in its natural form and therefore not suitable for derivatization with noncleavable linkers (35). Conversely, monomethyl auristatin F (MMAF) retains its efficacy even when attached to a simple alkyl chain *in vitro* and *in vivo* (36). One proposed mechanism for the decreased efficacy of noncleavable linked ADCs is that drugs with charged amino acids are always affected by reduced membrane permeability (35). This does affect diffusion into the cell as, in this case, drugs should pass the plasma membrane and thus limiting their ability to kill nearby cells. However, this also accounts for internalized drugs as they should pass the endosomal/lysosomal membrane. Therefore, a great incentive for using noncleavable linkers is to make the "bystander" effect better (37).

Cleavable linkers

Cleavable linkers are the major class of ADC linkers. The main feature of cleavable linkers is that they are cleaved by environmental differences (such as redox potential, pH) and specific lysosomal enzymes in response to extracellular and intracellular environments (32). There are different kinds of cleavable linkers used in ADC design as mentioned below.

Acid-sensitive or acid-labile linkers: These are a group of linkers that are sensitive to the acidic environment but are stable in the alkaline environment such as systemic circulation. Upon internalization into the targeted tumor cells, the acid-sensitive hydrazone group in acid-labile linkers gets hydrolyzed in lysosomal (pH 4.8) and endosomal (pH 5–6) acidic tumor microenvironment (38). However, these linkers have been associated with the nonspecific release of the drug in clinical studies (39). One successful example of ADC design using

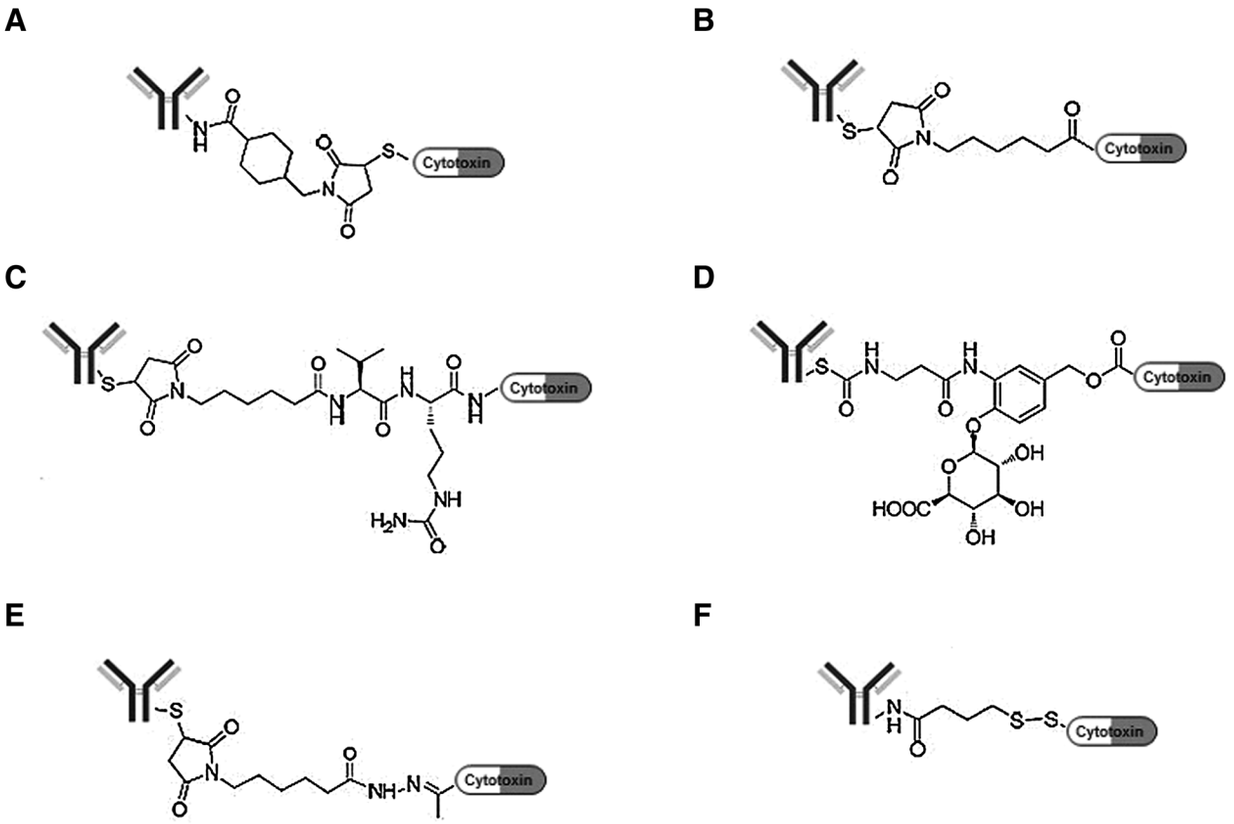

Figure 1.
Chemical structures of noncleavable and cleavable linkers. **A,** SMCC linker. **B,** Maleimidocaproyl linker. **C,** Peptide-based linker. **D,** β-Glucuronide linker. **E,** Acid-sensitive linker. **F,** Disulfide linker.

acid-sensitive linker is the IMMU-110. This ADC is composed of a humanized anti-CD74 mAb conjugated to doxorubicin via acid-labile hydrazone. IMMU-110 displayed improved activity against multiple myeloma and appeared to be safe in a monkey model of multiple myeloma cells (40).

Lysosomal protease–sensitive linkers: Lysosomal protease–sensitive linkers, also known as peptide-based linkers, are the most common linkers used in ADC design. As tumor cells exhibit high expression of lysosomal proteases like cathepsin B compared with the normal cells, therefore, cathepsin B–sensitive peptide linker ADCs are selectively bound to and transformed into cancerous cells through receptor-mediated endocytosis (41). In addition, peptide-based linkers are stable in unsuitable pH condition and different serum protease inhibitors; therefore, these peptide linkers are stable in the systemic circulation and only unleash the drug in the target cells (42). Valine-citrulline (v-c) is the most commonly used peptide linker in current clinical research. One example of successful use of the Valine-citrulline linker in ADC design is the Adcetris. Valine-alanine (v-a) and phenylalanine-lysine (p-l) have also been utilized in some other ADCs, such as SGN-CD70A and labetuzumab-SN-38 (43).

β-glucuronide linker: Another protease-sensitive linker is the β-glucuronide linker that is recognized and hydrolyzed by β-glucuronidase for the drug release (44). Lysosomes and tumor necrotic regions are rich in β-glucuronidase, which is inactive at physiologic pH and active at lysosomal pH (45). This selective site of action allows for the cleavage of the glycosidic linkage of the β-glucuronidase–sensitive β-glucuronide linker, thereby enabling the selective release of cytotoxic payloads. Therefore, an ADC with a glucuronic acid–based linker may improve the stability of the ADC in the blood circulation (46). Furthermore, the hydrophilicity of the glucuronic acid–based linker shows a higher solubility of the intact ADC compared with the dipeptide-based ADC, and its efficacy is comparable with that of the ADC coupled to the v-c linker (47).

Glutathione-sensitive disulfide linkers: Another most commonly cleavable linkers used in ADC design are the glutathione-sensitive disulfide linkers. Glutathione is a low molecular weight thiol that is found in the intracellular compartment (0.5–10 mmol/L in the cytoplasm) and extracellular environment (2–20 mmol/L in plasma; ref. 48). The main principle of this linker is the difference in reduction potential in the cytoplasm in contrast to plasma (49). Glutathione is highly released during cell survival, tumor growth, and cell stress conditions such as hypoxia; therefore, a high concentration of glutathione can be found in cancer cells than normal cells (50). In addition, the tumor cells also contain enzymes from the isomerase family of protein sulfide that may assist in the decrease of the disulfide bond in cellular compartments (51). Therefore, glutathione-sensitive linkers are stable in the blood flow and particularly chopped by the elevated intracellular concentration of glutathione in the tumor cell, releasing the active drugs at the tumor sites from the nontoxic prodrugs (52).

Cytotoxic payloads or warheads

The cytotoxic payload or warhead is another important component of ADC design that gets activated after release from ADC inside the cytoplasm of tumor cell (53), and the potency of warheads should be acceptable to destroy the tumor cells, even at low doses (54). The cytotoxic warhead used in ADCs should be of high stability in the systemic circulation and lysosomes. An ideal warhead for an ADC design should have an *in vitro* subnanomolar half maximal inhibitory concentration (IC_{50}) value for cancer cell lines and sufficient solubility in the aqueous environment of antibody (55). Low immunogenicity, small molecular weight, and a long half-life are also crucial aspects of the warheads (56). Moreover, the chemistry of warheads should also allow conjugation to the linker while maintaining the internalization property of the mAb and promoting its antitumor effects (57). There are 16 known drug classes integrated into clinical-stage ADCs, 11 of which are small-molecule–based, and the other five are derived from the proteins (11). In general, there are two main classes of warheads that are widely used in ADC design that are mentioned below.

Microtubule-disrupting agents

Auristatin: Auristatin is a synthetic antineoplastic agent derived from the natural product dolastatin 10 (58). The dolastatin 10 is a nonspecific toxic agent, and because of this reason, it does not use as a cytotoxic warhead in ADC design. However, the synthetic analogues of this class of drug such as MMAE and MMAF are presently being used as a cytotoxic payload in ADCs (59). MMAE is an antimitotic agent that exerts its action by blocking the tubulin polymerization process resulting in cell-cycle arrest and apoptosis (60). The main function of MMAF is the same as that of MMAE; however, it has reduced activity compared with MMAE due to the presence of a charged C-terminal phenylalanine. The auristatin is the most widely used payload in ADC design. One of the auristatin-based ADC is the Brentuximab vedotin, commercially available as Adcetris (61). It is composed of anti-CD30 chimeric IgG1 mAb (Brentuximab or cAC10), conjugated to 3–5 molecules of the warhead MMAE by a cathepsin-cleavable linker (62). It is used to treat CD30-positive lymphoproliferative disorders, including anaplastic large-cell lymphoma (ALCL) and Hodgkin lymphoma (62).

Maytansinoids: Maytansinoids represent a second major class of microtubule-disrupting agents isolated from the maytansine, a benzoansamacrolide (63). These drugs inhibit tubulin polymerization resulting in mitotic arrest and cell death (64). The function of maytansinoids is the same as that of Vinca alkaloids. However, the maytansinoids demonstrated cytotoxicity almost 100 times higher than the Vinca alkaloids (65). Because of the lack of tumor specificity and severe systemic toxicity, maytansinoids failed in human clinical trials as an anticancer drug. Although, the powerful cytotoxicity of maytansinoids can be utilized as targeted delivery access, particularly as antibody–maytansinoid–conjugates (AMC). Preclinical studies indicate that AMCs have significantly improved potential as anticancer agents in comparison with the unconjugated maytansinoids (66). A derivative of maytansine, DM1 and DM4 have already been used in ADC design such as Trastuzumab emtansine, which is commercially available under the name Kadcyla (67). It is composed of an antibody (Trastuzumab or Herceptin) conjugated with the warhead DM1 (maytansine derivative) using a noncleavable thioether linker against the HER2 receptor. This ADC is used to treat patients with HER2-positive metastatic breast cancer that are resistant to other treatments (68).

DNA-damaging agents

Calicheamicin: Calicheamicins are a class of enediyne antitumor antibiotics derived from the bacterium *Micromonospora echinospora* (69). Calicheamicin recognizes the minor groove of the TCCTAGGA sequence of DNA and halts DNA replication (70). N-acetyl-calicheamicin, a derivative of calicheamicin, is used in the ADC design as the payload (71). This ADC is named as the Gemtuzumab ozogamicin, commercially available as Mylotarg. It is composed of a humanized IgG4 mAb that is conjugated to a calicheamicin payload directed against a surface antigen CD33 that is present in 85%–90% of patients with acute myeloid leukemia (AML; ref. 72). Mylotarg was originally approved as a monotherapy for patients with CD33-positive AML in 2000 under the FDA's accelerated approval program. These patients were 60 years of age or older, had experienced their first relapse, and were not considered candidates for other cytotoxic chemotherapy (73).

Duocarmycin: Duocarmycin is a natural product derivative extracted from the bacteria *Streptomyces* strains (74). Duocarmycins are another class of DNA minor groove–binding alkylating agents. This class of drugs shows its action by binding to the minor groove of DNA and subsequently cause irreparable alkylation of DNA that disrupts the nucleic acid architecture and structural integrity (75). One of the examples of duocarmycin use in ADC design is recently reported in Yu and colleagues' study (76). In this study, they described a novel ADC against CD56 called promiximab-DUBA. This ADC consists of an anti-CD56 hIgG1 antibody that is linked to the payload duocarmycin with the help of a reduced interchain disulfide linker. This new ADC exhibited potent cytotoxic activity against cancer cells *in vitro* and *in vivo*.

Doxorubicin: Doxorubicin shows its action by intercalation of DNA that inhibits DNA synthesis (77). One prominent example of doxorubicin-based ADC design is the milatuzumab-conjugated doxorubicin ADC (IMMU-110) that has undergone phase I/II clinical trials for CD74-positive relapsed multiple myelomas (78). Another example of this class payload is the work of Ma and colleagues (79). In this study, the doxorubicin-based ADC was able to suppress tumor growth and improve the survival of hepatocellular carcinoma–bearing nude mice. Moreover, it showed less toxicity compared with single-agent doxorubicin or G7mAb.

Mechanism of Action of ADCs

The idea behind ADCs is the optimal delivery of a highly potent payload to its target using a specific carrier. Administration of ADCs is done intravenously into the bloodstream to avoid gastric acid and proteolytic enzyme degradation of the mAb (80). Ideally, exclusive expression of the target antigens on tumor cells, but not on normal cells, is the prerequisite for the mAb component of ADCs to find and bind to it (81).

Upon recognition and attaching to its target, internalization of the ADC–antigen complex into the cell takes place via receptor-mediated endocytosis (44, 82). Internalization takes place via three different routes: (i) clathrin-mediated endocytosis (the major route of intracellular uptake of ADCs), (ii) caveolae-mediated endocytosis, and (iii) pinocytosis (83). Clathrin- and caveolae-mediated endocytosis are antigen-dependent while pinocytosis is antigen-independent (84). The rate and efficiency of the ADC–antigen complex to be internalized depends on the type of target and the cytotoxic compounds. Insufficient affinity (K_d > 10 nmol/L), in case of low binding affinity that

does not result in binding to the receptor, may result in inefficient internalization leading to the off-target release of ADC and therefore results in systemic toxicity (39).

Internalization results in inward budding of the cell membrane and passage of proton ions into the early endosomes. These proton ions provide an acidic environment that creates an interaction between the mAb component of ADCs and human neonatal FcRs (FcRns; 18). A portion of the ADC binds to FcRns in endosomes and circulates back extracellularly, where the physiologic pH of 7.4 assists the release of the ADC from the FcRn (85). This recycling mechanism acts as a buffer to prevent normal cell death in the event of misdelivery. As we know that the recycling of ADCs by FcRn-mediated internalization mechanism may lead to drug release, therefore, such Fc-tail–lacking ADCs might have an advantage.

Finally, ADCs retained in the early endosome are transformed into the late endosome stage where they lose the proteins involved in recycling. The late endosome then couples to lysosomes that results in low pH. The acidic environment and lysosomes rich in proteases such as cathepsin-B and plasmin then cleave the ADC that subsequently undergoes optimal release of the free cytotoxic warheads into the cytoplasm (18, 23), where they interfere with the cellular mechanisms, induce apoptosis, and ultimately cell death (27, 86). The pathway of cell death depends on the type of warhead used. For example, auristatins and maytansinoids cause cellular apoptosis via interfering with microtubulins, while calicheamicins and duocarmycins induce cell death by intercalation of DNA (87). The success of ADC in inducing cytotoxicity depends on various factors such as the characteristics of the target antigen, selecting a specific antibody, engineering a stable linker, and conjugating potent payloads.

Resistance to ADCs

ADCs provide an ideal delivery method for cytotoxic payloads to treat different kinds of cancers (32). However, resistance to a cancer cell is still the main hurdle in all cancer therapies. Cancer cells under any therapeutic pressure develop a mechanism of resistance that allows them to survive. Such failure/reduction may have evolved after treatment with the drug (secondary or acquired resistance) or may be present from the start of the treatment (primary or *de novo* resistance). In general, resistance mechanisms to ADCs are subject to arise from each component of the ADCs, namely the mAb, the cytotoxic drug, or by triggering survival signaling pathways (54). Garcia-Alonso and colleagues (88) beautifully summarize various mechanisms of resistance to ADCs in his recent review. According to him, resistance to ADCs could be related to target antigens, ADC internalization, trafficking pathways, changes in the cell cycle and its regulating signaling pathways, drug efflux pumps, lysosomal function, target alteration of the cytotoxic compound, as well as apoptotic dysregulation (for details see ref. 88).

Antigen-related resistance includes alteration in the levels of the antigen recognized by the mAb component of an ADC system. Previously, it has been demonstrated that cancer cell lines exposed to multiple cycles of treatment with various ADCs result in a marked decrease in target antigen levels (89). On the contrary, a high antigen expression may reduce ADC efficacy because of reduced drug exposure (90). Moreover, truncation of the antigen ectodomain or its masking by components of the extracellular matrix as well as the existence of the antigens ligands can also impair ADC sensitivity (91, 92). In a study conducted by Sung and colleagues (93), it has been reported that trastuzumab-ADCs (T-DM1) internalization via caveolin-1 (CAV1) pathway leads to the decreased response of the ADC in a panel of $HER2^+$ cell lines. The decrease in response was due to the insufficient delivery of T-DM1 to lysosomes. Another method of ADC resistance is the impaired lysosomal function. Chemical or enzymatic cleavage in lysosomes is the prerequisite for ADCs to release the cytotoxic payload. It has been shown that the accumulation of T-DM1 in lysosomes makes cells resistant to T-DM1 through long-term exposure to drugs (94). These cells had low therapeutic efficacy in comparison with the sensitive cells. The decrease in therapeutic efficacy was due to increased lysosomal pH, which in turn inhibited lysosomal proteolytic enzymes. One prominent phenomenon of ADC resistance is the drug efflux pumps as the cytotoxic drug is eliminated from the cellular cytoplasm by the ATP-binding cassette transporters such as PgP/multidrug resistance 1 (MDR1; refs. 95–97). Similarly, cell-cycle dynamics also display a crucial role in the sensitivity of ADCs. It has been publicized that the levels of cyclin B, a cell-cycle protein that participates in G_2–M transition, were higher in $HER2^+$ breast cancer cells sensitive to T-DM1 in comparison with the cells made resistant to T-DM1 (98). Resistance to ADCs may also arise from the activation of downstream signaling pathways. Previous literature shows that activated signaling pathways such as PI3K/AKT and deletion in PTEN signaling has been associated with Gemtuzumab ozogamicin and trastuzumab resistance (99, 100). In line with these resistance mechanisms, dysregulation in apoptosis may also contribute to ADC sensitivity. It has been demonstrated that proapoptotic proteins BAX and BAK plays an important role in Gemtuzumab ozogamicin sensitivity (101). In addition, overexpression of antiapoptotic proteins Bcl-2 and Bcl-x are associated with Gemtuzumab ozogamicin resistance (102).

Optimization Strategies for ADCs

For ADCs to have better safety and efficacy profile, there are a number of factors that need to be optimized some of which are listed as follows.

Optimization of antigen

In the development of ADCs for cancers, one of the major issues is the recognition and affirmation of sufficient antigenic substrates for the mAb moiety. In antigen selection, several aspects are required to be evaluated.

First, the targeting maB component of an ADC must bind to a tumor-specific antigen that is present either substantially or abundantly expressed on tumor cells or remarkably overexpressed on tumor cells in comparison with the normal cells (14). Second, high target antigen expression on the cell surface is critically important for the circulating mAb to be accessible (12). Third, it should be an internalizing antigen so that the ADC rapidly internalizes into the cell after binding to the target antibody, where the cytotoxic payload can produce its effects on intracellular targets (15). The homogeneity of target antigen expression within the tumor type and among target-positive patients is also an important aspect to consider (103). Shedding or secreting of antigens is another feature that may reduce ADC binding to the targets, resulting in a significantly elevated risk of toxicity (104). Therefore, the optimization of these factors is important for antigen selection. Besides these, the identification of new target antigens is one of the ways to improve ADC research. Recently, Weber and colleagues (105) identified a new antigen called OR10H1 that is one of the olfactory receptors and is primarily expressed in the urinary bladder of humans with a predominantly higher expression of protein and mRNA amount in bladder cancer tissues. They also demonstrate that it responds to sandalwood scents, namely sandranol. Results show

that after the application of sandranol, the cancerous bladder cells changed their structure; they became rounder, with less frequently occurring cell multiplication and cell motility poorer. Data findings show that tumor growth is inhibited by the sandalwood scent; this process was boosted by the fact that receptor activation results in the production of interleukins as well as ATP, thus switching on the immune system's natural killer cells in the tissue. This research study suggests that OR10H1 receptor is a potential tumor biomarker and a valid target for therapy. Kodack and colleagues (106) also discovered a new antigen called Her3 antigen. The Her3 antigen is overexpressed in the metastasized brain cancer cells. Blocking Her3 function produced significant tumor growth delay and improved mouse survival. It shows that Her3 might be an effective antigen for targeting Her2-resistant tumor cells as they start to metastasize. Thus, using these new target antigens may provide novel ADCs that could provide better treatment approaches.

Optimization of antibody

In the development of therapeutic ADCs, strategies like enhancing specificity, affinity, and pharmacokinetics are of great importance for the optimization of therapeutic mAbs. Both naked antibodies and ADCs require improving antibody homogeneity and developability to minimize the rate of attrition of drug candidates (107). At present, there is a huge trend regarding publishing several hundred papers on the structural characterization and analysis of mAbs. In the last 10 years, there has been a literal explosion of a novel bispecific antibodies and half antibody technologies and approaches to evaluate the therapeutic functionality (108). Bispecific immunoglobulins contain two different antigen-binding sites. The bispecific antibody-based ADCs are being tested at the preclinical investigations (109). Andreev and colleagues (110) demonstrated a bispecific antibody–based new ADC. They generated a bispecific antibody–based ADC that was able to bind to HER2 and PRLR antigens expressed on the breast cancer cells. Their results showed that HER2×PRLR bispecific ADC destroys breast cancer cells more effectively than PRLR or HER2 ADCs alone. It is because bispecific antigens provide two different antigen-binding sites that result in improved therapeutic efficacy as compared with the first-, second-, and third-generation ADCs that provide only one antigen-binding site. This study proved the importance of the bispecific antibody used in ADC design resulting in enhanced treatment efficacy of ADCs.

In another interesting study, Herbener and colleagues (111) generated four different ADC bases of the ADC Indatuximab ravtansine or BT062 against CD138. The first antibody nBT062 was a wild-type; the second was a stable and half-antibody exchange–resistant nBT062. The third antibody was deficient in covalent binding between two heavy chains and it was a half nBT062, while the fourth one was a stable and bispecific nBT062-natalizumab antibody. Herbener and colleagues then compared the antitumor activity of these four different ADCs. Interestingly, the fourth ADC displayed the minimum efficiency as it might be because they only target CD138 antigen instead of targeting two antigens as bispecific gives the best result for two target antigens. In contrast, wild-type nBT062, stable nBT062, and half nBT062-DM4 models displayed high anticancer properties. IgG reduced the potency of wild-type and half nBT062-DM4, whereas stable nBT062-DM4 was only slightly affected. This study demonstrated the benefits of using half-antibody exchange-blocking mutations into therapeutic IgG4-based ADCs. From these studies, we can conclude that bispecific and half antigens give better results than first-, second-, and third-generation ADCs as in the latter case we can only target one antigen while in case of bispecific antigens we can target two antigens. Therefore, there is a need to search for more novel bispecific and half antigens in the future to target multiantigens at the same time.

Optimization of linkers

Premature release of drugs in the circulation results in systemic toxicity and a lower therapeutic window. The linker of an ADC design plays a crucial role in ADC outcomes. Its molecular design and properties are the key characteristics that substantially impact the efficacy, pharmacokinetics/pharmacodynamics, and therapeutic index of the ADC (19, 108).

Cancer cell resistance occurs via the upregulation of MDR1 expression. MDR1 has a high affinity for transporting hydrophobic compounds compared with the hydrophilic compounds. It has been demonstrated that nonpolar or noncharged linkers, Mytansinoid-based ADCs, have lower *in vitro* potency in $MDR1^+$ cells as compared with the $MDR1^-$ cells (107). Because of this drawback, efforts were made to developed hydrophilic or charged linkers. The resulting ADCs showed improved potency against $MDRI^+$ cells with highly polar or charged metabolites. Examples are the mal-PEG4-*N*-hydroxysuccinimide and *N*-Hydroxysuccinimidyl-4- (2-pyridyldithio)-2-sulfobutanoate (sulfo-SPDB; refs. 112, 113).

It is known that reducing hydrophobicity results in improved ADCs outcomes (114). The increase in drug antibody ratio (DAR) has a direct impact on the *in vitro* ADC potency. However, with the rise of DAR, the plasma clearance of ADC can increase that reduces exposure and *in vivo* efficacy (115). Increased ADC hydrophobicity has been demonstrated to be associated with increased clearance of ADC that can be modified by linker-drug design. This was confirmed using auristatin-based hydrophilic linker–drug constructs and pegylated ADCs, resulting in superior *in vivo* performance (114).

In addition, several new linkers are currently being tested in preclinical trials. Singh and colleagues (116) designed ADC with a new triglycyl peptide linker CX that needs cleavage of the single peptide bond to unleash the cytotoxic warhead in lysosomes. They compared the ADC complex consisting of the maytansinoid payload linked to the anti-EpCAM, and anti-EGFR mAbs via triglycyl peptide linker CX and noncleavable SMCC linker. The ADC composed of triglycyl peptide linker CX and noncleavable SMCC linker showed similar cytotoxic activity *in vitro* for several cancer cell lines; however, in other cell lines, especially a multidrug-resistant cell line CX ADC showed more cytotoxic activity (5–100 fold lower IC_{50}) than the SMCC ADC. Another example of a new ADC linker is the peptidomimetic linker. Wei and colleagues (117) discovered 3 series of peptidomimetic ADC linkers, including the cBu series that displayed similar degradation activity to that of the dipeptide-containing linkers. *In vivo*, cBu-Cit–containing ADC and v-c–containing ADCs showed a similar rate of inhibition of tumor cell growth and intracellular release of the payload. Moreover, the cBu-Cit- and v-c–containing ADCs exhibited equal efficacy in multiple mouse tumor models. They indicated that the cBu-Cit linker was primarily degraded by cathepsin B because intracellular cleavage of the cBu-Cit–containing ADC was halted by a cathepsin B-specific inhibitor; however, a cathepsin B–specific inhibitor could not inhibit intracellular cleavage of the v-c peptide containing ADC. Therefore, the novel peptidomimetic linkers allow the ADC to cleave by tumor-specific/enhanced proteases.

Despite previous extensive efforts to improve conjugation efficiency and ADC homogeneity, most of the ADC linkers developed so far only load single payloads. However, recent studies suggest that mAb can be linked to more than two payloads. Anami and colleagues (118) recently reported their work on branched linkers, which can load multiple molecules of payload. They constructed an ADC composed of anti-

HER2 antibody conjugated to the MMAF payload via branched linkers and compared with the ADC composed of linear linkers. Their results demonstrated that branched linkers have greater *in vitro* cytotoxicity than the linear linkers, revealing the effectiveness of the branched linker-based payload delivery. They also demonstrated that branched ADC was highly stable in the human plasma, having high cell specificity and antigen-binding efficiency, and more significant *in vitro* cell killing potency than the ADC containing linear linker with a DAR of 1.9.

New payloads used in ADC design

One of the new payloads used in ADCs design is the pyrrolobenzodiazepine class (PBD). PBDs belong to the class of natural products with antibacterial or anticancer characteristics that are produced by several actinomycetes and are sequence-selective DNA-alkylating compounds (119). The mode of action of PBDs to kill cancer cells is to bind and cross-link with a specific target of cancer cell DNA. As a consequence, this prevents the tumor cells' multiplication without deforming its DNA helix, thus potentially avoiding the emergence of drug resistance phenomenon (120). Several ADCs containing PBD are now in phase I clinical trials such as SGN-33A (121), and SC16LD6.5 (120). Besides these, amatoxin, spliceostatin C, and thailanstatin A are new payloads that work as RNA polymerase inhibitors (122). Amatoxin is the RNA polymerase II inhibitor that kills tumor cells by inhibiting DNA transcription, which is required by all cells (123). Puthenveetil and colleagues (122) reported the identification of a spliceostatin C and thailanstatin A as a novel natural product payload for ADC that can be utilized to produce potent cytotoxic ADCs. Thailanstatin A and Spliceostatin C are ultra-potent eukaryotic RNA-splicing inhibitors. These ADCs can specifically kill tumor cells expressing both high and low antigen levels but do not target antigen-negative cells. Moreover, these spliceostatin ADCs are capable of overcoming MDR phenotype in comparison with microtubule inhibitors such as MMAE and maytansinoids (124). Another example of new payload is nitric oxide (NO). Fumou and colleagues generated an anti-CD24 (cluster of differentiation 24) antibody–NO conjugate (ANC) using a new NO donor compound as a payload (125). The ANC showed more effective efficacy and lower toxicity than either component, G7mAb or NO donor. In light of these studies, it is evident that the future clinical development of ADCs could benefit from the identification of such payloads that can prove to be more effective, safe, and have the capability of overcoming a MDR phenomenon.

Overcoming ADC resistance

In clinics, ADCs are increasingly used, and the clinical success of these drugs has been limited due to the development of resistance. The factors contributing to the heterogeneity of resistance mechanisms are the increased expression of MDR1, alteration in the microtubule composition, downregulation of antigen expression, and antigen–ADC internalization as well as reduced intracellular transport or drug release (54).

The modular structure of the ADC provides the possibility to modify some of its components to develop new compounds that can overcome the resistance. "Classic" mechanism of resistance to certain payloads, such as microtubule-disrupting agents can be circumvented by changing the cytotoxic payload for drugs that are poor efflux substrates. For example, it was demonstrated that trastuzumab emtansine resistance is related to the increased MDR expression (126). However, vadastuximab talirine, an anti-CD33 antibody conjugated to PBD, exhibited robust activity in animal models of AML, including those in which Gemtuzumab ozogamicin had the least effect (121). In addition, the replacement of anthracycline-based ADCs with auristatin-based ADC also demonstrated improved outcomes in acquired resistant NHL tumor models (96). Another way of overcoming ADC resistance is based on the modification of the linker to increase its hydrophilicity, which can reduce MDR because MDR1 transports hydrophobic compounds more efficiently than hydrophilic compounds. Famous examples are Sulfo-SPDB (113) and mal-PEG4-N-hydroxysuccinimide that contains polar linkers with improved potency against MDR1Þ models (127).

Another strategy can be modifications of the linker-cytotoxic structure (107). One of the main issues in cancer is the heterogeneity within tumors, which may result in ADC not killing low antigen-expressing cells. It was demonstrated that trastuzumab emtansine resistance is associated with a decrease in HER2 expression (126). The ADC can be designed to eradicate not only antigen-positive cells but also other surrounding cells by a phenomenon called bystander effect regardless of the target antigen expression on their surface. This process depends on the charge of the linker-payload. For example, an ADC system containing a payload such as MMAE or are linked through a cleavable disulfide bond, such as the maytansinoid tubulin inhibitor DM4, release catabolites that are neutral and cross biomembranes killing neighboring cells (128, 56).

Improved tumor penetration

One of the major hurdles and critical factor for effective ADC delivery is the tumor and antigen accessibility. Because of the limited penetration of antibodies into the tumor, drug delivery is reduced; therefore, highly toxic warheads are of paramount importance. It has been demonstrated that when an ADC is injected into the human, only a small fraction, 0.001%–0.01%, of an injected ADC actually binds to the tumor-specific cells (129). In recent years, tremendous effort has been put toward increasing the tumor penetrability of an ADC (130). In this regard, designed ankyrin repeat proteins (DARPin), non-IgG scaffolds and noninternalizing mAb scaffolds are linked to cytotoxic payloads through disulfide linkers, which are then selectively delivered and cleaved in the tumor microenvironment (131, 132).

Tumor penetration of an ADCs can be affected by several factors such as the molecular size of an antibody, the binding site barrier effect, and biodistribution. One way to improve tumor penetration is to use smaller ADCs, which can be based on smaller binding units such as diabodies, nanobodies, affibodies, DARPins, etc. Of particular interest, nanobodies that are 12- to 15-kDa, and nonimmunogenic antigen-binding single-domain fragments, it can quickly clear from blood and normal tissue and quickly penetrate into the tumor (133). These small size antibodies allow the ADC to improve tumor penetration. Another factor that may contribute to poor tumor penetration is the binding-site barrier effect. It is a phenomenon in which antibodies having high target affinity for the target antigen near blood vessels may have less distribution away from blood vessels due to the rapid and tight binding of antigens (104). Small size ADCs that are capable of improved permeability and distribution may use the "bystander effect" and may be beneficial in heterogeneous target expressing tumors. Moreover, the biodistribution properties of an ADC may also affect tumor penetration. For the determination of ADC biodistribution, like antibodies, both biophysical and biological perspectives are of paramount importance. With the help of biophysical mechanism tissue penetration and diffusion characteristics of an ADC can be studied as ADC exhibit nanoparticle-like properties (134). Also, it is believed that vascular and lymphatic systems are involved in the trafficking of ADCs, similar to antibodies. In addition, the charge of the ADC, alterations in the ADC avidity or binding affinity to FcRn, or variations in the ADC

physiochemical properties are some of the factors that may also interfere with the tissue distribution of an ADC (135–137).

Surface modifications

For the drug to be better tolerated by our body and to have a better therapeutic window, it is a common practice as a method to modify the structure and formulation of drug carriers. These modifications can be performed on the linker or antibody moiety, which may reduce the clearance rate. Glycosylation and PEGylation are the two most commonly used techniques to modify these therapeutic agents.

Glycosylation is the process in which posttranslation modifications of proteins or antibodies occurs through the addition of carbohydrates (glycan) to the amino acid side chains. This process is dependent on both the amount and location of glycosylation, which may dramatically affect the disposition of these therapeutic agents, such as regulating receptor binding and Fc effector functions (138, 139).

Another suitable method of modification is PEGylation. In this method, a non-immunogenic PEG polymer is added to another biological molecule to overcome certain disadvantages. In general, this modification provides decreased immunogenicity, improved solubility, and prolonged-time of residence in the body (140–142). However, due to steric limitations, this method can also influence potency/binding affinity to its target; thus, individual conjugation sites and control of conjugation should be evaluated. This method can be used to improve linker solubility and limit aggregation.

ADCs in Clinics

At present, four ADCs are in clinical use approved by the FDA and European Union (EU) for treating different kinds of cancer (**Table 1**).

Gemtuzumab ozogamicin

Gemtuzumab ozogamicin (Mylotarg , Pfizer/Wyeth) is composed of a recombinant humanized immunoglobulin G4 (IgG4) mAb, a pH-sensitive hydrazone linker, and a calicheamicin derivative (*N*-acetyl-gamma-calicheamicin-dimethyl hydrazide) payload. The approval of this ADC by the FDA in 2000 was founded on the outcomes of three single-arm phase II clinical studies in patients with $CD33^+$ relapsed AML (73, 143). In the initial approval, Gemtuzumab ozogamicin (two 9-mg/ m^2 doses given on days 1 and 15 and a 28-day follow-up) was indicated as a monotherapy for treating individuals with $CD33^+$ AML in first relapse who were aged $\geq$ 60 years and not fit for cytotoxic chemotherapy (98, 144). However, in a phase III SWOGS0106 (Southwest Oncology Group, S0106) randomized comparative study, the effect of Gemtuzumab ozogamicin plus conventional induction therapy (daunorubicin and cytosine arabinoside) versus conventional induction therapy alone was studied in newly diagnosed 637 patients with AML (<60 years of age; ref. 145). In this postapproval study, patients showed no benefit in response rates or relapse-free survival with high liver toxicity and a long duration of cytopenia (73). Likewise, high mortality rates were observed in the combination therapy with Gemtuzumab ozogamicin in comparison with the standard arm (6% vs. 1%; ref. 145). Moreover, no survival benefits were observed in Gemtuzumab ozogamicin treatment versus standard treatments (146, 147). Because of these safety and efficacy concerns, the manufacturer of this drug voluntarily withdrew the U.S. New Drug Application in 2010 (148).

On September 1, 2017, FDA reapproved the Gemtuzumab ozogamicin–based on new data showing improved efficacy and tolerability with a lower suggested dose and a diverse dosing schedule (3 mg/m^2 on days 1, 4, and 7). The reapproval of Gemtuzumab ozogamicin was grounded on the outcomes of clinical trials, including AML-19 (149), ALFA-0701 (150), and MyloFrance-1 (151) that contributed to the favorable reassessment of Gemtuzumab ozogamicin. This ADC is indicated for treating patients with newly diagnosed $CD33^+$ AML (150, 152, 153) and individuals with relapsed/refractory $CD33^+$ AML over 2 years of age (32).

Metabolic profile

To assess the pharmacokinetic properties of Gemtuzumab ozogamicin, it is essential to quantify the pharmacokinetics of the entire Gemtuzumab ozogamicin molecule, as well as the total and unbound calicheamicin metabolites. Upon internalization, Gemtuzumab ozogamicin undergo hydrolysis resulting in the release of the calicheamicin derivative. Afterward, the derivative experiences a

Table 1. Currently approved ADCs in the market.

ADC	Antibody	Linker	Payload	Target	Action	Indication	Approval Year
Gemtuzumab Ozogamicin (Mylotarg)	Humanized IgG4	Cleavable, Hydrazone	Calicheamicin	CD33	DNA damaging agent	Single-agent and combination therapy for adults and pediatric patients (age $\geq$ 2) with relapsed or refractory AML	2000-approved 2010-withdraw 2018-reapproved
Brentuximab Vedotin (Adcetris)	Chimeric IgG1	Cleavable, Peptide	MMAE	CD30	Microtubule disrupting agent	Monotherapy in patients with relapsed or refractory classical HL and sALCL; First salvage therapy prior to auto-HSCT; First-line therapy in early and advanced stage HL; combination therapy for adults with previously untreated stage III or IV classical HL	2011
Trastuzumab Emtansine (Kadcyla)	Humanized IgG1	Non-cleavable, Thioether	DM1	HER2	Microtubule disrupting agent	Adults with unresectable or metastatic breast cancer	2013
Inotuzumab Ozogamicin (Besponsa)	Humanized IgG4	Cleavable, Hydrazone	Calicheamicin	CD22	DNA damaging agent	Monotherapy in adults with relapsed/refractory B-cell ALL	2017

Abbreviations: ALL, acute lymphocytic leukemia; sALCL, Systemic anaplastic large-cell lymphoma.

nonenzymatic intramolecular disulfide bond reduction. However, low amounts of unbound calicheamicin in the blood (average C_{max} of 1.5 ng/mL after the third dose) restricted the measurement of its pharmacokinetic studies (147, 151, 153). For the original dose regimen of 9 mg/m^2, the total predicted area under the curve (AUC) is 25%, and the value for the C_{max} of Gemtuzumab ozogamicin is 24% during the treatment. On days 1, 4, and 7 and with a dose of 3 mg/m^2, 0.38 mg/L and 0.63 mg/L after the first and third dose were predicted for the C_{max} of Gemtuzumab ozogamicin. Approximately 25 L was estimated as the total volume of distribution.

After the first dose, the Gemtuzumab ozogamicin clearance predicted from plasma was valued to be 3 L/hour, followed by 0.3 L/hour. At the suggested dose of 3 mg/m^2, the terminal plasma half-life was about 160 hours. In patients having hepatic or severe renal impairment, no proper pharmacokinetic properties of Gemtuzumab ozogamicin have been established. No alterations in pharmacokinetics for Gemtuzumab ozogamicin have been reported in mild-to-moderate renal dysfunction patients (creatinine clearance > 30 mL/minute; refs. 147, 151, 153).

Adverse events

When Gemtuzumab ozogamicin is used as a monotherapy, then pyrexia, chills, nausea, infection, hemorrhage, fatigue, headache, vomiting, abdominal pain, thrombocytopenia, neutropenia, stomatitis, and diarrhea, are the most frequent adverse events (AE) observed in ≥30% of cases (154). However, serious adverse effects related to Gemtuzumab ozogamicin usage included neutropenia (34.3%), thrombocytopenia (21.7%), and infusion-related reactions (IRR; 2.5%). Patients receiving Gemtuzumab ozogamicin as monotherapy have an increased risk of developing venous occlusive disease (VOD) before or after hematopoietic stem cell transplantation (HSCT). Also, patients with moderate to severe liver impairment were 8.7 times more likely to develop VOD. The factors that favor discontinuation in monotherapy studies are hemorrhage, infection, VOD, and multiorgan failure (154). In combination therapy, the most common (> 30%) and relevant AEs were a severe infection (41.2%), hemorrhage (9.9%), hepatotoxicity, including VOD/SOS (3.8%) and tumor lysis syndrome (1.5%; ref. 145). Grade 3–4 neutropenia, thrombocytopenia, and leukopenia were observed in 96.1%, 100%, and 100%, respectively. In patients with untreated *de novo* AML, myelosuppression was a very common adverse effect associated with Gemtuzumab ozogamicin in combination therapy.

Drug interactions

It has been demonstrated that Gemtuzumab ozogamicin and its cytotoxic warhead, *N*-acetyl gamma calicheamicin dimethyl hydrazide had a little inhibitory effect on CYP1A2, CYP2B6, CYP2A6, CYP2C8, CYP2C19, CYP2C9, and CYP2D6 at clinically relevant concentrations *in vitro* (147, 155). GO and its payload, calicheamicin, possess a low affinity for the induction of CYP2B6, CYP1A2, and CYP3A4 activities. Gemtuzumab ozogamicin payload, calicheamicin, demonstrated low inhibitory potential for P-glycoprotein (P-gp), MDR-associated protein 2, breast cancer resistance protein, bile salt export pump, organic anion transporter 1 and 2, multidrug and toxin extrusion protein 1 and 2, organic anion transporter 1B3, organic cation transporter 1 and 2, organic anion transporting polypeptide (OATP) 1B1, and UGT enzymes activities at clinically relevant levels *in vitro*. No alteration in phramacokinetics of Gemtuzumab ozogamicin coadministered with daunorubicine and cytarabine was observed (147).

Brentuximab vedotin

Brentuximab vedotin (SGN-35; Adcetris), a potent ADC in clinical practice, is composed of humanized immunoglobulin (Ig) G1 antibody that is linked to the payload MMAE, through a protease-cleavable linker (156). Brentuximab vedotin utilizes a v-c dipeptide linker capable of conditional cleavage, discharge of fully active drug and high stability in serum (157). Brentuximab vedotin exerts its anticancer activity by conjugating with CD30 on the surface of the cancer cell where endosomal internalization occurs and then transported to the lysosomes. Lysosomal internalization results in proteases cleavage of the linker peptide and subsequent release of MMAE to the cytoplasm, where it inhibits microtubule polymerization and induces apoptosis via cell-cycle arrest (158, 159). Besides direct binding to the CD30$^+$ lymphocytes, Brentuximab vedotin also exerts its antitumor activity via antibody-dependent cellular phagocytosis, immunogenic cell death, and the so-called bystander killing regardless of CD30 expression, as released MMAE easily diffuses to the surrounding tissue through the cell membrane (160).

On August 19, 2011, the FDA approved Brentuximab vedotin for the treatment of patients suffering from Hodgkin lymphoma and ALCL (160). This approval of Brentuximab vedotin was based on the assessment of two-phase II clinical studies. The first study included 102 patients with refractory or relapsed Hodgkin lymphoma. In this study, the objective response was 75%, with 34% complete remissions (CR; ref. 161). In the second trial, a total number of 58 patients were included with relapsed or refractory ALCL. Among these, 86% of the patients achieved an objective response with 53% CRs (162). This drug is approved in more than 65 countries for improved patient outcomes with relapsed or refractory systemic ALCL or relapsed or refractory classical Hodkin lymphoma (133, 163, 164). Retreatment with Brentuximab vedotin is often effective in patients who once had a benefit from Brentuximab vedotin (165). Brentuximab vedotin is used as a first-line therapy prior to auto-HSCT (166–168) and as consolidation postautologous transplant in Hodgkin lymphoma (169). Moreover, it is also used as first salvage therapy in early and advanced stage Hodgkin lymphoma (170, 171) and as second-line therapy together with multidrug chemotherapy for patients with refractory or relapsed Hodgkin lymphoma prior to auto-HSCT (172). In addition, in March 2018, FDA also approved Brentuximab vedotin coadministered with chemotherapy (doxorubicin, dacarbazine, and vinblastine), for treating adult patients who had previously untreated stage III or IV classical Hodgkin lymphoma (173).

Metabolic profile

The data about the pharmacokinetics of Brentuximab vedotin were evaluated in phase I clinical studies and population analysis of data from 314 patients (161, 174). The maximum concentration of Brentuximab vedotin was noted closest to the end of intravenous infusion, and the terminal half-life was noted to be 4–6 days. Within 21 days, the ADC and MMAE achieved its steady state, and there was no Brentuximab vedotin accumulation even after repeated doses given every 3 weeks (174). A single dose of 1.8 mg/kg Brentuximab vedotin results in 31.98 μg/mL C_{max} and AUC of 79.41 μg/mL/day, respectively. The median C_{max} of MMAE was found to be 4.97 ng/mL. Time to C_{max} and AUC of MMAE were recorded to be 2.09 days, and 37.03 ng/mL/day, respectively. A value of 7.37 L for central and 36.4 L for peripheral volumes of distribution was observed for MMAE.

Brentuximab vedotin is catabolized as a protein and eliminated from the body with a typically estimated clearance of 1.457 L/day and a half-life of 4–6 days, respectively (174). *In vivo* studies have shown that only a small portion of MMAE is catabolized by CYP3A, which is

released by the Brentuximab vedotin. Therefore, special attention should be given to the patients in case of receiving CYP3A inhibitors. The typical apparent clearance of 19.99 L/day and a half-life of 3–4 days was observed for MMAE, respectively. An excretion study indicated about 24% of the total MMAE recovered in both urine and feces over a 1-week period being administered as part of the ADC (174). Approximately 28% was excreted in urine and the rest 72% was found in the feces. Because kidneys and liver are the main pathways of elimination for MMAE, therefore, dose adjustment is mandatory for renal and hepatic impaired patients. It was found that MMAE exposure was approximately two times higher in patients with severe renal impairment (creatinine clearance < 30 mL/minute; ref. 175). Therefore, it is advisable to avoid the use of Brentuximab vedotin in patients with severe renal dysfunction.

AEs

The most common AEs ($\geq$ 10%) with the use of Brentuximab vedotin were infections, nausea, fatigue, diarrhea, peripheral sensory neuropathy, neutropenia, peripheral motor neuropathy, rash, cough, vomiting, myalgia, pyrexia, abdominal pain, arthralgia, pruritus, constipation, dyspnea, loss of weight, and upper respiratory tract infection (176). Peripheral motor neuropathy was most commonly noticed in patients retreated with Brentuximab vedotin but was primarily grade 2 in comparison with phase II studies (28% vs. 9%; ref. 176). Infusion-related reactions were found to be 13% in patients (177). These reactions include headache, chills, nausea, vomiting, rash, back pain, dyspnea, pruritus, and cough. Abdominal pain and anaphylactic reactions have also been reported in Brentuximab vedotin–treated patients (176, 178). Moreover, in patients treated with Brentuximab vedotin, pancreatitis was previously unrecognized severe AE before a case of fatal pancreatitis was reported in a clinical trial. Gandhi and colleagues reported an additional case of pancreatitis associated with the use of Brentuximab vedotin (179). Some pulmonary toxicities and John Cunningham polyomavirus reactivation that can cause fatal progressive multifocal leukoencephalopathy have also been reported with Brentuximab vedotin treatment (163, 176, 180).

Drug interactions

Brentuximab vedotin is a CYP3A4 substrate and possibly CYP2D6. The payload, MMAE, is metabolized by CYP3A4/5 via oxidation (177). The inhibitors of CYP3A4 and P-gp such as itraconazole, ketoconazole, indinavir, nefazodone, clarithromycin, saquinavir, telithromycin, atazanavir, nelfinavir, ritonavir, and voriconazole were involved in the increasing exposure of MMAE by 73%, but no change was observed in the plasma exposure to Brentuximab vedotin (175). In the case of Brentuximab vedotin administration together with strong inhibitors, a high risk of neutropenia may be observed. Rifampicin, a strong inducer of CYP3A4, found to reduce the plasma concentrations of MMAE metabolites, but did not alter the plasma exposure to Brentuximab vedotin. Midazolam, a CYP3A4 substrate, did not alter the metabolism of Brentuximab vedotin when coadministered (175).

Trastuzumab emtansine

Trastuzumab emtansine (T-DM1; Kadcyla, Roche) is another highly potent ADC that was first approved by the FDA and the EU in 2013 for the HER2-positive breast cancer treatment (181). It is composed of the mAb trastuzumab, the cytotoxic payload mertansine (DM1), and a nonreducible thioether linker MCC (4-[N-maleimidomethyl] cyclohexane-1-carboxylate) (182, 183). DM1 is a highly potent cytotoxic payload that binds microtubules in the same way as that of vinca alkaloids. *In vitro*, DM1 showed 11 to 25 times higher cytotoxicity than maytansine and a potency of 24 to 270 times more than taxanes. The DAR of DM1 is 3.5 (183). Binding of T-DM1 to HER2 receptors results in a T-DM1–HER2 complex formation that enters the target cells through receptor-mediated endocytosis (184, 83). This leads to proteolytic degradation of the antibody portion of T-DM1 in lysosomes, the release of the lysine-MCCDM1 into the cytosol and subsequent cell-cycle arrest and apoptosis induction (67, 185).

T-DM1 was first approved on the basis of the outcomes of two-phase III randomized clinical studies (EMILIA and TH3RESA) that proved its safety and efficacy. In the phase III EMILIA clinical trial, a total number of 991 patients with metastatic breast cancer were evaluated for T-DMI with the combination of capecitabine and lapatinib (186). Patients treated with T-DM1 displayed a significant difference in progression-free survival (PFS; median PFS, 9.6 months) in comparison with the lapatinib plus capecitabine median PFS (6.4 months). The overall survival (OS) rate of T-DM1 was also improved compared with lapatinib- and capecitabine-treated patients [29.9 vs. 25.9 months; HR, 0.75 (95% confidence interval (CI), 0.64–0.88); $P < 0.001$]. Thrombocytopenia (14%), elevated levels of aspartate aminotransferase (5%), and anemia (4%) were the most common grade 3 or worse AEs reported in the T-DM1 group.

Similarly, in phase III TH3RESA clinical study, T-DM1 was also found to be superior versus physician's choice in previously HER2-positive advanced breast cancer–treated patients (187). T-DM1 demonstrated significant improvements in PFS [median 6.2 vs. 3.3 months; HR, 0.528; (95% CI, 0.422–0.661)] and OS [median 22.7 vs. 15.8 months; HR, 0.68; (95% CI, 0.54–0.85)] in T-DM1–treated patients versus physician's choice treatment (187, 188). The data obtained from the EMILIA and TH3RESA clinical trials indicate that T-DM1 has a clearly better safety profile in comparison with the control arm group with fewer grade 3 or more AEs (189, 190).

Metabolic profile

The pharmacokinetics of T-MD1 indicated that a single intravenous injection of 3.6 mg/kg T-DM1 every 3 weeks gives a mean C_{max} of 83.4 μg/mL (133, 191). Metabolism of DM1 occurs mainly via CYP3A4/5and is a P-gp substrate (133). T-DM1 displayed the central volume of distribution of 3.13 L. Low levels of T-DM1 metabolites such as DM1, MCC-DM1, and Lys-MCC-DM1 were observed in human plasma (191). The T-DM1 half-life was approximately 4 days and a clearance of 0.68 L/day. No ADC accumulation was observed after repeated dosing every 3 weeks. Bile was the primary route of excretion for T-DM1 metabolites while minimal elimination was observed in the urine. Phramacokinetic characteristics of T-DM1 were not altered by mild-to-moderate renal impairment. Patients with creatinine clearance <30 mL/minute were unable to obtain recommendations (133). In patients with moderate-to-severe hepatic impairment, the AUC of the first cycle T-DM1 was approximately 38% versus 67% in patients with normal hepatic function, respectively (191).

AEs

The most common AEs associated with the use of T-DM1 included severe thrombocytopenia (54.2%), fatigue (37.5%), increased levels of transaminases (41.7%), anemia (29.2%), and nausea (25.0%) (191). Other AEs were hemorrhage, abdominal pain, pyrexia, musculoskeletal pain, vomiting, and dyspnea (133, 192). The majority of them were generally grade 1–2 and reversible. Some studies also reported serious hepatobiliary disorders (188, 189, 192) and left ventricular dysfunction in patients receiving T-DM1 (193). An increase in the serum transaminases indicates liver toxicity, which is usually asymptomatic;

therefore, T-DM1 should be permanently discontinued in case of elevation in serum transaminases level more than three folds. If the total bilirubin increases more than twice the normal upper limit, the T-DM1 should also be discontinued. Administration of T-DM1 to a pregnant woman can pose harm to a fetus (194). Some clinical trials also indicated the appearance of interstitial lung disease (192).

Drug interactions

As stated above, the metabolism of DM1, the payload of T-DM1, mainly occurs via CYP3A4 and up to some extent, by CYP3A5 (133). Therefore, it is recommended that T-DM1 should be avoided with strong CYP3A4 inhibitors (e.g., indinavir, ketoconazole, nefazodone, clarithromycin, itraconazole, atazanavir, ritonavir, nelfinavir, voriconazole, telithromycin, and saquinavir) as it may potentiate DM1 exposure and toxicity (195, 196).

Inotuzumab ozogamicin

Inotuzumab ozogamicin (CMC-544; Besponsa , Pfizer/ Wyeth) is a humanized CD-22–targeting ADC comprising IgG4 antibody, a cytotoxic payload calicheamicin [N-acetyl-c-calicheamicin dimethyl hydrazide (Calich-DMH)] that are covalently linked together by an acid-labile 4-(40-acetylphenoxy) butanoic acid linker (57, 155, 197). Calicheamicin is a highly potent DNA-alkylating agent produced by a soil microorganism; Micromonospora echinospora (175). Inotuzumab ozogamicin has high affinity and rapidly internalized into the cells that express CD22. Upon binding, Inotuzumab ozogamicin/CD22 complex is rapidly internalized into the lysosomal compartment, where calicheamicin is released to bind to the minor groove of DNA, resulting in double-strand cleavage with subsequent apoptosis and cell-cycle arrest (97, 198).

In the United States (199), Japan (200), EU (201), and several other countries (202), Inotuzumab ozogamicin is used as monotherapy for the treatment of adult patients having relapsed/refractory B-cell acute lymphoblastic leukemia (ALL; refs. 199, 201). Inotuzumab ozogamicin approval was mainly based on a global, open-label, phase III randomized INO-VATE ALL clinical trial (203). In this study, a total number of 326 patients with CD22-positive B-cell ALL in first or second relapse were randomized to the inotuzumab ozogamicin arm and standard-therapy arm (ST) receiving FLAG (fludarabine, cytarabine, and granulocyte-colony-stimulating factor), high-dose cytarabine, or mitoxantrone plus cytarabine in a 1:1 ratio. Overall, the CR rate was significantly higher for the Inotuzumab ozogamicin [80.7% (95% CI, 72.1–87.7) vs. 29.4% (95% CI, 21.0–38.8), $P < 0.001$] for the ST. The duration of remission (DOR) was significantly higher for the Inotuzumab ozogamicin arm versus ST arm [4.6 (95% CI, 3.9–5.40) vs. 3.1 (95% CI, 1.4–4.9) months]. The PFS and OS were also considerably longer with Inotuzumab ozogamicin in comparison with the ST arm (5.0 vs 1.8 months; $P < 0.001$) and (7.7 vs 6.7 months; $P = 0.04$). In addition, a large population was able to proceed to stem cell transplantation in the Inotuzumab ozogamicin arm versus ST arm (41% vs. 11%; $P < 0.001$).

It is recommended that Inotuzumab ozogamicin should be administered in 3- to 4-week cycles. A total dose of 1.8 mg/m^2 per cycle, administered as three divided doses (0.8, 0.5 and 0.5 mg/m^2 on days 1, 8, and 15), is recommended for the first cycle. For subsequent cycles, the recommended dose is 1.5 mg/m^2 per cycle, given as 0.5 mg/m^2 doses on days 1, 8 and 15. If the patient proceeds to HSCT, the recommended treatment duration should include two cycles or up to six cycles as the only therapy.

Metabolic profile

Pharmacokinetic properties of inotuzumab ozogamicin displayed nonlinear disposition in the initial phase I studies (204). There was increased drug exposure with either an increased number of doses or higher doses of the drug. Multiple doses of Inotuzumab ozogamicin results in a 5.3-fold accumulation between the first and fourth cycles and steady-state concentration were achieved by the fourth cycle (199, 201). At steady state, the total AUC per cycle was 100 μg/h/mL and the mean C_{max} was 308 ng/mL. *In vitro*, Calich-DMH was found to be approximately 97% bound to human plasma proteins, and the total volume of distribution was approximately 12 L (199, 201). Inotuzumab ozogamicin was mainly metabolized by nonenzymatic reduction and was a substrate of P-gp. At steady state, Inotuzumab ozogamicin demonstrated clearance of 0.0333 L/hour and at the end of the fourth cycle, the terminal elimination half-life was around 12.3 days.

AEs

Inotuzumab ozogamicin had a manageable tolerability profile in adult patients with relapsed or refractory ALL. However, the most commonly observed treatment-related hematologic AEs were fever (59%), thrombocytopenia (51%), neutropenia (49%), increased transaminases (26%), nausea (31%), headache (28%), fatigue (35%), infection (48%), anemia (36%), hemorrhage (33%), increased gamma-glutamyltransferase (21%), leukopenia (35%), pyrexia (32%), febrile neutropenia (26%), abdominal pain (23%), and hyperbilirubinemia (21%; refs. 203, 205). Inotuzumab ozogamicin was also associated with veno-occlusive disease/sinusoidal obstruction syndrome who underwent HSCT (203, 205). Moreover, increased transaminases and hyperbilirubinemia were also reported in patients treated with Inotuzumab ozogamicin, respectively (194).

Drug interactions

The data obtained from the *in vitro* studies revealed that the administration of Inotuzumab ozogamicin together with inducers or inhibitors of UGT drug-metabolizing enzymes or cytochrome P450 did not change the exposure to *N*-acetylgamma-calicheamicin dimethyl hydrazide (155, 201). Also, the exposure of CYP enzyme-substrate, major drug transporters or UGT enzymes is unlikely to be affected by the Inotuzumab ozogamicin and its calicheamicin derivatives.

ADCs in Clinical Development

The field of ADC poses a promising therapeutic option for malignant patients. More than 60 ADCs are at different clinical stages and their results have sparked significant interest in the rapidly growing number of ADC candidates. **Table 2** represents a few examples of the ADCs that are currently under clinical development stage.

Conclusions and Future Perspectives

Despite the approval of only four ADCs, there has been a significant improvement in the ADC design. The versatility of antibodies, exploration of new antigens and cytotoxic payloads, and the growing intricacy methods have made ADCs a frontier for the next generation of therapeutic treatments for a variety of diseases. A systemic evaluation of each component of an ADC design is necessary to enhance its efficacy. Moreover, improved understanding of the mechanistic pathways involved in ADC resistance will enable the rational design of ADCs and better treatment outcomes. At present, there are more than 60 ADCs in clinical development and the clinical data emerging from

Table 2. Summary table of some of the ADCs in clinical development.

ADC	Antibody	Linker	Payload	Target	Action	Indication	Phase	ClinicalTrials.gov Identifier
Trastuzumab Deruxtecan (DS-8201a)	Humanized-HER2	Cleavable	Camptothecin	HER2	DNA damaging Agent	Breast Cancer	3	NCT03529110 NCT03523585 NCT03734029
Sacituzumab govitecan	Humanize-IgG1	Cleavable	SN-38	TROP2	DNA damaging agent	Breast cancer	3	NCT03901339 NCT02574455
Mirvetuximab Soravtansine (IMGN853)	Humanized-IgG1	Cleavable	DM4	FOLR1	Microtubule disrupting agent	Ovarian cancer	3	NCT02631876
Enfortumab Vedotin	Humanized-IgG1	Cleavable	MMAE	Nectin-4	Microtubule disrupting agent	Urethelial cancer	3	NCT03474107
Trastuzumab Duocarmazine (SYD985)	Humanized-IgG1	Cleavable	Duocarmycin	HER2	DNA damaging agent	Breast cancer	3	NCT03262935
GSK2857916	Humanized-IgG1	Non-cleavable	MMAF	BCMA	Microtubule disrupting agent	Multiple Myeloma	2	NCT03544281 NCT03544281
Glembatumumab Vedotin	Humanized-IgG2	Cleavable	MMAE	GPNMB	Microtubule disrupting agent	Recurrent Osteosarcoma	2	NCT02487979 NCT02363283
Anetumab Ravtansine	Humanized-IgG1	Cleavable	DM4	Mesothelin	Microtubule disrupting agent	Mesothelioma	2	NCT02610140 NCT03023722
Labetuzumab Govitecan	Humanized-IgG1	Cleavable	SN-38	CEACAM5	DNA damaging agent	Colorectal cancer	2	NCT01915472 NCT01605318
PSMA ADC	Humanized-IgG1	Cleavable	MMAE	PSMA	Microtubule disrupting agent	Prostate cancer	2	NCT01695044 NCT02020135
Coltuximab ravtansine (SAR3419)	Humanized-IgG1	Cleavable	DM4	CD19	Microtubule disrupting agent	B-cell lymphoma	2	NCT01472887 NCT01470456 NCT00796731
Depatuxizumab mafodotin (ABT-414)	Humanized-IgG1	Non-cleavable	MMAF	EGFR	Microtubule disrupting agent	Glioblastoma	2	NCT02343406 NCT02573324 NCT02590263
AGS-16C3F	Humanized-IgG2	Non-cleavable	MMAF	ENPP3	Microtubule disrupting agent	Renal cell carcinoma	2	NCT02639182
SYD985	Humanized-IgG1	Cleavable	Duocarmycin	HER2	DNA damaging agent	Breast cancer	1	NCT02277717
IMGN779	Humanized-IgG1	Cleavable	DGN462	CD33	DNA damaging agent	AML	1	NCT02674763
PF-06647263	Humanized-IgG1	Cleavable	Calicheamicin	EFNA4	DNA damaging agent	Advanced malignancies	1	NCT02078752
U3-1402	Humanized-IgG1	Cleavable	DX-8951 (DXd)	HER3	DNA damaging agent	NSCLC	1	NCT03260491
MEDI3726	Humanized-IgG1	Cleavable	PBD	PSMA	DNA damaging agent	Prostate cancer	1	NCT02991911
SGN-CD352A	Humanized-IgG1	Cleavable	PBD	CD352A	DNA damaging agent	MM	1	NCT02991911
SAR408701	Humanized-IgG1	Cleavable	DM4	CEACAM5	Microtubule disrupting agent	Solid tumors	1	NCT03324113
AGS67E	Humanized-IgG2	Cleavable	MMAE	CD37	Microtubule disrupting agent	AML	1	NCT02175433
AGS-62P1	Humanized-IgG1	Cleavable	Amberstatin269	FLT3	DNA damaging Agent	AML	1	NCT02864290
MEDI4276	Humanized-IgG1	Cleavable	Tubulysin	HER2	Microtubule disrupting agent	Breast cancer Gastric cancer	1	NCT02576548

Abbreviations: BCMA, B-cell maturation antigen; CEACAM5, carcinoembryonic antigen related cell adhesion molecule 5; EFNA4, Ephrin-A4; ENPP3, ectonucleotide pyrophosphatase/phosphodiesterase 3; FLT3, FMS-like tyrosine kinase 3; FOLR1, folate receptor alpha; GPNMB, glycoprotein non-metastatic b; MM, multiple myeloma; NSCLC, non-small cell lung cancer; PSMA, prostate specific membrane antigen; TROP2, Trophoblast antigen 2.

these next-generation ADCs will provide important insights into the mechanistic basis of ADC design, and the opportunity to better understand the impact of changes in ADC properties on therapeutic activity and safety.

Combination therapies have the capability of reducing drug resistance, improving drug efficacy, shrinking tumor metastasis, and growth and increasing cancer survival rates (206). Findings of studies indicate that ADC provides even better efficacy and less toxic when combine with other drugs. One of the examples of such ADC combination therapy is the AXL-107-MMAE and BRAF/MEK (207). The results of this study indicate that AXL-107-MMAE alone has a little effect rather than the AXL-107-MMAE and BRAF/MEK combination. These inhibitors in combination enhance each other's activity to supportively inhibit growing colonies of drug-resistant tumor cells. Similarly, Schönfeld and colleagues (208) also enhanced the activity of Indatuximab ravtansine (BT062) as a combination treatment in multiple myeloma. *In vivo* and *in vitro* investigation of Indatuximab ravtansine showed great antitumor activity in combination with lenalidomide and dexamethasone. The combination therapy displayed a stronger effect on tumor growth as compared to the monotherapy when Indatuximab ravtansine 4 mg/kg was combined with lenalidomide and dexamethasone.

Further clinical research indicates that ADC can also be combined with checkpoint inhibitors that may increase the incidence of CD8Þ T effector cells to cancer tissues improving the clinical response. For instance, the combination of trastuzumab emtansine with pembrolizumab (a PD-1 inhibitor) and Brentuximab vedotin with PD-L1 or PD-1 inhibitors are being studied in metastatic breast cancer individuals and patients with refractory or relapsed Hodgkin lymphoma (Clinical Trial identifiers: NCT02924883, NCT03032107, and NCT01896999). On the basis of these observations, it is obvious that it is the need of time to combine ADCs with other drugs that will open a new window for ADC design research.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. World Health Organisation. Cancer; 2019. Available from: http://www.who.int/mediacentre/factsheets/fs297/en/.
2. DeVita VT Jr, Chu E. A history of cancer chemotherapy. Cancer Res 2008;68:8643–53.
3. Loadman P. Anticancer drug development. Br J Cancer 2002;86:1665–6.
4. Goodman LS, Wintrobe MM, Dameshek W, Goodman MJ, Gilman A, Mclennan MT. Nitrogen mustard therapy: use of methyl-bis(beta-chloroethyl)amine hydrochloride and tris(beta-chloroethyl)amine hydrochloride for hodgkin's disease, lymphosarcoma, leukemia and certain allied and miscellaneous disorders. J Am Med Assoc 1946;132:126–32.
5. Goldman B. Multidrug resistance: can new drugs help chemotherapy score against cancer? J Natl Cancer Inst 2003;95:255–7.
6. Maleki LA, Baradaran B, Majidi J, Mohammadian M, Shahneh FZ. Future prospects of monoclonal antibodies as magic bullets in immunotherapy. Hum Antibodies 2013;22:9–13.
7. Gordon MR, Canakci M, Li L, Zhuang J, Osborne B, Thayumanavan S. Field guide to challenges and opportunities in antibody-drug conjugates for chemists. Bioconjug Chem 2015;26:2198–215.
8. Strebhardt K, Ullrich A. Paul Ehrlich's magic bullet concept: 100 years of progress. Nat Rev Cancer 2008;8:473–80.
9. Ford CH, Newman CE, Johnson JR, Woodhouse CS, Reeder TA, Rowland GF, et al. Localisation and toxicity study of a vindesine-anti-CEA conjugate in patients with advanced cancer. Br J Cancer 1983;47:35–42.
10. Trail PA, Willner D, Lasch SJ, Henderson AJ, Hofstead S, Casazza AM, et al. Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. Science 1993;261:212–5.
11. Strohl WR. Current progress in innovative engineered antibodies. Protein Cell 2018;9:86–120.
12. Damelin M, Zhong W, Myers J, Sapra P. Evolving strategies for target selection for antibody-drug conjugates. Pharm Res 2015;32:3494–507.
13. Diamantis N, Banerji U. Antibody-drug conjugates–an emerging class of cancer treatment. Br J Cancer 2016;114:362–7.
14. Tipton TR, Roghanian A, Oldham RJ, Carter MJ, Cox KL, Mockridge CI, et al. Antigenic modulation limits the effector cell mechanisms employed by type I anti-CD20 monoclonal antibodies. Blood 2015;125:1901–9.
15. Donaghy H. Effects of antibody, drug and linker on the preclinical and clinical toxicities of antibody-drug conjugates. mAbs 2016;8:659–71.
16. Casi G, Neri D. Noninternalizing targeted cytotoxics for cancer therapy. Mol Pharm 2015;12:1880–4.
17. Golfier S, Kopitz C, Kahnert A, Heisler I, Schatz CA, Stelte-Ludwig B, et al. Anetumab ravtansine: a novel mesothelin-targeting antibody-drug conjugate cures tumors with heterogeneous target expression favored by bystander effect. Mol Cancer Ther 2014;13:1537–48.
18. Peters C, Brown S. Antibody-drug conjugates as novel anti-cancer chemotherapeutics. Biosci Rep 2015;35:pii: e00225.
19. Hughes B. Antibody-drug conjugates for cancer: poised to deliver? Nat Rev Drug Discov 2010;9:665–7.
20. Mehrling T, Soltis D. Challenges in optimising the successful construction of antibody drug conjugates in cancer therapy. Antibodies 2018;7:11.
21. Payés CJ, DanielsWells TR, Maffía PC, Penichet ML, Morrison SL, Helguera G. Genetic engineering of antibody molecules. Rev Cell Biol Mol Med 2015;1:1–52.
22. Maynard J, Georgiou G. Antibody engineering. Annu Rev Biomed Eng 2000;2:339–76.
23. Panowski S, Bhakta S, Raab H, Polakis P, Junutula JR. Site-specific antibody drug conjugates for cancer therapy. mAbs 2014;6:34–45.
24. Wang R, Li L, Zhang S, Li Y, Wang X, Miao Q, et al. A novel enediyne-integrated antibody-drug conjugate shows promising antitumor efficacy against CD30(+) lymphomas. Mol Oncol 2018;12:339–55.
25. Carter PJ. Potent antibody therapeutics by design. Nat Rev Immunol 2006;6:343–57.
26. Almagro JC, Fransson J. Humanization of antibodies. Front Biosci 2008;13:1619–33.
27. Chalouni C, Doll S. Fate of antibody-drug conjugates in cancer cells. J Exp Clin Cancer Res 2018;37:20.
28. Vankemmelbeke M, Durrant L. Third-generation antibody drug conjugates for cancer therapy – a balancing act. Ther Deliv 2016;7:141–4.
29. Gallery M, Zhang J, Bradley DP, Brauer P, Cvet D, Estevam J, et al. A monomethyl auristatin E-conjugated antibody to guanylyl cyclase C is cytotoxic to target-expressing cells in vitro and in vivo 2018;13:e0191046.
30. Alley SC, Benjamin DR, Jeffrey SC, Okeley NM, Meyer DL, Sanderson RJ, et al. Contribution of linker stability to the activities of anticancer immunoconjugates. Bioconjug Chem 2008;19:759–65.
31. Nagayama A, Ellisen LW, Chabner B, Bardia A. Antibody-drug conjugates for the treatment of solid tumors: clinical experience and latest developments. Target Oncol 2017;12:719–39.
32. Tsuchikama K, An Z. Antibody-drug conjugates: recent advances in conjugation and linker chemistries. Protein Cell 2018;9:33–46.
33. Ducry L, Stump B. Antibody-drug conjugates: linking cytotoxic payloads to monoclonal antibodies. Bioconjug Chem 2010;21:5–13.

34. Lewis Phillips GD, Li G, Dugger DL, Crocker LM, Parsons KL, Mai E, et al. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. Cancer Res 2008;68:9280–90.
35. McCombs JR, Owen SC. Antibody drug conjugates: design and selection of linker, payload and conjugation chemistry. AAPS J 2015;17:339–51.
36. Doronina SO, Mendelsohn BA, Bovee TD, Cerveny CG, Alley SC, Meyer DL, et al. Enhanced activity of monomethylauristatin F through monoclonal antibody delivery: effects of linker technology on efficacy and toxicity. Bioconjug Chem 2006;17:114–24.
37. Hamilton GS. Antibody-drug conjugates for cancer therapy: The technological and regulatory challenges of developing drug-biologic hybrids. Biologicals 2015;43:318–32.
38. Dan N, Setua S, Kashyap VK, Khan S, Jaggi M, Yallapu MM, et al. Antibody-drug conjugates for cancer therapy: chemistry to clinical implications. Pharmaceuticals 2018;11:32.
39. Senter PD. Potent antibody drug conjugates for cancer therapy. Curr Opin Chem Biol 2009;13:235–44.
40. Lu J, Jiang F, Lu A, Zhang G. Linkers having a crucial role in antibody-drug conjugates. Int J Mol Sci 2016;17:561.
41. Bryden F, Martin C, Letast S, Lles E, Vieitez-Villemin I, Rousseau A, et al. Impact of cathepsin B-sensitive triggers and hydrophilic linkers on in vitro efficacy of novel site-specific antibody-drug conjugates. Organic Biomol Chem 2018;16:1882–9.
42. Dubowchik GM, Firestone RA, Padilla L, Willner D, Hofstead SJ, Mosure K, et al. Cathepsin B-labile dipeptide linkers for lysosomal release of doxorubicin from internalizing immunoconjugates: model studies of enzymatic drug release and antigen-specific in vitro anticancer activity. Bioconjug Chem 2002;13:855–69.
43. Wang YJ, Li YY, Liu XY, Lu XL, Cao X, Jiao BH. Marine antibody-drug conjugates: design strategies and research progress. Marine Drugs 2017;15:18.
44. Jaracz S, Chen J, Kuznetsova LV, Ojima I. Recent advances in tumor-targeting anticancer drug conjugates. Bioorg Med Chem 2005;13:5043–54.
45. de Graaf M, Boven E, Scheeren HW, Haisma HJ, Pinedo HM. Beta-glucuronidase-mediated drug release. Curr Pharm Des 2002;8:1391–403.
46. Bargh JD, Isidro-Llobet A, Parker JS, Spring DR. Cleavable linkers in antibody-drug conjugates. Chem Soc Rev 2019;48:4361–74.
47. Jeffrey SC, Andreyka JB, Bernhardt SX, Kissler KM, Kline T, Lenox JS, et al. Development and properties of beta-glucuronide linkers for monoclonal antibody-drug conjugates. Bioconjug Chem 2006;17:831–40.
48. Griffith OW. Biologic and pharmacologic regulation of mammalian glutathione synthesis. Free Radic Biol Med 1999;27:922–35.
49. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. J Nutr 2004;134:489–92.
50. Balendiran GK, Dabur R, Fraser D. The role of glutathione in cancer. Cell Biochem Funct 2004;22:343–52.
51. Turell L, Carballal S, Botti H, Radi R, Alvarez B. Oxidation of the albumin thiol to sulfenic acid and its implications in the intravascular compartment. Brazilian J Med Biol Res 2009;42:305–11.
52. Sapra P, Hooper AT, O'Donnell CJ, Gerber HP. Investigational antibody drug conjugates for solid tumors. Expert Opin Investig Drugs 2011;20:1131–49.
53. McLaughlin J, LoRusso P. Antibody-drug conjugates (ADCs) in clinical development: fundamentals, drug development, and clinical outcomes to target cancer. New Haven: Wiley; 2016. p. 321–44.
54. Shefet-Carasso L, Benhar I. Antibody-targeted drugs and drug resistance–challenges and solutions. Drug resistance updates: reviews and commentaries in antimicrobial and anticancer chemotherapy. 2015;18:36–46.
55. Nasiri H, Valedkarimi Z, Aghebati-Maleki L, Majidi J. Antibody-drug conjugates: promising and efficient tools for targeted cancer therapy. J Cell Physiol 2018;233:6441–57.
56. Li F, Emmerton KK, Jonas M, Zhang X, Miyamoto JB, Setter JR, et al. Intracellular released payload influences potency and bystander-killing effects of antibody-drug conjugates in preclinical models. Cancer Res 2016;76:2710–9.
57. Bouchard H, Viskov C, Garcia-Echeverria C. Antibody-drug conjugates-a new wave of cancer drugs. Bioorg Med Chem Lett 2014;24:5357–63.
58. Tai YT, Mayes PA, Acharya C, Zhong MY, Cea M, Cagnetta A, et al. Novel afucosylated anti-B cell maturation antigen-monomethyl auristatin F antibody-drug conjugate (GSK2857916) induces potent and selective anti-multiple myeloma activity. Blood 2014;122:877.
59. Maderna A, Leverett CA. Recent advances in the development of new auristatins: structural modifications and application in antibody drug conjugates. Mol Pharm 2015;12:1798–812.
60. Betella I, Birrer MJ, Moore KN, Bates RC. Antibody-drug conjugate-based therapeutics: state of the science. J Natl Cancer Inst 2019;111:538–49.
61. van de Donk NW, Dhimolea E. Brentuximab vedotin. mAbs 2012;4:458–65.
62. U.S. Food and Drug Administration. FDA expands approval of Adcetris for first-line treatment of Stage III or IV classical Hodgkin lymphoma in combination with chemotherapy (Press release). FDA. 20 March 2018. Retrieved 20 March 2018 [press release]. 2018. Available from: https://www.fda.gov/news-events/press-announcements/fda-expands-approval-adcetris-first-line-treatment-stage-iii-or-iv-classical-hodgkin-lymphoma.
63. Kumar A, White J, James Christie R, Dimasi N, Gao C. Chapter 12: antibody-drug conjugates. In: Goodnow RA, editor. Annual reports in medicinal chemistry, Volume 50: Gaithersburg: Academic Press; 2017. p. 441–80.
64. Lopus M, Oroudjev E, Wilson L, Wilhelm S, Widdison W, Chari R, et al. Maytansine and cellular metabolites of antibody-maytansinoid conjugates strongly suppress microtubule dynamics by binding to microtubules. Mol Cancer Ther 2010;9:2689–99.
65. Oroudjev E, Lopus M, Wilson L, Audette C, Provenzano C, Erickson H, et al. Maytansinoid-antibody conjugates induce mitotic arrest by suppressing microtubule dynamic instability. Mol Cancer Ther 2010;9:2700–13.
66. Chari RV, Martell BA, Gross JL, Cook SB, Shah SA, Blattler WA, et al. Immunoconjugates containing novel maytansinoids: promising anticancer drugs. Cancer Res 1992;52:127–31.
67. Barok M, Joensuu H, Isola J. Trastuzumab emtansine: mechanisms of action and drug resistance. Breast Cancer Res 2014;16:209.
68. Yardley DA, Krop IE, LoRusso PM, Mayer M, Barnett B, Yoo B, et al. Trastuzumab emtansine (T-DM1) in patients with HER2-positive metastatic breast cancer previously treated with chemotherapy and 2 or more HER2-targeted agents: results from the T-PAS expanded access study. Cancer J 2015;21:357–64.
69. Maiese WM, Lechevalier MP, Lechevalier HA, Korshalla J, Kuck N, Fantini A, et al. Calicheamicins, a novel family of antitumor antibiotics: taxonomy, fermentation and biological properties. J Antibiot 1989;42:558–63.
70. Kim EG, Kim KM. Strategies and advancement in antibody-drug conjugate optimization for targeted cancer therapeutics. Biomol Ther 2015;23:493–509.
71. Kern JC, Cancilla M, Dooney D, Kwasnjuk K, Zhang R, Beaumont M, et al. Discovery of pyrophosphate diesters as tunable, soluble, and bioorthogonal linkers for site-specific antibody-drug conjugates. J Am Chem Soc 2016;138:1430–45.
72. Tsimberidou AM, Giles FJ, Estey E, O'Brien S, Keating MJ, Kantarjian HM. The role of gemtuzumab ozogamicin in acute leukaemia therapy. Br J Haematol 2006;132:398–409.
73. Bross PF, Beitz J, Chen G, Chen XH, Duffy E, Kieffer L, et al. Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. Clin Cancer Res 2001;7:1490–6.
74. Crane EA, Gademann K. Capturing biological activity in natural product fragments by chemical synthesis. Angew Chem Int Ed 2016;55:3882–902.
75. Elgersma RC, Coumans RG, Huijbregts T, Menge WM, Joosten JA, Spijker HJ, et al. Design, synthesis, and evaluation of linker-duocarmycin payloads: toward selection of HER2-targeting antibody–drug conjugate SYD985. Mol Pharm 2015;12:1813–35.
76. Yu L, Lu Y, Yao Y, Liu Y, Wang Y, Lai Q, et al. Promiximab-duocarmycin, a new CD56 antibody-drug conjugates, is highly efficacious in small cell lung cancer xenograft models. Oncotarget 2017;9:5197–207.
77. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol 2013;65:157–70.
78. Govindan SV, Cardillo TM, Sharkey RM, Tat F, Gold DV, Goldenberg DM. Milatuzumab-SN-38 conjugates for the treatment of CD74+ cancers. Mol Cancer Ther 2013;12:968–78.
79. Ma Z, He H, Sun F, Xu Y, Huang X, Ma Y, et al. Selective targeted delivery of doxorubicin via conjugating to anti-CD24 antibody results in enhanced antitumor potency for hepatocellular carcinoma both in vitro and in vivo. J Cancer Res Clin Oncol 2017;143:1929–40.
80. Nolting B. Linker technologies for antibody-drug conjugates. Methods Mol Biol 2013;1045:71–100.
81. O'Mahony D, Bishop MR. Monoclonal antibody therapy. Front Biosci 2006;11:1620–35.
82. Chari RV. Targeted cancer therapy: conferring specificity to cytotoxic drugs. Acc Chem Res 2008;41:98–107.

83. Kovtun YV, Goldmacher VS. Cell killing by antibody-drug conjugates. Cancer Lett 2007;255:232–40.
84. Conner SD, Schmid SL. Regulated portals of entry into the cell. Nature 2003; 422:37–44.
85. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol 2007;7:715–25.
86. Kalim M, Chen J, Wang S, Lin C, Ullah S, Liang K, et al. Intracellular trafficking of new anticancer therapeutics: antibody-drug conjugates. Drug Des Devel Ther 2017;11:2265–76.
87. Sievers EL, Senter PD. Antibody-drug conjugates in cancer therapy. Annu Rev Med 2013;64:15–29.
88. Garcia-Alonso S, Ocana A, Pandiella A. Resistance to antibody-drug conjugates. Cancer Res 2018;78:2159–65.
89. Chen R, Hou J, Newman E, Kim Y, Donohue C, Liu X, et al. CD30 downregulation, MMAE resistance, and MDR1 upregulation are all associated with resistance to brentuximab vedotin. Mol Cancer Ther 2015;14:1376–84.
90. van der Velden VH, Boeckx N, Jedema I, te Marvelde JG, Hoogeveen PG, Boogaerts M, et al. High CD33-antigen loads in peripheral blood limit the efficacy of gemtuzumab ozogamicin (Mylotarg®) treatment in acute myeloid leukemia patients. Leukemia 2004;18:983–8.
91. Nagy P, Friedländer E, Tanner M, Kapanen AI, Carraway KL, Isola J, et al. Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. Cancer Res 2005;65: 473–82.
92. Scaltriti M, Rojo F, Ocana A, Anido J, Guzman M, Cortes J, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. J Natl Cancer Inst 2007;99:628–38.
93. Sung M, Tan X, Lu B, Golas J, Hosselet C, Wang F, et al. Caveolae-mediated endocytosis as a novel mechanism of resistance to trastuzumab emtansine (T-DM1). Mol Cancer Ther 2018;17:243–53.
94. Ríos-Luci C, García-Alonso S, Díaz-Rodríguez E, Nadal-Serrano M, Arribas J, Ocaña A, et al. Resistance to the antibody–drug conjugate T-DM1 is based in a reduction in lysosomal proteolytic activity. Cancer Res 2017;77:4639–51.
95. Yu M, Ocana A, Tannock IF. Reversal of ATP-binding cassette drug transporter activity to modulate chemoresistance: why has it failed to provide clinical benefit? Cancer Metastasis Rev 2013;32:211–27.
96. Yu SF, Zheng B, Go M, Lau J, Spencer S, Raab H, et al. A novel anti-CD22 anthracycline-based antibody–drug conjugate (ADC) that overcomes resistance to auristatin-based ADCs. Clin Cancer Res 2015;21:3298–306.
97. Takegawa N, Nonagase Y, Yonesaka K. DS-8201a, a new HER2-targeting antibody-drug conjugate incorporating a novel DNA topoisomerase I inhibitor, overcomes HER2-positive gastric cancer T-DM1 resistance 2017;141:1682–9.
98. Sabbaghi M, Gil-Gómez G, Guardia C, Servitja S, Arpí O, García-Alonso S, et al. Defective Cyclin B1 induction in trastuzumab-emtansine (T-DM1) acquired resistance in HER2-positive breast cancer. Clin Cancer Res 2017;23:7006–19.
99. Rosen DB, Harrington KH, Cordeiro JA, Leung LY, Putta S, Lacayo N, et al. AKT signaling as a novel factor associated with in vitro resistance of human AML to gemtuzumab ozogamicin. PLoS One 2013;8:e53518.
100. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 2007;12: 395–402.
101. Haag P, Viktorsson K, Lindberg ML, Kanter L, Lewensohn R, Stenke L. Deficient activation of Bak and Bax confers resistance to gemtuzumab ozogamicin-induced apoptotic cell death in AML. Exp Hematol 2009;37:755–66.
102. Walter RB, Raden BW, Cronk MR, Bernstein ID, Appelbaum FR, Banker DE. The peripheral benzodiazepine receptor ligand PK11195 overcomes different resistance mechanisms to sensitize AML cells to gemtuzumab ozogamicin. Blood 2004;103:4276–84.
103. Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature 2013;501:346.
104. Lucas AT, Price LSL, Schorzman AN, Storrie M, Piscitelli JA, Razo J, et al. Factors affecting the pharmacology of antibody–drug conjugates. Antibodies 2018;7:10.
105. Weber L, Schulz WA, Philippou S, Eckardt J, Ubrig B, Hoffmann MJ, et al. Characterization of the olfactory receptor OR10H1 in human urinary bladder cancer. Front Physiol 2018;9:456.
106. Kodack DP, Askoxylakis V, Ferraro GP, Sheng Q, Badeaux M, Goel S, et al. The brain microenvironment mediates resistance in luminal breast cancer to PI3K inhibition through HER3 activation. Sci Transl Med 2017;9:pii: eaal4682.
107. Beck A, Goetsch L, Dumontet C, Corvaïa N. Strategies and challenges for the next generation of antibody-drug conjugates. Nat Rev Drug Discov 2017;16: 315.
108. Sau S, Alsaab HO, Kashaw SK, Tatiparti K, Iyer AK. Advances in antibody-drug conjugates: a new era of targeted cancer therapy. Drug Discov Today 2017;22: 1547–56.
109. Sedykh SE, Prinz VV, Buneva VN, Nevinsky GA. Bispecific antibodies: design, therapy, perspectives. Drug Des Devel Ther 2018;12:195.
110. Andreev J, Thambi N, Perez Bay AE, Delfino F, Martin J, Kelly MP, et al. Bispecific antibodies and antibody–drug conjugates (ADCs) bridging HER2 and prolactin receptor improve efficacy of HER2 ADCs. Mol Cancer Ther 2017; 16:681–93.
111. Herbener P, Schönfeld K, König M, Germer M, Przyborski JM, Bernöster K, et al. Functional relevance of in vivo half antibody exchange of an IgG4 therapeutic antibody-drug conjugate. PLoS One 2018;13:e0195823.
112. Chari RV, Miller ML, Widdison WC. Antibody–drug conjugates: an emerging concept in cancer therapy. Angew Chem Int Ed 2014;53:3796–827.
113. Ab O, Whiteman KR, Bartle LM, Sun X, Singh R, Tavares D, et al. IMGN853, a folate receptor-α (FRα)–targeting antibody–drug conjugate, exhibits potent targeted antitumor activity against FRα-expressing tumors. Mol Cancer Ther 2015;14:1605–13.
114. Lyon RP, Bovee TD, Doronina SO, Burke PJ, Hunter JH, Neff-LaFord HD, et al. Reducing hydrophobicity of homogeneous antibody-drug conjugates improves pharmacokinetics and therapeutic index. Nat Biotechnol 2015;33: 733.
115. Hamblett KJ, Senter PD, Chace DF, Sun MM, Lenox J, Cerveny CG, et al. Effects of drug loading on the antitumor activity of a monoclonal antibody drug conjugate. Clin Cancer Res 2004;10:7063–70.
116. Singh R, Setiady YY, Ponte J, Kovtun YV, Lai KC, Hong EE, et al. A new triglycyl peptide linker for antibody–drug conjugates (ADCs) with improved targeted killing of cancer cells. Mol Cancer Ther 2016;15:1311–20.
117. Wei B, Gunzner-Toste J, Yao H, Wang T, Wang J, Xu Z, et al. Discovery of peptidomimetic antibody–drug conjugate linkers with enhanced protease specificity. J Med Chem 2017;61:989–1000.
118. Anami Y, Xiong W, Gui X, Deng M, Zhang CC, Zhang N, et al. Enzymatic conjugation using branched linkers for constructing homogeneous antibody–drug conjugates with high potency. Organic Biomol Chem 2017;15: 5635–42.
119. Hartley JA. The development of pyrrolobenzodiazepines as antitumour agents. Expert Opin Investig Drugs 2011;20:733–44.
120. Saunders LR, Bankovich AJ, Anderson WC, Aujay MA, Bheddah S, Black K, et al. A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. Sci Transl Med 2015;7: 302ra136.
121. Kung Sutherland MS, Walter RB, Jeffrey SC, Burke PJ, Yu C, Kostner H, et al. SGN-CD33A: a novel CD33-targeting antibody–drug conjugate using a pyrrolobenzodiazepine dimer is active in models of drug-resistant AML. Blood 2013;122:1455–63.
122. Puthenveetil S, Loganzo F, He H, Dirico K, Green M, Teske J, et al. Natural product splicing inhibitors: a new class of antibody–drug conjugate (ADC) payloads. Bioconjug Chem 2016;27:1880–8.
123. Moldenhauer G, Salnikov AV, Lüttgau S, Herr I, Anderl J, Faulstich H. Therapeutic potential of amanitin-conjugated anti-epithelial cell adhesion molecule monoclonal antibody against pancreatic carcinoma. J Natl Cancer Inst 2012;104:622–34.
124. Flygare JA, Pillow TH, Aristoff P. Antibody-drug conjugates for the treatment of cancer. Chem Biol Drug Des 2013;81:113–21.
125. Sun F, Wang Y, Luo X, Ma Z, Xu Y, Zhang X, et al. Anti-CD24 antibody-nitric oxide conjugate (ANC) selectively and potently suppresses hepatic carcinoma. Cancer Res. 2019;79:3395–405.
126. Loganzo F, Tan X, Sung M, Jin G, Myers JS, Melamud E, et al. Tumor cells chronically treated with a trastuzumab–maytansinoid antibody–drug conjugate develop varied resistance mechanisms but respond to alternate treatments. Mol Cancer Ther 2015;14:952–63.
127. Kovtun YV, Audette CA, Mayo MF, Jones GE, Doherty H, Maloney EK, et al. Antibody-maytansinoid conjugates designed to bypass multidrug resistance. Cancer Res 2010;70:2528–37.
128. Golfier S, Kopitz C, Kahnert A, Heisler I, Schatz CA, Stelte-Ludwig B, et al. Anetumab ravtansine: a novel mesothelin-targeting antibody–drug conjugate cures tumors with heterogeneous target expression favored by bystander effect. Mol Cancer Ther 2014;13:1537–48.

129. Epenetos AA, Snook D, Durbin H, Johnson PM, Taylor-Papadimitriou J. Limitations of radiolabeled monoclonal antibodies for localization of human neoplasms. Cancer Res 1986;46:3183–91.
130. Deonarain MP, Yahioglu G, Stamati I, Marklew J. Emerging formats for next-generation antibody drug conjugates. Expert Opin Drug Discov 2015; 10:463–81.
131. Merten H, Brandl F, Plückthun A, Zangemeister-Wittke U. Antibody–drug conjugates for tumor targeting. Novel conjugation chemistries and the promise of non-IgG binding proteins. Bioconj Chem 2015;26:2176–85.
132. Gébleux R, Wulhfard S, Casi G, Neri D. Antibody format and drug release rate determine the therapeutic activity of noninternalizing antibody–drug conjugates. Mol Cancer Ther 2015;14:2606–12.
133. Pruszynski M, Koumarianou E, Vaidyanathan G, Revets H, Devoogdt N, Lahoutte T, et al. Improved tumor targeting of anti-HER2 nanobody through N-Succinimidyl 4-Guanidinomethyl-3-iodobenzoate radiolabeling. J Nuclear Med 2014;55:650–6.
134. Schmidt MM, Wittrup KD. A modeling analysis of the effects of molecular size and binding affinity on tumor targeting. Mol Cancer Ther 2009;8:2861–71.
135. Boswell CA, Tesar DB, Mukhyala K, Theil FP, Fielder PJ, Khawli LA. Effects of charge on antibody tissue distribution and pharmacokinetics. Bioconjug Chem 2010;21:2153–63.
136. Boylan NJ, Zhou W, Proos RJ, Tolbert TJ, Wolfe JL, Laurence JS. Conjugation site heterogeneity causes variable electrostatic properties in Fc conjugates. Bioconjug Chem 2013;24:1008–16.
137. Yip V, Palma E, Tesar DB, Mundo EE, Bumbaca D, Torres EK, et al. Quantitative cumulative biodistribution of antibodies in mice: effect of modulating binding affinity to the neonatal Fc receptor. mAbs 2014;6:689–96.
138. Shibata-Koyama M, Iida S, Misaka H, Mori K, Yano K, Shitara K, et al. Nonfucosylated rituximab potentiates human neutrophil phagocytosis through its high binding for FcγRIIIb and MHC class II expression on the phagocytotic neutrophils. Exp Hematol 2009;37:309–21.
139. Zhou Q, Stefano JE, Manning C, Kyazike J, Chen B, Gianolio DA, et al. Site-specific antibody-drug conjugation through glycoengineering. Bioconjug Chem 2014;25:510–20.
140. Gefen T, Vaya J, Khatib S, Harkevich N, Artoul F, Heller ED, et al. The impact of PEGylation on protein immunogenicity. Int Immunopharmacol 2013;15: 254–9.
141. Veronese FM, Mero A. The impact of PEGylation on biological therapies. BioDrugs 2008;22:315–29.
142. Burke PJ, Hamilton JZ, Jeffrey SC, Hunter JH, Doronina SO, Okeley NM, et al. Optimization of a PEGylated glucuronide-monomethylauristatin E linker for antibody-drug conjugates. Mol Cancer Ther 2017;16:116–23.
143. Larson R, Boogaerts M, Estey E, Karanes C, Stadtmauer E, Sievers E, et al. Antibody-targeted chemotherapy of older patients with acute myeloid leukemia in first relapse using Mylotarg (gemtuzumab ozogamicin). Leukemia 2002; 16:1627.
144. Clarke WT, Marks PW. Gemtuzumab ozogamicin: is there room for salvage? Blood 2010;116:2618–9.
145. Petersdorf SH, Kopecky KJ, Slovak M, Willman C, Nevill T, Brandwein J, et al. A phase 3 study of gemtuzumab ozogamicin during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. Blood 2013; 121:4854–60.
146. Burnett AK, Hills RK, Milligan D, Kjeldsen L, Kell J, Russell NH, et al. Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: results of the MRC AML15 trial. J Clin Oncol 2010;29:369–77.
147. Caron PC, Jurcic JG, Scott AM, Finn RD, Divgi CR, Graham MC, et al. A phase 1B trial of humanized monoclonal antibody M195 (anti-CD33) in myeloid leukemia: specific targeting without immunogenicity. Blood 1994;83:1760–8.
148. Jefferson E. FDA: Pfizer voluntarily withdraws cancer treatment mylotarg from US Market. Silver Spring, MD:U.S. Food and Drug Administration; 2010. Available from: https://www.fiercepharma.com/pharma/fda-pfizer-voluntarily-withdraws-cancer-treatment-mylotarg-from-u-s-market.
149. Amadori S, Suciu S, Selleslag D, Aversa F, Gaidano G, Musso M, et al. Gemtuzumab ozogamicin versus best supportive care in older patients with newly diagnosed acute myeloid leukemia unsuitable for intensive chemotherapy: results of the randomized phase III EORTC-GIMEMA AML-19 trial. J Clin Oncol 2016;34:972–9.
150. Castaigne S, Pautas C, Terré C, Raffoux E, Bordessoule D, Bastie J-N, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. Lancet 2012;379:1508–16.
151. Taksin A, Legrand O, Raffoux E, De Revel T, Thomas X, Contentin N, et al. High efficacy and safety profile of fractionated doses of Mylotarg as induction therapy in patients with relapsed acute myeloblastic leukemia: a prospective study of the alfa group. Leukemia 2007;21:66.
152. Delaunay J, Recher C, Pigneux A, Witz F, Vey N, Blanchet O, et al. Addition of gemtuzumab ozogamycin to chemotherapy improves event-free survival but not overall survival of AML patients with intermediate cytogenetics not eligible for allogeneic transplantation. Results of the GOELAMS AML 2006 IR study. Blood 2011;118:79.
153. Burnett AK, Russell NH, Hills RK, Kell J, Freeman S, Kjeldsen L, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy improves survival in older patients with acute myeloid leukemia. J Clin Oncol 2012;30:3924–31.
154. Sievers EL, Larson RA, Stadtmauer EA, Estey E, Lowenberg B, Dombret H, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. J Clin Oncol 2001;19:3244–54.
155. Ricart AD. Antibody-drug conjugates of calicheamicin derivative: gemtuzumab ozogamicin and inotuzumab ozogamicin. Clin Cancer Res 2011;17:6417–27.
156. Berger GK, McBride A, Lawson S, Royball K, Yun S, Gee K, et al. Brentuximab vedotin for treatment of non-Hodgkin lymphomas: a systematic review. Crit Rev Oncol Hematol 2017;109:42–50.
157. Sutherland MS, Sanderson RJ, Gordon KA, Andreyka J, Cerveny CG, Yu C, et al. Lysosomal trafficking and cysteine protease metabolism confer target-specific cytotoxicity by peptide-linked anti-CD30-auristatin conjugates. J Biol Chem 2006;281:10540–7.
158. Francisco JA, Cerveny CG, Meyer DL, Mixan BJ, Klussman K, Chace DF, et al. cAC10-vcMMAE, an anti-CD30-monomethyl auristatin E conjugate with potent and selective antitumor activity. Blood 2003;102:1458–65.
159. Hsu PL, Hsu SM. Autocrine growth regulation of CD30 ligand in CD30-expressing Reed-Sternberg cells: distinction between Hodgkin's disease and anaplastic large cell lymphoma. Lab Invest 2000;80:1111–9.
160. Senter PD, Sievers EL. The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. Nat Biotechnol 2012;30:631–7.
161. Younes A, Gopal AK, Smith SE, Ansell SM, Rosenblatt JD, Savage KJ, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. J Clin Oncol 2012;30:2183–9.
162. Pro B, Advani R, Brice P, Bartlett NL, Rosenblatt JD, Illidge T, et al. Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. J Clin Oncol 2012;30:2190–6.
163. Connors JM, Jurczak W, Straus DJ, Ansell SM, Kim WS, Gallamini A, et al. Brentuximab vedotin with chemotherapy for stage III or IV Hodgkin's Lymphoma. N Engl J Med 2018;378:331–44.
164. US Food and Drug Administration. In: ADCETRIS (brentuximab vedotin). Highlights of prescribing information 2016. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/125388s000,125399s 000lbl.pdf.
165. Bartlett NL, Chen R, Fanale MA, Brice P, Gopal A, Smith SE, et al. Retreatment with brentuximab vedotin in patients with CD30-positive hematologic malignancies. J Hematol Oncol 2014;7:24.
166. Moskowitz AJ, Schöder H, Gavane S, Thoren KL, Fleisher M, Yahalom J, et al. Baseline metabolic tumor volume is an independent prognostic factor for relapsed and refractory Hodgkin lymphoma patients receiving PET-adapted salvage therapy with brentuximab vedotin and augmented ICE. Hematol Oncol 2017;35:36–7.
167. Herrera AF, Palmer J, Martin P, Armenian S, Tsai NC, Kennedy N, et al. Autologous stem-cell transplantation after second-line brentuximab vedotin in relapsed or refractory Hodgkin lymphoma. Ann Oncol 2018;29:724–30.
168. Herrera AF, Moskowitz AJ, Bartlett NL, Vose JM, Ramchandren R, Feldman TA, et al. Interim results of brentuximab vedotin in combination with nivolumab in patients with relapsed or refractory Hodgkin lymphoma. Blood 2018; 131:1183–94.
169. Moskowitz CH, Nademanee A, Masszi T, Agura E, Holowiecki J, Abidi MH, et al. Brentuximab vedotin as consolidation therapy after autologous stem-cell transplantation in patients with Hodgkin's lymphoma at risk of relapse or progression (AETHERA): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2015;385:1853–62.
170. Eichenauer DA, Plutschow A, Kreissl S, Sokler M, Hellmuth JC, Meissner J, et al. Incorporation of brentuximab vedotin into first-line treatment of advanced

classical Hodgkin's lymphoma: final analysis of a phase 2 randomised trial by the German Hodgkin Study Group. Lancet Oncol 2017;18:1680–7.
171. Radford J, Illidge T, Counsell N, Hancock B, Pettengell R, Johnson P, et al. Results of a trial of PET-directed therapy for early-stage Hodgkin's Lymphoma. N Engl J Med 2015;372:1598–607.
172. Garcia-Sanz R, Sureda A, Gonzalez AP, De la Cruz F, Sanchez-Gonzalez B, Rodriguez A, et al. Brentuximab vedotin plus ESHAP (BRESHAP) is a highly effective combination for inducing remission in refractory and relapsed Hodgkin Lymphoma patients prior to autologous stem cell transplant: a trial of the spanish group of lymphoma and bone marrow transplantation (GELTAMO). Blood 2016;128:1109.
173. O'Connor OA, Lue JK, Sawas A, Amengual JE, Deng C, Kalac M, et al. Brentuximab vedotin plus bendamustine in relapsed or refractory Hodgkin's lymphoma: an international, multicentre, single-arm, phase 1–2 trial. Lancet Oncol 2018;19:257–66.
174. Younes A, Bartlett NL, Leonard JP, Kennedy DA, Lynch CM, Sievers EL, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med 2010;363:1812–21.
175. Wolenski FS, Xia CQ, Ma B, Han TH, Shyu WC, Balani SK. CYP suppression in human hepatocytes by monomethyl auristatin E, the payload in brentuximab vedotin (Adcetris((R))), is associated with microtubule disruption. Eur J Drug Metab Pharmacokinet 2018;43:347–54.
176. Oak E, Bartlett NL. A safety evaluation of brentuximab vedotin for the treatment of Hodgkin lymphoma. Expert Opin Drug Saf 2016;15:875–82.
177. Han TH, Gopal AK, Ramchandren R, Goy A, Chen R, Matous JV, et al. CYP3A-mediated drug-drug interaction potential and excretion of brentuximab vedotin, an antibody-drug conjugate, in patients with CD30-positive hematologic malignancies. J Clin Pharmacol 2013;53:866–77.
178. DeVita MD, Evens AM, Rosen ST, Greenberger PA, Petrich AM. Multiple successful desensitizations to brentuximab vedotin: a case report and literature review. J Natl Compr Canc Netw 2014;12:465–71.
179. Gandhi MD, Evens AM, Fenske TS, Hamlin P, Coiffier B, Engert A, et al. Pancreatitis in patients treated with brentuximab vedotin: a previously unrecognized serious adverse event. Blood 2014;123:2895–7.
180. Carson KR, Newsome SD, Kim EJ, Wagner-Johnston ND, von Geldern G, Moskowitz CH, et al. Progressive multifocal leukoencephalopathy associated with brentuximab vedotin therapy: a report of 5 cases from the Southern Network on Adverse Reactions (SONAR) project. Cancer 2014;120:2464–71.
181. Amiri-Kordestani L, Blumenthal GM, Xu QC, Zhang L, Tang SW, Ha L, et al. FDA approval: ado-trastuzumab emtansine for the treatment of patients with HER2-positive metastatic breast cancer. Clin Cancer Res 2014;20:4436–41.
182. Widdison WC, Wilhelm SD, Cavanagh EE, Whiteman KR, Leece BA, Kovtun Y, et al. Semisynthetic maytansine analogues for the targeted treatment of cancer. J Med Chem 2006;49:4392–408.
183. Phillips GD, Li G, Dugger DL, Crocker LM, Parsons KL, Mai E, et al. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody–cytotoxic drug conjugate. Cancer Res 2008;68:9280–90.
184. Ritchie M, Tchistiakova L, Scott N. Implications of receptor-mediated endocytosis and intracellular trafficking dynamics in the development of antibody drug conjugates. MAbs 2013;5:13–21.
185. Lambert JM, Chari RV. Ado-trastuzumab Emtansine (T-DM1): an antibody–drug conjugate (ADC) for HER2-positive breast cancer. J Med Chem 2014;57:6949–64.
186. Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012;367:1783–91.
187. Krop IE, Kim SB, Gonzalez-Martin A, LoRusso PM, Ferrero JM, Smitt M, et al. Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA): a randomised, open-label, phase 3 trial. Lancet Oncol 2014;15:689–99.
188. Kim SB, Wildiers H, Krop IE, Smitt M, Yu R, Lysbet de Haas S, et al. Relationship between tumor biomarkers and efficacy in TH3RESA, a phase III study of trastuzumab emtansine (T-DM1) vs. treatment of physician's choice in previously treated HER2-positive advanced breast cancer. Int J Cancer 2016;139:2336–42.
189. Diéras V, Miles D, Verma S, Pegram M, Welslau M, Baselga J, et al. Trastuzumab emtansine versus capecitabine plus lapatinib in patients with previously treated HER2-positive advanced breast cancer (EMILIA): a descriptive analysis of final overall survival results from a randomised, open-label, phase 3 trial. Lancet Oncol 2017;18:732–42.
190. Krop IE, Kim S, Martin AG, LoRusso PM, Ferrero JM, Badovinac-Crnjevic T, et al. Trastuzumab emtansine versus treatment of physician's choice in patients with previously treated HER2-positive metastatic breast cancer (TH3RESA): final overall survival results from a randomised open-label phase 3 trial. Lancet Oncol 2017;18:743–54.
191. Krop IE, Beeram M, Modi S, Jones SF, Holden SN, Yu W, et al. Phase I study of trastuzumab-DM1, an HER2 antibody-drug conjugate, given every 3 weeks to patients with HER2-positive metastatic breast cancer. J Clin Oncol 2010;28:2698–704.
192. Shen K, Ma X, Zhu C, Wu X, Jia H. Safety and efficacy of trastuzumab emtansine in advanced human epidermal growth factor receptor 2-positive breast cancer: a meta-analysis. Sci Rep 2016;6:23262.
193. Moilanen T, Jokimaki A, Tenhunen O, Koivunen JP. Trastuzumab-induced cardiotoxicity and its risk factors in real-world setting of breast cancer patients 2018;144:1613–21.
194. National Toxicology Program. NTP Monograph: developmental effects and pregnancy outcomes associated with cancer chemotherapy use during pregnancy. NTP Monogr 2013;2:i–214.
195. Haddley K. Trastuzumab emtansine for the treatment of HER2-positive metastatic breast cancer. Drugs Today 2013;49:701–15.
196. Davis JA, Rock DA, Wienkers LC, Pearson JT. In vitro characterization of the drug-drug interaction potential of catabolites of antibody-maytansinoid conjugates. Drug Metab Dispos 2012;40:1927–34.
197. Thota S, Advani A. Inotuzumab ozogamicin in relapsed Bcell acute lymphoblastic leukemia. Eur J Haematol 2017;98:425–34.
198. Shor B, Gerber HP, Sapra P. Preclinical and clinical development of inotuzumab-ozogamicin in hematological malignancies. Mol Immunol 2015;67:107–16.
199. U.S. Food and Drug Administration. Besponsa (inotuzumab ozogamicin) for injection, for intravenous use: US prescribing information 2017; 2019. Available from: https://www.accessdata.fda.gov.
200. Pfizer Japan Inc. Besponsa (inotuzumab ozogamicin): Japanese prescribing information; 2018. Available from: https://www.pmda.go.jp.
201. European Medicines Agency. Besponsa (inotuzumab ozogamicin): EU summary of product characteristics; 2017. Available from: https://www.ema.europa.eu; 2019.
202. Lamb YN. Inotuzumab ozogamicin: first global approval. Drugs 2017;77:1603–10.
203. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. N Engl J Med 2016;375:740–53.
204. Advani A, Coiffier B, Czuczman MS, Dreyling M, Foran J, Gine E, et al. Safety, pharmacokinetics, and preliminary clinical activity of inotuzumab ozogamicin, a novel immunoconjugate for the treatment of B-cell non-Hodgkin's lymphoma: results of a phase I study. J Clin Oncol 2010;28:2085–93.
205. DeAngelo DJ, Stock W, Stein AS, Shustov A, Liedtke M, Schiffer CA, et al. Inotuzumab ozogamicin in adults with relapsed or refractory CD22-positive acute lymphoblastic leukemia: a phase 1/2 study. Blood Adv 2017;1:1167–80.
206. Bayat Mokhtari R, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, et al. Combination therapy in combating cancer. Oncotarget 2017;8:38022–43.
207. Boshuizen J, Koopman LA, Krijgsman O, Shahrabi A, van den Heuvel EG, Ligtenberg MA, et al. Cooperative targeting of melanoma heterogeneity with an AXL antibody-drug conjugate and BRAF/MEK inhibitors 2018;24:203–12.
208. Schonfeld K, Zuber C, Pinkas J, Hader T, Bernoster K, Uherek C. Indatuximab ravtansine (BT062) combination treatment in multiple myeloma: pre-clinical studies 2017;10:13.