Antibody-Drug Conjugates: A Comprehensive Review

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Running title: Antibody-Drug Conjugates in Cancer Immunotherapy.

Keywords: Cancer; antibody-drug conjugates; antibody; linker; payload; treatment

Declaration of interests
All authors declare no competing interests.
Abstract

Antibody-drug conjugates (ADCs) are one of the fastest-growing anti-cancer drugs. This approach comprises a monoclonal antibody conjugated to the cytotoxic payload via a chemical linker that directed towards 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, a monoclonal antibody, 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 US Food and Drug Administration (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 cancer/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. Based on 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 withstand 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 maximum tolerated dose (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 identify their target themselves without harming the organism (8). Ehrlich also anticipates attaching toxin to the antibodies to improve their therapeutic specificity. 45 years later, in accordance with the concept of Paul Ehrlich, methotrexate (MTX) was attached to an antibody against leukemia cells (9). In the 1980s, clinical trials of ADCs grounded on mouse immunoglobulin G (IgG) molecules were performed. The first ADCs based on chimeric and humanized monoclonal antibodies (mAbs) were testified in the 1990s (10). This technology consists of highly specific monoclonal antibodies (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 monoclonal antibody (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 higher expressed in the tumor cell compared to the healthy cell (13). Second, the target antigen should be displayed on the surface of the tumor cell to be available to the circulated
monoclonal antibody (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 cytotoxic agent (15). Though some studies have demonstrated that non-internalized 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 mesothelin that is overexpressed in mesothelioma, ovarian, pancreatic, and lung adenocarcinomas found to be low expressed in the healthy cell. Sven Golfier et al (17) developed new ADC against mesothelin that showed great efficacy in patient-derived xenograft tumor models. Though it also displayed a bystander effect on neighboring mesothelin-negative tumor cells.

Selection of antibody moiety

The antibody is the main component of 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 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 aids 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 Rong Wang et al. This ADC consists of a chimeric anti-CD30 monoclonal antibody (mAb) linked to Lidamycin, a potent cytotoxic agent, via a non-protease 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 forwarded to design humanized monoclonal antibody. Humanized antibody contains only the CDRs 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 monoclonal antibody against HER-2 positive cells linked to the DM1 cytotoxic agent. This ADC is used for the treatment of HER-2 positive metastatic breast cancer patients that do not respond to antibody treatment alone or to chemotherapy (27). Recently, next-generation or 3rd generation ADCs are replacing the second generation ADCs as in this generation; a fully human antibody is used instead of 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. Melissa Gallery et al (29) successfully demonstrated fully human antibody-based ADC. In their study, they used ADC consisting of a fully human anti-GCC monoclonal antibody conjugated to a highly cytotoxic drug monomethyl auristatin E 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 monoclonal antibody. 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, non-toxic 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 above-mentioned conditions: non-cleavable and cleavable linkers (figure 1).

*Figure 1. Chemical structures of non-cleavable and cleavable linkers.*

**Non-cleavable linkers**

Non-cleavable linkers consist of stable bonds that resist proteolytic degradation and provide higher stability than the cleavable linkers (32). The mechanism of action of non-cleavable 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 non-cleavable linkers make it possible to modify the chemical properties of small molecules in order to modulate the affinity of the transporter and to improve the efficiency.
Gail D et al (34) demonstrated that trastuzumab conjugated via non-reducible thioether linkage (SMCC) to the DM1 cytotoxic drug, exhibited superior activity in comparison to 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 to different conjugates, and toxicity in rats was insignificant as compared to 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 non-cleavable linker. For non-cleavable 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 anti-mitotic drug that is most effective in its natural form and therefore not suitable for derivatization with non-cleavable linkers (35). Conversely, 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 non-cleavable 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 non-cleavable linkers is to make better the “bystander” effect (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 ADCs design such as:
**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 get hydrolyzed in lysosomal (PH 4.8) and endosomal (PH 5–6) acidic tumor microenvironment (38). However, these linkers have been associated with the non-specific release of the drug in clinical studies (39). One successful example of ADC design using acid-sensitive linker is the IMMU-110. This ADC is composed of a humanized anti-CD74 monoclonal antibody conjugated to the doxorubicin (DOX) via acid-labile hydrazone. IMMU-110 displayed improved activity against multiple myeloma (MM) and appeared to be safe in a monkey model of MM 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 exhibited high expression of lysosomal proteases like cathepsin B compared to 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 physiological 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 to the dipeptide-based ADC, and its efficacy is comparable to that of the ADC coupled to the vc 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) (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 non-toxic 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 IC50 (half maximal inhibitory concentration) 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 sixteen known drug classes integrated into clinical-stage ADCs, eleven 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;

**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 analogs of this class of drug such as monomethyl auristatin E (MMAE) and monomethyl auristatin F (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 monomethyl auristatin F (MMAF) is the same as that of MMAE, however, it has reduced activity compared to 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 monomethyl auristatin E (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 (HL) (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 cytotoxicity of maytansinoids demonstrated 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. Though, the powerful cytotoxicity of maytansinoids can be utilized as targeted delivery access, particularly as antibody-maytansinoid-conjugates (AMCs). Preclinical studies indicate that AMCs have significantly improved potential as anti-cancer agents in comparison to 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 non-cleavable thioether linker against the HER2 receptor. This ADC is used to treat HER2-positive metastatic breast cancer patients 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 (GO), 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 acute myeloid leukemia (AML) patients (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 irreversible 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 Lin Yu et al 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 Zhaoxiong Ma et al (79). In this study, the doxorubicin based ADC was able to suppress tumor growth and improves the survival of hepatocellular carcinoma (HCC) 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 in order to avoid gastric acids and proteolytic enzymes 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; (1) clathrin-mediated endocytosis (the major route of intracellular uptake of ADCs), (2) caveolae-mediated endocytosis, and (3) 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 (kD > 10 nM), 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 monoclonal antibody component of ADCs and human neonatal FcRs (FcRns) (18). A portion of the ADC binds to FcRns in endosomes and circulates back to the extracellular, where the physiological 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 (86, 87). 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 (88). 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 (89). 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 monoclonal antibody, the cytotoxic drug, or by triggering survival signaling pathways (54). Garcia-Alonso et al (90) 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 reference 90).

Antigen related resistance includes alteration in the levels of the antigen recognized by the monoclonal antibody 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 (91). On the contrary, a high antigen expression may reduce ADC efficacy because of reduced drug exposure (92). 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 ADCs sensitivity (93, 94). In a study conducted by Sung and colleagues (95), 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 (96). These cells had low therapeutic efficacy in comparison to 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 (ABC) transporters such as PgP/MDR1 (97-99). 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 G2–M transition, were higher in HER2+ breast cancer cells sensitive to T-DM1 in comparison to the cells made resistant to T-DM1 (100). 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 GO and trastuzumab resistance (101, 102). In line with these resistance mechanisms, dysregulation in apoptosis may also contribute to ADC sensitivity. It has been demonstrated that pro-apoptotic proteins BAX and BAK plays an important role in GO sensitivity (103). In addition, overexpression of anti-apoptotic proteins Bcl-2 and Bcl-x are associated with Go resistance (104).

**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;

1. **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 monoclonal antibody 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 to 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 (105). Shedding or secreting of antigens is another feature that may reduce ADCs binding to the targets, resulting in a significantly elevated risk of toxicity (106). 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, Lea Weber et al (107) 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 predominant 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. David P. Kodack and his team (108) also discovered a new antigen called Her3 antigen. The Her3 antigen overexpressed in the metastasized brain cancer cells.
Blocking Her-3 function produced significant tumor growth delay and improved mouse survival. It shows that Her-3 might be an effective antigen for targeting Her-2-resistant tumor cells as they start to metastasize. Thus using these new target antigens may provide novel ADCs that could provide better treatment approaches.

2. Optimization of antibody

In the development of therapeutic ADCs, strategies like enhancing specificity, affinity, and pharmacokinetics (PK) are of great importance for the optimization of therapeutic mAbs. Both naked antibodies and ADCs require improving antibody homogeneity and developability in order to minimize the rate of attrition of drug candidates (109). 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 Abs and half Abs technologies and approaches to evaluate the therapeutic functionality (110). Bispecific immunoglobulins contain two different antigen-binding sites. The bispecific antibody based ADCs are being tested at the preclinical investigations (111). Julian Andreev et al (112) 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 HER2xPRLR 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 compare to the 1st, 2nd and 3rd generation ADCs that provides 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, Peter Herbener and his team (113) generated four different ADC bases of the antibody-drug conjugate 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. Peter Herbener et al 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 antibody drug-conjugates. From these studies, we can conclude that bispecific, and half antigens give better results than 1st, 2nd, and 3rd 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 multi-antigens at the same time.

3. 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 (PK)/pharmacodynamics (PD), and therapeutic index of the ADC (19, 110).
Cancer cell resistance occurs via the upregulation of multidrug resistance 1 (MDR1) expression. MDR1 has a high affinity for transporting hydrophobic compounds compared to the hydrophilic compounds. It has been demonstrated that nonpolar or non-charged linkers, Mytansinoid-based ADCs, have lower in vitro potency in MDR1+ cells as compared to the MDR1− cells (114). Due to this drawback, efforts were made to develop hydrophilic or charged linkers. The resulting ADCs showed improved potency against MDR1+ cells with highly polar or charged metabolites. Examples are the mal-PEG4-N-hydroxysuccinimide and N-Hydroxysuccinimidyld-4-(2-pyridyldithio)-2-sulfobutanoate (sulfo-SPDB) (115, 116).

It is known that reducing hydrophobicity results in improved ADCs outcomes (117). The increase in drug antibody ratio (DAR) has a direct impact on the in vitro ADCs potency. However, with the rise of DAR, the plasma clearance of ADC can increase that reduces exposure and in vivo efficacy (118). 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 (117).

Additionally, several new linkers are currently being tested in preclinical trials. Rajeeva Singh et al (119) 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 consists of the maytansinoid payload linked to the anti-EpCAM, and anti-EGFR monoclonal antibodies via triglycyl peptide linker CX and non-cleavable SMCC linker. The ADC composed of triglycyl peptide linker CX and non-cleavable SMCC linker showed similar cytotoxic activity in vitro for several cancer cell lines; however, in other cell lines especially a multi-drug resistant cell line CX ADC showed more cytotoxic activity (5-100 fold lower IC50) than the SMCC ADC.
Another example of a new ADC linker is the peptidomimetic linker. BinQing Wei and his team (120) 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 Val-Cit containing ADCs showed a similar rate of inhibition of tumor cell growth and intracellular release of the payload. Moreover, the cBu-Cit and Val-Cit 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 Val-Cit peptide containing ADC. Therefore, the novel peptidomimetic linkers allow the ADC to cleavage 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 monoclonal antibody can be linked to more than two payloads. Yasuaki Anami et al (121) 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 monomethyl auristatin F 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 drug-antibody ratio (DAR) of 1.9.

4. New payloads used in ADC design
One of the new payloads used in ADCs design is the Pyrrolobenzodiazepine class (PBDs). Pyrrolobenzodiazepines belong to the class of natural products with antibacterial or anti-cancer characteristics that are produced by several actinomycetes and are sequence-selective DNA alkylating compounds (122). 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’ multiplications without deforming its DNA helix, thus potentially avoiding the emergence of drug resistance phenomenon (123). Several ADCs containing PBD are now in Phase I clinical trials such as SGN-33A (124), and SC16LD6.5 (123). Besides these, amatoxin, spliceostatin C, and thailanstatin A are new payloads that work as RNA polymerase inhibitors (125). Amatoxin is the RNA polymerase II inhibitor that kills tumor cells by inhibiting DNA transcription, which is required by all cells (126). Sujet Puthenveetil et al (125) 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 multi-drug resistant (MDR) phenotype in comparison to microtubule inhibitors such as monomethyl auristatin E (MMAE) and maytansinoids (127). Another example of new payload is NO (nitric oxide). Fumou, et al generated an anti-CD24 (cluster of differentiation 24) antibody-nitric oxide conjugate (ANC) using a new NO donor compound as a payload (128). 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 provide more effective, safe, and having the capability of overcoming a multi-drug resistant phenomenon.

5. 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, down-regulation 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 (129). 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 (124). In addition, the replacement of anthracycline-based ADCs with auristatin-based ADC also demonstrated improved outcomes in acquired resistant NHL tumor models (98). 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 (116) and mal-PEG4-N-hydroxysuccinimide that contains polar linkers with improved potency against MDR1þ models (130).
Another strategy can be modifications of the linker-cytotoxic structure (109). 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 (129). 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 (131, 132).

6. 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 (133). In recent years, tremendous effort has been put toward increasing the tumor penetrability of an ADC (134). In this regard, designed ankyrin repeat proteins (DARPins), non-IgG scaffolds and non-internalizing monoclonal antibody scaffolds are linked to cytotoxic payloads through disulfide linkers, which are then selectively delivered and cleaved in the tumor microenvironment (135, 136).

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-kD, and nonimmunogenic antigen-binding single-domain fragments, it can quickly clear from blood and normal tissue and quickly penetrate into the tumor (137). 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 (106). 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 (138). 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 (139-141).

7. 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 post-translation 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 (142, 143).

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 (144-146). 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 EU for treating different kinds of cancer (Table 1).

1) **Gemtuzumab Ozogamicin**

Gemtuzumab ozogamicin (GO; Mylotarg ®, Pfizer/Wyeth, USA), is composed of a recombinant humanized immunoglobulin G4 (IgG4) monoclonal antibody, 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 acute myeloid leukemia (AML) (147, 148). In the initial approval, GO (two 9-mg/ m² 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 ≥ 60 years and not fit for cytotoxic chemotherapy (100, 149). However,
in a phase III SWOGS0106 (Southwest Oncology Group, S0106) randomized comparative study, the effect of GO plus conventional induction therapy (daunorubicin and cytosine arabinoside) versus conventional induction therapy alone was studied in newly diagnosed 637 AML patients (<60 years of age) (150). In this post-approval study, patients showed no benefit in response rates or relapse-free survival with high liver toxicity and a long duration of cytopenia (147). Likewise, high mortality rates were observed in the combination therapy with GO in comparison to the standard arm (6% vs 1%) (150). Moreover, no survival benefits were observed in GO treatment vs standard treatments (151, 152). Because of these safety and efficacy concerns, the manufacturer of this drug voluntarily withdrew the US New Drug Application in 2010 (153).

On September 1, 2017, FDA re-approved the GO-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 re-approval of GO was grounded on the outcomes of clinical trials, including AML-19 (154), ALFA-0701 (155), and MyloFrance-1 (156) that contributed to the favorable reassessment of GO. This ADC is indicated for treating newly diagnosed CD33-positive AML patients (155, 157, 158) and individuals with relapsed/refractory CD33-positive AML over 2 years of age [32].

**Metabolic Profile**

In order to assess the pharmacokinetic (PK) properties of GO, it is essential to quantify the PK of the entire GO molecule, as well as the total and unbound calicheamicin metabolites. Upon internalization, GO undergo hydrolysis resulting in the release of the calicheamicin derivative. Afterward, the derivative experiences a non-enzymatic intramolecular disulfide bond reduction. However, low amounts of unbound calicheamicin in the blood [average $C_{\text{max}}$ of 1.5 ng/mL after the third dose] restricted the measurement of its PK studies (152, 156, 158). For the original dose
regimen of 9-mg/m², the total predicted area under the curve (AUC) is 25%, and the value for the Cmax of GO is 24% during the treatment. On days 1, 4, and 7 and with a dose of 3 mg/m², 0.38 mg/L and 0.63 mg/L after the first and third dose were predicted for the Cmax of GO. Approximately 25 L was estimated as the total volume of distribution.

After the first dose, the GO clearance predicted from plasma was valued to be 3 L/h, followed by 0.3 L/h. At the suggested dose of 3 mg/m², the terminal plasma half-life was about 160 h. In patients having hepatic or severe renal impairment, no proper PK properties of GO have been established. No alterations in pharmacokinetics for GO have been reported in mild-to-moderate renal dysfunction patients (creatinine clearance > 30 mL/min) (152, 156, 158).

**Adverse Events**

When GO 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 (AEs) observed in ≥30% of cases (159). However, serious adverse effects related to GO usage included neutropenia (34.3%), thrombocytopenia (21.7%), and infusion-related reactions (IRRs) (2.5%). Patients receiving GO 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 multi-organ failure (159). In combination therapy, the most common (> 30%) and relevant adverse events were a severe infection (41.2%), hemorrhage (9.9%), hepatotoxicity, including VOD/SOS (3.8%) and tumor lysis syndrome (1.5%) (150). 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 GO in combination therapy.

**Drug Interactions**

It has been demonstrated that GO 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 (152, 160). GO and its payload, calicheamicin, possess a low affinity for the induction of CYP2B6, CYP1A2, and CYP3A4 activities. GO payload, calicheamicin, demonstrated low inhibitory potential for P-glycoprotein (P-gp), multidrug resistance-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 PK of GO co-administered with daunorubicine and cytarabine was observed (152).

2) **Brentuximab Vedotin**

Brentuximab vedotin (BV, SGN-35; Adcetris, Seattle Genetics, USA), a potent ADC in clinical practice, is composed of humanized immunoglobulin (Ig) G1 antibody that is linked to the payload monomethyl auristatin E (MMAE), through a protease-cleavable linker (161). BV utilizes a valine-citrulline dipeptide linker capable of conditional cleavage, discharge of fully active drug and high stability in serum (162). BV 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 (163, 164). Besides direct binding to
the CD30+ lymphocytes, BV 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 (165).

On August 19, 2011, the FDA approved Brentuximab vedotin for the treatment of patients suffering from Hodgkin lymphoma (HL) and anaplastic large cell lymphoma (ALCL) (165). This approval of BV was based on the assessment of two-phase II clinical studies. The first study included 102 patients with refractory or relapsed HL. In this study, the objective response was 75%, with 34% complete remissions (CRs) (166). 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 (167). This drug is approved in more than 65 countries for improved patient outcomes with relapsed or refractory systemic ALCL or relapsed or refractory classical HL (137, 168, 169). Retreatment with BV is often effective in patients who once had a benefit from BV (170). BV is used as a first-line therapy prior to auto-HSCT (171-173) and as consolidation post-autologous transplant in Hodgkin lymphoma (174). Moreover, it is also used as first salvage therapy in early and advanced stage HL (175, 176) and as second-line therapy together with multidrug chemotherapy for patients with refractory or relapsed HL prior to auto-HSCT (177). Additionally, in March 2018, FDA also approved BV co-administered with chemotherapy (doxorubicin, dacarbazine, and vinblastine), for treating adult patients who had previously untreated stage III or IV classical HL (178).

**Metabolic Profile**

The data about the PKs of BV were evaluated in phase I clinical studies and population analysis of data from 314 patients (166, 179). The maximum concentration of BV 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 BV accumulation even after repeated doses given every 3 weeks (179). A single dose of 1.8 mg/kg BV results in 31.98 μg/mL Cmax and AUC of 79.41 μg/mL × day, respectively. The median Cmax of MMAE was found to be 4.97 ng/mL. Time to Cmax 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.

BV 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 (179). In vivo studies have shown that only a small portion of MMAE is catabolized by CYP3A, which is released by the BV. 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 (179). Approximately 28% was excreted in urine and the rest 72% was found in the feces. Since 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/min) (180). Therefore, it is advisable to avoid the use of BV in severe renal dysfunction patients.

**Adverse Events**

The most common AEs (≥ 10%) with the use of BV 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 (181). Peripheral motor neuropathy was most commonly noticed in patients retreated with BV but was primarily grade 2 in comparison with phase II studies (28% vs. 9%) (181). Infusion-related reactions were found to be 13% in patients (182). These reactions include headache, chills, nausea, vomiting, rash, back pain, dyspnea, pruritus, and cough. Abdominal pain and anaphylactic reactions have also been reported in BV treated patients (181, 183). Moreover, in patients treated with BV pancreatitis was previously unrecognized severe AE before a case of fatal pancreatitis was reported in a clinical trial. Gandhi et al. reported an additional case of pancreatitis associated with the use of BV (184). Some pulmonary toxicities and JC polyomavirus reactivation that can cause fatal progressive multifocal leukoencephalopathy have also been reported with BV treatment (168, 181, 185).

**Drug Interactions**

Brentuximab vedotin is a CYP3A4 substrate and possibly CYP2D6. The payload, MMAE, is metabolized by CYP3A4/5 via oxidation (182). 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 BV (180). In the case of BV 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 BV. Midazolam, a CYP3A4 substrate, did not alter the metabolism of BV when co-administered (180).

3) **Trastuzumab Emtansine**
Trastuzumab emtansine (T-DM1; Kadcyla®, Roche, Switzerland) is another highly potent ADC that was first approved by the FDA and the EU in 2013 for the HER2-positive breast cancer (BC) treatment (186). It is composed of the monoclonal antibody trastuzumab, the cytotoxic payload mertansine (DM1), and a non-reducible thioether linker MCC (4-[N-maleimidomethyl] cyclohexane-1-carboxylate) (187, 188). 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× higher cytotoxicity than maytansine and a potency of 24× to 270× more than taxanes. The drug antibody ratio (DAR) of DM1 is 3.5 (188). Binding of T-DM1 to HER2 receptors results in a T-DM1-HER2 complex formation that enters the target cells through receptor-mediated endocytosis (189, 190). 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, 191).

T-DM1 was first approved based on 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 (192). Patients treated with T-DM1 displayed a significant difference in progression-free survival (PFS) (median PFS, 9.6 months) in comparison to 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 versus 25.9 months; hazard ratio (HR) 0.75 (95% 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 (193). 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 (193, 194). The data obtained from the EMILIA and TH3RESA clinical trials indicate that T-DM1 has a clearly better safety profile in comparison to the control arm group with fewer grade 3 or more adverse events (195, 196).

**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_{\text{max}} of 83.4 μg/mL (137, 197). Metabolism of DM1 occurs mainly via CYP3A4/5 and is a P-gp substrate (137). 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 (197). 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. PK characteristics of T-DM1 were not altered by mild-to-moderate renal impairment. Patients with creatinine clearance <30 mL/min were unable to obtain recommendations (137). In patients with moderate-to-severe hepatic impairment, the AUC of the first cycle T-DM1 was approximately 38% vs 67% in patients with normal hepatic function, respectively (197).

**Adverse Events**

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%) (197). Other AEs were hemorrhage, abdominal pain, pyrexia, musculoskeletal pain, vomiting, and dyspnea (137, 198). The majority of them were generally grade 1-2 and reversible. Some studies also reported serious hepatobiliary disorders (194, 195, 198) and left ventricular dysfunction in patients receiving T-DM1 (199). 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 (200). Some clinical trials also indicated the appearance of interstitial lung disease (198).

**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 (137). 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 (201, 202).

**4) Inotuzumab Ozogamicin**

Inotuzumab ozogamicin (IO, CMC-544; Besponsa ®, Pfizer/ Wyeth) is a humanized CD-22 targeting antibody-drug conjugate comprising IgG4 antibody, a cytotoxic payload calicheamicin [Nacetyl-c-calicheamicin dimethyl hydrazide (Calich-DMH)] that are covalently linked together by an acid-labile 4-(40-acetylphenoxy) butanoic acid linker (203-205). Calicheamicin is a highly potent DNA alkylating agent produced by a soil microorganism; Micromonospora echinospora (180). IO has high affinity and rapidly internalized into the cells that express CD22. Upon binding, IO/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 (204, 206).

In the USA (207), Japan (208), EU (209), and several other countries (210), IO is used as monotherapy for the treatment of adult patients having relapsed/refractory B-cell acute lymphoblastic leukemia (ALL) (207, 209). IO approval was mainly based on a global, open-label, phase III randomized INO-VATE ALL clinical trial (211). 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 IO (80.7% [95% confidence interval (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 IO arm vs ST arm [4.6 (CI; 3.9–5.40) vs 3.1 (CI; 1.4–4.9)] months]. The progression-free survival (PFS) and overall survival (OS) were also considerably longer with IO in comparison to the ST arm (5.0 vs 1.8 months; p<0.001) and (7.7 vs 6.7 months; p=0.04). Additionally, a large population was able to proceed to stem-cell transplantation in the IO arm vs standard therapy arm (41% vs 11%; p < 0.001).

It is recommended that IO should be administered in 3- to 4-week cycles. A total dose of 1.8 mg/m² per cycle, administered as three divided doses (0.8, 0.5 and 0.5 mg/m² on days 1, 8 and 15), is recommended for the first cycle. For subsequent cycles, the recommended dose is 1.5 mg/m² per cycle, given as 0.5 mg/m² doses on Days 1, 8 and 15. If the patient proceeds to hematopoietic stem cell transplantation (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 (212). There was increased drug exposure with either an increased number of doses or higher doses of the drug. Multiple doses of IO results in a 5.3-fold accumulation between the first and fourth cycles and steady-state concentration were achieved by the fourth cycle (207, 209). At steady state, the total AUC per cycle was 100 μg/h/mL and the mean Cmax was 308 ng/mL. In vitro, Calich-DMH was found to be ≈ 97% bound to human plasma proteins, and the total volume of distribution was ≈ 12 L (207, 209). IO was mainly metabolized by non-enzymatic reduction and was a substrate of P-gp. At steady state, IO demonstrated clearance of 0.0333 L/h and at the end of the fourth cycle, the terminal elimination half-life was around 12.3 days.

**Adverse Events**

Inotuzumab ozogamicin had a manageable tolerability profile in adult patients with relapsed or refractory ALL. However, the most commonly observed treatment-related hematological adverse events 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%) (211, 213). IO was also associated with Veno-occlusive disease/sinusoidal obstruction syndrome who underwent HSCT (211, 213). Moreover, increased transaminases and hyperbilirubinemia were also reported in patients treated with IO, respectively (200).

**Drug Interactions**

The data obtained from the in vitro studies revealed that the administration of IO together with inducers or inhibitors of UGT drug-metabolizing enzymes or cytochrome P450 did not change
the exposure to N-acetylgamma- calicheamicin dimethyl hydrazide (160, 209). Also, the exposure of CYP enzyme-substrate, major drug transporters or UGT enzymes is unlikely to be affected by the IO 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 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 (214). 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 (215). 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, Kurt Schönfeld et al (216) 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 CD8⁺ T effector cells to cancer tissues improving the clinical response. For instance, the combination of trastuzumab emtansine with pembrolizumab (a PD-1 inhibitor) and BV with PD-L1 or PD-1 inhibitors are being studied in metastatic breast cancer individuals and patients with refractory or relapsed HL (Clinical trials identifiers: NCT02924883, NCT03032107, and NCT01896999). Based on 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 ADCs design research.

**Contributors**

Puregmaa Khongorzul and Cai Jia Ling did the literature search and selected published work for the report. Puregmaa Khongorzul and Cai Jia Ling wrote the manuscript. Farhan Ullah Khan and Awais Ullah Ihsan made the tables and final draft of the report. Juan Zhang designed the study and approved the final report.

**Acknowledgments**
This work was supported in part by the Double First-Class University project (CPU2018PZQ12 and CPU2018GY14), The National Natural Science Foundation of China (81973223), Natural Science Foundation of Jiangsu Province (BK20161459).

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http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/125388s000,125399s000lbl.pdf (accessed March 15, 2019). [Internet].


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(22):1109--.


Figure Legends

**Figure 1.** Chemical structures of non-cleavable and cleavable linkers. A. SMCC linker. B. Maleimidocaproyl linker C. Peptide-Based Linker. D. β-Glucuronide linker. E. Acid-sensitive linker. F. Disulfide linker.
Table 1. Currently approved antibody-drug conjugates (ADCs) in the market.

<table>
<thead>
<tr>
<th>ADC</th>
<th>Antibody</th>
<th>Linker</th>
<th>Payload</th>
<th>Target</th>
<th>Action</th>
<th>Indication</th>
<th>Approval Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemtuzumab</td>
<td>Humanized IgG4 Cleavable, Hydrazone Calicheamicin CD33 DNA damaging agent</td>
<td>Single-agent and combination therapy for adults and pediatric patients (age ≥ 2) with relapsed or refractory AML</td>
<td>2000-approved</td>
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<tr>
<td>Ozogamicin</td>
<td>(Mylotarg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2010-withdraw</td>
</tr>
<tr>
<td>Brentuximab</td>
<td>Chimeric IgG1 Cleavable, Peptide MMAE CD30 Microtubule disrupting agent</td>
<td>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</td>
<td>2011</td>
<td></td>
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<tr>
<td>Vedotin</td>
<td>(Adcetris)</td>
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</tr>
<tr>
<td>Trastuzumab</td>
<td>Humanized IgG1 Non-cleavable, Thioether DM1 HER2 Microtubule disrupting agent</td>
<td>Adults with unresectable or metastatic breast cancer</td>
<td>2013</td>
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<td>Emtansine</td>
<td>(Kadcyla)</td>
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<td>Inotuzumab</td>
<td>Humanized IgG4 Cleavable, Hydrazone Calicheamicin CD22 DNA damaging agent</td>
<td>Monotherapy in adults with relapsed/ refractory B-cell ALL</td>
<td>2017</td>
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<tr>
<td>Ozogamicin</td>
<td>(Besponsa)</td>
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</table>

**Abbreviations:** AML: Acute myeloid leukemia; MMAE: Monomethyl auristatin E; HL: Hodgkin leukemia; sALCL: Systemic anaplastic large-cell lymphoma; auto-HSCT: Autologous-hematopoietic stem cell transplantation; HER2: Human epidermal growth factor 2; ALL: Acute lymphocytic leukemia
Table 2. Summary table of some of the antibody-drug conjugates (ADCs) in clinical development.

<table>
<thead>
<tr>
<th>ADC</th>
<th>Antibody</th>
<th>Linker</th>
<th>Payload</th>
<th>Target</th>
<th>Action</th>
<th>Indication</th>
<th>Phase</th>
<th>ClinicalTrials.gov Identifier</th>
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<td>Breast Cancer</td>
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**Abbreviations:**
- HER2: Human epidermal growth factor 2
- TROP2: Trophoblast antigen 2
- FOLR1: Folate receptor alpha
- MMAE: Monomethyl auristatin E
- MMAF: Monomethyl auristatin F
- BCMA: B-cell maturation antigen
- GPNMB: Glycoprotein non-metastatic b
- CEACAM5: carcinoembryonic antigen related cell adhesion molecule 5
- PSMA: Prostate specific membrane antigen
- FGFR: Fibroblast growth factor receptor
- ENPP3: Ectonucleotide pyrophosphatase/phosphodiesterase 3
- AML: Acute myeloid leukemia
- EFNA4: Ephrin-A4
- NSCLC: Non-small cell lung cancer
- PBD: Pyrrolobenzodiazepine
- MM: Multiple myeloma
- AML: Acute myeloid leukemia
- FLT3: FMS-like tyrosine kinase 3
Molecular Cancer Research

Antibody-Drug Conjugates: A Comprehensive Review
Puregmaa Khongorzul, Cai Jia Ling, Farhan Ullah Khan, et al.
Mol Cancer Res Published OnlineFirst October 28, 2019.

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Access the most recent version of this article at:
doi:10.1158/1541-7786.MCR-19-0582

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