REVIEW

Recent advances of antibody drug conjugates for clinical applications

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\textbf{KEY WORDS}

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\textbf{Abstract} Antibody drug conjugates (ADCs) normally compose of a humanized antibody and small molecular drug via a chemical linker. After decades of preclinical and clinical studies, a series of ADCs have been widely used for treating specific tumor types in the clinic such as brentuximab vedotin (Adcetris\textsuperscript{®}) for relapsed Hodgkin’s lymphoma and systemic anaplastic large cell lymphoma, gemtuzumab ozogamicin (Mylotarg\textsuperscript{®}) for acute myeloid leukemia, ado-trastuzumab emtansine (Kadcyla\textsuperscript{®}) for HER2-positive metastatic breast cancer, inotuzumab ozogamicin (Besponsa\textsuperscript{®}) and most recently polatuzumab vedotin-piiq (Polivy\textsuperscript{®}) for B cell malignancies. More than eighty ADCs have been investigated in different clinical stages from approximately six hundred clinical trials to date. This review summarizes the key elements of ADCs and highlights recent advances of ADCs, as well as important lessons learned from clinical data, and future directions.

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1. Introduction
Chemotherapy is one of the major treatment options for cancer therapy. Although a number of chemotherapy drugs have been widely used in the clinic, serious hurdles still remain such as adverse effects and drug resistance. Extensive efforts have been made to increase the efficacy of cytotoxic drugs, such as combining different chemotherapeutic drugs and using highly potent agents such as auristatin and maytansine. However, systemic toxicity and narrow therapeutic window limit their clinical use. The advances of monoclonal antibody provide opportunities to use their specific binding property for targeted drug delivery. Based on this concept, antibody drug conjugates (ADCs) are designed and developed through conjugation of antibodies and cytotoxic drugs in the past decades. As illustrated in Fig. 1, ADCs selectively bind to the receptors of tumor cells. After that, the receptor–ADC complex is usually internalized through the endocytosis pathway. The linker is cleaved, and cytotoxic drugs are released. Consequently, these drugs induce cytotoxic effects through various mechanisms of action such as binding to the minor groove of deoxyribonucleic acid (DNA) or interacting with tubulin.

In the first-generation ADCs such as BR96–doxorubicin and KS1/4–methotrexate, chemotherapy drugs are usually conjugated to murine antibodies via a non-cleavable linker. However, these ADCs are generally less potent than free drugs. Then, researchers developed gemtuzumab ozogamicin (GO) with improved efficacy, because GO is consisted of a potent calicheamicin derivative and a humanized antibody to reduce immunogenicity. Yet, GO has several disadvantages, including an unstable linker, a high percentage of unconjugated antibody, poor CMC (chemistry, manufacturing, and control) properties, as well as high toxicity. Limitations of the first-generation ADCs lead to the development of the second-generation ADCs. The monoclonal antibody (mAb) technology has been established with high tumor cell targeting. Furthermore, many potent chemotherapy drugs have been discovered. Therefore, compared with the first-generation ADCs, the second-generation ADCs showed better CMC characteristics. For instance, brentuximab vedotin, ado-trastuzumab emtansine, and inotuzumab ozogamicin are typical second-generation ADCs on the market. Drawbacks of the second-generation ADCs include off-target toxicity, fast clearance, and competition with unconjugated antibodies. The lessons learned from the previous ADCs expedite the development of the third-generation ADCs. Site-specific conjugation has been created in the design of ADCs, which could result in homogeneous ADCs with drug–antibody ratio (DAR) of two or four, as well as improved pharmacokinetics.

In this review article, we will discuss the key elements of ADCs, overview their preclinical and clinical development, as well as future directions of ADCs.

2. Key elements in antibody drug conjugates
2.1. Antigen selection
The selection of an appropriate antigen is one of the major challenges in the development of ADCs. Three aspects should be considered in antigen selection. (i) High-level expression in
tumors while low-level expression in healthy tissues. For example, ado-trastuzumab emtansine targets human epidermal growth factor receptor 2 (HER2), whose expression reaches the level of $2 \times 10^6$ in tumor cells compared with $2 \times 10^4$ in healthy cells\(^{16}\). (ii) Target antigens express on the tumor cell surface, so that they can be accessible to the antibody\(^{13}\). (iii) The rate of internalization and route of intracellular trafficking\(^{13,17,18}\). It is worth mentioning that non-internalized ADCs can also display therapeutic effects through a strong “bystander effect”, that is, a membrane-permeable drugs are able to induce cell death to the neighboring cells\(^{19}\).

2.2. Antibody selection

ADCs are composed of three parts, including antibody, drug and linker. To design effective ADCs, all three components are essential and important (Fig. 2\(^{20}\)).

Among them, antibodies with a molecular weight of around 150 kDa are a major component of ADCs\(^{21}\). Besides target specificity, antibodies also need to bind with suitable affinity, thereby increasing accumulation and retention in tumor sites\(^{3}\). Most ADCs have binding affinities with $K_D$ values ranging from 0.1 to 1.0 nmol/L. Of note, previous studies reported that if the binding affinity is too high, the delivery of the antibody in solid tumors may be affected, which is called the binding-site barrier\(^{22,23}\).

Most antibodies used in the clinic are selected from human immunoglobulin G (IgG), about 150 kDa consisted of two heavy and two light chains\(^{24,25}\). Nowadays, there are many ongoing studies for the use of antibody derived from IgGs\(^{20}\). Generally, antibody derivatives can be classified as antigen-binding fragments (Fab), single-chain variable fragments (scFv) and variable domains (VHH, also named as nanobodies)\(^{24,26}\). Fab and scFv include both the heavy and light domain of the parental IgGs, and retain the size and affinity of the area binding the antigen\(^{24}\). Because of the smaller size compared with regular IgGs, they show improved pharmacokinetics for tumor penetration\(^{27}\). The nanobodies do not have CH1 domain but possess a long complementary determining region 3 (CDR3)\(^{26}\), which displays high stability because of their resistance to denaturing factors\(^{28}\). Moreover, the nanobodies are smaller than the filtration size of kidney, thereby they are excreted through the kidneys with a higher clearance and relatively lower toxicity\(^{28–30}\). For example, Ploegh and co-workers conjugated a nanobody with oligoglycine-modified cytotoxic payloads, which exhibited higher specificity and lower cytotoxicity towards tumor cells compared with traditional ADCs\(^{31}\).

2.3. Chemotherapy drugs

Several criteria are important for choosing suitable chemotherapy drugs. First, these drugs display high cytotoxicity to tumor cells (normally half maximal inhibitory concentration (IC\(_{50}\)) in the nanomolar and picomolar range)\(^{5,13,17}\). Because only nearly 2% of the injected ADCs will distribute into tumors after intravenous administration, thereby resulting in low intracellular concentrations\(^{32}\). Second, these drugs have a functional group or can be derived to be conjugated with the antibody. Third, these drugs are stable in physiological conditions\(^{33}\). Therefore, a relatively small number of cytotoxic drug families are used in current clinical trials. Most of them are derivatives of auristatins or maytansine, which are both microtubule inhibitors\(^{34,35}\). Others are DNA damaging drugs, including (i) drugs inducing double-strand DNA break (e.g., calicheamicin)\(^{36}\) (ii) drugs alkylating DNA (e.g., duocarmycin)\(^{37}\); and (iii) drugs crosslinking with DNA (e.g., pyrrolobenzodiazepine dimers)\(^{38}\). Several representative cytotoxic drugs are discussed in this section.

2.3.1. Auristatin

Auristatin derivatives, including monomethyl analogs monomethyl auristatin E/F (MMAE and MMAF), are the largest class of ADCs in clinical development\(^{13}\). MMAE and MMAF are both derived from dolastatin 10, which is isolated from sea hare\(^{39,40}\). Dolastatin 10 is highly toxic to both tumors and healthy tissues, which leads to its failure in clinical trials\(^{41}\). However, its derivatives MMAE and MMAF are presently used as cytotoxic drugs in ADCs. MMAE can permeate cell membranes, thereby displaying the bystander effect. In contrast, MMAF is more hydrophilic and cannot permeate cell membranes. The lack of bystander effect makes MMAF derived ADCs less efficient compared with MMAE derived ADCs. Meanwhile, MMAF derived ADCs are relatively less toxic\(^{42}\). In 2015, the U.S. Food and Drug Administration (FDA) approved brentuximab vedotin, a MMAE conjugate, to treat Hodgkin lymphoma and anaplastic large cell

![Figure 2](https://example.com/figure2.png)  
**Figure 2**  
Rational design of ADCs components\(^{20}\).
lymphoma. In 2019, another MMAE derived ADC, polatuzumab vedotin-piiq, was approved to treat relapsed or refractory diffuse large B-cell lymphoma.

2.3.2. Maytansinoids

Other kinds of ADCs in clinical development are derivatives of maytansine (maytansinoids, DMs). Maytansine is a natural product isolated from African shrub Maytenus ovatus, whose mechanism is to disrupt microtubule polymerization. Meanwhile, maytansine is one of the first cytotoxic drugs that have a picomolar IC₅₀ value to tumor cells. However, due to its systemic toxicity, maytansines also failed in clinical trials. Incorporation of maytansine derivatives into ADCs significantly improved its therapeutic index. In 2013, ado-trastuzumab emtansine, a DM1 derived ADC, was approved by the FDA to treat HER-2-positive metastatic breast cancer. Additionally, two more maytansine derivatives, DM1 and DM4-based ADCs are presently in clinical trials.

2.3.3. Calicheamincs

Calicheamicins that are isolated from the actinomycete Microamonospora echinospora can induce DNA double-strand cleavage through binding to the minor groove of DNA. Calicheamicins were among the first DNA damaging drugs incorporated in ADCs, but their narrow therapeutic windows and serious side effects limited their clinical applications. These shortcomings have now been largely overcome because of the advances of ADCs technologies especially the linker chemistry and the optimization of dosing approaches. Two of the five ADCs on market, gemtuzumab ozogamicin (GO) and inotuzumab ozogamicin (InO), are calicheamicin derived ADCs. GO is the first ADC drug on market to treat acute myeloid leukemia. However, GO was withdrawn from the US and European markets because of its adverse effects. After dose fractionation, in which patients receive three doses of 3 mg/m² GO instead of one dose of 9 mg/m² GO, the FDA re-approved GO in 2017. In addition, InO was approved by FDA in 2017 to treat B cell acute lymphoblastic leukemia and other B cell malignancies.

2.4. Linkers

An effective linker needs to be stable during circulation because the release of drugs in the blood stream will affect ADCs’ pharmacokinetics, thus leading to toxicity and lower therapeutic index. Once the ADCs are internalized into tumor cells, the linker needs to be cleaved, rapidly releasing drugs. One critical factor that should be taken into consideration is the DAR. Too few drug molecules on each antibody result in decreased efficacy, while excessive DAR will lead to poor pharmacokinetics of ADCs because of higher hydrophobicity and lower solubility. It has been reported that the DAR of most clinical trials ADCs are in the range of 2.0–4.0. Linkers used in typical ADCs can be divided into noncleavable and cleavable linkers. Noncleavable linkers usually rely on the lysosomal degradation to release the cytotoxic drugs which are attached to the linker and an amino acid residue of the antibody. For example, brentuximab vedotin was designed to use a noncleavable linker (succinimidyl trans-4-(maleimidylmethyl)cyclohexane-1-carboxylate, SMCC) to crosslink the maytansinoid to the HER2 antibody. The structure of cleavable linkers includes a position of cleavage between the antibody and the drug. Usually, based on the cleavage mechanisms, cleavable linkers can be classified into three groups: (i) Acid sensitive, such as hydrazone linkers, that are cleaved in the lysosome because of low pH environment. For example, the hydrazone linker is used in both GO and InO. In addition, although acid cleavable linkers are designed for maintaining stability during circulation and release drugs in the acidic environment, it has been reported that acid cleavable linkers could be associated with nonspecific release of the drugs. (ii) Lysosomal protease sensitive, such as valine–alanine and valine–citrulline peptide linkers, that are designed to release drugs after cleavage by intracellular proteases. For example, cathepsin B, a lysosomal protease, cleaves the dipeptide bond in the tumor cells. In addition, a cathepsin B-sensitive dipeptide linkage (valine–citrulline) is used in brentuximab vedotin. (iii) Redox sensitive, such as disulfide linkers, that takes advantage of higher glutathione concentration in tumor microenvironment. Optimizing the steric hindrance of disulfide bridges can decrease premature drug release. For example, this method is applied in the case of anetumab ravtansine and coltuximab ravtansine using a disulfide linker N-hydroxysuccinimidyld-4-(2-pyridydithio) butanoate (SPDB) to crosslink DM4.

3. Site-specific conjugation

As previously described, a suitable DAR is important to the design of ADCs. Site-specific conjugation can produce a consistent generation of relatively homogeneous ADCs products without altering the antigen binding affinity. Three strategies are mainly used for site-specific conjugation on the antibody: (i) engineered cysteines; (ii) enzymatic conjugations; and (iii) incorporation of unnatural amino acids.

3.1. Engineered cysteine

The thiol group in the cysteine side chain can be used for site-specific modification, because of its high nucleophilicity. Companies such as Genentech, Seattle Genetics, Pfizer have developed different ADCs with engineered cysteines. These ADCs have a uniform DAR of 2 or 4. Furthermore, ADCs constructed by this method showed encouraging in vivo results, including higher efficacy and better toleration compared with conventional ADCs. For example, vadastuximab talirine, which consists of anti-CD33 engineered cysteine and pyrrolobenzodiazepine (PBD) dimer through a cleavable dipeptide linker (valine-alanine), is the first ADC with site-specific conjugation.

3.2. Enzymatic conjugations

Several enzymes such as the bacterial derived formyl glycine generating enzyme (FGE), transglutaminases, glycotransferases, and sorthases have been used for conjugating the antibodies. The reaction sites of antibodies are designed to react specifically to the corresponding functional groups. Therefore, the enzymatic conjugation method leads to site-specific conjugation and homogeneous DARs. For example, SMARTag® is a technology that uses FGE. FGE can insert the antibody after a sequence of specific modification, because of its high nucleophilicity. Complementary to the reactive group of antibodies, FGE will insert the corresponding functional groups. Therefore, the enzymatic conjugation method leads to site-specific conjugation and homogeneous DARs.
3.3. Incorporation of unnatural amino acid

Incorporation of unnatural amino acids (UAAs) with bio-orthogonal groups are also used on site-specific conjugation. The most common method of UAAs incorporation is to engineer transfer RNA (tRNA) synthetases and recognize UAAs, thus resulting the genetic coding of the UAAs.1, For instance, Tian et al.7 reported a site-specific ADC using UAAs. Compared to traditional cysteine conjugated ADCs, this ADC may possess better selectivity and efficacy both in vitro and in vivo7. Yet, the UAAs-based methodology needs special techniques and reagents for preparation and manufacturing37.

4. Preclinical development of antibody drug conjugates

In current clinical trials, calicheamicins, auristatin and maytansinoid are the most commonly used cytotoxic drugs in ADCs. Meanwhile, several other types of drugs are in the stage of preclinical development, such as microtubule inhibitors37, anthracyclines74 and amatoxins75.

4.1. Microtubule inhibitors

The approval of ADCs based on auristatin and maytansinoid accelerates the development of new microtubule inhibitors drugs. Tubulysins, a series of peptidic compounds, are representative examples. Tubulysins are originally isolated from myxobacteria and show potent inhibition through tubulin polymerization75. Among different types of tubulysins, tubulysin D is the most effective one with cytotoxic activity in the range of picomolar in various tumor cell lines76. In 2014, Cohen et al.78 developed ADCs that are consisted of trastuzumab and the stable tubulysin analogs Tub-OH or Tub-OMOM. Both 131I-labeled and unlabeled versions of the tubulysins are conjugated to the surface lysines through a non-cleavable N-hydroxysuccinimide linker. These ADCs showed favorable therapeutic effects both in vitro and in vivo79.

4.2. Anthracyclines

Recent studies on anthracyclines such as nemorubicin and its major metabolite, PNU-159682, indicate that these agents might overcome the limitations of doxorubicin such as drug resistance and cardiac toxicity74. Furthermore, compared with doxorubicin, PNU-159682 showed three orders of magnitude more cytotoxic activity against different tumor cell lines including doxorubicin resistant cells79. These features are associated with tight and stable bindings of PNU-159682 to DNA80. Yu et al.74 conjugated PNU-159682 to anti-CD22 antibody through a maleimidocaproyl-diethylamine linker. This ADC is 20-fold more effective than pinatuzumab vedotin in vitro and displays therapeutic effects in four types of xenograft tumors. Furthermore, it may overcome the drug resistance induced by the p-glycoprotein81.

4.3. Amatoxins

Amatoxins are a class of peptide toxins. α-Amanitin, a representative example was originally isolated from the amanita phalloides mushroom and was found to be an inhibitor of the eukaryotic RNA polymerase II74, inducing transcriptional arrest and leading to tumor cells death82. Moldenhauer et al.83 conjugated α-amanitin to chiHEA125 (a chimerized anti-human epithelial cell adhesion molecule monoclonal antibody) via a glutarate linker. This ADC has a picomolar IC50 value in Colo205 and MCF-7 tumor cells83. Moreover, it also displayed tumor inhibition in a BxPc-3 pancreatic xenograft model83.

5. Antibody drug conjugates in clinical trials

ADCs have become an important class of anti-cancer drugs, with a dramatically increasing number of ADCs in clinical studies for treating hematologic malignancies and solid tumors over the past 5 years.33,34. Table 1 lists the approved ADCs. Four of these ADCs are designed to treat hematologic malignancies, in which the target antigens are more accessible for circulating ADCs compared to solid tumors84. Table 2 lists the ADCs presently in phase II or phase III clinical studies. A mass of ADCs are in phase I clinical trials, which are not listed here. The clinical results of ADCs that are approved or in phase III clinical trials are further discussed in this section.

5.1. Gemtuzumab ozogamicin

Gemtuzumab ozogamicin (GO; Mylotarg®, Fig. 3A) is the first ADC approved by the FDA85. GO is consisted of a CD33 monoclonal antibody and calicheamicin via a cleavable hydrazone linker85. In 2000, Based on three phase II trials, GO received accelerated approval for treating patients aged 60 and older with CD33-positive acute myeloid leukemia (AML) who are unable to use other cytotoxic chemotherapy86. The overall response rates (ORR) of GO were 26%—30% and the side effects contained hepatic veno-occlusive disease and delayed hematopoietic recovery86,87. Meanwhile, one phase III trial (NCT00085709) tested the addition of GO during induction therapy in patients under the age of 61 and no significant benefit of GO was observed87. Moreover, toxic effects were observed including hepatotoxicity, infusion reactions and pulmonary toxicity88. These clinical results lead to Pfizer’s voluntary withdrawal of GO in 2010. After dose optimization (patients receive three doses of 3 mg/m2 GO instead of one dose of 9 mg/m2 GO before), the FDA re-approved GO in 201789.

5.2. Brentuximab vedotin

The second ADC approved by the FDA is brentuximab vedotin (BV; Adcetris®, Fig. 3B), which is made by conjugation of MMAE and an anti-CD30 antibody through a protease-cleavable dipeptide linker90. Because of the results of phase II trials, 75% ORR in relapsed Hodgkin’s lymphoma91 and 86% ORR in systemic anaplastic large cell lymphoma90, BV received accelerated approval in 2011. Adverse events mainly contained neuropathy, neutropenia, anemia and thrombocytopenia90,91. Among them, neuropathy was the most frequent adverse event which happened in patients treated with BV90. According to the encouraging results of the phase III trial (AETHERA, NCT01100502) that investigated the utilization of BV as consolidation treatment in Hodgkin’s lymphoma, BV received the full approval in 201592.

5.3. Ado-trastuzumab emtansine

Ado-trastuzumab emtansine (T-DM1; Kadcyla®, Fig. 3C) is the third ADC on market introduced in 201393. T-DM1 is consisted of
maytansinoid DM1 and the anti-HER2 antibody. T-DM1 received approval according to the phase III trial (EMILIA, NCT00829166)\textsuperscript{93,94}. In T-DM1 arm, the median duration of progression-free survival (PFS) was 9.6 months and in active comparator, the median duration of PFS was 6.4 months ($P < 0.001$). The overall survival (OS), which is 30.9 months versus 25.1 months ($P < 0.001$), and the ORR, which is 43.6\% versus 30.8\% ($P < 0.001$) also supported the T-DM1 over comparator\textsuperscript{93}. Furthermore, the overall rate of adverse events was lower in the T-DM1 arm (40.8\%) than in the comparator arm (57.0\%), as well as the rate of serious adverse events (15.5\% versus 18.0\%)\textsuperscript{93}.

5.4. Inotuzumab ozogamicin

Inotuzumab ozogamicin (InO; Besponsa\textsuperscript{8}, Fig. 3D) is the fourth approved ADC drug introduced in 2017\textsuperscript{51}. InO is composed of calicheamicin derivative and the anti-CD22 antibody through a cleavable hydrazone linker\textsuperscript{51}. InO received the FDA approval according to the results of the phase III trial (INO-VATE, NCT01564784)\textsuperscript{95}. In this trial, acute lymphocytic leukemia patients were randomized and treated with InO or a defined investigator’s choice\textsuperscript{94}. The complete remission was 80.7\% in the InO arm vs 29.4\% in the comparator arm ($P < 0.001$). In the InO arm, the PFS was 5.0 months, while only 1.7 months in the comparator arm ($P < 0.001$)\textsuperscript{94,95}. Several other phase III studies are currently ongoing including the combination with frontline therapy (NCT03150693) and post-induction chemotherapy (NCT03959085).

5.5. Polatuzumab vedotin-piiq

The most recent ADC on market (in June 2019) is polatuzumab vedotin-piiq (Polivy\textsuperscript{8}, Fig. 3E), prepared by conjugation of MMAE to an anti-CD79b antibody through a protease-cleavable dipeptide linker\textsuperscript{96}. According to the results of the phase Ib/II GO29365 study (NCT02257567), polatuzumab vedotin-piiq received the accelerated approval\textsuperscript{97}. In this trial, large B-cell lymphoma patients were randomized and treated with polatuzumab vedotin plus bendamustine and rituximab (BR) or BR alone\textsuperscript{97}. The complete response rate was 40\% in polatuzumab vedotin plus BR arm, compared to 18\% in BR alone arm\textsuperscript{97}. Objective response rate was 45\% in the polatuzumab vedotin plus BR arm, compared to 18\% in the BR alone arm\textsuperscript{97}.

5.6. Rovalpituzumab tesirine

Rovalpituzumab tesirine (Rova-T) is an ADC that utilizes a cleavable dipeptide linker for conjugating PBD dimer to the anti-delta-like protein 3 (DLL3) antibody\textsuperscript{8}. In a phase I trial (NCT01901653), dose escalation test of pharmacokinetics, safety and preliminary efficacy of Rova-T were evaluated in recurrent small cell lung cancer patients\textsuperscript{99,100}. The maximum tolerated dose (MTD) was 0.4 mg/kg, the ORR was 17\%, the duration of response (DOR) was 2.89 months, the clinical benefit rate (CBR) was 58\%, the PFS was 2.79 months and the OS was 4.76 months\textsuperscript{8}. Until now, two phase III studies about Rova-T are active including the comparison study with topotecan (NCT03061812) and a maintenance treatment for small cell lung cancer (NCT03033511).
Table 2 Antibody drug conjugates (ADCs) in phase III and phase II development.

| ADC                      | Target antigen | Linker               | Cytotoxin | Developer       | Indication(s)                                      | Phase | NCT number                |
|--------------------------|----------------|----------------------|-----------|-----------------|---------------------------------------------------|-------|---------------------------|
| Rovalpituzumab tesirine  | DLL3           | Cleavable dipeptide  | PBD dimer | AbbVie (Stemcentrx) | Small-cell lung cancer                            | III    | NCT03061812 (ongoing)     |
|                          |                |                      |           |                 |                                                   |       | NCT03033511 (ongoing)     |
| Mirvetuximab soravtansine| FOLR1          | Cleavable disulfide  | DM4       | ImmunoGen       | Ovarian, endometrial, non-small cell lung cancer | III    | NCT02631876 (ongoing)     |
| Depatuxizumab mafodotin  | EGFR           | Noncleavable (mc)    | MMAF      | AbbVie          | Glioblastoma and other EGFR-positive tumors       | III    | NCT02573324 (ongoing)     |
| Sacituzumab govitecan    | Trop-2         | Acid-labile ester    | SN-38     | Immunomedics    | Triple-negative breast cancer, urothelial and other cancers | III    | NCT02574455 (ongoing)     |
|                          |                |                      |           |                 |                                                   |       | NCT03901339 (ongoing)     |
| Naratuximab emtansine    | CD37           | Noncleavable (SMCC)  | DM1       | ImmunoGen       | Diffuse large B cell lymphoma and follicular lymphoma | II     | NCT01534715 (ongoing)     |
| Lorvotuzumab mertansine  | CD56           | Cleavable disulfide  | DM1       | ImmunoGen       | Leukemia                                           | II     | NCT01237678 (completed)   |
| Coltuximab ravtansine    | CD19           | Cleavable disulfide  | DM4       | ImmunoGen       | Diffuse large B cell lymphoma, acute lymphocytic leukaemia | II     | NCT01472887 (completed)   |
|                          |                |                      |           |                 |                                                   |       | NCT01440179 (terminated)  |
|                          |                |                      |           |                 |                                                   |       | NCT01470456 (completed)   |
| Indatuximab ravtansine   | CD138          | Cleavable disulfide  | DM4       | Biotest         | Multiple myeloma                                   | II     | NCT01638936 (completed)   |
|                          |                |                      |           |                 |                                                   |       | NCT01001442 (completed)   |
| Anetumab ravtansine      | Mesothelin     | Cleavable disulfide  | DM4       | Bayer Health Care | Mesothelioma and other solid tumors                | II     | NCT03023722 (ongoing)     |
|                          |                |                      |           |                 |                                                   |       | NCT02839681 (terminated)  |
| SAR566658                 | CA6            | Cleavable disulfide  | DM4       | Sanofi           | Triple-negative breast cancer                      | II     | NCT02984683 (completed)   |
| Glembatumumab vedotin     | gpNMB          | Cleavable dipeptide  | MMAE      | Celllex         | Metastatic breast cancer and melanoma              | II     | NCT01997333 (completed)   |
| PSMA ADC                 | PSMA           | Cleavable dipeptide  | MMAE      | Progenics/Seattle Genetics/Genentech/Roche        | Diffuse large B-cell lymphoma, follicular non-Hodgkin lymphoma | II     | NCT02020135 (completed)   |
| Pinatuzumab vedotin       | CD22           | Cleavable dipeptide  | MMAE      |                 |                                                   | II     | NCT01695044 (completed)   |
|                          |                |                      |           |                 |                                                   |       | NCT01691898 (completed)   |
| Telisotuzumab vedotin     | ABT-700        | Cleavable dipeptide  | MMAE      | AbbVie/Pierre Fabre | Advanced solid tumors cancer and non-small cell lung cancer | II     | NCT02099058 (ongoing)     |
| SGN-LIV1A                 | LIV-1          | Cleavable dipeptide  | MMAE      | Seattle Genetics | Breast cancer, lung cancer                         | II     | NCT01042379 (ongoing)     |
| AGS-16C3F                 | ENPP3          | Noncleavable (mc)    | MMAF      | Agensys/Astellas | Renal cell carcinoma                               | II     | NCT02639182 (ongoing)     |
5.7. Mirvetuximab soravtansine

Mirvetuximab soravtansine comprises an anti-folate receptor alpha (FRα) antibody conjugating to DM4 through a cleavable disulfide linker\(^{101}\). In a phase I trial, activity and safety of mirvetuximab soravtansine were evaluated in ovarian or peritoneal cancer patients\(^{102}\). The confirmed ORR was 26%, the median PFS was 4.8 months and the median DOR was 19.1 weeks\(^{102}\). Especially, for the patients who received three or fewer prior lines of treatment, the ORR, PFS and DOR were 39%, 6.7 months and 19.6 weeks respectively\(^{102}\). Furthermore, the adverse events including fatigue (30%), nausea (37%), blurred vision (41%) and diarrhea (44%) were mainly grade 1 or 2\(^{103}\). Currently, one phase III study is active to compare with investigator’s choice of chemotherapy (NCT02631876).

5.8. Depatuxizumab mafodotin

Depatuxizumab mafodotin is prepared by conjugation of MMAF to an anti-epidermal growth factor receptor (EGFR) antibody through a non-cleavable linker\(^{103}\). In a phase I study (NCT01800695), pharmacokinetics, effect and safety of depatuxizumab mafodotin plus temozolomide were evaluated in patients with glioblastoma multiforme\(^{104}\). The most frequent adverse events were photophobia (35%), fatigue (38%) and blurred vision (63%)\(^{104}\). The 6-month OS rate was 69.1%, the 6-month PFS rate was 25.2% and the ORR was 14.3%\(^{104}\). Based on the encouraging results, a phase II trial (NCT02343406) and a phase III trial (NCT02574455) are ongoing to test the therapeutic effect in newly diagnosed or recurrent glioblastoma.

5.9. Sacituzumab govitecan

Sacituzumab govitecan is consisted of an anti-tumor-associated calcium signal transducer 2 (Trop-2) antibody and the SN-38 via an acid-labile ester linker\(^{105}\). A phase I/II trial (NCT01631552) showed that the median PFS was 5.5 months, the response rate was 33.3%, the CBR was 45.4%, the median DOR was 7.7 months, and the OS was 13.0 months\(^{106}\). Besides, frequent grade ≥3 adverse events included anemia and neutropenia\(^{106}\). Furthermore, two phase III trials are currently active to cure triple-negative breast cancer (NCT02574455) and HR\(^+\)/HER2\(^-\) metastatic breast cancer (NCT03901339).

6. Challenges for clinical applications of ADCs

The approval of GO, BV, T-DM1, InO, and polatuzumab vedotin-piiq have boosted the quantity of ADCs in clinical trials. Up to now, more than 80 ADCs were examined in a wide variety of clinical trials\(^{107}\). However, the clinical trials for approximately 55 ADCs have been terminated\(^{107}\). There are many challenges for the clinical applications of ADCs, among which toxic effects are most formidable\(^{5,107}\). The toxicity of ADCs is mainly caused by the chemotherapeutic drugs\(^{15}\). For example, MMAE conjugated drugs induce neutropenia and peripheral neuropathy, MMAF causes ocular toxicities and thrombocytopenia; DM1 is associated with neutropenia, gastrointestinal effects and thrombocytopenia, DM4 mainly causes ocular toxicity; calicheamicin conjugated drugs suggest thrombocytopenia and hepatic dysfunction as frequent toxicity\(^{7}\). There are several approaches to decrease toxic effects. The most practical method is to tune the dosing regimen\(^{107}\). For instance, after a phase III trial with fractionated dosing, the FDA re-approved GO\(^{50}\). Another way to maximize the therapeutic index is to use biomarkers to select the right patient population\(^{108,109}\), monitor response signals in early stage\(^{107,110}\), or guide the combination therapy\(^{111,112}\).

Another challenge is the specificity of antibodies\(^{113}\). Based on the rationale, target antigens need to express high levels in tumors and minimal expression in healthy tissues, thus making the target antigen tumor-specific\(^{15}\). However, most tumor antigens also...
express in normal tissues, which makes antigens tumor-associated rather than tumor-specific\textsuperscript{11,12}. For example, the major toxicity of SGN-15 (also known as BR-96 doxorubicin), which is consisted of doxorubicin and anti-Lewis Y antibody, is hemorrhagic gastritis\textsuperscript{13,14}. The primary cause of hemorrhagic gastritis is the expression of Lewis Y antigen in the gastric mucosa cells\textsuperscript{11,14}. Another example is the bivatuzumab mertansine, which targets the CD44v6 antigen\textsuperscript{15}. In a phase I trial (NCT02254018), fatal exfoliate of skin toxicity was observed because the target antigen was also expressed in the deep layers of skin\textsuperscript{11,15}.

Lastly, current preclinical models cannot predict ADCs’ activity in human patients\textsuperscript{11,15}. Although a large number of ADCs show therapeutic benefits in rodent tumor models, many of them are not effective in the clinic. One reason is the difference between rodent and human antigens\textsuperscript{16,17}. To solve this challenge, it is essential to comprehensively characterize human antigen and carefully select its corresponding antibody\textsuperscript{79}.

7. Future directions of antibody drug conjugates

ADCs represent a rapidly increasing field in cancer therapy. Various ADCs technologies developed over the past decade have created a large variety of possibilities for designing new ADCs\textsuperscript{13}. For instance, promising antigen targets are uncovered for both solid and hematologic tumors\textsuperscript{57,117}. Plenty of highly potent drugs have been discovered including microtubule inhibitors, anacryclines, and amatoxins, which may become important complements of auristatins and maytansinoids\textsuperscript{75}. New generation linkers have been characterized in order to improve the therapeutic window of ADCs\textsuperscript{13,57,118–120}. Future directions include bispecific ADCs that are designed to increase both potency and selectivity\textsuperscript{121–123}, or deliver multiple classes of payloads\textsuperscript{124}. Furthermore, the combination strategies are currently explored in many clinical trials, such as combining with checkpoint inhibitors (NCT02605915, NCT01896999, NCT02581631, NCT02684292, and NCT02572167) and traditional chemotherapies (NCT03959085, NCT03187210, NCT01476410, and NCT01771107). Although there remain many obstacles to overcome, the development of new ADCs provides tremendous opportunities for future cancer treatment.

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Author contributions

Pengxuan Zhao wrote the paper. Yuebao Zhang, Wenqing Li, and Christopher Jeanty edited the paper. Guangya Xiang provided constructive advice and edited the paper. Yizhou Dong conceived the review topic and wrote the paper.

Conflicts of interest

The authors declare no conflicts of interest.

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