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REVIEW ARTICLE

# Potential drugs used in the antibody–drug conjugate (ADC) architecture for cancer therapy

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## Abstract

Cytotoxic small-molecule drugs have a major influence on the fate of antibody–drug conjugates (ADCs). An ideal cytotoxic agent should be highly potent, remain stable while linked to ADCs, kill the targeted tumor cell upon internalization and release from the ADCs, and maintain its activity in multidrug-resistant tumor cells. Lessons learned from successful and failed experiences in ADC development resulted in remarkable progress in the discovery and development of novel highly potent small molecules. A better understanding of such small-molecule drugs is important for development of effective ADCs. The present review discusses requirements making a payload appropriate for antitumor ADCs and focuses on the main characteristics of commonly-used cytotoxic payloads that showed acceptable results in clinical trials. In addition, the present study represents emerging trends and recent advances of payloads used in ADCs currently under clinical trials.

KEYWORDS

antibody–drug Conjugate (ADC), auristatin, calicheamicin, cytotoxic small molecules, maytansine, payloads, warheads

## 1 | INTRODUCTION

Paul Ehrlich, the famous German physician and scientist, was the first to describe the term "chemotherapy" in the early 1900s for the use of chemical agents to treat diseases. However, the modern application of chemotherapy was introduced in the mid-1900s by nitrogen mustard, a cytotoxic chemical that targets rapidly dividing cancer cells (DeVita and Chu, 2008; Goodman et al., 1946; Peters & Brown, 2015). Since then, a great number of anticancer agents have been introduced for the treatment of cancer patients, including methotrexate (MTX), 6-mercaptopurine (6-MP), taxanes, vinca alkaloids, nitrogen mustard, and anthracyclines (DeVita & Chu, 2008). These chemotherapeutic agents not only have a small therapeutic index (maximum tolerated dose/minimum efficacious dose [MTD/MED]), but also target both normal and cancer cells. The off-target toxicity, as well as the small therapeutic index, leads to severe side effects in patients receiving chemotherapy, representing a major drawback and limiting their usage. To circumvent the limitations of the chemotherapeutic agents, a large body of research has been devoted to find new drugs capable of specifically fighting cancer and improving patient's life, leading to evolution of targeted cancer therapies (E. G. Kim and K. M. Kim, 2015).

Monoclonal antibodies (mAbs), a distinct class of targeted anticancer therapeutics, offer a number of advantages compared with traditional chemotherapeutic agents, importantly including long half-life and great selectivity, which result in diminished off-target toxicity. The application of mAbs, as a promising strategy to treat malignancies in clinical practice, dates back to 1997, when the first mAb *rituximab* was successfully approved for the treatment of low-grade B-cell lymphoma. These successes were followed by a number of other mAbs approved by the United States Food and Drug Administration (FDA) for the treatment of solid tumors and hematological malignancies (Boyiadzis & Foon, 2008; Scott, Wolchok, & Old, 2012). In spite of enormous successful experiences, there are still drawbacks associated with the anticancer efficiency of unarmed (or naked) mAbs, encouraging efforts to further increase the potency of therapeutic mAbs (Sassoon & Blanc, 2013).

Covalently linking toxins or drugs to mAbs, as a targeted therapy, promises the increased enrichment of toxin or drug molecules in tumor cells by simultaneously sparing normal cells from the off-target effects, enhanced solubility of hydrophobic compounds, and the elongation of plasma half-life through prevention of renal clearance, which in turn leads to an increased therapeutic window (Beck, Senter, & Chari, 2011; Teicher & Chari, 2011). Over the years, investigators have improved mAb effectiveness through several strategies in which mAbs directly deliver the cytotoxic agents to cancer cells, including antibody-radionuclide conjugates (ARCs; the conjugation of radionuclides to antibodies), recombinant immunotoxins (RITs; antibody- or antibody fragment-protein toxin fusion), antibody–enzyme conjugates (conjugation of enzymes to antibodies), and antibody–drug conjugates (ADCs; conjugation of small-molecule drugs to antibodies), among which only ARCs and ADCs have achieved clinical and regulatory successes (Choudhary, Mathew, & Verma, 2011; Kreitman & Pastan, 2011; Steiner & Neri, 2011; Teicher & Chari, 2011; Winston, Fuller, Evelegh, & Hurrell, 2001). The three former conjugates are beyond the scope of this review and have been extensively covered elsewhere. The present review first provides a brief introduction to ADCs and a summary of their historical development against cancer. Then, we mainly discuss the cytotoxic payloads used in ADC architecture and the requirements making a payload compound suitable for development of an ADC, particularly by focusing on their structural and mechanistic features. Lastly, the present review highlights the emerging trend of using payloads for ADCs and recent advances in promising ongoing clinical studies.

## 2 | ANTIBODY–DRUG CONJUGATES (ADCS)

ADCs represent a new class of protein-based therapeutic agents which combine the targeting capabilities, high selectivity, and stability of mAbs with the cancer-killing potential of highly potent payloads (300–1,000 Da, with sub-nanomolar [nM] IC50 values) to increase precise drug delivery in cancer cells (Beck, Wurch, Bailly, & Corvaia, 2010b; Behrens et al., 2015; Doronina et al., 2003; Dubowchik & Walker, 1999; Jackson et al., 2014a; Sievers & Senter, 2013; Wagner-Rousset et al., 2014). In an ADC, the mAb is covalently conjugated to a variable number of small-molecule payloads through a linker, serving as a targeted delivery agent to an antigen-positive tumor cell detected by the mAbs that allows for discrimination between healthy and cancerous cells (Anderl, Faulstich, Hechler, & Kulke, 2013; DiJoseph et al., 2004; Mullard, 2013; Peters & Brown, 2015). Figure 1 indicates an ADC consisting of a mAb, a potent payload, and a linker. Such immunoconjugates, as compared with traditional cytotoxic drugs, lead to decreased toxicity and increased efficacy of the payloads, leading to the decreased MED and increased MTD (Beck, Goetsch, Dumontet, & Corvaia, 2017). ADCs generally mediate cancer cell death through the following steps: (a) reorganization and binding to tumor antigens

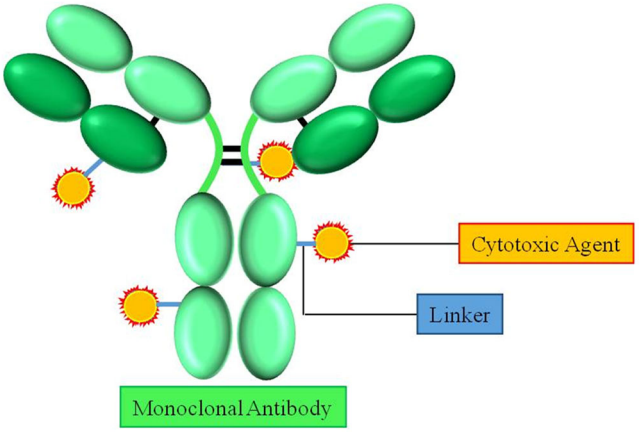



**FIGURE 1** Schematic structure of an antibody–drug conjugate (ADC). A typical ADC comprises a monoclonal antibody conjugated with a potent payload by a linker. Such a molecule can serve as a potent anticancer agent able to selectively target and kill cancer cells

through the mAb moiety, (b) the internalization of ADC–antigen complex, and (c) the release of cytotoxic payload following ADC degradation in the lysosome, allowing the payload to kill the target cell. In this way, the payloads are delivered specifically into the target cells through the mAb moiety with minimized unwanted off-target toxicity (Abdollahpour-Alitappeh et al., 2019; Diamantis & Banerji, 2016). Ideally, following their administration in blood, the ADCs, as a prodrug, are nontoxic but, when binding to the target cell and internalized into the target cell, the active drug is then released from the ADCs and eradicates the cancer cells.

## 3 | THE HISTORY OF ADC PAYLOADS

The use of mAbs, as a vehicle, is not a new concept; conjugated mAbs, antibodies armed with cytotoxic molecules, were first described in the 1970s in preclinical models, but failed to translate into clinical benefits. The clinical trials with murine IgG-based ADCs, albeit with limited success, were first reported in the 1980s. Early ADCs, known as first-generation ADCs, used classical chemotherapy drugs, including N-acetyl melphalan, idarubicin, mitomycin C, anthracycline, vinca alkaloids, methotrexate, and doxorubicin linked to murine mAbs (Diamantis & Banerji, 2016; Dosio, Brusa, & Cattel, 2011; Endo et al., 1987; Kato, Tsukada, Hara, & Hirai, 1983; Pimm, Paul, Ogumuyiwa, & Baldwin, 1988; Rowland, Pietersz, & McKenzie, 1993; Shefet-Carasso & Benhar, 2015; Smyth, Pietersz, & McKenzie, 1987; Spearman, Goodwin, Apelgren, & Bumol, 1987). BR96-doxorubicin and KS1/4-methotrexate were developed in the first-generation ADCs, consisting of doxorubicin and methotrexate as payloads for the treatment of metastatic breast cancer and non-small–cell lung cancer, respectively (Elias et al., 1990; Trail et al., 1993). Despite the successful development of ADCs, these early conjugates showed limited efficacy, moderate potency, and low activity in clinical trials, when compared with the parent drug, mainly due to lack of drug potency (Chari, 1998). This is because of the fact that drugs used in the first-generation ADCs were not highly potent and their concentrations in the serum were not in the optimal range. In general, the real number of ADCs that are internalized is frequently lower than ones binding to the surface of the target cells (Bakhtiar, 2016; Chari, 2008), showing a need for more drug molecules per cell or more potent drug molecules. It soon turned out that a highly potent payload is essentially required for development of a successful ADC.

Multiple promising efforts have been conducted to improve therapeutic benefits and decrease adverse side effects of the anticancer drugs (Panowski, Bhakta, Raab, Polakis, & Junutula, 2014; Peters & Brown, 2015). The lack of successful clinical results in first-generation ADCs, although initially discouraging, led to discovery of more potent small-molecule drugs. In the past 10 years, innovations have led to the discovery of novel payloads able to overcome the limitations encountered by first-generation ADCs. The payload potency was improved by using new drugs, including microtubule-targeting agents (maytansinoids and auristatins) or DNA-targeting agents (calicheamicins) that showed 100–1,000-fold more potency as compared with previously-used payloads (Teicher & Chari, 2011). The introduction of such novel drugs, accompanied by improvement of mAbs and linker technologies, not only improved all aspects of ADCs, but also resulted in new interest in the ADC field, followed by introduction of second- and third-generation ADCs (Abdollahpour-Alitappeh et al., 2019; Chari, 1998; Teicher & Chari, 2011).

By using a highly potent drug calicheamicin, Wyeth and Celltech could develop an anti-CD33 ADC, gemtuzumab ozogamicin (Mylotarg) (Sievers & Linenberger, 2001). However, gemtuzumab ozogamicin, although approved in 2000 by the FDA, was later withdrawn from the market in a required post approval study due to growing concerns about clinical benefits and safety (Panowski et al., 2014; Ravandi, 2011). In fact, gemtuzumab ozogamicin, in combination with chemotherapy, not only failed to show improved survival but, quite the contrary, exhibited increased levels of fatal toxicity when compared with chemotherapy alone (Beck et al., 2010a). Nowadays, a variety of potent payloads can be used for development of highly efficient ADCs, paving the way for the selection of rational, modern, and next generation payloads in the ADC architecture.

## 4 | CYTOTOXIC PAYLOAD CHARACTERISTICS

The cytotoxic drug, also known as "cytotoxic small molecule, warhead, or payload," is an important factor which influences the properties and activities of ADCs. Although there are a great number of known cellular toxins, only a few number of toxic structures and, even lower, modes of actions have been identified to be suitable for the ADC concept (Anderl et al., 2013). Of note, this is because of the fact that the toxin, as an ADC payload, must fulfill a number of requirements, including a potent cell toxicity, defined mechanism of action, appropriate modified site where the original drug is released from the conjugate into the tumor cell, the maintenance of potency after conjugation, as well as acceptable stability and solubility in aqueous formulation and physiological conditions. What's more, the drug should be obtainable and synthetically accessible under conditions of good manufacturing practice (GMP) by a safe, efficient, and cost-effective process (Beck et al., 2017; Lu, Jiang, Lu, & Zhang, 2016; Schrama, Reisfeld, & Becker, 2006; Teicher & Chari, 2011). Here, we focus on some of the key features involved in payload requirements.

### 4.1 | Drug structure

In spite of limited possibilities in their structures, the payloads have to allow the conjugation through a linker. In this light, payloads used in ADC architectures must contain a functional group in their structure suitable for the coupling to the antibody. The nature and site of the modification have to be carefully selected to preserve the potency of the parental drug. What's more, the payloads must

maintain their potency when modified for conjugation or bound to amino acids after mAb degradation in noncleavable ADCs.

Payloads must contain appropriate water solubility in aqueous buffers, to facilitate conjugation to the antibody and ensure sufficient solubility of the conjugate under physiological conditions. At the same time, the payload must contain a sufficient stability in plasma taking into account the long half-life of the antibody moiety in circulation. Importantly, the molecular size of the payload should be small to reduce the immunogenicity risk (Anderl et al., 2013; Chari, Miller, & Widdison, 2014). Last but not least, a great number of cytotoxic small-molecule drugs used in ADC structure are hydrophobic; their hydrophobic nature leads to induced antibody aggregation. The circumvent of this issue not only limits fast clearance rates and immunogenicity, but also guarantees a long shelf life of ADCs (Diamantis & Banerji, 2016).

### 4.2 | Drug potency

The inherent cytotoxic potency of a payload must be extremely high, because of a low penetration of mAbs in tumors, limited expression of antigens, ineffective internalization, and linker metabolization; these may lead to a very limited number of payloads in the target cell (Dosio et al., 2011). Studies using radiolabeled antibodies in cancer patients demonstrated that as little as 0.003–0.08% of an injected antibody dose may accumulate per gram of tumor (Bosslet et al., 1998; Poli et al., 2013), highlighting the need for payloads capable of cell killing at extremely low concentrations. Such potent payloads, which affect critical cellular targets present in low copy numbers, will only guarantee high cytotoxic activities against a genetically-heterogeneous environment of a tumor tissue as well as the prevention of cancer cell escape through resistance mechanisms. Based on above evidence, drug developers focus progressively on the application of potent small-molecule drugs able to kill cells at sub-nM concentrations. In addition, the importance of using very potent drugs stems from economic considerations; antibodies are large molecules (150 kDa), much larger than drugs, and it is not economical to administer several grams of ADCs per patient.

### 4.3 | Intracellular drug targets

The target of the ADC payload should be placed inside the cell, as a vast majority of newly-introduced ADCs rely upon internalization of the drug conjugates, beginning with the endocytosis of the ADC–antigen complex, degradation of antibody or linker moieties in the lysosome, and, eventually, release of the payload into the cytoplasm of the target cell (Abdollahpour-Alitappeh et al., 2019). The targets of a majority of highly toxic agents from plants, animals, and microorganisms are located outside the cells, for example, on neuronal cells through blockage of ion channels or on disturbances of blood clotting, therefore being unsuitable to be used as ADC payloads. Based on above, the majority of ADCs described in literature rely mainly on a small number of payloads able to target one of the three cellular structures, including DNA, tubulin filaments, or RNA. However, not all of the toxins belonging to these three classes proved successful, as discussed below.

## 5 | CYTOTOXIC PAYLOADS USED IN ADC ARTITECTURES

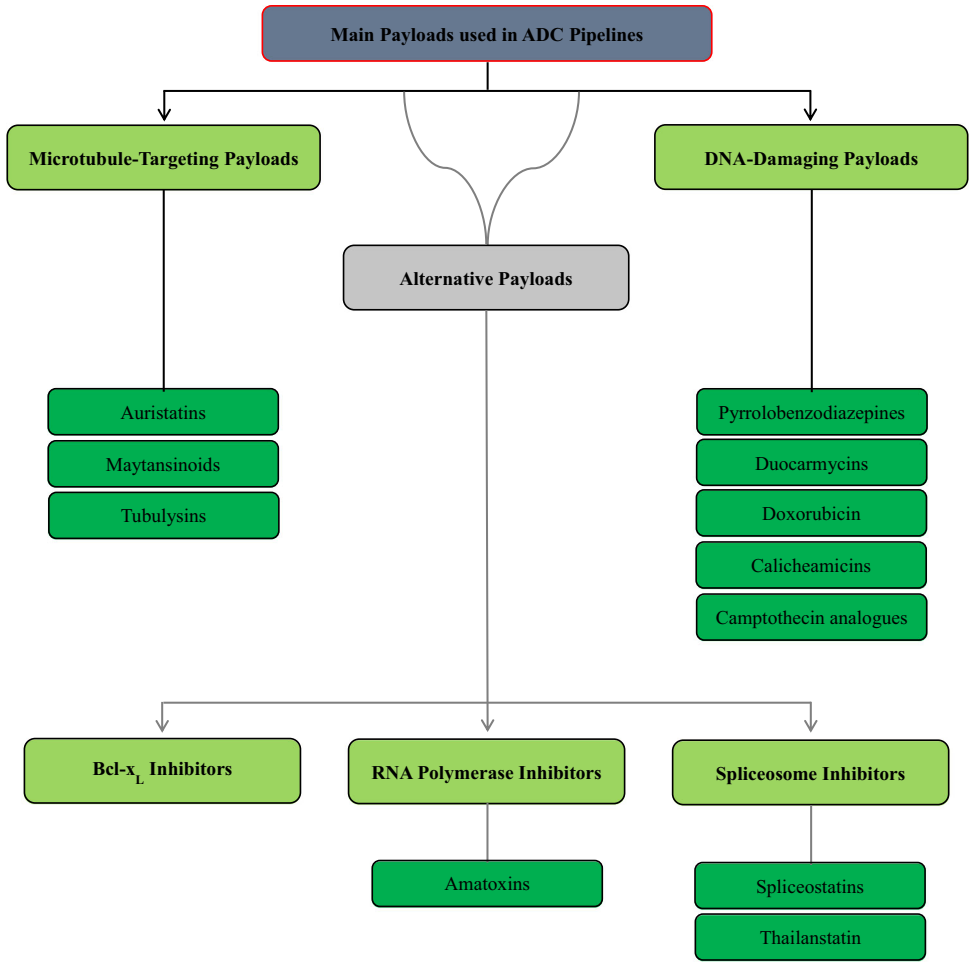
Among a wide variety of toxins known in nature, considering the technical requirements mentioned above, there are only few toxic agents suitable for ADC applications. Despite their different intracellular targets, a great number of drugs, as well as, most probably, their cognate ADCs, show a similar scenario: cell-cycle arrest (either in S or G2/M, depending upon the drug) and subsequent apoptosis (Abdollahpour-Alitappeh et al., 2019; Kovtun & Goldmacher, 2007; Naito et al., 2000). These documents propose that nondividing cells, which rest in the G0 phase, are most likely less sensitive as compared with dividing cells, including cancer cells, to these drugs as well as their cognate ADCs. In fact, nondividing cells were demonstrated to have resistance to tubulin-, microtubule-, or DNA-targeting drugs (Drewinko, Patchen, Yang, & Barlogie, 1981; Jedema et al., 2004; Rao, Freireich, Smith, & Loo, 1979), representing that ADCs containing such drugs would also preferentially kill dividing cancer cells (Kovtun & Goldmacher, 2007).

The payloads used in approved ADCs or currently being used in ADC research and development are far more potent than previously-used ones and can generally fall into two distinct categories, corresponding to distinct intracellular targets: microtubule-targeting agents and DNA-damaging agents (Table 1; Diamantis & Banerji, 2016). In addition, there is a third group, called alternative payloads, including an RNA polymerase II inhibitor α-amanitin, which is under investigation and development. Microtubule-targeting agents, including maytansinoids (LoRusso, Weiss, Guardino, Girish, & Sliwkowski, 2011) and auristatins (Senter & Sievers, 2012), and DNA-disrupting agents, including calicheamicin (Ricart, 2011), with potencies several-fold greater than conventional chemotherapeutic agents, exhibited promising outcomes as ADC payloads in a variety of clinical studies.

### 5.1 | Microtubule-targeting agents as ADC payloads

Tubulin polymerization is essential for a variety of cellular processes, including intracellular transport, mitosis, and structural integrity maintenance. There are five known binding sites for microtubules, including vinca alkaloid-, colchicine-, taxane-, maytansine-, and laulimalide-binding sites. Microtubule/tubulin targeting agents, according to their mechanisms of action, can fall into two major categories, including (a) tubulin polymerization promoters that stabilize microtubule structures and (b) tubulin polymerization inhibitors that destabilize microtubule structures (Chen, Lin, Arnst, Miller, & Li, 2017). Microtubule-targeting agents impede the capacity of mitotic spindles for the segregation of chromosomes, lead to the altered cytoskeletal architecture of cells, induce cell-cycle arrest in the G2/M phase, and cause cell death, making them a potential and

**TABLE 1** Two main categories of cytotoxic payloads used for antibody–drug conjugate (ADC) development: microtubule-targeting agents and DNA-damaging agents




striking target for drug discovery (Dumontet & Jordan, 2010). Vinca alkaloids (including vinblastine and vincristine) and taxoids (including paclitaxel and docetaxel) are examples of microtubule-targeting agents, acting by disrupting normal microtubule formation and stabilizing altered microtubule structures, respectively, in a way that interferes with normal microtubular degradation during mitosis (Abal, Andreu, & Barasoain, 2003).

Blockage of tubulin polymerization has provided a fundamental basis for development of ADCs recently entering clinical development. Maytansinoids and auristatins, as highly potent microtubule-targeting agents, have been effectively used as payloads for a number of clinically-approved ADCs. Both payloads are powerful inhibitors of microtubule assembly, which bind to tubulin in the proximity of the vinblastine-binding site (Bhattacharyya & Wolff, 1977; Gebleux & Casi, 2016), and lead to G2/M cell-cycle arrest and eventually apoptosis. This biocidal mechanism was demonstrated to have high efficiency at killing rapidly-proliferating cells (Gebleux & Casi, 2016). Figure 2 indicates the mechanisms of action of auristatins or maytansinoids on microtubule formation (Peters & Brown, 2015; Steinmetz & Prota, 2018).

The widespread application of microtubule-targeting agents is due to their moderately selective toxicity for rapidly-proliferating cells. This not only gives an added measure of safety but also reflects the importance of tubulin in the mitosis process. Nonetheless, a general disadvantage of ADCs that use microtubule-targeting agents as their payload is that the payloads unfold their cytotoxic effect primarily on proliferating cells because of their inherent mechanism of action. In this way, some rapidly dividing noncancerous cells may be killed, resulting in common side effects. In addition, quiescent and nondividing cells have more likely less sensitivity to the drug action and are likely to escape the drug mechanism, possibly opening the way for development of resistance. Importantly, this can be a disadvantage because some of the tumor types, such as cancer stem cells (CSCs) or tumor initiating cells (TICs), are inherently slow growing. However, microtubule-targeting agents are particularly cyotoxic to cancer cells that divide and grow faster than most normal cells (Abdollahpour-

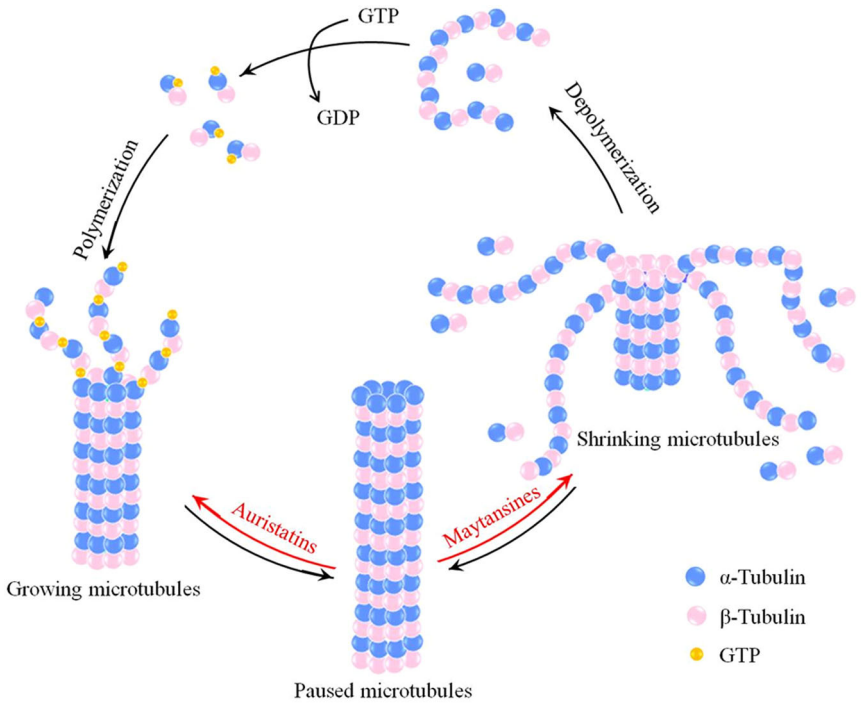



**FIGURE 2** Effects of auristatins and maytansines on microtubule formation. Auristatins interfere with the formation of microtubules through binding to the β-subunit of α-β tubulin dimers, causing continuous and excessive growth of microtubules. Maytansines block the polymerization of tubulin dimmers, preventing the formation of mature microtubules

Alitappeh, Hashemi Karouei, Lotfinia, Amanzadeh, & Habibi-Anbouhi, 2018; Chari, 1998; Chari et al., 2014; Hollander, Kunz, & Hamann, 2008; Lee et al., 1989). Of note, it is important to remember that most mouse xenograft models contain tumors that grow much faster than normal human tumors, therefore presumably providing a false indication of efficacy for such agents that are extremely specific for rapidly-proliferating cells.

### 5.1.1 | Auristatins

A series of studies, started by Pettit et al. in the mid-1960s to explore potential effects of marine life forms as an anticancer drug source, resulted in the discovery of dolastatin peptides from the shell-less mollusk *Dolabella auricularia* (sea hare): dolastatins 1–15, showing the ability to strongly kill cancer cell lines (Anderl et al., 2013; Luesch, Moore, Paul, Mooberry, & Corbett, 2001; Pettit et al., 1981, 1982, 1987, 1987, 1989a, 1989b, 1989c, 1990, 1993). In addition, the parent compound was identified in cyanobacteria *Symploca hydnoides* and *Lyngbya majuscula*, which are nourishment to the sea hare (Dan et al., 2018). In further studies, dolastatins 10 and 15 were found to have high cytotoxic activities against human cancer cell lines at extremely low concentrations (with an average IC50 value in the sub-nM range), exhibiting the most promising peptides within the dolastatin family capable of binding powerfully to tubulin, inhibiting polymerization, and causing cell death (Anderl et al., 2013; Bai, Friedman, Pettit, & Hamel, 1992; Bai, Pettit, & Hamel, 1990a; Bai, R. Pettit, & Hamel, 1990b; Bai et al., 1993; Doronina et al., 2006; Doronina et al., 2003; Quentmeier, Brauer, Pettit, & Drexler, 1992; Steube et al., 1992). Lastly, in the 1990s, dolastatin 10 successfully passed several Phase I clinical trials, and entered Phase II trials (Pitot et al., 1999); however, dolastatin 10 was later withdrawn from clinical studies because of disappointing results, including insufficient activity, high systemic toxicity, and severe side effects, dampening the hope of any therapeutic benefits (Banerjee, Wang, Mohammad, Sarkar, & Mohammad, 2008; Doronina et al., 2003, 2006). Efforts to address this issue and to establish the drug class in large quantities, along with encouraging observations in the positive therapeutic index and high potency found in preclinical models, resulted in development of the potent water-soluble synthetic dolastatin analogs: termed as auristatins (Anderl et al., 2013).

Auristatins are potent microtubule-targeting agents capable of blocking tubulin assembly and leading to G2/M phase cell-cycle arrest, which result in the dividing cells to undergo apoptosis. They impede the microtubule formation through binding to the β-subunit of α-β tubulin dimers in the cytoplasm. The drug then acts by inhibiting the GTP hydrolysis on the β-subunit, leading to excessive and continuous growth of microtubules (Figure 2; Bouchard, Viskov, & Garcia-Echeverria, 2014; Diamantis & Banerji, 2016; Sapra & Shor, 2013). As the microtubules lose their capacity to shorten and separate sister chromatids during anaphase, the cell is frozen in the metaphase stage of mitosis (Francisco et al., 2003). Auristatin PE (also known as soblidotin or TZT-1027) was the first described synthetic dolastatin 10 analog that structurally differs in the absence of the thiazole ring from the original dolaphenine residue, leading to a terminal benzylamine moiety (Kobayashi et al., 1997). Auristatin PE successfully entered Phase I and II clinical trials but eventually failed to demonstrate significant anticancer benefits or any confirmed response in patients suffering from cancer (Anderl et al., 2013; Patel et al., 2006; Riely et al., 2007).

As a more effort to enhance in vivo efficiency, Seattle Genetics has developed two novel auristatin derivatives, including monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF),

fully synthetic drugs prepared from SAR (structure–activity relationship) studies, showing no degradation in plasma and human liver lysosomal environment, as well as in the presence of proteases (Carter and Senter, 2008; Chen et al., 2017; Doronina et al., 2006; Senter & Sievers, 2012). Despite the lack of adverse effects seen in previous clinical trials with auristatins as well as increased therapeutic index, MMAF and MMAE were found to be too toxic to be used in their native form and then derivatized for use as payloads in ADC development (Anderl et al., 2013; Carter & Senter, 2008; Doronina et al., 2006; Senter & Sievers, 2012). MMAE and MMAF, which are currently being used as payloads in a large number of ADCs by conjugating to the mAb cysteine residues, have been chosen among a wide variety of candidates due to their great potency, water solubility, physiological stability, and suitability for the attachment of stable linkers (Beck et al., 2017; Maderna et al., 2014; Rouleau et al., 2015). The fully synthetic nature of MMAE and MMAF may give them a significant advantage as compared with other payloads used for ADCs. Of note, the peptide-like structure of MMAE and MMAF can limit the conjugation effect on the physical properties of the mAb (Anderl et al., 2013; Carter & Senter, 2008; Doronina et al., 2006, 2003).

The main difference between MMAE and MMAF is the presence of a phenylalanine residue at the C-terminus of MMAF which contributes to membrane impermeability. Because of its hydrophobic nature, MMAE can diffuse out of the target cell and mediate bystander effects, the killing of nearby cells. This feature, although leading to MMAE to be more potent than MMAF as shown by in vitro studies, seems to be a potential disadvantage for the application of MMAE in ADCs targeting nonsolid hematological malignancies containing homogenous antigen expression (Chen et al., 2017; Okeley et al., 2010; Peters & Brown, 2015). Auristatins, specifically MMAE and MMAF, constitute most of the commonly used payloads in ADC architecture currently investigated, accounting for a majority of cytotoxic payloads in ADC clinical trials. Other auristatin analogs are also being studied by a variety of companies, such as Ambrx, Novartis, Bayer, Pierre Fabre, Pfizer, and Sanofi/Genzyme (Beck et al., 2017; Maderna et al., 2014; Rouleau et al., 2015).

### 5.1.2 | Maytansinoids

Maytansinoids, a family of cytotoxins with a macrolide structure, are derivatives of natural cytotoxic agents known as maytansines originally isolated in 1972 from the bark of an Ethiopian shrub *Maytenus serrata* by Kupchan et al. (1972). In the following years, a number of maytansine derivatives were isolated from bacteria, mosses, and higher plants (Anderl et al., 2013; Cassady, Chan, Floss, & Leistner, 2004; Rinehart & Shield, 1976). Maytansine and maytansinoids prevent microtubule assembly similar to auristatins, but are mechanistically similar to vinca alkaloids (Hamel, 1992); they strongly prevent microtubule polymerization and the formation of mature microtubules through binding to β-subunit of tubulin at or near the vinblastine-binding site, mediating mitotic arrest in the cells (Bhattacharyya & Wolff, 1977; Mandelbaum-Shavit, Wolpert-DeFilippes, & Johns, 1976; Remillard, Rebhun, Howie, & Kupchan, 1975). The hydrolysis of the GTP molecule on the β-subunit leads to further disassembly of existing microtubules, which again freezes the cell in metaphase and prevents cell division (Figure 2; Oroudjev et al., 2010). The antimitotic effect of maytansines at sub-nM concentrations with an ED50 (effective dose) between 0.1 and 0.01 ng/ml (Cassady et al., 2004) has made them a promising candidate for anticancer drugs (Anderl et al., 2013; Cassady et al., 2004). Nevertheless, maytansines failed to demonstrate therapeutic benefits in human clinical trials because of their potential systemic toxicity, low therapeutic index, as well as no significant response and induced severe side effects in patients with cancers, largely because of the lack of tumor specificity (Chari et al., 1992; Issell & Crooke, 1978; Ravry, Omura, & Birch, 1985).

However, their extremely high potency, excellent and acceptable stability, as well as suitable solubility in aqueous solutions made maytansines an attractive candidate for the development of ADCs (Anderl et al., 2013; Beck et al., 2017; Chari et al., 1992). First attempts to establish antibody-conjugated maytansine derivatives have been undertaken in the 1980s and early 1990s. Since then, a number of disulfide-containing maytansinoids, containing a methyl-dithiopropanoyl group instead of the native N-acetyl group, have been evaluated and entered clinical trials (Chari et al., 1992). Maytansinoids and their derivatives showed approximately 1,000-fold more in vitro cytotoxicity as compared with conventional clinically-used anticancer drugs (Kupchan et al., 1977). In addition to a general drawback of all ADCs based on microtubule-targeting agents as mentioned above, maytansinoid-based ADCs suffer from additional disadvantages, including the hydrophobic characters of the drug and the linker to be used. The free drug is membrane permeable and can elicit uncontrollable and severe side effects. The hydrophobic nature of the linkers used for maytansinoid-based ADCs leads to increased conjugate aggregation or diminished binding capacity of the antibody particularly at high drug loads, exhibiting a high effect on the applicability of maytansine-based ADCs (Anderl et al., 2013; Chari, 1998; Hollander et al., 2008). More importantly, drug transporters mainly facilitate the efflux of hydrophobic compounds (Szakacs, Paterson, Ludwig, Booth-Genthe, & Gottesman, 2006; Takeshita et al., 2009). Therefore, maytansinoid-based ADCs are substrates for multidrug resistance protein 1 (MDR1; also called permeability glycoprotein 1, P-glycoprotein 1 [P-gp or Pgp], ATP-binding cassette subfamily B member 1 [ABCB1], or cluster of differentiation 243 [CD243]) that is a critical protein of the cell membrane having the ability to pump a wide variety of foreign substances out of cells. Therefore, several highly water-soluble hydrophilic linkers (including Sulfo-SPDB and Mal-PEG4-NHS) are under development to increase their solubility and mediate the preparation of more hydrophilic ADCs. These hydrophilic linkers result in production of ADCs with higher drug loads, deliver of higher drug concentrations to the target cell, and more polar maytansinoid metabolites inside the cell which are a poor substrate for MDR efflux pumps and therefore overcome MDR (Zhao et al., 2011).

Maytansines, however, possessed no obvious functional group for conjugation to an antibody molecule (Chari et al., 2014). The first step to overcome the problem was to develop maytansine analogs incorporating a thiol-containing substituent, which were able to undergo disulfide exchange with a suitably modified antibody to give a conjugate. DM1 and DM4 are thiol-bearing maytansinoids containing methyl disulfide substitutions at the C3 N-acyl-N-methyl-L-alanyl ester side chain of maytansine (Dan et al., 2018). DM1, which was described first in 1992 and linked to antibodies via different linkers, is an extremely potent maytansinoid developed by Immunogen, showing a free drug IC50 of 0.1–1.0 nM. The ADC trastuzumab-DM1 (T-DM1), in which DM1 is coupled with trastuzumab through a noncleavable linker, was the third FDA-approved ADC for cancer therapy in human beings. The drug is thought to bind to the antibody outside the cell until the entire ADC is transported through endocytosis into the cytoplasm (Lewis Phillips et al., 2008). After intracellular processing of the ADC, a lysyl-modified but still cytotoxic form of DM1 is released, leading to antitubulin-associated cell death. The charged form of the drug shows no membrane permeability and therefore no abovementioned bystander effect of killing neighboring cells (Kovtun et al., 2006; Lewis Phillips et al., 2008). A study carried out by Pillow showed that site-specific conjugation of maytansinoid payloads could improve the effectiveness of the resultant ADCs (Pillow et al., 2014). In a study, Widdison et al. (2015) indicated that an anilino-linked maytansinoid leads to improved bystander effects when compared with traditional disulfide-linked maytansinoids. In collection, maytansinoid-based ADCs are an attractive approach which is believed to be greatly validated following the FDA-approval of ado-trastuzumab emtansine (T-DM1).

### 5.1.3 | Tubulysins

Tubulysins are a series of naturally occurring antimitotic tetrapeptides first isolated from myxobacteria by by Höfle et al. in 2000 (Sasse et al., 2000). When screening myxobacterial culture extracts for biologically active compounds, Höfle et al. showed the isolation of four members of the tubulysin family (A, B, D, and E) (Murray, Peterson, & Fecik, 2015; Sasse et al., 2000). These natural products are functionally similar to dolastatins, representing approximately 100- to 1,000-fold more potent in comparison with traditional chemotherapeutic agents with EC50 in the nM to sub-nM range (Chen et al., 2017).

Tubulysins, similar to auristatins and maytansines, are microtubule-disrupting agents, which inhibit microtubule polymerization, prevent cell growth and division during mitosis, and induce cell death. Their known biological activities can be summarized as follows: Tubulysins, upon release, bind to the vinca domain of tubulin with higher affinity than vinblastine, and rapidly decompose the cytoskeleton and mitotic machinery of dividing cancer cells, resulting in tubulin depolymerization, G2/M accumulation, and apoptosis (Kaur et al., 2006). The exceptional antiproliferative activity of tubulysins has resulted in a great deal of interest in evaluating their clinical potential and studying their mechanism of action. In growth inhibition assays, tubulysins were found to be inactive against bacteria and yeasts, and weakly active against fungi. However, because of their remarkable ability to inhibit tubulin polymerization, tubulysins have been exploited to target human cancer cell lines, showing extremely potent antiproliferative activity against a great number of human cancer cells including breast, cervix, colon, leukemia, lung, melanoma, ovarian, and prostate cancer cells. Tubulysins demonstrated not only to have a degree of selectivity against human cancer cells because of their rapid division rates, but also to be effective in MDR cancer cells that either overexpress P-glycoprotein pumps or have tubulin mutations. Therefore, they may bypass the obstacles associated with the efflux pumps for DM1 (Balasubramanian, Raghavan, Begaye, Sackett, & Fecik, 2009; Kaur et al., 2006; Khalil, Sasse, Lunsdorf, Elnakady, & Reichenbach, 2006; Li et al., 2016; Murray et al., 2015; Sasse et al., 2000; Steinmetz et al., 2004).

So far, approximately 14 different tubulysin isoforms have been reported with a conserved core structure consisting of an L-isoleucine (Ile), a tubuvaline (Tuv), and an N-methylD-pipecolic acid (Mep) unit. All natural tubulysins have a special N,O-acetal and either a tubutyrosine (Tut) or a tubuphenylalanine (Tup) at the C-termini for their biological function. What's more, it has been demonstrated that N,O-acetal can be replaced by a plain alkyl group to offer N-14-desacetoxytubulysin H with no loss in potency.

Taken together regarding their high cytotoxic potency against a wide variety of cancer cells, especially MDR cancer cells, tubulysins have been a favored choice as payloads for the selective targeting of cancer cells through ADCs. Based on the SAR study of the tubulysins, this class allows many conjugation and targeting strategies, and is well-suited for any kind of conjugation to polymers or biomolecules including mAbs. Taking advantage of the high folate receptor expression in a number of cancers, tubulysin B–folic acid conjugate (EC0305) was the first targeted drug involving tubulysin. Since then, multiple ADCs carrying tubulysin as a payload have been exploited. Tubulysin D, the most potent member of the tubulysin family with IC50 values between 0.01 and 10 nM, has the ability to cause multipolar spindles, which was initially conjugated with polymers to offer proof-of-concept studies in preclinical models. Currently, several ADCs bearing tubulysin D, as a payload, are under active development, which deliver tubulysin selectively to cancer cells and, therefore, avert toxic effects on normal tissues.

Importantly, computer-assisted drug design and biological electronic principles have mediated the synthesis of several tubulysin derivatives with appropriate biological activity. However, the clinical pharmacological data of these compounds have not yet been documented (Chen et al., 2017; Cohen et al., 2014; Diamantis & Banerji, 2016). In collection, tubulysins are projected to be an attractive new class of tubulin inhibitors, whose analogs can be successfully conjugated to mAbs for the development of a potent and stable ADC.

### 5.1.4 | Other microtubule-targeting agents as ADC payloads

In addition to the abovementioned microtubule-targeting payloads, there are other payloads targeting microtubules under investigation.

Although they have not yet been successfully used as anticancer agents, such compounds may have the great potential to be applied as ADC payloads. However, there are little data to date supporting their effectiveness and advantages.

Cryptophycins are a class of dioxadiazacyclohexadecenetetrone cytotoxins with more potency than MMAE and DM1, first isolated in the early 1990s from the cultures of *Nostoc cyanobacteria*. Cryptophycins can bind to microtubules at the vinca-binding site and induce tubulin depolymerization, eventually leading to mitotic arrest. Cryptophycin-1 is the most abundant component, demonstrating to be highly potent against a wide range of solid tumors as well as MDR cancer cells. Cryptophycin-52 (also known as Cr-52 and LY355703), a highly potent synthetic version of cryptophycin, successfully passed Phase I and reached Phase II clinical trials but was withdrawn because of side effects. Cryptophycins show relative hydrophilicity, high potency, and lack of P-gp susceptibility, making them an attractive payload for ADC architecture (Chen et al., 2017; Steinkuhler et al., 2016). Hemiasterlin is a small family of naturally occurring tripeptides derived from marine sponges, representing a potent inhibitor of cell growth. Hemiasterlin binds to the vinca-peptide site in tubulin, disrupts normal microtubule dynamics, and inhibits tubulin polymerization. HTI-286 (also known as taltobulin), a fully synthetic analog of hemiasterlin, was demonstrated to be active against various MDR cancer cells. Hemiasterlin-based ADCs demonstrated to have reduced toxicity, suitable therapeutic window, and excellent cytotoxicity against a wide variety of tumor cells (Chen et al., 2017; Loganzo et al., 2003). Cemadotin, also known as LU103793, is a synthetic, pentapeptide, water-soluble analog of dolastatin 15, representing potent antiproliferative and antitumor activities through inhibiting microtubule assembly and tubulin polymerization. Cemadotin exerts its antitumor activity by suppressing spindle microtubule dynamics through binding at a new site in tubulin, showing to be effective payloads for ADC synthesis (Bernardes et al., 2012). Rhizoxin is a macrocyclic lactone compound isolated from the pathogenic plant fungus *Rhizopus microspores* which is capable of binding to tubulin and inhibiting microtubule assembly. Rhizoxin showed the preclinical antitumor activity against several human tumor cell lines and xenograft models (McLeod et al., 1996; Prota et al., 2014). Discodermolide is the most potent natural promoter of tubulin assembly, showing to be promising candidates for ADC synthesis. Other tubulin inhibitors under investigation include taccalonolide A or B, taccalonolide AF or AJ, epothilone A and B, taccalonolide AI-epoxide, colchicine, CA-4, laulimalide, paclitaxel, and docetaxel, as well as their synthetic derivatives (Chen et al., 2017).

## 5.2 | DNA-damaging agents as ADC payloads

DNA-damaging agents have a long history in cancer chemotherapy for either reducing tumor growth or eliminating tumor cells. DNA-damaging agents are a set of cytotoxic payloads which exert their cytotoxic effects through DNA binding in the double-helix minor groove, and lead to the scission, alkylation, intercalation, or cross-linking of the nucleic acid strands (Figure 3; Gebleux & Casi, 2016). Table 1 indicates some of the important DNA-damaging agents used in ADC architecture. Representative examples of this class include camptothecin and anthracycline agents (DNA-intercalators), calicheamicin and uncialamycin (DNA double-strand breakers), as well as pyrrolobenzodiazepine and duocarmycin payloads (DNA alkylators), each of which may be tailored as a mono-alkylator or a bis-alkylator (DNA-cross linker). Drugs belonging to this class are highly potent, with free drug IC50 < 1.0 nM, and cause cell death (Lee et al., 1989; Thorson et al., 2000). Growing evidence suggested that DNA-damaging agents have suitable activity in a number of MDR cancer cells and are more efficient in killing tumor cells as compared with microtubule-targeting agents, specifically in solid tumors.

It is believed that DNA-damaging agents have two potential advantages over microtubule-targeting agents, as ADC payloads: (a) DNA-damaging agents (picomolar [pM] IC50 values) show higher potency as compared with microtubule-targeting agents (sub-nM IC50 values), enabling ADCs to target tumor cells with low expressed antigens, and (b) DNA-damaging agents were shown to have excellent activity throughout the various cell-cycle phases, demonstrating exquisite potency against dividing and nondividing cells, particularly nondividing CSCs (Fu & Ho, 2018; Maugeri-Sacca, Bartucci, & De Maria, 2012).

### 5.2.1 | Pyrrolobenzodiazepines

Pyrrolobenzodiazepines (PBDs) are a series of natural products with antibiotic or antitumor properties originally isolated from *Streptomyces*

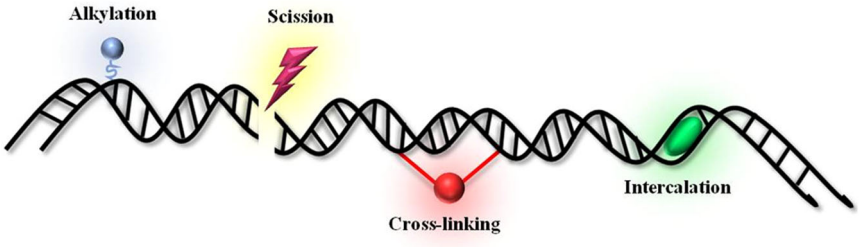



**FIGURE 3** Mechanism of actions of DNA-damaging agents. DNA-damaging agents can fall into roughly four mechanistic categories: DNA double-strand breakers, DNA alkylators, DNA intercalators, and DNA cross-linkers. DNA-damaging agents, compared with microtubule-targeting agents, can kill target cells at any point in their life cycle

species in the 1960s. After discovery of anthramycin as the first PBD, investigations led to identification of more than 12 naturally occurring PBDs (Antonow & Thurston, 2011; Leimgruber, Stefanovic, Schenker, Karr, & Berger, 1965). PBD molecules represent a significant class of sequence-specific DNA-alkylating agents which covalently bind to the C2-amino groups of a guanine residue in the minor groove of double-stranded DNAs (Antonow & Thurston, 2011; Cipolla, Araujo, Airoldi, & Bini, 2009; Gerratana, 2012; Hartley, 2011; Kamal, Reddy, Devaiah, Shankaraiah, & Reddy, 2006; Mantaj, Jackson, Karu, Rahman, & Thurston, 2016). PBDs are unable to bind to single-stranded DNA (or RNA), representing extremely selective in the requirement of a minor groove structure for covalent binding to duplex or hairpin DNA (Rahman, Corcoran, Bui, Jackson, & Thurston, 2014; Rahman et al., 2009a). In addition, they were demonstrated to have a kinetic preference for a three-base-pair recognition sequence, 59-Py–G–Py-39 (in which Py = pyrimidine and G = reacting guanine) (Rahman, Vassoler, James, & Thurston, 2010). PBDs, when bound, remain unattached in the DNA minor groove forming the PBD/DNA adduct, which lead to avoiding DNA repair via slight distortion of the helix and inhibiting several biological processes, such as binding of transcription factors to DNA and some enzyme functions including RNA polymerase and endonucleases (Brucoli et al., 2013; Clingen et al., 2005; Hsieh et al., 2011; Jackson, James, Jenkins, Rahman, & Thurston, 2014b; Kopka et al., 1994; Kotecha et al., 2008; Puvvada et al., 1997; Puvvada, Hartley, Jenkins, & Thurston, 1993; Rahman et al., 2013; Wells et al., 2006). These block cell division with no distortion of the DNA helix, therefore potentially causing lethal lesions. PBDs showed strong antitumor or antibiotic activities as compared with chemotherapeutic agents. Importantly, tumor cells frequently display deficiency in one or more related DNA repair pathways, therefore resulting in selective antitumor activities (Farmer et al., 2005; Mantaj et al., 2016). Most importantly, PBD adducts were demonstrated to be preferentially repaired in healthy cells in comparison with tumor cells (Andreassen & Ren, 2009).

There are currently two subfamilies of PBDs, including naturally occurring PBD monomers capable of forming singly-alkylated DNA-adducts and synthetic PBD dimers consisting of two PBD units coupled via a C8/C8′-linker capable of forming intrastrand or interstrand DNA cross-links in addition to monoadducts (Gregson et al., 2001; Mantaj et al., 2016; Rahman, James, & Thurston, 2011b; Rahman, James, Bui, Drake, & Thurston, 2011a; Rahman, Thompson, James, Narayanaswamy, & Thurston, 2009b). The PBD monomer includes the agents first isolated from *Streptomyces* species (e.g., tomaymycin and anthramycin) and a number of recently-introduced synthetic analogs developed over the last 50 years (including Limazepines A-F) (Antonow & Thurston, 2011; Fotso et al., 2009; Mantaj et al., 2016). The PBD monomers exhibit antibacterial and antitumor properties (Kotecha et al., 2008). To investigate PBDs with sequence-binding selectivity, Thurston developed a PBD dimer through linking two PBDs, resulting in production of PBD dimers with 600-fold activity in vitro (Mantaj, Jackson, Rahman, & Thurston, 2016). This makes PBD dimers a promising payload for use in ADC architecture. PBD dimers bind specially to guanidine residues on various positions in the double-stranded DNA helix and drive DNA strand cross-linking. Of important note, dimerization improves the PBD binding affinity, sequence specificity, and efficacy. PBD dimers showed potent in vitro antitumor cytotoxicity (IC50 values with the mid to low pM ranges) against a broad range of tumor cell lines. Most importantly, PBD dimers indicate acceptable activity in MDR1 and refractory tumors because of the fact that they are normally not substrates of MDR1 (Mantaj, Jackson, Rahman, & Thurston, 2017), significantly avoiding the commonly-observed drug resistance. The cytotoxicity of PBD dimers seems to stem from the inability of repair proteins to appropriately recognize DNA damage, leading to slow-progressing repair rates of monoadducts, and cross-links present in the minor groove of DNA (Clingen et al., 2005). Although interstrand crosslink, intrastrand cross-links, and monoadducts can be formed by PBD dimers (Rahman et al., 2009b), the interstrand cross-linked adduct is believed to be the main toxic form in cells.

Unlike microtubule-targeting agents, PBD monomers and dimers can lead to cell death in both dividing and nondividing cells. Due to their high potency, great intracellular targets, completely different cellular mechanism (as compared with tubulin inhibitors), different mode of DNA interaction (as compared with other DNA-damaging agents such as calicheamicin), as well as a small tendency for the development of drug-resistant phenotypes, natural or synthetic PBDs and PBD dimers can serve as appropriate payloads for the synthesis of ADCs. PBD monomers and dimers are becoming established as important next-generation payloads in the ADC therapeutic field. Of note, PBD dimers can even be more attractive cytotoxic payloads for use in ADCs because of their higher potency and interesting mechanism of action (Gregson et al., 2001).

### 5.2.2 | Duocarmycins

Duocarmycins, a family of naturally occurring antibiotic metabolites, are extremely powerful antineoplastic compounds originally isolated from *Streptomyces* bacteria in the late 1970s (Yasuzawa et al., 1988). They consist of an indole moiety (serving as a DNA-binding component) and an unprecedented spirocyclopropylcyclohexadienone moiety (serving as a pharmacophore group), which lead to selective alkylation of DNA sequences (Hurley et al., 1988). Duocarmycins represent powerful cytotoxic DNA minor groove-alkylating agents which exert their high potency through the formation of DNA adduct. Duocarmycins selectively prefer a five-base-pair AT-rich sequence that better accommodates the central pyrroloindole subunit. In terms of mode of action, duocarmycins bind to the DNA minor groove, N3 of adenine attacks the cyclopropane moiety within the DNA minor groove thus forming a DNA adduct, and alkylation of adenines occurs at the N3 position. The induced irreversible DNA alkylation hinders DNA architecture and structural integrity, and leads to DNA cleavage and, eventually, cell death through apoptosis (Boger & Johnson, 1995). A recent controversial theory claimed that duocarmycins could also act through the inhibition of aldehyde dehydrogenase 1, an enzyme playing critical

roles in cancer cell detoxification and viability (Tercel et al., 2013; Wirth et al., 2013; Wirth, Schmuck, Tietze, & Sieber, 2012).

Adozelesin, bizelesin, and carzelesin, members of the cyclopropylpyrroloindole family, are artificially synthesized analogs of duocarmycins. These drugs have achieved high research and clinical attention and have advanced into clinical studies for cancer treatments. Adozelesin is an alkylating small groove DNA-binding agent which quickly restrains the replication of DNA in treated cells via a trans-acting mechanism, primarily arresting cells in the S phase. Bizelesin targets the DNA small groove and causes DNA cross-linking, thereby restraining DNA replication and RNA synthesis. It also strengthens the induction of p21 and p53 and induces G2/M cell-cycle arrest, leading to slow cell death but without any apoptosis. Carzelesin is a cyclopropylpyrroloindole prodrug consisting of a nonreactive chloromethyl forebody that is functional upon activation. It is activated by hydrolysis of the phenylurethane substituent to generate U-76073 and a subsequent ring closure step yields the cyclopropyl-containing U-76074 form that is active in DNA binding (Dokter et al., 2014; Li et al., 1992; Sugiyama, Lian, Isomura, Saito, & Wang, 1996; Tietze, Krewer, von Hof, Frauendorf, & Schuberth, 2009). CC-1065 and duocarmycin SA are the most widely used molecules among duocarmycin analogs (Dokter et al., 2014; Li et al., 1992; Sugiyama et al., 1996; Tietze et al., 2009).

Duocarmycin and its analogs display impressively high cytotoxicity against the growing cancer cells in culture, showing strong cytotoxic properties with IC50 values in the pM range against a variety of cell lines (Tietze & Schmuck, 2011). Duocarmycins are capable of applying their mode of action at any stages of cell-cycle, representing better antitumor activities as compared with microtubule-targeting agents that only attack tumor cells during the mitotic state. What's more, duocarmycin analogs have also been demonstrated to be effective on solid tumors and various MDR models (Diamantis & Banerji, 2016; Dokter et al., 2014; Li et al., 1992; Sugiyama et al., 1996; Tietze et al., 2009). However, despite their high antitumor activity, duocarmycins can not directly used for cancer chemotherapy, making them excellent candidates as payloads for ADC synthesis.

A duocarmycin analog DUBA (duocarmycin–hydroxybenzamide–azaindole), the final active drug form, has been developed into several new-generation ADCs for in vitro or in vivo efficacy evaluations. SYD983, an anti-HER2 ADC, is a leading ADC derived from this platform, showing decreased tumor growth in a BT-474 mouse xenograft and acceptable stability in the plasma of human and cynomolgus monkey (Dokter et al., 2014; Li et al., 1992; Sugiyama et al., 1996; Tietze et al., 2009). In collection, the high potency of duocarmycins and their analogs not only makes them an appropriate candidate for maximizing ADC cell-killing potency, but also may be effective against MDR cancer cells.

### 5.2.3 | Doxorubicins

Doxorubicin, an actinomycete-derived antimitotic anticancer agent often regarded by the trade name Adriamycin, is a member of the anthracycline compounds originally isolated in the 1970s from *Streptomyces peucetius* (Arcamone et al., 1969; Di Marco, Gaetani, & Scarpinato, 1969; Yang, Teves, Kemp, & Henikoff, 2014). Doxorubicin is a 14-hydroxylated version of daunorubicin which has been used with great efficacy to treat a broad range of solid and nonsolid tumors, representing as one of the most impactful antitumor chemotherapeutic agents widely used in the clinic (Yang et al., 2014).

Although extensively used in the clinics, the molecular mechanism(s) of doxorubicin driving cardiotoxicity or cell death remains to be elusive. However, two seemingly conflicting models have been proposed for doxorubicin-mediated cell death through inducing DNA damage, including (a) DNA helix intercalation and disruption of topoisomerase II-mediated DNA repair and (b) development of free radicals and subsequent damage to cellular membranes, DNA and proteins (Gewirtz, 1999). Briefly, the oxidization of doxorubicin first leads to the formation of an unstable metabolite, semiquinone, which is then converted back to doxorubicin in a process releasing reactive oxygen species. The reactive oxygen species can in turn result in lipid peroxidation, membrane and DNA damage, increased cellular oxidative stress, and induction of cell death through apoptosis (Doroshow, 1986; Thorn et al., 2011). Otherwise, doxorubicin can enter the nucleus and poison topoisomerase II, causing DNA damage and subsequent cell death (Tewey, Rowe, Yang, Halligan, & Liu, 1984). There is also evidence supporting that doxorubicin leads to DNA adduct formation, free radical generation, and ceramide overproduction (Gewirtz, 1999; Minotti, Menna, Salvatorelli, Cairo, & Gianni, 2004; Senchenkov, Litvak, & Cabot, 2001). As a DNA intercalator, doxorubicin forms hydrogen-bonds with guanine and intercalates into the DNA strand at sites with adjacent GC base pairs, therefore inhibiting DNA replication and, eventually, protein synthesis. This process has been demonstrated to trigger DNA damage responses and induce cell death (Bouchard et al., 2014; Brown, Sandhu, & Herrmann, 2015; Yang et al., 2014). DNA intercalation and induced topoisomerase II poisoning are thought to be major doxorubicin mode of actions which result in DNA damage and cell death. Nevertheless, the most well-known mechanism of doxorubicin seems to be through topoisomerase II poisoning, where it traps the topoisomerase II at the DNA breakage sites, resulting in an increased and stabilized cleavable enzyme-DNA linked complex during DNA replication and subsequent prevention of the nucleotide strand ligation after double-strand breaks. Simply stated, Topoisomerase II poisoning by doxorubicin is thought to disrupt DNA replication and transcription, leading to apoptosis-mediated cell death. Additionally, the oxidation of doxorubicin quinone structure leads to semiquinone radical formation and subsequent superoxide and $H_2O_2$ generation, resulting in elevated oxidative stress and eventually DNA damage. Along with increased cellular oxidative stress, doxorubicin treatment also results in an increased cellular ceramide level, triggering processes such as growth arrest, apoptosis, and senescence. However, some of these functions, such as inhibition of DNA and RNA synthesis, are only observed at doses higher than the clinical dose (approximately 40–60 $mg/m^2$; Bouchard et al., 2014; Brown et al., 2015; Gewirtz, 1999; Yang et al., 2014).

Despite their clinical importance, the occurrence of drug resistance and side effects lead to a narrow therapeutic window, representing the main drawbacks for successful cancer treatment (Thorn et al., 2011). For this regard, ADCs based on doxorubicin and other doxorubicin derivatives have been designed and synthesized to improve its unfavorable parameters and therapeutic window. An ADC consisting of a chimeric anti-LewisY cBR96 mAb coupled with doxorubicin was shown to release the payload inside the cytosome of targeted cancer cells, thereby, increasing the total efficacy of doxorubicin (Bouchard et al., 2014; Brown et al., 2015; Yang et al., 2014). BMS-182248 was the first doxorubicin-based ADC to reach Phase II clinical trials in patients with non-small-cell lung cancer. However, Seattle Genetics later made a decision to cease its development, although the data were encouraging (Beck et al., 2017).

### 5.2.4 | Calicheamicins

Calicheamicins, also known as LL-E33288 antibiotics, represent a class of enediyne-containing DNA-cleaving antitumor agents originally discovered by the Lederle Laboratories (American Cyanamid Co.) in the mid-1980s when conducting a research for development of novel fermentation-derived antitumor antibiotics in *Micromonospora echinospora*. In the meantime, scientists found that the bacterium produced a compound in the culture media which is an incredibly potent cytotoxic agent. This class of drugs was found to be related structurally to other enediynes such as neocarzinostatin, esperamicins, kedarcidin, C-1027, maduropeptin, dynemicins, shishijimicin, and namenamicin. The compound was then found to have excellent activity in biochemical induction assays at concentrations lower than 1 pg/ml, showing to be highly potent against Gram-negative and -positive bacteria. More interestingly, calicheamicin was demonstrated to have extraordinary potency against tumor cells, showing a roughly 4,000 and 1,000–10,000 times more potency than adriamycin and clinically-used anticancer drugs, respectively, with an optimal dose at 0.5–1.5 μg/kg (Anderl et al., 2013; Dosio et al., 2011; Edo et al., 1988; Golik et al., 1987a, 1987b; Lee et al., 1987a, 1987b, 1989; McDonald et al., 1996; Oku, Matsunaga, & Fusetani, 2003; Smith & Nicolaou, 1996).

Once inside the cells, calicheamicins, functionally similar to anthracyclines, diffuse into the cell nucleus, target and bind to the DNA minor groove, and site-specifically induce double-strand DNA breaks, resulting in the cell death through apoptosis; in fact, reactive diradical species, formed by calicheamicins, eventually result in DNA strand cleavage at different locations, leading to rapid cell death via apoptosis (Boger & Johnson, 1995; Gebleux & Casi, 2016; Jenkins, Hurley, Neidle, & Thurston, 1994; Shor, Gerber, & Sapra, 2015; Walker, Landovitz, Ding, Ellestad, & Kahne, 1992). In addition, calicheamicins were demonstrated to result in altered expression of various central cell elements at transcriptional levels, including ribosomal and nuclear proteins, stress response-related proteins, various genes playing a role in DNA repair/synthesis, as well as biosynthetic and metabolic genes (Dan et al., 2018; Watanabe, Supekova, & Schultz, 2002). Most importantly, the drug less depends upon cell-cycle progression, making calicheamicin effectively appropriate against CSCs (Gupta, Chaffer, & Weinberg, 2009; Sapra, Hooper, O'Donnell, & Gerber, 2011).

Among a variety of calicheamicin analogs identified in *M. echinospora ssp.* including $\alpha_2^I$, $\alpha_3^I$, $\beta_1^I$, and $\delta_1^I$ (iodinated analogs), and $\beta_1^{Br}$ and $\gamma_1^{Br}$ (bromine-containing analogs), Calicheamicin $\gamma_1^I$ (hereafter called calicheamicin) is the most intensively studied calicheamicin, exhibiting highly potent cytotoxic effects (in vivo potency: 0.15 μg/kg) (Dosio et al., 2011). Calicheamicin consists of two structurally-different areas, each containing a particular role in the biological activity of the drug. The bigger part of the two areas contains one extended sugar residue, consisting of a hexasubstituted benzene ring and four monosaccharide units linked together via glycosidic thioester and hydroxylamine linkages. The second structural region, the aglycon (also known as calicheamicinone), has a condensed, highly-functionalized bicyclic core harboring a strained enediyne unit within a bridging 10-member ring. The aryltetrasaccharide is thought to deliver the drug to its target and strongly bind it to the minor groove of double-helix DNA (Dosio et al., 2011; Nicolaou, Smith, & Yue, 1993).

Nevertheless, in spite of promising initial experiments showing a potent activity at sub-pM concentrations in vitro, their further evaluation in preclinical models revealed that calicheamicins also destroy the DNA of normal cells, have a low therapeutic index, and cannot be used as a single therapeutic agent for cancer treatment. This, thus, precluded further development for their application in clinical settings. Nonetheless, the highly cytotoxic activity, the small molecular size, and the defined mechanism of action turned calicheamicins into an attractive payload for ADC synthesis (Anderl et al., 2013). To this end, the natural trisulfide found in the calicheamicin structure was converted to a disulfide, thus incorporating a functional group allowing its linkage to mAbs. In addition, a hydrazide functionality has been introduced into calicheamicins to conjugate them to mAbs through acid-labile bonds (Frei, Elias, Wheeler, Richardson, & Hryniuk, 1998). N-acetyl-calicheamicin $\gamma_1^I$ was selected as an ADC payload due to its appropriate stability as compared with the calicheamicin $\gamma_1^I$ (Zein, Poncin, Nilakantan, & Ellestad, 1989). Calicheamicin is currently being studied as a payload in a variety of ADCs, importantly including FDA-approved ADCs gemtuzumab ozogamicin and inotuzumab ozogamicin. However, it is important to note that calicheamicin has an extremely hydrophobic nature, and only a few molecules can be conjugated to an antibody.

### 5.2.5 | Camptothecin analogs

Camptothecin (CPT) is a natural inhibitor of the nuclear enzyme topoisomerase I with the potent anticancer activity initially isolated in the 1980s from *Camptothecaacuminata*, the Chinese ornamental tree. CPT molecules were demonstrated to have strong cytotoxic activity against a broad range of experimental tumors and preliminary clinical trials, inhibiting both DNA and RNA synthesis in mammalian cells. Such naturally occurring products exert their cytotoxic activity via binding to the topoisomerase I and DNA

complex, preventing DNA religation and, therefore, resulting in DNA damage and subsequent cell death through apoptosis (Pommier, 2006). Nevertheless, the drug activity is limited at physiological pH because the lability of the CPT E ring lactone induces the formation of the inactive hydroxy acid at this pH range. Of note, at acidic pH, the reaction is reversible, providing a potential opportunity for selectively targeting a variety of solid tumors that are surrounded by acidic extracellular environment while maintaining normal intracellular pH (Dancey & Eisenhauer, 1996; Hsiang, Hertzberg, Hecht, & Liu, 1985).

Although CPTs show broad-spectrum antitumor activity, their low solubility and adverse effects are considered to be an important pitfall. To circumvent these limitations, tremendous efforts have been made toward the production of clinical CPT analogs. So far, the FDA has approved two water-soluble analogs: topotecan (TPT) and irinotecan (camptothecin-11, CPT-11), demonstrating significant antitumor activities in the clinic (Adams et al., 2000; Burke et al., 2009). TPT and CPT-11 have the same mechanism of action through the topoisomerase I inhibitory activity and are thought to make use of their toxic effects in the S-phase of DNA synthesis, interfering with cancer cell growth followed by cell death. TPT, a semi-synthetic derivative of CPT, which is primarily used to treat non-small-cell lung cancer and advanced ovarian cancer, has been recently approved for the treatment of cervical cancer. CPT-11, a semi-synthetic analog of CPT approved by the FDA in 1996 which is used initially for the treatment of colorectal cancer, is a prodrug converted into a more potent CPT analog, SN-38, under carboxylesterase action (Garcia-Carbonero & Supko, 2002; Satoh et al., 1994).

TPT, unlike CPT-11, is not a prodrug and exists mostly in the inactive carboxylate type at neutral pH. Clinical investigations using CPT indicated that optimal efficacy of CPT-11, TPT, and other CPT analogs is obtained after continuous and extended exposure to low concentrations of CPT (Gerrits, de Jonge, Schellens, Stoter, & Verweij, 1997). This proposed that CPT or its CPT analogs can be preferably suitable for ADC approaches, because of the fact that antibodies show prolonged circulation half-lives, often several days to weeks (Burke et al., 2009; Carter & Senter, 2008; Wu & Senter, 2005). In addition, and importantly, topoisomerase I seems to be a promising target for ADC development due to its role in cellular DNA replication and transcription.

SN-38 and DX-8951f (DX-8951; also known as exatecan mesylate) are two analogs of CPT used as ADC payloads. SN-38, the active metabolite of CPT-11, exerts its anticancer effects through inhibition of DNA topoisomerase I (Starodub et al., 2015). SN-38 has nearly 1,000-fold potency than CPT-11, as a result of which the drug cannot be administrated directly to patients due to high toxicity and poor solubility (Starodub et al., 2015). The higher potency of SN-38 makes the drug suitable for ADC synthesis. DX-8951f is a CPT analog with favorable characteristics as compared with TPT and CPT-11, including water solubility, stronger DNA topoisomerase I inhibitory, lack of esterase-dependent activation, and broad antitumor activity. Importantly, DX-8951f not only has greater antitumor activities as compared with the other CPT analogs as well as SN-38 (Nakada et al., 2016), but also is not an MDR1 substrate, demonstrating to be effective against MDR1 cancer cells (Beck et al., 2017; Ogitani et al., 2016; Takegawa et al., 2017). DX-8951f prevents topoisomerase I activity through stabilizing a cleavable complex between DNA and topoisomerase I and preventing religation of DNA breaks, therefore leading to inhibition of DNA replication and induction of cell death. This drug requires no enzymatic activation and displays greater cytotoxic activity as compared with CPT and other CPT analogs.

### 5.2.6 | Other DNA-damaging agents

Besides the abovementioned payloads, other molecules, which can be used as a DNA-damaging agent in ADC synthesis, include SGD-1882 (a cytotoxic DNA minor groove cross-linking derivative of PBD dimers which is not an MDR1 substrate) (Kim & Kim, 2015), centanamycin (an indolecarboxamide synthesized as a less toxic analog of CC-1065 and duocarmycin which binds to DNA and alkylates or intercalates into the DNA) (Beck et al., 2011; Kim & Kim, 2015), PNU-159682 (a highly potent metabolite of the anthracyclines which shows three folds more cytotoxicity compared with doxorubicin) (Yu et al., 2015), and uncialamycin (an enediyne natural product isolated from the *Streptomyces uncialis*) (Chowdari et al., 2018), all showing acceptable potency against a broad range of cancer cell lines. Indolinobenzodiazepine dimers, also known as IGNs, are an indolino-benzodiazepine dimer consisting of a mono-imine moiety, representing a novel set of cytotoxic agents with highly potent activity in vitro (an IC50 value of low pM) against a variety of cancer cells. IGNs, like the PBD dimer SJG-136, are derived from the natural anthramycin family. IGNs bind to the DNA minor groove and their two imine functionalities are covalently reacted with guanine residues, leading to DNA cross-linking. It was demonstrated that the substitution of the PBD pyrrolo group with an indolino moiety leads to approximately 10-fold more potent activity in vitro than SJG-136, presumably because of a faster rate of adduct formation with DNA.

## 5.3 | Alternative payloads

Most payloads used in ADC architectures, as mentioned above, have been designed to directly disrupt important cellular machineries, including DNA replication, DNA transcription, or tubulin polymerization. All the abovementioned compounds, both microtubule-targeting payloads and DNA-damaging payloads, have the following characteristics: (a) a significantly higher cytotoxic potency (with an IC50 value ranging from 0.01 to 0.1 nM) as compared with traditional chemotherapeutic agents, such as doxorubicin that kills cells in the 100–1,000 nM concentration range, representing 100–1,000-fold more potent than payloads used in the first-generation ADCs; (b) cancer cell death through induction of apoptosis regardless of the cell-killing mechanism; (c) an appropriate functional group for conjugation to an antibody (in the absence of a functional group, the desired substituent can be introduced at an appropriate site to maintain parent drug potency); (d) rational solubility in aqueous solutions to enable the reaction with antibodies; and (e) extended

stability in aqueous formulations commonly used for antibodies (Abdollahpour-Alitappeh et al., 2019; Chari, 2008; Dosio et al., 2011; Smets, 1994).

However, in addition to the payloads discussed above, a variety of recent investigations have assessed different pathways, and opened interesting avenues into studying new mechanisms for cytotoxicity, including direct induction of apoptosis, inhibition of spliceosome, and inhibition of RNA polymerase.

### 5.3.1 | Bcl-xL inhibitors

Cancer is generally nonresponsive to apoptosis-associated signaling. One of the mechanisms by which cancer cells acquire resistance to apoptosis is the overexpression of antiapoptotic Bcl-2 family members, including Bcl-xL. Agents capable of blocking the BH3-binding domain present on Bcl-$x_L$ were demonstrated to prevent unsuitable apoptotic functions and, most likely, trigger apoptosis in cancer cells. In this regard, tremendous studies have been devoted to investigate the possibility of ADC-mediated delivery of Bcl-$x_L$ inhibitors. There are two anti-EGFR Bcl-$x_L$ ADCs that were demonstrated to have acceptable activity in xenograft studies, representing to be synergistic with docetaxel (Hennessy, 2016).

### 5.3.2 | Spliceosome inhibitors

RNA splicing is a key mechanism involved in translation of eukaryotic genes. New pre-mRNAs (messenger RNAs) are processed in the spliceosome, a large ribonucleoprotein complex involved in mRNA processing in eukaryotic cells. Misregulation or mutations in the mRNA splicing machinery have been reported in several cancers. Targeting the spliceosome using small molecules offers a promising therapeutic option for targeted cancer therapy (Bonnal, Vigevani, & Valcarcel, 2012; Butler, 2013; Kaida, Schneider-Poetsch, & Yoshida, 2012). There are several natural products capable of inhibiting RNA splicing through binding to different spliceosome subunits. Thailanstatin A can bind to the SF3b subunit of the sliceosome, thus preventing RNA splicing. In a study, an anti-Her2 thailanstatin ADC was demonstrated to exhibit the low nM activity in various Her2-expressing cell lines. Spliceostatins, a potent spliceosome inhibitor, are bacterial natural products which exhibit promising anticancer activity against a variety of cancer cell lines. The mechanism of action as well as powerful cytotoxicity of such agents have resulted in efforts to develop spliceosome inhibitors as appropriate antitumor drugs (Eustaquio, Janso, Ratnayake, O'Donnell, & Koehn, 2014).

### 5.3.3 | RNA polymerase inhibitors

The application of transcription inhibitors has paved a new strategic avenue in the ADC field. RNA polymerase II is a critical enzyme involved in DNA transcription into precursors of messenger RNA. RNA polymerase inhibitors are effective cytotoxins capable of directly blocking DNA transcription into mRNA. Amatoxins, a set of macrocyclic peptides generated by mushrooms mainly by the genus *Amanita*, are the most well-known class of powerful and selective inhibitors of RNA polymerase II, which inhibit protein synthesis (Hallen, Luo, Scott-Craig, & Walton, 2007; Lindell, Weinberg, Morris, Roeder, & Rutter, 1970). Approximately nine naturally occurring amatoxins have been identified so far, two of which, α-amanitin and β-amanitin, account for approximately 90% of all amatoxins. In a study, β-amanitin was conjugated to a MUC1-targeting mAb, which showed specific cytotoxicity against T47D cells (Danielczyk et al., 2006). α-amanitin, a very water-soluble mushroom-derived octapeptide, is currently under investigation as an ADC payload in preclinical studies (Lindell et al., 1970). In a study, α-amanitin was effectively delivered to the cancer cells via an HER2-targeting mAb, showing IC50 values in a pM range (Dan et al., 2018). In a recently developed ADC, chiHEA125-Ama, α-amanitin has been conjugated to an EpCAM-targeting mAb, demonstrating potent in vitro and in vivo antiproliferative activities (Moldenhauer et al., 2012). More recently, an improvement has been observed in the in vivo antitumor efficiency of anti-PSMA-α-amanitin, when coupled through a stable and cleavable linker (Dan et al., 2018). Taken together, the main advantage for amatoxins, as a payload in ADCs, is their hydrophilic character than other cytotoxic payloads, yielding the following values: (a) the increased solubility and uniformity in aqueous conditions, facilitating the conjugation reaction; (b) a decrease in ADC aggregation, a phenomenon commonly seen with hydrophobic payloads; (c) low molecular weight of the released drug from disintegrated tumor cells, leading to low accumulation of the drug in other tissues but quick excretion in urine; and (d), most importantly, highly active in MDR cancer cells because of poor substrates for MDR processes (Anderl et al., 2013; Kim & Kim, 2015).

## 6 | PAYLOADS IN THE MARKET AND CLINICAL PIPELINES

ADC development has gained worldwide attention following the recent approval of two ADCs (Besponsa and re-approval of Mylotarg). In fact, we are in an age of "ADC boom" with a large number of novel and emerging ADCs under development or in clinical trials. Of important note, the increasing number of ADCs in the clinics, clinical trials, and research settings not only reflects the growing interest and confidence of physicians and pharmaceutical companies in the area, but also highlights the value and benefits that ADCs provide to cancer patients.

However, despite the considerable growth in ADC pipelines over the last 10 years, a significant portion of ADCs (approximately 30%) has been discontinued for various reasons, a limited number of ADCs have reach successful late stage trials, and only four ADCs have received FDA approval, all for oncological indications. The four FDA-approved ADCs currently used to treat cancer patients include gemtuzumab ozogamicin (Mylotarg), brentuximab vedotin (Adcetris), trastuzumab emtansine (Kadcyla), and inotuzumab ozogamicin (Besponsa) with calicheamicin, MMAE, DM1, and calicheamicin as

**TABLE 2** FDA-approved ADCs in the market

| Payload | Antibody–drug conjugate | Developer | Indication | Status |
|---|---|---|---|---|
| Calicheamicin | Gemtuzumab ozogamicin (Mylotarg) | Pfizer | CD33 + AML | Conditionally approved in 2000, withdrawn in 2010, and reintroduced into the US market in 2017 |
| | Inotuzumab ozogamicin (CMC-544; Besponsa) | Pfizer | ALL and CLL | Approved in 2017 by the European Commission and FDA (pre-registration) |
| MMAE | Brentuximab vedotin (SGN-35; Adcetris) | Seattle Genetics/ Takeda | ALCL and HL | Conditionally approved and entered market in 2011 |
| DM1 | Trastuzumab emtansine (Ado-trastuzumab Emtansine, T-DM1; Kadcyla) | Genentech/ Roche | HER2 + metastatic breast cancer | Fully approved and entered market in 2013 |

Abbreviations: ALL, acute lymphoblastic leukemia; ALCL, anaplastic large-cell lymphoma; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; DM1, thiol-containing maytansinoids; HL, Hodgkin's lymphoma; HER2, human epidermal growth factor receptor 2; MMAE, monomethyl auristatin E.

payloads, respectively (Table 2) (Abdollahpour-Alitappeh et al., 2019). All of the abovementioned FDA-approved ADCs were demonstrated to be effective in patients with cancer. Although withdrawn from the market because of concerns about safety and clinical outcomes, gemtuzumab ozogamicin, an anti-CD33-calicheamicin ADC, has been recently reintroduced into the US market in 2017. The re-approval of gemtuzumab ozogamicin has demonstrated that ADCs offer a clinically-validated opportunity for the treatment of patients with cancer.

Tables 3 and 4 summarize the ADCs in clinical trials, by focusing on their payloads, sponsors, therapeutic indications, and status. The data were retrieved from the clinical trials (https://clinicaltrials.gov). The number of ADCs in clinical trials is quickly growing, so that more than 100 different ADCs are currently in different phases of clinical trials (approximately more than 250) for the treatment of various cancers with more than 90 active ADCs (recruiting) under evaluation. As shown in Tables 3,4, major pharmaceutical companies worldwide contribute to the current flourishing number of ADCs in clinical studies, which raises a new hope in the battle against different types of cancers.

The majority of ADCs currently in clinical trials, although differing in the mAb moiety targeting various cancers, only employ a small number and common cytotoxic payloads, a great number of which target microtubules or DNA and have high anticancer activity (with an $IC_{50}$ value of around 0.1 to 0.001 nM). More than 70% of the ADCs in clinical trials utilize microtubule-targeting payloads and only 29 ADCs utilize DNA-disrupting agents in their structure. The majority of the ADC payloads currently in clinical trials make use of auristatins (MMAE and MMAF) and maytansinoids (DM1 and DM4) as a payload. The recent approval of brentuximab vedotin and trastuzumab emtansine shows that their payloads, an auristatin and a maytansinoid, respectively, fulfill the criteria required for an appropriate payload. The remaining payloads are based upon tubulysins, PBDs, duocarmycins, doxorubicins, calicheamicins, IGNs, and CPT-11 derivatives. Calicheamicin, as a DNA-disrupting agent, was the first payload used in gemtuzumab ozogamicin, the first-commercially available ADC.

Auristatins are potent microtubule-targeting agents which constitute a majority of cytotoxic currently investigated payloads used in ADCs. As mentioned above, MMAE and MMAF constitute the largest class of ADCs in clinical trials, followed by maytansinoids (DM1 and DM4) as the second largest one in clinical trials, which have been used successfully in the ADC development.

A great number of PBDs and PBD dimers have been developed for use in ADCs, some of which have been conjugated to different mAbs. Since 2013, more than 10 ADCs consisting of PBD dimmers as payloads have successfully entered clinical trials, making PBD molecules the third most important payloads after auristatins and maytansinoids. Though PBD molecules do not significantly interrupt the DNA structure, vital DNA functions, such as transcription and translation, are prevented through the formation of DNA-PBD adduct (Dan et al., 2018). There are two and three doxorubicin- and duocarmycin-ADCs tested in clinical trials, respectively. Five

**TABLE 3** Clinical pipeline of ADCs based on microtubule-targeting agents that have reached clinical trials

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| *Auristatins* | | | | | |
| MMAE | Glembatumumab vedotin (CDX-011) | Celldex Therapeutics | Metastatic GPNMB overexpressing TNBC | II (Completed) | NCT01997333 |
| | | | Advanced GPNMB expressing breast cancer | II (Completed) | NCT01156753 |
| | | National Cancer Institute (NCI) | Recurrent or refractory osteosarcoma | II (Completed) | NCT02487979 |
| | | | Metastatic or locally-recurrent uveal melanoma | II (Completed) | NCT02363283 |
| | | CuraGen Corporation | Unresectable Stage III or IV melanoma | I/II (Completed) | NCT00412828 |
| | | PrECOG, LLC. | Advanced or metastatic GPNMB expressing SCC of the lung | I/II (Terminated) | NCT02713828 |
| MMAE | CDX-014 | Celldex Therapeutics | RCC and OCCC | I (Terminated) | NCT02837991 |
| MMAE | PSMA ADC | Progenics Pharmaceuticals, Inc. | mCRPC | II (Completed) | NCT02020135 |
| | | | | | NCT01695044 |
| | | | Progressive mCRPC | I (Completed) | NCT01414283 |
| | | | Progressive CMPC | I (Completed) | NCT01414296 |
| | | Heinrich Elinzano, MD | Advanced brain tumors (GBM and GS) | II (Completed) | NCT01856933 |
| MMAE | Polatuzumab Vedotin (RG7596, DCDS4501A) | Hoffmann-La Roche | DLBCL | III (Recruiting) | NCT03274492 |
| | | | r/r FL or DLBCL | I/II (Recruiting) | NCT02257567 |
| | | | | I (Recruiting) | NCT02611323 |
| | | | | I (Recruiting) | NCT02600897 |
| | | | r/r FL | I/II (Active, not recruiting) | NCT02729896 |
| | | | B-NHL | I/II (Recruiting) | NCT03677141 |
| | | | | I (Recruiting) | NCT03671018 |
| | | Genentech, Inc. | B-NHL | I/II (Completed) | NCT01992653 |
| | | | r/r B-NHL and CLL | I (Completed) | NCT01290549 |
| | | | r/r FL and r/r DLBCL | I/II (Active, not recruiting) | NCT01691898 |
| MMAE | RG7841 (DLYE5953A) | Genentech, Inc. | Incurable, locally advanced, or metastatic solid tumors | I (Completed) | NCT02092792 |
| MMAE | RG7882 (DMUC4064A) | Genentech, Inc. | PROC and unresectable pancreatic cancer | I (Completed) | NCT02146313 |
| MMAE | RG7986 (DCDS0780A) | Hoffmann-La Roche | r/r B-NHL | I (Active, not recruiting) | NCT02453087 |
| MMAE | Ladiratuzumab Vedotin (SGN-LIV1A) | Seattle Genetics, Inc. | Locally advanced or metastatic TNBC | I/II (Recruiting) | NCT03310957 |

(Continues)

**TABLE 3** (Continued)

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| | | | Metastatic breast cancer | I (Recruiting) | NCT01969643 |
| | | QuantumLeap Healthcare Collaborative | Breast cancer | II (Recruiting) | NCT01042379 |
| | | Hoffmann-La Roche | Metastatic or inoperable locally advanced TNBC | I/II (Recruiting) | NCT03424005 |
| MMAE | Enfortumab Vedotin (ASG-22ME; ASG-22CE; [formerly AGS-22M6E]) | Astellas Pharma Global Development, Inc. | Previously treated locally advanced or metastatic UC | III (Recruiting) | NCT03474107 |
| | | | Locally advanced or metastatic urothelial bladder cancer | II (Recruiting) | NCT03219333 |
| | | | Urothelial bladder cancer | I (Recruiting) | NCT03288545 |
| | | | Metastatic UC and other nectin-4 expressing solid tumors | I (Recruiting) | NCT02091999 |
| | | Astellas Pharma Inc | Nectin-4 expressing solid tumors | I (Completed) | NCT01409135 |
| | | | Locally advanced or metastatic UC | I (Active, not recruiting) | NCT03070990 |
| MMAE | ASG-5ME | Astellas Pharma Inc | CRPC | I (Completed) | NCT01228760 |
| | | Seattle Genetics, Inc. | Pancreatic adenocarcinoma or GC | I (Completed) | NCT01166490 |
| MMAE | AGS15E (ASG-15ME) | Astellas Pharma Global Development, Inc. | Metastatic UC | I (Active, not recruiting) | NCT01963052 |
| MMAE | Bay 79-4620 (3ee9-IC, CA9-ADC, or CAIX-ADC) | Bayer | Advanced solid tumors | I (Completed) | NCT01028755 |
| | | | | I (Terminated) | NCT01065623 |
| MMAE | AGS67E | Astellas Pharma Global Development, Inc. | r/r Lymphoid malignancies | I (Active, not recruiting) | NCT02175433 |
| | | | AML | I (Terminated) | NCT02610062 |
| MMAE | CX-2029 | CytomX Therapeutics | Metastatic or locally advanced unresectable solid tumors or DLBCL | I/II (Recruiting) | NCT03543813 |
| MMAE | Telisotuzumab vedotin (ABBV-399) | AbbVie | Previously treated c-Met positive NSCLC | II (Recruiting) | NCT03539536 |
| | | | Advanced solid tumors | I (Recruiting) | NCT02099058 |
| | | Southwest Oncology Group | c-MET positive lung SCC, c-MET positive squamous cell tumors | II (Recruiting) | NCT03574753 |
| MMAE | Azintuxizumab vedotin (ABBV-838) | AbbVie | r/r MM | I (Terminated) | NCT02462525 |
| MMAE | ABBV-221 | AbbVie | EGFR overexpressing advanced solid tumors | I (Terminated) | NCT02365662 |
| MMAE | ABBV-085 (M15–394) | AbbVie | Advanced solid tumors | I (Active, not recruiting) | NCT02565758 |
| MMAE | Tisotumab vedotin (HuMax-TF-ADC) | Genmab | Solid tumors (including PC, and ovary, cervix, endometrium, bladder, esophagus, and lung cancers) | I/II (Completed) | NCT02552121 |

(Continues)

**TABLE 3** (Continued)

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| | | | Previously treated, recurrent, or metastatic cervical cancer | II (recruiting) | NCT03438396 |
| | | | Solid tumors (including ovary, cervix, endometrium, bladder, and esophagus cancers, as well as PC, NSCLC, and HNSCC) | II (recruiting) | NCT03245736 |
| | | | Recurrent or Stage IVB cervical cancer | I/II (recruiting) | NCT03786081 |
| | | | Solid tumors (including CRPC, NSCLC, HNSCC, and ovary, cervix, endometrium, bladder, and esophagus cancers) | I/II (Active, not recruiting) | NCT02001623 |
| | | Seattle Genetics, Inc. | Solid tumors (including CRC, NSCLC, exocrine pancreatic cancer, and HNSCC | II (recruiting) | NCT03485209 |
| | | | PROC, fallopian tube cancer, and peritoneal cancer | II (Recruiting) | NCT03657043 |
| MMAE | Enapotamab vedotin (HuMax-Axl-ADC) | Genmab | Multiple solid tumors (including NSCLC, melanoma, sarcoma, and ovarian, cervical, endometrial, and thyroid cancers | I /II (Recruiting) | NCT02988817 |
| MMAE | BA3011 (CAB-AXL-ADC) | BioAtla, LLC | Advanced solid tumors (including NSCLC, melanoma, and pancreatic Cancer) | I /II (Recruiting) | NCT03425279 |
| MMAE | RC48-ADC (hertuzumab-vc-MMAE) | RemeGen | HER2 positive advanced breast cancer | I/II (Recruiting) | NCT03052634 |
| | | | | I (Recruiting) | NCT02881138 |
| | | | | I (Unknown) | NCT02881190 |
| | | | HER2 overexpressing locally advanced or metastatic UC | II (Recruiting) | NCT03809013 |
| | | | HER2 positive metastatic or unresectable UC | II (Recruiting) | NCT03507166 |
| | | | HER2 overexpressing locally advanced or metastatic GC | II (Recruiting) | NCT03556345 |
| | | | HER2 positive metastatic breast cancer | II (Recruiting) | NCT03500380 |
| MMAE | Pinatuzumab vedotin (RG7593 or DCDT2980S) | Genentech, Inc. | r/r FL and r/r DLBCL | I/II (Active, not recruiting) | NCT01691898 |
| | | | r/r B-NHL and CLL | I (Completed) | NCT01209130 |
| MMAE | Lifastuzumab vedotin (RG7599 or DNIB0600A) | Genentech, Inc. | PSOC or non-squamous NSCLC | I (Completed) | NCT01995188 |
| | | | NSCLC and PROC | I (Completed) | NCT01363947 |
| | | | PROC, OC, primary peritoneal, and fallopian tube cancers | II (Terminated) | NCT01991210 |
| MMAE | Indusatumab vedotin (MLN-0264 or TAK-264) | Millennium Pharmaceuticals, Inc. | Advanced or metastatic GCC positive pancreatic cancer | II (Terminated) | NCT02202785 |
| | | | Stomach or gastroesophageal junction cancers | II (Terminated) | NCT02202759 |
| | | | Advanced GI carcinoma, or metastatic or recurrent GCC expressing gastroesophageal junction cancer or GC | I (Terminated) | NCT02391038 |
| | | | Advanced GCC expressing GI malignancies and | I (Completed) | NCT01577758 |

(Continues)

**TABLE 3** (Continued)

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| MMAE | SGN-CD48A | Seattle Genetics, Inc. | MM | I (Recruiting) | NCT03379584 |
| MMAE | Vandortuzumab vedotin (RG7450 or DSTP3086S) | Genentech, Inc. | mCRPC | I (Completed) | NCT01283373 |
| MMAE | Sofituzumab vedotin (RG7458 or DMUC5754A) | Genentech, Inc. | PROC or unresectable pancreatic cancer | I (Completed) | NCT01335958 |
| MMAE | RG7600 (DMOT4039A) | Genentech, Inc. | PROC or unresectable pancreatic cancer | I (Completed) | NCT01469793 |
| MMAE | RG7636 (DEDN6526A) | Genentech, Inc. | metastatic or unresectable melanoma | I (Completed) | NCT01522664 |
| MMAE | ALT-P7 (HM2-MMAE) | Alteogen, Inc. | HER2 positive metastatic breast cancer | I (Enrolling by invitation) | NCT03281824 |
| MMAF | GSK2857916 (J6M0-mcMMAF) | GlaxoSmithKline | r/r MM | II (Recruiting) | NCT03525678 |
| | | | | II (Recruiting) | NCT03544281 |
| | | | | I (Not yet recruiting) | NCT03828292 |
| | | | r/r MM or BCMA-expressing hematologic malignancies | I (Active, not recruiting) | NCT02064387 |
| | | Myeloma Canada Research Network | r/r MM | I/II (Not yet recruiting) | NCT03715478 |
| MMAF | Denintuzumab mafodotin (SGN-CD19A) | Seattle Genetics, Inc. | r/r B-ALL, B-LBL, and Burkitt lymphoma or leukemia | I (Completed) | NCT01786096 |
| | | | r/r B-NHL | I (Completed) | NCT01786135 |
| | | | DLBCL or grade 3b FL | II (Terminated) | NCT02855359 |
| | | | r/r DLBCL or grade 3b FL | II (Terminated) | NCT02592876 |
| MMAF | Depatuxizumab mafodotin (ABT-414) | AbbVie (prior sponsor, Abbott) | Squamous cell tumors | I (Completed) | NCT01741727 |
| | | AbbVie | GBM | I (Completed) | NCT01800695 |
| | | | | III (Recruiting) | NCT03419403 |
| | | | Newly-diagnosed EGFR amplified GBM and GS | III (Recruiting) | NCT02573324 |
| | | | GBM (in children) | II (Recruiting) | NCT02343406 |
| | | | Newly-diagnosed and recurrent Grade III or IV malignant glioma and GBM | I/II (Recruiting) | NCT02590263 |
| MMAF | AGS-16C3F | Agensys, Inc. | RCC | I (Completed) | NCT01672775 |
| | | Astellas Pharma Global Development, Inc. | Metastatic RCC | II (Active, not recruiting) | NCT02639182 |
| MMAF | | Astellas Pharma Inc | RCC | I (Completed) | NCT01114230 |

(Continues)

**TABLE 3** (Continued)

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| | AGS-16M8F (the hybridoma version of AGS 16C3F) | | | | |
| MMAF | Vorsetuzumab mafodotin (SGN-75) | Seattle Genetics, Inc. | CD70 positive r/r NHL or metastatic RCC | I (Completed) | NCT01015911 |
| | | | CD70 positive metastatic RCC | I (Terminated) | NCT01677390 |
| MMAF | MEDI-547 | MedImmune LLC | r/r solid tumors | I (Terminated) | NCT00796055 |
| MMAF | PF-06263507 (ADC 5T4) | Pfizer | advanced solid tumors | I (Terminated) | NCT01891669 |
| *Navel auristatins* | | | | | |
| Auristatin F | XMT-1522 | Mersana Therapeutics | Advanced HER2- expressing breast cancer, advanced HER2-positive NSCLC, and GC | I (Active, not recruiting) | NCT02952729 |
| | | | PROC, NSCLC, metastatic papillary RCC, papillary thyroid cancer, and salivary gland cancer | I (Recruiting) | NCT03319628 |
| Auristatin F is a slightly modified MMAF | | | | | |
| Amberstatin269 | ARX788 | Zhejiang Medicine Co., Ltd. | Advanced HER2 expressing breast cancer and GC | I (Recruiting) | NCT02512237 |
| | | Ambrx, Inc. | HER2 positive breast and stomach cancers | I (Recruiting) | NCT03255070 |
| Amberstatin269, also known as AS269, is an MMAF derivative serving as a potent cytotoxic tubulin inhibitor | | | | | |
| AGD-0182 | AGS62P1 | Astellas Pharma Global Development, Inc. | AML | I (Recruiting) | NCT02864290 |
| AGD-0182 is a synthetic analog of the naturally occurring tubulin-binding molecule Dolastatin 10 | | | | | |
| Aur0101 | PF-06647020 | Pfizer | Advanced solid tumors | I (Recruiting) | NCT02222922 |
| | | Kathy Miller | Metastatic TNBC | I (Recruiting) | NCT03243331 |
| Auristatin W | Lupartumab amadotin (BAY1129980) | Bayer | Neoplasms | I (Terminated) | NCT02134197 |
| Auristatin W | Aprutumab ixadotin (BAY1187982) | Bayer | FGFR2 positive advanced solid tumors | I (Terminated) | NCT02368951 |
| Auristatin derivative | PF-06650808 | Pfizer | Advanced solid tumors and breast cancer | I (Completed) | NCT02129205 |
| Auristatin derivative | W0101 | Pierre Fabre Medicament | Advanced or metastatic solid tumors | I/II (Recruiting) | NCT03316638 |
| PF06380101 | PF-06664178 (RN927C) | Pfizer | Advanced solid tumors | I (Terminated) | NCT02122146 |
| PF06380101 is a dolastatin 10 analog | | | | | |
| *Maytansinoids* | | | | | |
| DM1 | Naratuximab emtansine (IMGN529; Debio 1562) | Debiopharm International SA | r/r DLBCL and B-NHL | II (Recruiting) | NCT02564744 |
| | | ImmunoGen, Inc. | r/r NHL and CLL | I (Completed) | NCT01534715 |
| DM1 | Lorvotuzumab mertansine (IMGN901) | M.D. Anderson Cancer Center | Leukemia | II (Completed) | NCT02420873 |
| | | Children's Oncology Group | | II (Active, not recruiting) | NCT02452554 |

(Continues)

**TABLE 3** (Continued)

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| | | | r/r wilms tumor, rhabdomyosarcoma, neuroblastoma, pleuropulmonary blastoma, malignant peripheral nerve sheath tumor, or synovial sarcoma | | |
| | | ImmunoGen, Inc. | Advanced solid tumors and extensive stage SCLC | I/II (Terminated) | NCT01237678 |
| | | | MM | I (Completed) | NCT00991562 |
| DM1 | Cantuzumab mertansine (SB-408075; huC242-DM1) | ImmunoGen | CRC and solid tumors | I (Terminated) | ---------- |
| DM1 | Laprituximab emtansine (IMGN289) | ImmunoGen, Inc. | Advanced EGFR positive solid tumors | I (Terminated) | NCT01963715 |
| DM1 | Bivatuzumab mertansine | Boehringer Ingelheim | Advanced HNSCC | I (Completed) | NCT02254018 |
| | | | CD44v6 positive metastatic breast cancer | I (Completed) | NCT02254005 |
| | | | CD44v6 positive recurrent or metastatic breast cancer | I (Terminated) | NCT02254031 |
| | | | Advanced HNSCC or esophagus | I (Terminated) | NCT02254044 |
| DM1 | MLN2704 | Millennium Pharmaceuticals, Inc. | Metastatic androgen-independent PC | I/II (Completed) | NCT00070837 |
| | | | | I (Completed) | NCT00052000 |
| DM1 | AMG 172 | Amgen | Refractory kidney cancer | I (Completed) | NCT01497821 |
| DM1 | AMG 595 | Amgen | recurrent GBM and/or AA | I (Completed) | NCT01475006 |
| DM1 | LOP 628 | Novartis Pharmaceuticals | cKIT positive solid tumors and AML | I (Terminated) | NCT02221505 |
| DM4 | Mirvetuximab soravtansine (IMGN853) | ImmunoGen, Inc. | Platinum-resistant FRα positive advanced EOC, primary peritoneal cancer, and/or fallopian tube cancer | III (Active, not recruiting) | NCT02631876 |
| | | | EOC, primary peritoneal cancer, and fallopian tube cancer | Ib/II (Recruiting) | NCT02606305 |
| | | | OC and other FOLR1 positive solid tumors | I (Active, not recruiting) | NCT01609556 |
| | | Alessandro Santin | FRα positive endometrial cancer | II (Not yet recruiting) | NCT03832361 |
| | | M.D. Anderson Cancer Center | Localized TNBC | II (Recruiting) | NCT03106077 |
| | | City of Hope Medical Center | FRα positive recurrent OC, TNBC, and primary peritoneal, fallopian tube, and endometrial cancers | I (Recruiting) | NCT02996825 |
| | | Ohio State University Comprehensive Cancer Center | Recurrent OC, and endometrial, fallopian tube or primary peritoneal cancers | I (Recruiting) | NCT03552471 |
| DM4 | CX-2009 | CytomX Therapeutics | Metastatic or locally advanced unresectable solid tumors | I/II (Recruiting) | NCT03149549 |
| DM4 | Coltuximab ravtansine (SAR3419) | Sanofi | r/r DLBCL | II (Completed) | NCT01470456 |
| | | | | II (Completed) | NCT01472887 |

(Continues)

**TABLE 3** (Continued)

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| | | | r/r B-NHL | I (Completed) | NCT00549185 |
| | | | r/r CD19 positive B-NHL | I (Completed) | NCT00796731 |
| | | | ALL | II (Terminated) | NCT01440179 |
| DM4 | Indatuximab ravtansine (BT-062) | Biotest Pharmaceuticals Corporation | r/r MM | I/II (Completed) | NCT01001442 |
| | | | | I/II (Active, not recruiting) | NCT01638936 |
| | | | | I (Completed) | NCT00723359 |
| DM4 | Anetumab ravtansine (BAY 94–9343) | Stacey Stein | Pretreated mesothelin-expressing pancreatic cancer | II (Recruiting) | NCT03023722 |
| | | Bayer | Mesothelioma | II (Active, not recruiting) | NCT02610140 |
| | | | Advanced refractory solid tumors | I (Completed) | NCT02485119 |
| | | | Mesothelin expressing advanced solid tumors | I (Recruiting) | NCT03102320 |
| | | | Recurrent PROC | I (Recruiting) | NCT02751918 |
| | | | Advanced solid tumors | I (Active, not recruiting) | NCT01439152 |
| | | | Mesothelin-expressing solid tumors | I (Active, not recruiting) | NCT02639091 |
| | | | Advanced solid tumors with different degrees of hepatic or renal impairment | I (Active, not recruiting) | NCT02696642 |
| | | | Mesothelin-expressing advanced solid tumors | I (Active, not recruiting) | NCT02824042 |
| | | Mayo Clinic | Mesothelin positive advanced Stage III, IIIA,IIIB, and IV NSCLC | I/II (Recruiting) | NCT03455556 |
| | | National Cancer Institute (NCI) | Refractory OC, fallopian tube, or primary peritoneal cancers | II (Recruiting) | NCT03587311 |
| | | | Mesothelin positive pleural mesothelioma | I/II (Recruiting) | NCT03126630 |
| | | | Mesothelin positive advanced or recurrent Stage II, IIA, IIB, and III pancreatic cancer | I (Not yet recruiting) | NCT03816358 |
| | | | Mesothelin expressing lung adenocarcinoma | II (Terminated) | NCT02839681 |
| DM4 | SAR408701 | Sanofi | Advanced solid tumors (escalation phase) | I/II (Recruiting) | NCT02187848 |
| | | | Advanced malignant solid tumors | I (Active, not recruiting) | NCT03324113 |
| DM4 | SAR428926 | Sanofi | Advanced solid tumors | I (Completed) | NCT02575781 |
| DM4 | HKT288 | Novartis Pharmaceuticals | Solid tumors (including EOC and RCC) | I (Terminated) | NCT02947152 |

(Continues)

**TABLE 3** (Continued)

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| DM4 | SAR566658 | Sanofi | CA6 positive metastatic TNBC | II (Completed) | NCT02984683 |
| | | | CA6 positive and refractory solid tumors | I (Completed) | NCT01156870 |
| DM4 | AVE9633 | Sanofi | r/r CD33 positive AML | I (Terminated) | NCT00543972 |
| DM4 | BIIB015 | Biogen | r/r solid tumors | I (Completed) | NCT00674947 |
| DM4 | IMGN388 | ImmunoGen, Inc. | Solid tumors | I (Completed) | NCT00721669 |
| DM4 | Cantuzumab ravtansine (HuC242-DM4; IMGN242) | ImmunoGen, Inc. | Solid tumors (including non-CRC and pancreatic cancer | I (Completed) | NCT00352131 |
| DM4 | LY3076226 | Eli Lilly and Company | Advanced or metastatic cancers | I (Completed) | NCT02529553 |
| DM4 | BMS-986148 | Bristol-Myers Squibb | Advanced solid tumors (including mesothelioma, NSCLC, OC, GC, and pancreatic cancer) | I/II (Active, not recruiting) | NCT02341625 |
| | | | Advanced and/or metastatic solid tumors | I (Completed) | NCT02884726 |
| DM4 | MEN1309 | Menarini Group | CD205 positive metastatic solid tumors and r/r NHL | I (Recruiting) | NCT03403725 |
| *Tubulysins* | | | | | |
| AZ13599185 | MEDI4276 (AZ13599185-Trastuzumab) | MedImmune LLC | HER2 expressing breast cancer or stomach cancers, or GC | I (Completed) | NCT02576548 |
| *Undisclosed tubulin inhibitors* | | | | | |
| Tubulin inhibitor | AbGn-107 | AbGenomics B.V Taiwan Branch | Chemo-refractory locally advanced, recurrent or metastatic CRC, GC, and pancreatic or biliary cancers | I (Recruiting) | NCT02908451 |
| Tubulin inhibitor | STRO-002 | Sutro Biopharma, Inc. | OC and endometrial cancers | I (Not yet recruiting) | NCT03748186 |
| Tubulin inhibitor | STRO-001 | Sutro Biopharma, Inc. | Advanced B-Cell malignancies | I (Recruiting) | NCT03424603 |

Abbreviations: AA, anaplastic astrocytomas; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BCMA, B-cell maturation antigen; B-ALL, B-lineage acute lymphoblastic leukemia; B-LBL, B-lineage lymphoblastic lymphoma; B-NHL, B-cell non-Hodgkin lymphoma; CRC, colorectal cancer; CLL, chronic lymphocytic leukemia; CCRCC, clear cell renal cell carcinoma; CRPC, castrate-resistant prostate cancer; CMPC, castrate metastatic prostate cancer; DLBCL, diffuse large B-cell lymphoma; EOC, epithelial ovarian cancer; FR, folate receptor ; FL, follicular lymphoma; GC, gastric cancer; GCC, guanylyl cyclase C; GI, gastrointestinal; GPNMB, glycoprotein NMB; GBM, glioblastoma multiforme; GS, gliosarcoma; HNSCC, head and neck squamous cell carcinoma; HER2, human epidermal growth factor receptor 2; HL, Hodgkin's lymphoma; MM, multiple myeloma; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin F; mCRPC, metastatic castration-resistant prostate cancer; NHL, non-Hodgkin lymphoma; NSCLC, non-small-cell lung cancer; OC, ovarian cancer; OCCC, ovarian clear cell carcinoma; PC, prostate cancer; PROC, platinum-resistant ovarian cancer; PSOC, platinum-sensitive ovarian cancer; RCC, renal cell carcinoma; r/r, relapsed or refractory; SCC, squamous cell carcinoma; SCLC, small cell lung cancer; TNBC, triple negative breast cancer; UC, urothelial carcinoma.
In fact, due to the highly competitive nature of the ADC field, the chemical structure of payloads used in a great number of ADCs in early clinical trials was not disclosed.

**TABLE 4** Clinical pipeline of ADCs based on DNA-Damaging agents that have reached clinical trials

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| *Benzodiazepines (PBDs)* | | | | | |
| SGD1882 | Vadastuximab talirine (SGN-CD33A; 33 A) | Seattle Genetics, Inc. | Newly-diagnosed AML | III (Terminated) | NCT02785900 |
| | | | Relapsed chemoresistant AML | I/II (Terminated) | NCT02614560 |
| | | | MDS | I/II (Terminated) | NCT02706899 |
| | | | AML | I (Completed) | NCT01902329 |
| | | | | I (Completed) | NCT02326584 |
| SGD1882 | SGN-CD70A | Seattle Genetics, Inc. | RCC, MCL, DLBCL, and grade 3 FL | I (Completed) | NCT02216890 |
| SGD1882 | SGN-CD19B | Seattle Genetics, Inc. | r/r aggressive B-NHL subtypes of DLBCL and grade 3 FL | I (Terminated) | NCT02702141 |
| SGD1882 | SGN-CD123A | Seattle Genetics, Inc. | AML | I (Terminated) | NCT02848248 |
| SGD1882 | SGN-CD352A | Seattle Genetics, Inc. | r/r MM | I (Active, not recruiting) | NCT02954796 |
| SG3199 | Rovalpituzumab Tesirine (Rova-T; SC16LD6.5) | Stemcentrx | r/r DLL3 expressing SCLC | II (Completed) | NCT02674568 |
| | | | Recurrent SCLC | I/II (Completed) | NCT01901653 |
| | | | DLL3 expressing advanced solid tumors | I/II (Recruiting) | NCT02709889 |
| | | | SCLC | I (Completed) | NCT02874664 |
| | | | DLL3 expressing extensive stage SCLC | I (Recruiting) | NCT02819999 |
| | | AbbVie | Extensive stage SCLC | III (Recruiting) | NCT03033511 |
| | | | Advanced or metastatic DLL3 expressing SCLC | III (Active, not recruiting) | NCT03061812 |
| | | | Cancer | II (Enrolling by invitation) | NCT03543358 |
| | | | Advanced, recurrent SCLC | I (Completed) | NCT03086239 |
| | | | Advanced solid tumors | I (Recruiting) | NCT03000257 |
| | | | Extensive stage SCLC | I (Active, not recruiting) | NCT03026166 |
| SG3199 | Camidanlumab tesirine (ADCT-301, HuMax-TAC-ADC) | ADC Therapeutics S.A. | r/r CD25 positive AML or CD25 positive ALL | I (Completed) | NCT02588092 |
| | | | r/r HL and NHL | I (Recruiting) | NCT02432235 |
| | | | Advanced solid tumors | I (Recruiting) | NCT03621982 |
| SG3199 | Loncastuximab tesirine (ADCT-402) | ADC Therapeutics S.A. | r/r DLBCL | II (Recruiting) | NCT03589469 |
| | | | r/r B-ALL | I (Completed) | NCT02669264 |

(Continues)

**TABLE 4** (Continued)

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| | | | Advanced DLBCL or MCL | I (Recruiting) | NCT03684694 |
| | | | Advanced DLBCL, MCL, or FL | I (Recruiting) | NCT03685344 |
| | | | r/r B-NHL | I (Active, not recruiting) | NCT02669017 |
| SG3199 | MEDI3726 (previously known as ADCT-401) | MedImmune LLC | mCRPC | I (Recruiting) | NCT02991911 |
| PBD | SC-002 | Stemcentrx | Elapsed SCLC or LCNEC | I (Terminated) | NCT02500914 |
| PBD | SC-003 | Stemcentrx | PSOC | I (Terminated) | NCT02539719 |
| PBD | MEDI2228 | MedImmune LLC | r/r MM | I (Recruiting) | NCT03489525 |
| PBD | ADCT-502 | ADC Therapeutics S.A. | Advanced HER2 expressing solid tumors (including NSCLC, and breast, gastroesophageal and bladder cancers) | I (Terminated) | NCT03125200 |
| PBD | SJG-136 (NSC 694501) | National Cancer Institute (NCI) | EOC, and primary peritoneal or fallopian tube cancers | II (Terminated) | NCT01200797 |
| | | | Advanced solid tumors | I (Completed) | NCT00103220 |
| | | | Metastatic solid tumors | I (Completed) | NCT00121290 |
| | | | r/r acute leukemia, MDS, blastic phase CML, or CLL | I (Terminated) | NCT00301769 |
| *Indolino-benzodiazepines (IGNs)* | | | | | |
| DGN462 | IMGN779 | ImmunoGen, Inc. | r/r CD33 positive AML | I (Recruiting) | NCT02674763 |
| DGN462 is a novel DNA-alkylating agent consisting of a mono-imine moiety | | | | | |
| DGN549 | TAK-164 | Millennium Pharmaceuticals, Inc. | Advanced GCC positive GI cancers | I (Recruiting) | NCT03449030 |
| DGN549 | IMGN632 | ImmunoGen, Inc. | r/r CD123 positive ALL, AML, BPDCN, and other CD123 positive malignancies | I (Recruiting) | NCT03386513 |
| *Duocarmycins* | | | | | |
| seco-DUBA | Trastuzumab duocarmazine (trastuzumab vc-seco-DUBA; SYD985) | Synthon Biopharmaceuticals BV | HER2 positive locally advanced or metastatic breast cancer | III (Recruiting) | NCT03262935 |
| | | | Solid tumors | I (Active, not recruiting) | NCT02277717 |
| seco-DUBA | MGC018 | MacroGenics | Advanced solid tumors | I /II (Recruiting) | NCT03729596 |
| MED-A | BMS-936561 (MDX-1203, αCD70_MED-A) | Bristol-Myers Squibb | Advanced or recurrent CCRCC or r/r B-NHL | I (Completed) | NCT00944905 |
| *Doxorubicins* | | | | | |
| Doxorubicin | Milatuzumab doxorubicin (IMMU-110 or hLL1-DOX) | Immunomedics, Inc. | Recurrent MM | I /II (Completed) | NCT01101594 |

(Continues)

**TABLE 4** (Continued)

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| | | | Relapsed NHL and CLL | I/II (Unknown) | NCT01585688 |
| Doxorubicin | cBR96-doxorubicin immunoconjugate (cBR96-Dox, BMS-182248, or SGN-15) | Seattle Genetics, Inc. | Hormone refractory PC | II (Completed) | NCT00031187 |
| | | | Advanced NSCLC | II (Completed) | NCT00086333 |
| | | | NSCLC | II (Completed) | NCT00051571 |
| | | | Metastatic or recurrent breast cancer | II (Terminated) | NCT00028483 |
| | | | Advanced OC | II (Terminated) | NCT00051584 |
| *Calicheamicins* | | | | | |
| CM1 | PF-06647263 (anti-EFNA4-ADC) | Pfizer | Advanced solid tumors (including previously treated metastatic TNBC) | I (Completed) | NCT02078752 |
| CM1 | CMD-193 | Ludwig Institute for Cancer Research | Advanced Lewis-Y antigen expressing tumors | I (Completed) | NCT00293215 |
| | | Pfizer | Advanced solid tumors | I (Completed) | NCT00161642 |
| | | | | I (Terminated) | NCT00257881 |
| CM1 | CMB-401 (hCTM01-calicheamicin) | Pfizer | Recurrent platinum-sensitive EOC | I (Terminated) | – |
| *Camptothecin analogs* | | | | | |
| SN-38 | Sacituzumab govitecan (IMMU-132) | Immunomedics, Inc. | r/r TNBC | III (Recruiting) | NCT02574455 |
| | | | Metastatic UC | II (Recruiting) | NCT03547973 |
| | | | TNBC | II (Unknown) | NCT02161679 |
| | | | Advanced epithelial cancer | I/II (Active, not recruiting) | NCT01631552 |
| | | University of Wisconsin, Madison | mCRPC | II (Recruiting) | NCT03725761 |
| SN-38 is the active metabolite of irinotecan prodrug | | | | | |
| SN-38 | Labetuzumab govitecan (IMMU-130) | Immunomedics, Inc. | Metastatic CRC | II (Unknown) | NCT01915472 |
| | | | | I/II (Active, not Recruiting) | NCT01605318 |
| | | | | I (Completed) | NCT01270698 |
| DX-8951 derivative | Trastuzumab Deruxtecan (DS-8201a) | Daiichi Sankyo, Inc. | HER2 positive, unresectable and/or metastatic breast cancer | III (Recruiting) | NCT03529110 |
| | | | Breast cancer | III (Recruiting) | NCT03523585 |
| | | | HER2 low-expressing breast cancer | III (Recruiting) | NCT03734029 |
| | | | HER2 overexpressing and/or HER2 mutated advanced NSCLC | II (Recruiting) | NCT03505710 |

(Continues)

**TABLE 4** (Continued)

| Payload | Antibody–drug conjugate | Sponsor | Conditions | Phase (status) | ClinicalTrials.gov identifier |
|---|---|---|---|---|---|
| | | | HER2 expressing breast cancer and UC | I (Recruiting) | NCT03523572 |
| | | | HER2 expressing advanced CRC | II (Recruiting) | NCT03384940 |
| | | | HER2 overexpressing advanced gastroesophageal junction adenocarcinoma or GC | II (Recruiting) | NCT03329690 |
| | | | HER2 positive breast cancer | II (Active, not recruiting) | NCT03248492 |
| | | | | I (Active, not recruiting) | NCT03366428 |
| | | | Her2 positive stomach and breast cancers | I (Active, not recruiting) | NCT03368196 |
| | | | Advanced solid tumors | I (Active, not recruiting) | NCT03383692 |
| | | | | I (Active, not recruiting) | NCT02564900 |
| DX-8951 derivative is an exatecan derivative (DXd) | | | | | |
| DX-8951 derivative | U3–1402 | Daiichi Sankyo, Inc. | HER3 positive metastatic breast cancer | I/II (Recruiting) | NCT02980341 |
| | | | Metastatic or unresectable EGFR mutant NSCLC | I (Recruiting) | NCT03260491 |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; B-ALL, B-lineage acute lymphoblastic leukemia; BPDCN, blastic plasmacytoid dendritic cell neoplasm ; B-LBL, B-lineage lymphoblastic lymphoma; BCMA, B-cell maturation antigen; B-NHL, B-cell non-Hodgkin lymphoma; CML, chronic myeloid leukemia; CLL, chronic lymphocytic leukemia; CCRCC, clear cell renal cell carcinoma; CRC, colorectal cancer; DLL3, delta-like protein 3; DLBCL, diffuse large B-cell lymphoma; EOC, epithelial ovarian cancer; FL, follicular lymphoma; FR, folate receptor; HER2, human epidermal growth factor receptor 2; HL, Hodgkin's lymphoma; GC, gastric cancer; GI, gastrointestinal; GPNMB, glycoprotein NMB; GCC, guanylyl cyclase C; LCNEC, large-cell neuroendocrine carcinoma; mCRPC, metastatic castration-resistant prostate cancer; MM, multiple myeloma; MDS, myelodysplastic syndrome; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; NSCLC, non-small-cell lung cancer; OC, ovarian cancer; PSOC, platinum-sensitive ovarian cancer; PBD, pyrrolobenzodiazepine; RCC, renal cell carcinoma; r/r, relapsed or refractory; SCLC, small cell lung cancer; seco-DUBA, seco-duocarmycin-hydroxybenzamide-azaindole; TNBC, triple negative breast cancer; UC, urothelial carcinoma.

In fact, due to the highly competitive nature of the ADC field, the chemical structure of payloads used in a great number of ADCs in early clinical trials was not disclosed.

ADCs utilizing calicheamicin as their payload are currently being evaluated in clinical studies, two of which have been approved by the FDA. Developments described in the clinical trials hold great promise for designing more diverse ADCs with significantly more successful clinical pipelines.

## 7 | CONCLUSIONS AND FUTURE DIRECTIONS

ADCs, as an attractive strategy to circumvent the limitations of a single-agent therapy, lead to preferential delivery of cytotoxic agents into targeted cancer cells, thereby decreasing cellular cytotoxicity to healthy tissues. Early ADCs used a variety of anticancer drugs, due to their ready accessibility and well-known toxicological characteristics; however, it soon turned out that ADCs with such chemotherapeutic agents, as a drug moiety, lack sufficient antitumor activity to be useful for clinical application. Tremendous efforts over the past 10 years have then been focused on exploring the use of highly potent molecules for ADC synthesis. This, in turn, leads to generation of effective second- and third-generation ADCs that take benefit of highly potent payloads. Currently, most of the ADCs utilize two families of highly toxic compounds as their payload moiety: microtubule-targeting agents and DNA-damaging agents. Tubulin inhibitors, such as auristatins and maytansines, are being widely used as ADC payloads for ADC development. Such payloads selectively target rapidly dividing cancer cells and are less susceptible to nondividing normal cells. Alternatively, DNA-damaging agents, such as calicheamicins and PBDs, have the ability to cause apoptosis in all cells, even in CSCs, but more likely have far more side effects.

However, despite advances in payload potency, the list of acceptable payloads for application in ADC architecture has not increased. Indeed, there are currently only a limited number of highly cytotoxic natural compounds, derivatives, or synthetic analogs with the potential to be used as a payload in the ADC architecture and to progress to the clinic. The absence of payload diversity in the clinical studies might explain the reason for dramatically high clinical failure rates.

Not surprisingly, in the next-generation ADCs, it is projected that the number of novel highly potent payloads with various mechanisms of action, greater therapeutic efficacy, and fewer side effects rises in the upcoming years for use in ADC architecture. Modern and emerging medicinal chemistry can potentially help biological scientists develop the next generation of ADC payloads with picomolar–-femtomolar toxicity against a wide range of cancer cells. However, challenges and difficulties involved in finding new drugs to be suited as ADC payloads should be considered, including higher potency, solubility, stability and hydrophilicity, as well as suitable activity against drug-resistant tumors and nontoxicity to normal cells/tissues.

Such new payloads with various mechanisms of action can overcome resistance to currently used drugs. In addition, hybrid payloads capable of targeting various binding sites on the tubulin molecule or targeting both DNA and microtubule simultaneously may circumvent ADC drug resistance. This is particularly important when next-generation ADCs are tailored to target solid tumors.

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## CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

## AUTHOR CONTRIBUTIONS

All the authors were involved in the conception and drafting of this review. In detail, S. Y. conceived and wrote this review. M. M-B., F. H., E. K., and M. K. researched, collected the data, and prepared the draft. M. L., T. G., and K. S S. retrieved data from the clinical trials (https://clinicaltrials.gov) and draw the figures. M. A-A., M. H K., and N. B. designed the study, reviewed, and edited the manuscript, and provided detailed feedback. All authors read and approved the final manuscript.

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