Antibody Therapeutics, 2018, Vol. 1, No. 2 ***43–53***
doi:10.1093/abt/tby007
Advance Access Publication on 30 August 2018

Review

DNA damaging agent-based antibody-drug conjugates for cancer therapy

Ying Fu*, and Mitchell Ho*

Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20892, USA

Received: June 1, 2018; Revised: August 15, 2018; Accepted: August 27, 2018

ABSTRACT

Currently, four antibody-drug conjugates (ADCs) are approved by the Food and Drug Administration or the European Medicine Agency to treat cancer patients. More than 60 ADCs are in clinical development for cancer therapy. More than 60% of ADCs in clinical trials employ microtubule inhibitors as their payloads. A better understanding of payloads other than microtubule inhibitors, especially DNA-damaging agents, is important for further development of ADCs. In this review, we highlight an emerging trend of using DNA-damaging agents as payloads for ADCs. This review summarizes recent advances in our understanding gained from ongoing clinical studies; it will help to define the utility of DNA-damaging payloads for ADCs as cancer therapeutics. Future directions of the development of ADCs are also discussed, focusing on targeting drug resistance and combination treatment with immunotherapy.

Statement of Significance: More than 60 antibody-drug conjugates (ADCs) are in clinical development, four are approved. This review summarizes recent advances in our understanding gained from ongoing clinical studies and highlights an emerging trend of using DNA-damaging agents as payloads for ADCs.

KEYWORDS: antibody-drug conjugate; DNA-damaging agent; cancer therapy; drug resistance

INTRODUCTION

The modern origin of antibody-drug conjugates (ADCs) dates back to the 'magic bullet' proposal from Paul Ehrlich in the 1900s [1]. The same concept was also documented in Chinese traditional herbal medicine 2000 years before Ehrlich's proposal. The Shennong (or Godly Farmer) reported a basic pharmacological philosophy: *Jun-Chen-Zuo-Shi* (emperor, minister, assistant and guide) indicating the first consideration of different functions within a prescription. The *jun* (emperor) treats the major symptoms. The *chen* (minister) serves to boost the effects of *jun* and relieves secondary symptoms. The *zuo* (assistant) helps in modulating the effects of *jun* and *chen*, and to counteract the toxic or side effects of the herbs. The *shi* (guide) ensures that all components are delivered to the target(s). By this definition, ADCs are *shi* (antibody)-*jun* (drug)-*zuo* (conjugates) (Fig. 1). In the simplest form, ADCs are comprised of a monoclonal antibody (mAb) linked to payloads (cytotoxic drugs). ADCs perfectly combine the high specificity of the antibodies with the strong potency of the payloads. The mechanism of action of ADCs can be summarized into three steps: (1) The ADCs recognize tumor antigen through antibody binding. (2) The target cell endocytoses the ADC-antigen complex. (3) The cytotoxic drug is released after lysosomal degradation of the ADC, which allows it to bind to its intracellular target (Fig. 1). In this way, payloads can be specifically delivered into target tumor cells by the antibody while minimizing undesired toxicity to normal cells [2].

To date, four ADCs are approved by the Food and Drug Administration (FDA) or the European Medicine Agency: gemtuzumab ozogamicin (developed by Wyeth/Pfizer), inotuzumab ozogamicin (developed by Wyeth/Pfizer), brentuximab vedotin ADCs (developed by Seattle Genetics) and trastuzumab emtansine (developed by Roche). Currently, there are more than 60 ADCs in clinical trials, almost all for oncological indications. ADCs have been discussed extensively on the design for the next generation (e.g. choice of antibody, linker, drug and conjugation strategy) [3–9]. In this review, we will focus on a summary of the historical development of ADCs against cancer, using DNA-damaging agent as their payloads, and discuss new ideas in the field that can be applied to those ADCs. We believe there

*To whom correspondence should be addressed. Ying Fu and Mitchell Ho. Tel: +01 240 760 7848; Email: ying.fu@nih.gov and homi@mail.nih.gov.

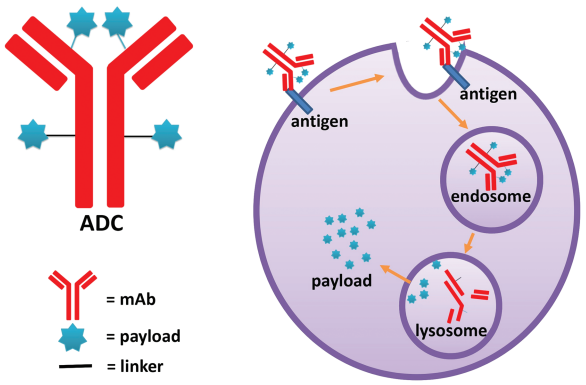

Figure 1. Proposed schematic of mechanism of action for ADCs: antigen binding, endocytosis, endosome formation, lysosome trafficking, lysosomal degradation and release of payload.

are two potential benefits of using DNA-damaging agent as the ADC payload: First, DNA-damaging agents (picomolar IC_{50} values) can provide higher potency than anti-microtubule payloads (sub-nanomolar IC_{50} values) and enable ADCs to target less abundant tumor antigens [10]. Secondly, they have the potential to kill non-dividing cancer stem cells in a combination with targeted agents directed against DNA repair effectors [11].

DNA-damaging agents as payloads of ADCs

One of the major hurdles for the development of the early ADCs (e.g. BR96-DOX) was the low potency of the payloads (e.g. doxorubicin only exhibits nanomolar activity *in vitro*) [1]. The current ADCs mainly employ two families of much more potent compounds as the payloads: microtubule inhibitors and DNA-damaging agents. Among the 60+ ADCs currently in clinical development, more than 60% utilize microtubule-targeting payloads (auristatins and maytansinoids); only 25 ADCs have DNA-damaging agent in their construct (Table 1). Microtubule-targeting agent-based ADCs have been reviewed elsewhere [12, 13]. There is a clear trend that the ADC field is moving towards treatments against solid tumors from liquid cancer [14–18]. We want to highlight that this trend may shift the choice of payload from microtubule-targeting agents to DNA-damaging agents, which usually exhibit higher potency.

Calicheamicins. Calicheamicin was identified in a search for new DNA-damaging agents in the 1980s. It was originally isolated from the bacterium *Micromonospora echinospora*. The calicheamicins were recognized as the most potent antitumor agents ever discovered [19]. Calicheamicin γ_1^I is the most promising member of this family, which is also used to construct ADCs. The mechanism of action is summarized in Figure 2A. In brief, the methyl trisulfide undergoes reductive bond cleavage by intracellular reducing components (e.g. glutathione). After spontaneous cyclization and Bergman cycloaromatization, 1,4-dehydrobenzene diradicals are generated, which subsequently form abstract hydrogen atoms from DNA, resulting in a double-strand diradical. In the presence of oxygen, DNA double strands are cleaved, followed by cell death. *N*-acetyl-calicheamicin γ_1^I was chosen as the payload of ADCs because it is more stable than the calicheamicin γ_1^I [20]. In total, there are five ADCs [Gemtuzumab ozogamicin/Mylotarg [21], Inotuzumab ozogamicin/CMC-544 [22], PF-06647263 [23], CMD-193 [24] and CMB-401 [25]] employing calicheamicin as their payload currently being tested in clinical trials; two are approved by the FDA. The most recent ADC advanced to clinical trial is PF-06647263, an anti-Ephrin-A4 ADC used to treat triple-negative breast cancer. The results of the phase I trial of PF-06647263 suggest that it is well tolerated, but no objective responses were observed in this trial [23].

Pyrrolobenzodiazepines. In the 1960s, pyrrolobenzodiazepine (PBD) monomers were isolated from Streptomyces bacteria. They were found to have highly potent antibiotic and antitumor activity. PBDs react through the N10-C11 imine/carbinolamine functionality with the amino group in C2 position of guanine residue to form a DNA adduct (Fig. 2B) [26]. To explore the sequence binding selectivity of PBD, Thurston *et al*. linked two PBDs to form a PBD dimer through a propyldioxy ether linker. It resulted in 600 times more potency *in vitro*, which makes the PBD dimer an attractive payload for ADCs. There are 13 ADCs [Vadastuximab talirine/SGN-CD33A [27–29],SGN-CD70A [30], SGN-CD19B, SGN-CD123A [31], SGN-CD352A [32], Rovalpituzumab tesirine/Rova-T [33–35], ADCT-301/HuMax-TAC-PBD [36], ADCT-402 [37], MEDI3726/ADC-401, IMGN779 [38], IMGN632 [39], SC-002 and SC-003] with PBD payloads currently being tested in clinical trials. There are two possible reasons that make PBDs the most prominent class of DNA-damaging payloads: (1) PBDs have picomolar activity *in vitro* and demonstrated therapeutic index in clinic [40]. (2) PBDs can avoid multi-drug resistance protein 1 (MDR1)-mediated drug resistance [41].

Duocarmycins. The duocarmycins were originally isolated from *Streptomyces zelensis* in the late 1970s. They consist of a series of three connected pyrroloindole subunits with one having an unprecedented spirocyclic cyclopropapyrroloindole (CPI) moiety. The mechanism of action is through the formation of DNA adducts (Fig. 2C): N3 of adenine attacks the cyclopropane moiety at the least substituted carbon atom; the alkylation ultimately leads to cell death [41]. The CPI moiety could be derivatized in its ring-open chloromethyl form in the phenolic state. This change allows the preparation of a variety of prodrugs that are currently used in ADCs. The structure was further modified by Boger and his colleagues to produce a more accessible version with equal potency and a more stable cyclopropabenzindole moiety [41]. There are two duocarmycin-ADCs [Trastuzumab duocarmazine/SYD985 [42] and BMS-936561/MDX-1203 [43]] tested in clinical trials.

Camptothecin analogues. Camptothecin was first isolated from the Chinese ornamental tree *Camptothecaacuminata* in the 1980s. It is a DNA topoisomerase I inhibitor with potent anticancer activity. Irinotecan (Camptothecin-11, CPT-11) is a semisynthetic analog of camptothecin that

Table 1. ADCs using DNA-damaging agents as payloads

Payload		ADC (developer)
Calicheamicin (ozogamicin)		Gemtuzumab ozogamicin/Mylotarg (Pfizer) Inotuzumab ozogamicin/CMC-544 (Pfizer) PF-06647263 (Pfizer/AbbVie) CMD-193 (Pfizer) CMB-401 (Pfizer)
Benzodiazepine (PBD)	Talirine	Vadastuximab talirine/SGN-CD33A (Seattle Genetics) SGN-CD70A (Seattle Genetics) SGN-CD19B (Seattle Genetics) SGN-CD123A (Seattle Genetics) SGN-CD352A (Seattle Genetics)
	Tesirine	Rovalpituzumab tesirine/Rova-T (AbbVie) Camidanlumab tesirine/ADCT-301 (ADC Therapeutics/Genmab) ADCT-402 (ADC Therapeutics) MEDI3726/ADC-401 (MedImmune)
	Indolinobenzodiazepines	IMGN779 (ImmunoGen) IMGN632 (ImmunoGen)
	NA	SC-002 (AbbVie) SC-003 (AbbVie)
Duocarmycin		Trastuzumab duocarmazine/SYD985 (Synthon) BMS-936561/MDX-1203(BMS)
Camptothecin analogues	SN38	Sacituzumab govitecan/IMMU-132 (Immunomedics) Labetuzumab govitecan/IMMU-130 (Immunomedics)
	DX-8951	DS-8201a (Daiichi Sankyo) U3-1402 (Daiichi Sankyo)
Doxorubicin		Milatuzumab doxorubicin/IMMU-110 (Immunomedics)

was approved by the FDA in 1996. The two analogues of camptothecin used as payloads for ADCs are SN38 and DX-8951f (also known as exatecan mesylate). SN38, the active metabolite of irinotecan, is approximately 1000 times more potent than its mother compound [44]. Compared to SN38, DX-8951f is a more water-soluble camptothecin analog [45]. An advantage is that DX-8951f is not an MDR1 substrate [46, 47]. Four ADCs [Sacituzumab govitecan/IMMU-132 [48], designated as a breakthrough therapy by the FDA; Labetuzumab govitecan/IMMU-130 [49]; DS-8201a [50] and U3-1402 [51]] that employed camptothecin analogues as their payload are being tested in clinical trials.

Doxorubicin. Doxorubicin (also known as Adriamycin) was originally discovered in *Streptomyces peucetius* in the 1970s. The mechanisms of action to damage DNA are mainly through DNA intercalation and generation of free radicals. Milatuzumab doxorubicin/IMMU-110 is the only ADC using doxorubicin as its payload tested in a phase I/II clinical trial, but was later discontinued [52].

Different ADCs employing DNA-damaging or anti-microtubule payload against the same antigen

Currently, 25 ADCs that use DNA-damaging agents as their payloads are either approved by the FDA (gemtuzumab ozogamicin/Mylotarg was withdrawn in 2010 but re-approved in 2017, inotuzumab ozogamicin was approved in 2017) or are being evaluated in clinical trials (Table 1). In this section, we summarize 10 ADCs using DNA-damaging payloads (2 out of 10 discontinued, 20%, Fig. 3) together with 13 other ADCs targeting the same antigens (5 out of 13 discontinued, 38%). It is not possible to conclude what the high discontinued rate of ADCs using anti-microtubule payload is because of the choice of the payload. However, we want to initiate this comparison, which requires additional time to follow the developmental process of the ADCs (Table 2). High competence is found in some 'star' targets such as human epidermal growth factor receptor 2 (HER2) and CD19.

HER2. HER2 is probably the most attractive target in the field of ADCs. Increased expression of HER2 is evident in 25–30% of breast cancers [53]. Patients with HER2-positive breast cancer have an aggressive form of the disease with significantly shortened cancer-free survival and overall survival [54]. Increased levels of HER2 have a direct role in the pathogenesis of these cancers, thereby a therapeutic agent directly targeted against HER2 can provide valuable benefit to those patients. Trastuzumab emtansine, anti-HER2 ADC (T-DM1) is the only approved ADC to target HER2 that employs an anti-microtubule agent (DM1) as its

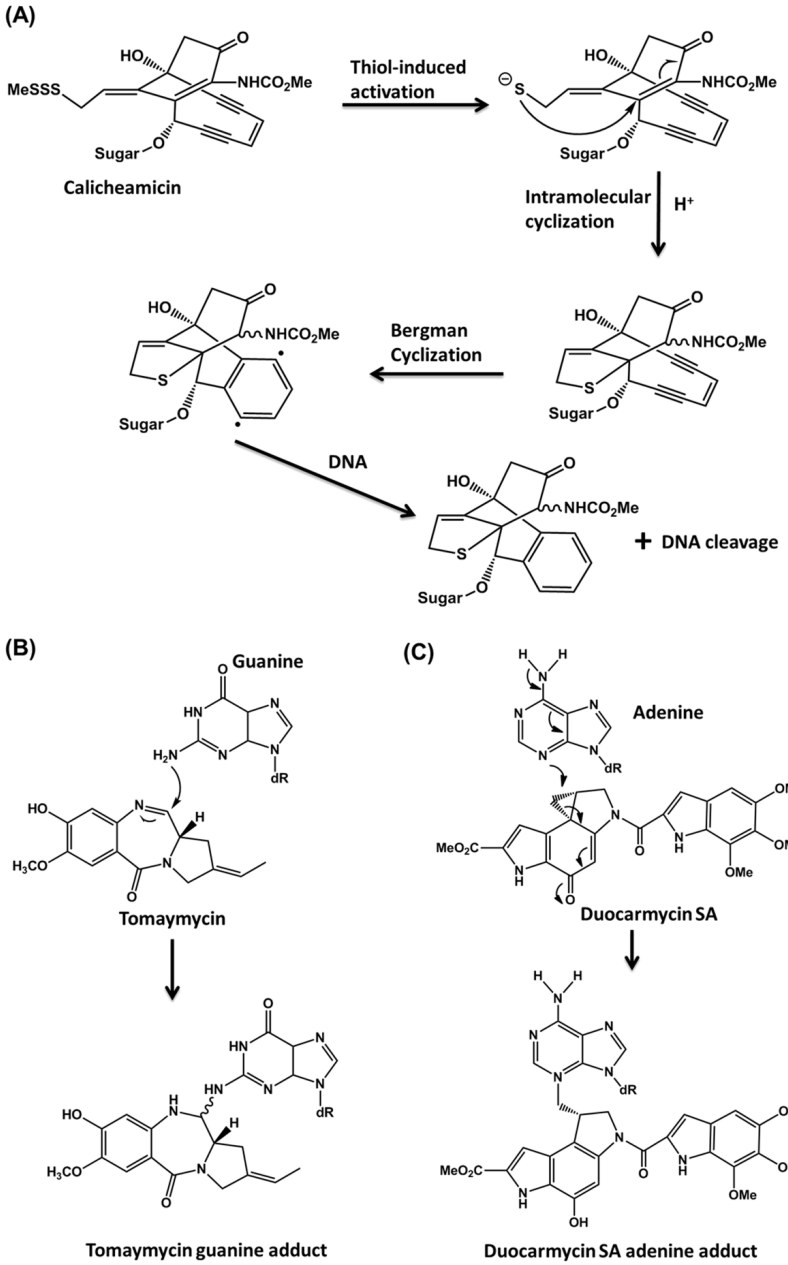

Figure 2. Mechanism of action for DNA-damaging agents used in ADCs. (A) Calicheamicin, (B) Pyrrolobenodiazepine monomer (Tomaymycin) and (C) Duocarmycin.

payload [55]. However, T-DM1 is still not effective enough to kill cancer cells expressing relatively low levels of HER2. Therefore, only ~20% of patients with breast cancer are eligible for HER2-targeted therapies, and a high relapse rate is observed in a majority of the patents with initial drug response, mainly because of intratumorally heterogeneous expression of HER2 [56]. Replacement of DM1 with a more potent duocarmycin payload (SYD985) is currently under clinical development to address the problem of the low eligibility proportion and high relapse rate of anti-HER2 ADC therapies [56, 57]. Different from T-DM1, SYD985 employs a cleavable linker that facilitates its bystander killing, especially against HER2-negative cancer cells [58]. DS-8201a is another promising anti-HER2 ADC equipped with DNA-damaging payload which has advanced to phase II clinical trial. Because SYD985 and DS-8201a show better efficacy than T-DM1 against breast cancer with low HER2 expression, there is optimism that they will be able to treat patients with low HER2 expression that cannot be treated by T-DM1 [47, 56, 57]. Regarding anti-HER2 ADCs with anti-microtubule payloads, two novel technologies are applied to generate XMT-1522 and MEDI4276, respectively. XMT-1522 is generated on the Dolaflexin ADC platform (polymer linker), which allows 12–15 auristatin payloads per antibody without harming the

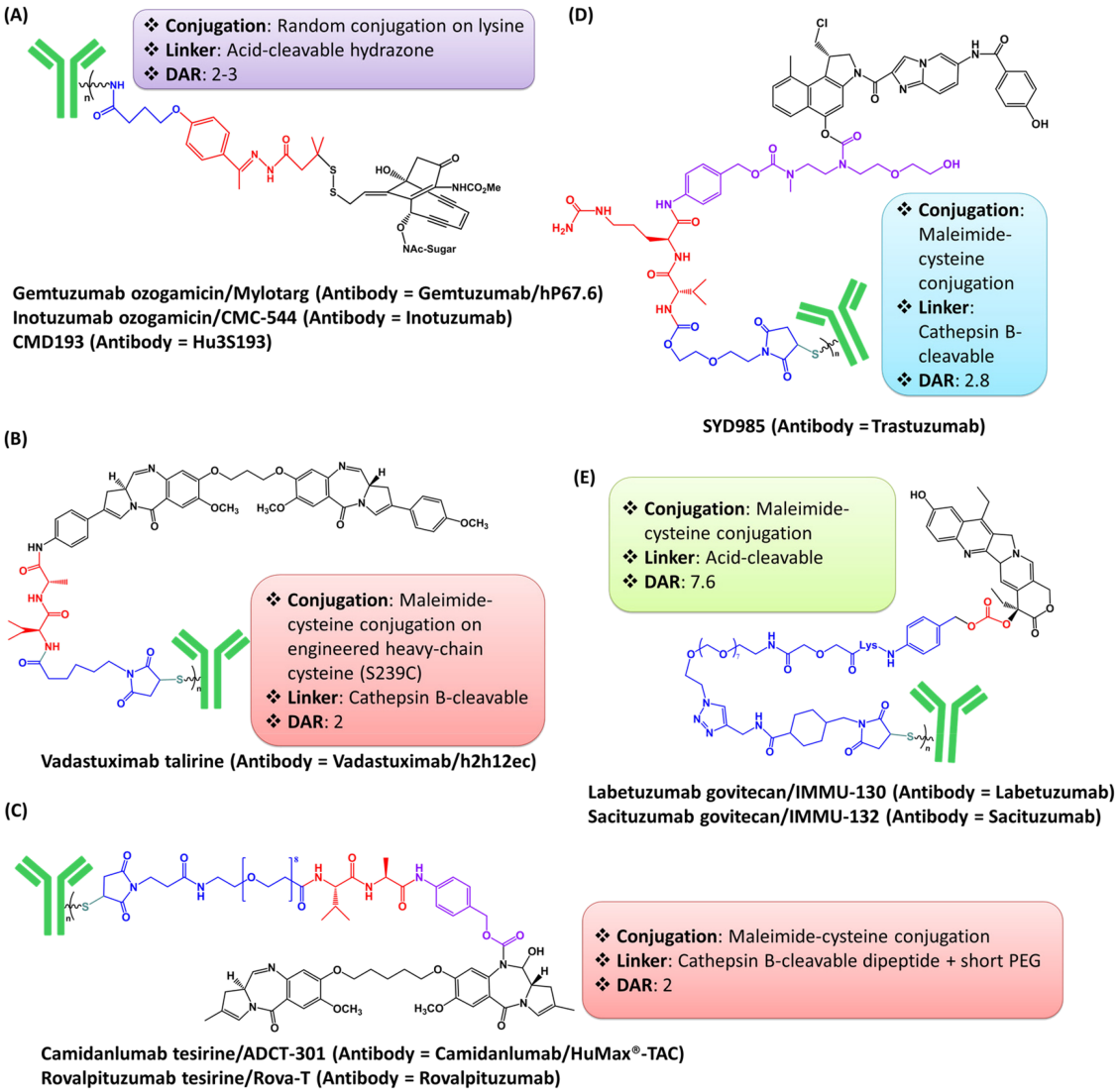

Figure 3. Representative structures of ADCs in clinical trials approved by the FDA using DNA-damaging agents as their payloads. (A) Gemtuzumab ozogamicin/Mylotarg, inotuzumab ozogamicin/CMC-544 and CMD193. (B) Vadastuximab talirine/SGN-CD33A. (C) Camidanlumab tesirine/ADCT-301, Rovalpituzumab tesirine/Rova-T. (D) SYD985. (E) Labetuzumab govitecan/IMMU-130, sacituzumab govitecan/IMMU-132. Color indication: (Blue) linker part conjugated on the antibody, (Red) cleavable linker section, (Pink) self-immolating group, (Black) payload of ADC.

pharmacokinetics [59]. MEDI4276 is a biparatopic ADC targeting two different epitopes on HER2 that induces robust internalization and better efficacy than T-DM1 *in vivo* [62, 63].

CD33. CD33 is a member of the immunoglobulin superfamily subset of sialic acid-binding immunoglobulin-related lectins. Two tyrosine residues are found in the cytoplasmic tail of CD33, which are phosphorylated upon pharmacological treatment (e.g. pervanadate) or receptor crosslinking. These phosphorylated tyrosine motifs are able to provide docking sites for recruitment and activation of Src homology-2 (SH2) domain-containing tyrosine phosphatases (SHP-1/2), which may result in transmission of inhibitory downstream signals, and affect the function of neighboring membrane receptors [60]. Approximately 85% of pediatric and adult acute myeloid leukemia (AML) cases are 'CD33$^+$', as defined by the presence of CD33 on greater than 20–25% of the leukemic blasts [61]. Two active anti-CD33 ADCs both use DNA-damaging agent as their payloads. Gemtuzumab ozogamicin was used to treat AML from 2000 to 2010. However, it was voluntarily withdrawn by Pfizer concerning its low response rate (RR) and high liver toxicity. In recent years, four randomized clinical trials have been completed and strongly support the efficacy of Gemtuzumab ozogamicin in newly diagnosed AML. Finally, the FDA approved Gemtuzumab ozogamicin for treating newly diagnosed AML in 2017 [62]. A second anti-CD33 ADC currently under investigation is IMGN779, which uses an indolinobenzodiazepine payload. IMGN779 has accomplished its phase I trial, the patients showed much better tolerance to IMGN779 [maximum tolerated dose (MTD) is larger than 0.7 mg/kg] than to vadastuximab talirine (MTD = 0.02 mg/kg; however, phase III trial was discontinued in 2017) [63].

CD70. CD70 is a member of the tumor necrosis factor superfamily transiently expressed on nascent antigen-activated T and B lymphocytes. The receptor for CD70 is CD27; CD70–CD27 interactions regulate T- and B-lymphocyte functions. High levels of CD70 expression are found in lymphomas such as renal cell carcinoma (RCC). Upregulated CD70 can directly induce T-cell death and exhaustion or indirectly promote regulatory T-cell proliferation to promote tumor immunotolerance. The functional diversity of CD70 may be one of the major reasons that all anti-CD70 ADCs are discontinued.

CD19. CD19 is the earliest differentiation antigen of the B cell lineage and is ubiquitously expressed on all types of B lymphocytes except plasma cells. It assembles with the antigen receptor of B lymphocytes in order to decrease the threshold for antigen receptor-dependent stimulation [64]. CD19 is an attractive target for B-cell non-Hodgkin lymphomas (NHL). Two anti-CD19 ADCs (SGN-CD19B and ADCT-402) using PBD dimer payload are currently in phase I clinical trials. ADCT-402 shows an encouraging complete RR of 34.3% so far, which is comparable to phase I data from denintuzumab mafodotin and coltuximab ravtansine (payloads = anti-microtubule agents) [65].

CD22. CD22 (Siglec-2) is expressed in a B-cell lineage-specific fashion, starting at the pre-B cell stage. CD22 is present on the surface of all stages of B cells, including activated B cells and memory B cells, but CD22 is lost after terminal differentiation into plasma cells. This broad expression profile during B-cell development has made CD22 an attractive target for leukemia. Inotuzumab ozogamicin was approved by the FDA in 2017. Similar to Mylotarg, it is composed of *N*-acetyl-calicheamicin γ_1^I conjugated to humanized IgG4 antibody [66]. Inotuzumab ozogamicin has significantly higher complete RR compared to standard chemotherapy (80.7% vs. 29.4%) [67]. Inotuzumab ozogamicin uses the same nonspecific conjugation chemistry as Mylotarg, which leads to a heterogeneous mixture of conjugate species. Different species potentially have differential stability, antigen-binding affinity and pharmacokinetics. The 2010 withdrawal of Mylotarg was believed largely due to the heterogeneous nature of the conjugate and the instability of the hydrazone linker, which leads to a narrow therapeutic window. However, it seems that the instability of hydrazone linker is antibody dependent. The same hydrazone linker is used in inotuzumab ozogamicin, in which good stability was found in human blood (~2%/day hydrolysis was found).

Carcinoembryonic antigen-related cell adhesion molecule 5. Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) belongs to the glycosylphosphatidylinositol-anchored carcinoembryonic antigen family [68]. CEACAM5 is overexpressed on a variety of solid tumors and is associated with adhesion and invasion. The first-in-human anti-CEACAM5 ADC, SAR408701 (payload = DM4), has advanced to phase II trial. In the phase I/II trial of labetuzumab govitecan (payload = SN38), 41 out of 86 patients showed stabilized disease [50, 73].

TROP2. Trop2 is a transmembrane glycoprotein associated with different types of cancers. Its function involves cancer cell proliferation, survival, self-renewal and invasion. Sacituzumab govitecan (IMMU-132, payload= SN38) is the only ADC currently under active clinical investigation against TROP2. Promising results were released in December 2017 showing sacituzumab govitecan has an objective RR at 31% in patients with triple negative breast cancer.

Linkers for DNA-damaging ADCs

The first generation of FDA-approved ADCs employed an acid-cleavable hydrazone linker, which is stable in blood at neutral pH, but hydrolyzed once the ADC is internalized into the acidic endosomes and lysosomes (pH = 4–6). The acid-labile hydrazone linker was found to be crucial for activity. It makes use of the low pH of endosomal and lysosomal compartments. The release of the unconjugated intermediate at pH = 4.5 is 97% after 24 h at 37°C, whereas only 6% was found at pH = 7.4 [66].

In comparison to calicheamicin-based ADCs, duocarmycin-, TOP inhibitors- and PBDs-based ADCs do not need a disulfide bond for their activity, which makes them feasible for cysteine conjugation. Conjugation to cysteine instead of lysine can significantly improve the homogeneity [69]. Under controlled reduction conditions, the interchain disulfide bond can be reduced and becomes available for excellent reactivity of maleimide-tagged payload. Regarding a typical IgG_1, four interchain disulfide bonds can be reduced to eight conjugation-available SH sites. Further purification using hydrophobic interaction chromatography can be used to narrow down the species this method generates. The dipeptide linker [valine-citrulline (vc) or valine-alanine (va)], which can be cleaved by cathepsin B (lysosomal protease), is employed in the duocarmycin- and PBD-based ADCs. New site-specific conjugation methods that have been reviewed recently include engineered mAbs (THIOMAB, enzyme directed, unnatural amino acid) and native mAbs to site-specific conjugate 2–8 payload molecules (glycan modification, functional re-bridging of native disulfides and conjugation of cysteines obtained from reducing native disulfides) [9, 75, 76].

Chemical [70], near-infrared light [71] and tumor microenvironment-triggered payload release strategies have also shown promising results. There is probably no universal conjugate-linkage strategy optimal for all antibody-drug conjugates. It is necessary that each pair of antibody/drug be optimized to obtain the best therapeutic outcome. New linkers can be designed to improve water solubility (e.g. PEGylation) and tumor selectivity (e.g. enzymatic-cleavable, photocleavable, proton cleavable) [72]. This will substantially reduce the heterogeneity of ADCs and toxicity. Based on current development of the ADCs using DNA-damaging agents, we summarize that the ideal properties for a linker are as follows: (1) stable during circulation and (2) cleavable after binding to the target or internalization.

Table 2. ADCs with DNA- and microtubule-targeting payload against the same antigens that have reached clinical trials

Antigen target	Lead indication (stage, ClinicalTrials.gov identifier)	ADC name (also known as another name)	Payload/Linker	MTD (mg/kg)
HER2	**Breast and gastric cancers (phase I, NCT02277717)**	**Trastuzumab duocarmazine (SYD985)**	**Duocarmycin/vc dipeptide**	**>2.4**
	Solid tumors (phase II, NCT02564900)	**DS-8201a***	**DX-8951/peptide**	**>6.4**
	Breast cancer (phase II, NCT03052634)	Hertuzumab-vc–MMAE (RC48-ADC)	MMAE/vc dipeptide	NA
	Breast and gastric cancers (phase I, NCT02512237)	ARX788	Amberstatin269/-	NA
	NSCLC, breast and gastric cancers (phase I, NCT02952729)	XMT-1522	Auristatin/Fleximer polymer	NA
	Solid tumors (phase I,NCT02576548)	MEDI4276	MMETA/maleimidocaproic acid	<0.9
	HER2+ metastatic breast cancer (approved by FDA in 2013)	Trastuzumab emtansine (Kadcyla, T-DM1)	DM1/SMCC	3.6
CD33	**AML (phase III, discontinued)**	**Vadastuximab talirine (SGN-CD33A)**	**Talirine/va dipeptide**	**0.02**
	AML (phase I, NCT02614560)	**IMGN779**	**Indolinobenzodiazepines/SPDB**	**>0.7**
	AML (approved by FDA in 2017)	**Gemtuzumab ozogamicin (Mylotarg)**	**Calicheamicin/hydrazone**	**>0.2**
	AML (phase I, discontinued)	AVE9633	DM4/SPDB	2-4
CD70	**Renal cancer (phase I, discontinued)**	**SGN-CD70A**	**Talirine/va dipeptide**	**NA**
	NHL and Renal cancer (phase I; discontinued)	Vorsetuzumab mafodotin (SGN-75)	MMAF/maleimidocaproic acid	3
	Renal cancer (phase I, discontinued)	AMG 172	DM1/SMCC	NA
CD19	**Relapsed NHL (phase I, NCT02702141)**	**SGN-CD19B**	**Talirine/va dipeptide**	**NA**
	B-ALL (phase I, NCT02669264)	**ADCT-402**	**Tesirine/va dipeptide**	**>0.12**
	B-NHL (phase II, NCT01786096)	Denintuzumab mafodotin (SGN-CD19A)	MMAF/maleimidocaproic acid	>6
	DLBCL (phase II, NCT01472887)	Coltuximab ravtansine (SAR3419)	DM4/SPDB	4.3
CD22	**ALL (approved by FDA in 2017)**	**Inotuzumab ozogamicin (CMC-544)**	**Calicheamicin/hydrazone**	**0.05**
	NHL (phase II; discontinued)	Pinatuzumab vedotin (RG7593 or DCDT2980S)	MMAE/vc dipeptide	>2.4
CEACAM5	**Metastatic colorectal cancer (phase II, NCT01915472)**	**Labetuzumab govitecan (IMMU-130)**	**SN38/CL2A**	**>16**
	Solid tumors (phase II,NCT02187848)	SAR408701	DM4/SPDB	NA
TROP2	**Triple negative breast cancer (phase III, NCT02574455)**	**Sacituzumab govitecan (IMMU-132)**	**SN38/CL2A**	**12**
	NSCLC, breast and ovarian cancers (phase I; discontinued)	PF-06664178 (RN927C)	Novel auristatin/vc dipeptide	<3.6

ADC, antibody–drug conjugate; ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; B-NHL, B-Cell Non-Hodgkin Lymphoma; CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; DLBCL, Diffuse large B-cell lymphoma; HER, human epidermal growth factor receptor; MMAE, monomethyl auristatin E; MTD, maximum tolerated dose, NHL, non-Hodgkin lymphoma; NSCLC, non-small-cell lung cancer; RCC, renal cell carcinoma; RR: response rate, SMCC, succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate; sulfo-SPDB, N-hydroxysuccinimidyl-4-(2-pyridyldithio)-2-sulfobutanoate; va, valine-alanine; vc, valine-citrulline, * FDA break through designation

Perspectives

In recent years, appreciable advances have been made against liquid tumor using ADCs, bispecific mAbs and chimeric antigen receptor (CAR) T-cell therapy [73–78]. More work on optimization of ADCs is needed to cure patients from solid tumors [79]. We believe that payload is an important consideration when designing the next generation of ADCs, especially against solid tumors [80]. Emerging new payloads have shown promising pre-clinical/clinical data. Here are a few examples: new tubulysin payloads [display pM potency [81–83] or retain potency against multidrug-resistant cancer cells [84]], PM050489 [one β-tubulin binder originally isolated from marine sponge [85]], new cryptophycin [a microtubule binder at the vinca site [86]], new CPIs [DNA alkylator that can generate toxic interstrand crosslinks [87]], D211 (an isoquinolidinobenzodiazepine payload that belongs to the PBD dimer family), kinesin spindle protein inhibitors [88], FGX-2-62 (pyridinobenzodiazepines, sequence-selective DNA mono-alkylating agent), bicyclic octapeptide amanitins (an RNA polymerase II inhibitor) [89], spliceostatins and thailanstatins (RNA spliceosome inhibitors) [90–92] and PNU-159682 (an anthracycline which is three orders of magnitude more potent than doxorubicin) [93].

Higher potency payloads (e.g. DNA-damaging payloads) will make it possible to target a tumor-specific/associated surface receptor at a relatively low expression level (less than 5000 receptors/cell). A more important criterion for the next generation of payloads is a higher therapeutic index than current payloads. To achieve this, it may require a shift from focusing on the mechanisms of action targeting the crucial survival pathways of cancer cells to other hallmarks of cancer: (1) reactivating growth suppressors, (2) inducing cell death, (3) blocking replicative immortality, (4) inhibiting angiogenesis and (5) hindering invasion and metastasis. The development of the next generation of ADC payloads also requires modern medicinal chemistry to achieve picomolar–femtomolar activity against targeted cells, which may require a combination of targeting two or several hallmarks of cancer.

Antibody engineering is another important consideration for the next generation of ADCs. In the 1990s, the 'binding site barrier' concept was introduced to illustrate that antibody binds to the target cell near the entry point after extravasation from blood capillaries, after which it further migrates to binding sites inside the tumor nodule [94]. High receptor density ($\sim 10^5$–10^6 receptors/cell) and high affinity antibodies ($\sim$nanomolar) can slow down the movement from the perivascular space. Mechanism-based generic pharmacokinetic/pharmacodynamic (PD) model is urgently needed to probe for potential solutions governing uniform penetration of an ADC in a solid tumor mass [95]. Therapeutic antibodies have been developed normally based on their binding to target cells. In order to improve ADC efficacy, new methods to screen for rapid internalizing antibodies by phage display technology have been explored [96]. To enhance solid tumor penetration ability, 3D-cancer cell culture can be readily utilized [96]. Bi-specific mAbs promote robust internalization [97] and allow a potential combination of ADC therapy and immunotherapy (e.g. CAR T-cell therapy). A biparatopic antibody shows improved internalization and is active to T-DM1 resistant cell lines [56]. Peptide-masked probody showed diminished antigen binding, but it can be activated by appropriate proteases from the tumor microenvironment [98]. This tumor-specific activation mechanism makes probody ADC with less systemic toxicity. Computational selection of cancer receptor targets for ADCs probably will play an important role in ADC design, although this method is still in an early stage of development.

Drug resistance limits the efficacy of cancer therapies. Tumor evolution or heterogeneity contributes to evasive mechanisms that limit durable responses [99]. Following recent approvals of ADCs, the following investigations on the resistance of ADCs potentially resulting in clinical treatment failure should be carried out in detail. Potential mechanism of resistance includes (1) escape by antigen-negative cancer cells or by limited surface antigen renewal, (2) hide by staying in resting (G_0) phase of cell cycle, (3) increased permeability glycoprotein (Pgp)-mediated drug efflux, (4) overexpression of drug efflux transporters such as multidrug resistance protein. (5) alternation of bypass of cell-death pathways, (6) increased anti-apoptotic effects of Bcl-2 and Bcl-X_L activity, (7) high circulating antigen to compromise ADC binding to cancer cells and (8) defects in ADC trafficking pathways. Recent work on anti-CD276/B7-H3 ADCs demonstrated replaying Monomethyl auristatin E (MMAE) payload with PBD dimer successfully overcomes the Pgp-mediated drug resistance in tumor-infiltrating vasculature cells [100]. To further address the drug resistance problem, models of acquired resistance to ADCs are needed. Recent studies have used different approaches: (1) continuous exposure of ADC at low dosage, followed by incremental dose increase and (2) cyclical treatment for a short duration at moderate to high dose. High-potent DNA-damaging agents may have the potential to remove those cells in resting (G_0) phase; however, this hypothesis still needs to be tested.

Using DNA-damaging agents as the payloads of ADCs has been well-established. Their safety/drug resistance clearly needs more investigation. Questions that need to be further addressed: (1) What are the mechanisms of ADC resistance?; (2) Can ADCs achieve targeting rare neoantigens in tumors?; (3) What are the suitable payloads for ADCs targeting immune cells? and (4) How do ADCs fit in combination treatments with immunotherapy?

AUTHOR CONTRIBUTIONS

Y.F. and M.H. contributed to the writing of the manuscript.

FUNDING

This research was funded by the Intramural Research Program of the National Institutes of Health (NIH), National Cancer Institute (NCI), Center for Cancer Research (Z01 BC 010891 and ZIA BC 010891 to M.H.) and the 2017 NCI Director's Intramural Innovation Award (Career Development Award to Y.F.). We thank the NIH and NCI Fellows Editorial Board for editorial assistance.

REFERENCES

1. Chari, RV, Miller, ML, Widdison, WC. Antibody-drug conjugates: an emerging concept in cancer therapy. *Angew Chem Int Ed Engl* 2014; 53: 3796–827.
2. Diamantis, N, Banerji, U. Antibody-drug conjugates—an emerging class of cancer treatment. *Br J Cancer* 2016; 114: 362–7.
3. Beck, A, Goetsch, L, Dumontet, C et al. Strategies and challenges for the next generation of antibody-drug conjugates. *Nat Rev Drug Discov* 2017; 16: 315–37.
4. Martin, C, Kizlik-Masson, C, Pelegrin, A et al. Antibody-drug conjugates: design and development for therapy and imaging in and beyond cancer, LabEx MAbImprove industrial workshop, July 27–28, 2017, Tours. *France. mAbs-Austin* 2018; 10: 210–21.
5. Joubert, N, Denevault-Sabourin, C, Bryden, F et al. Towards antibody-drug conjugates and prodrug strategies with extracellular stimuli-responsive drug delivery in the tumor microenvironment for cancer therapy. *Eur J Med Chem* 2017; 142: 393–415.
6. Sau, S, Alsaab, HO, Kashaw, SK et al. Advances in antibody-drug conjugates: a new era of targeted cancer therapy. *Drug Discov Today* 2017; 22: 1547–56.
7. Thomas, A, Teicher, BA, Hassan, R. Antibody-drug conjugates for cancer therapy. *Lancet Oncol* 2016; 17: e254–62.
8. Gerber, HP, Sapra, P, Loganzo, F et al. Combining antibody-drug conjugates and immune-mediated cancer therapy: what to expect? *Biochem Pharmacol* 2016; 102: 1–6.
9. Nanna, AR, Li, X, Walseng, E et al. Harnessing a catalytic lysine residue for the one-step preparation of homogeneous antibody-drug conjugates. *Nat Commun* 2017; 8: 1112.
10. Gijs, V, Beusker, P, Ubink, R et al. Toward clinical development of SYD985, a novel HER2-targeting antibody-drug conjugate (ADC). *J Clin Oncol* 2015; 32, 626–626
11. Maugeri-Sacca, M, Bartucci, M, De Maria, R. DNA damage repair pathways in cancer stem cells. *Mol Cancer Ther* 2012; 11: 1627–36.
12. Chen, H, Lin, Z, Arnst, KE et al. Tubulin inhibitor-based antibody-drug conjugates for cancer therapy. *Molecules* 2017: 22; 1281–1309.
13. Klute, K, Nackos, E, Tasaki, S et al. Microtubule inhibitor-based antibody-drug conjugates for cancer therapy. *Onco Targets Ther* 2014; 7: 2227–36.
14. Goss, GD, Vokes, EE, Gordon, MS et al. Efficacy and safety results of depatuxizumab mafodotin (ABT-414) in patients with advanced solid tumors likely to overexpress epidermal growth factor receptor. *Cancer* 2018; 124: 2174–83.
15. Phillips, AC, Boghaert, ER, Vaidya, KS et al. ABT-414, an antibody-drug conjugate targeting a tumor-selective EGFR epitope. *Mol Cancer Ther* 2016; 15: 661–9.
16. Willuda, J, Linden, L, Lerchen, HG et al. Preclinical antitumor efficacy of BAY 1129980—a novel auristatin-based anti-C4.4A (LYPD3) antibody-drug conjugate for the treatment of non-small cell lung cancer. *Mol Cancer Ther* 2017; 16: 893–904.
17. Rose, AA, Annis, MG, Frederick, DT et al. MAPK pathway inhibitors sensitize BRAF-mutant melanoma to an antibody-drug conjugate targeting GPNMB. *Clin Cancer Res* 2016; 22: 6088–98.
18. Sommer, A, Kopitz, C, Schatz, CA et al. Preclinical efficacy of the auristatin-based antibody-drug conjugate BAY 1187982 for the treatment of FGFR2-positive solid tumors. *Cancer Res* 2016; 76: 6331–9.
19. Zein, N, Sinha, AM, Mcgahren, WJ et al. Calicheamicin-gamma-1-I—an antitumor antibiotic that cleaves double-stranded DNA site specifically. *Science* 1988; 240: 1198–201.
20. Zein, N, Poncin, M, Nilakantan, R et al. Calicheamicin gamma 1I and DNA: molecular recognition process responsible for site-specificity. *Science* 1989; 244: 697–9.
21. Loke, J, Khan, JN, Wilson, JS et al. Mylotarg has potent anti-leukaemic effect: a systematic review and meta-analysis of anti-CD33 antibody treatment in acute myeloid leukaemia. *Ann Hematol* 2015; 94: 361–73.
22. Kantarjian, HM, DeAngelo, DJ, Stelljes, M et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. *New Engl J Med* 2016; 375: 740–53.
23. Garrido-Laguna, I, Krop, IE, Burris, H et al. A phase I study of PF-06647263, a novel EFNA4-ADC, in patients with metastatic triple negative breast cancer. *J Clin Oncol* 2017; 35: 2511–2511.
24. Herbertson, RA, Tebbutt, NC, Lee, FT et al. Phase I biodistribution and pharmacokinetic study of Lewis Y-targeting immunoconjugate CMD-193 in patients with advanced epithelial cancers. *Clin Cancer Res* 2009; 15: 6709–15.
25. Chan, SY, Gordon, AN, Coleman, RE et al. A phase 2 study of the cytotoxic immunoconjugate CMB-401 (hCTM01-calicheamicin) in patients with platinum-sensitive recurrent epithelial ovarian carcinoma. *Cancer Immunol Immunother* 2003; 52: 243–8.
26. Smellie, M, Bose, DS, Thompson, AS et al. Sequence-selective recognition of duplex DNA through covalent interstrand cross-linking: kinetic and molecular modeling studies with pyrrolobenzodiazepine dimers. *Biochemistry* 2003; 42: 8232–9.
27. Stein, AS, Walter, RB, Advani, AS et al. SGN-CD33A (vadastuximab talirine) followed by allogeneic hematopoietic stem cell transplant (AlloHSCT) results in durable complete remissions (CRs) in patients with acute myeloid leukemia (AML). *Biol Blood Marrow Tr* 2016; 22: S211–2.
28. Stein, AS, Walter, RB, Erba, HP et al. A phase 1 trial of SGN-CD33A as monotherapy in patients with CD33-positive acute myeloid leukemia (AML). *Blood* 2018; 131: 387–96.
29. Kennedy, DA, Alley, SC, Zhao, BT et al. SGN-CD33A: preclinical and phase 1 interim clinical trial results of a CD33-directed PBD dimer antibody-drug conjugate for the treatment of acute myeloid leukemia (AML). *Cancer Res* 2015; 75. 10.1158/1538-7445.AM2015-DDT02-04.
30. Sandall, S, Anderson, M, Jonas, M et al. SGN-CD70A, a novel and highly potent anti-CD70 ADC, induces double-strand DNA breaks and is active in models of MDR plus renal cell carcinoma (RCC) and non-Hodgkin lymphoma (NHL). *Cancer Res* 2014; 74. 10.1158/1538-7445.AM2014-2647.
31. Li, F, Sutherland, MK, Yu, C et al. Characterization of SGN-CD123A, a potent CD123-directed antibody-drug conjugate for acute myeloid leukemia. *Mol Cancer Ther* 2018; 17: 554–64.
32. Lewis, T, Olson, DJ, Gordon, KA et al. SGN-CD352A: a novel humanized anti-CD352 antibody -drug conjugate for the treatment of multiple myeloma. *Cancer Res* 2016; 76. http://cancerres.aacrjournals.org/content/76/14_Supplement/1195.short.
33. Mansfield, A, Aggarwal, R, Beltran, H et al. Preliminary safety and efficacy of rovalpituzumab tesirine in patients with delta-like protein 3-expressing advanced solid tumors. *Neuroendocrinology* 2018; 106: 298–8.
34. Komarnitsky, P, Lee, HJ, Shah, M et al. A phase 3 trial of rovalpituzumab tesirine vs topotecan in patients with advanced small cell lung cancer following frontline platinum-based chemotherapy. *Ann Oncol* 2017; 28. https://academic.oup.com/annonc/article/28/suppl_5/mdx386.010/4109533.
35. Komarnitsky, PB, Lee, HJ, Shah, M et al. A phase III study of rovalpituzumab tesirine maintenance therapy following first-line platinum-based chemotherapy in patients with extensive disease small cell lung cancer (ED SCLC). *J Clin Oncol* 2017; 35. http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.15_suppl.TPS8583
36. Tallman, MS, Feingold, JM, Spira, AI et al. A phase 1, open-label, dose-escalation, multicenter study to evaluate the tolerability, safety, pharmacokinetics, and activity of ADCT-301 in patients with relapsed or refractory CD25-positive acute myeloid leukemia. *J Clin Oncol* 2016; 34. http://ascopubs.org/doi/abs/10.1200/JCO.2016.34.15_suppl.TPS7071.
37. Chung, KY, Hamadani, M, Kahl, BS et al. A phase 1 adaptive dose-escalation study to evaluate the tolerability, safety, pharmacokinetics, and antitumor activity of ADCT-402 in patients with relapsed or refractory B-cell lineage non Hodgkin lymphoma (B-NHL). *J Clin Oncol* 2016; 34. http://ascopubs.org/doi/abs/10.1200/JCO.2016.34.15_suppl.TPS7580.
38. Cortes, J, DeAngelo, D, Wang, E et al. Initial results from a first-in-human study of Imgn779, a Cd33-targeting antibody-drug conjugate (Adc) with novel DNA alkylating activity, in patients with relapsed or refractory Aml. *Haematologica* 2017; 102: 217–8.
39. Kovtun, Y, Jones, G, Audette, C et al. A CD123-targeting antibody-drug conjugate (ADC), IMGN632, designed to eradicate acute myeloid leukemia (AML) cells while sparing normal bone marrow cells. *Blood* 2016; 128. http://www.bloodjournal.org/content/128/22/768?sso-checked=true.
40. Hartley, JA. The development of pyrrolobenzodiazepines as antitumour agents. *Expert Opin Investig Drugs* 2011; 20: 733–44.

41. Boger, DL, Johnson, DS. CC-1065 and the duocarmycins: understanding their biological function through mechanistic studies. *Angew Chem Int Ed Engl* 1996; 35: 1438–74.
42. Aftimos, PG, van Herpen, CM, Mommers, EC et al. SYD985, a novel anti-HER2 ADC, shows promising activity in patients with HER2-positive and HER2-negative metastatic breast cancer. *Cancer Res* 2017; 77. http://cancerres.aacrjournals.org/content/77/4_Supplement/P6-12-02.
43. Owonikoko, TK, Hussain, A, Stadler, WM et al. First-in-human multicenter phase I study of BMS-936561 (MDX-1203), an antibody-drug conjugate targeting CD70. *Cancer Chemother Pharmacol* 2016; 77: 155–62.
44. Starodub, AN, Ocean, AJ, Shah, MA et al. First-in-human trial of a novel anti-Trop-2 antibody-SN-38 conjugate, sacituzumab govitecan, for the treatment of diverse metastatic solid tumors. *Clin Cancer Res* 2015; 21: 3870–8.
45. Nakada, T, Masuda, T, Naito, H et al. Novel antibody drug conjugates containing exatecan derivative-based cytotoxic payloads. *Bioorg Med Chem Lett* 2016; 26: 1542–5.
46. Takegawa, N, Nonagase, Y, Yonesaka, K et al. DS-8201a, a new HER2-targeting antibody-drug conjugate incorporating a novel DNA topoisomerase I inhibitor, overcomes HER2-positive gastric cancer T-DM1 resistance. *Int J Cancer* 2017; 141: 1682–9.
47. Ogitani, Y, Aida, T, Hagihara, K et al. DS-8201a, a novel HER2-targeting ADC with a novel DNA topoisomerase I inhibitor, demonstrates a promising antitumor efficacy with differentiation from T-DM1. *Clin Cancer Res* 2016; 22: 5097–108.
48. Tagawa, ST, Faltas, B, Lam, E et al. Sacituzumab govitecan (IMMU-132) for patients with pretreated metastatic urothelial uancer (UC): interim results. *Ann Oncol* 2017; 28. https://oncologypro.esmo.org/Meeting-Resources/ESMO-2017-Congress/Sacituzumab-govitecan-IMMU-132-for-patients-with-pretreated-metastatic-urothelial-uancer-UC-interim-results.
49. Dotan, E, Cohen, SJ, Starodub, AN et al. Phase I/II trial of labetuzumab govitecan (Anti-CEACAM5/SN-38 antibody-drug conjugate) in patients with refractory or relapsing metastatic colorectal cancer. *J Clin Oncol* 2017; 35: 3338–46.
50. Modi, S, Tsurutani, J, Takahashi, S et al. Safety and efficacy results from a phase 1 study of DS-8201a in patients with HER2 expressing breast cancers. *Cancer Res* 2018; 78. http://cancerres.aacrjournals.org/content/78/4_Supplement/PD3-07.
51. Kogawa, T, Yonemori, K, Naito, Y et al. Phase 1/2, multicenter, non-randomized, open-label, multiple-dose first-in-human study of U3-1402 (anti-HER3 antibody drug conjugate) in subjects with HER3-positive metastatic breast cancer. *J Clin Oncol* 2017; 35. http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.15_suppl.TPS1116.
52. Stein, R, Smith, MR, Chen, S et al. Combining milatuzumab with bortezomib, doxorubicin, or dexamethasone improves responses in multiple myeloma cell lines. *Clin Cancer Res* 2009; 15: 2808–17.
53. Coussens, L, Yang-Feng, TL, Liao, YC et al. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science* 1985; 230: 1132–9.
54. Slamon, DJ, Clark, GM, Wong, SG et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987; 235: 177–82.
55. Junttila, TT, Li, GM, Parsons, K et al. Trastuzumab-DM1 (T-DM1) retains all the mechanisms of action of trastuzumab and efficiently inhibits growth of lapatinib insensitive breast cancer. *Breast Cancer Res Tr* 2011; 128: 347–56.
56. Li, JY, Perry, SR, Muniz-Medina, V et al. A biparatopic HER2-targeting antibody-drug conjugate demonstrates potent antitumor activity in primary tumor models that are refractory to or ineligible for HER2-targeted therapies. *Cancer Res* 2016; 76. http://cancerres.aacrjournals.org/content/76/4_Supplement/PD5-08.
57. Dokter, W, Ubink, R, van der Lee, M et al. Preclinical profile of the HER2-targeting ADC SYD983/SYD985: introduction of a new duocarmycin-based linker-drug platform. *Mol Cancer Ther* 2014; 13: 2618–29.
58. van der Lee, MM, Groothuis, PG, Ubink, R et al. The preclinical profile of the duocarmycin-based HER2-targeting ADC SYD985 predicts for clinical benefit in low HER2-expressing breast cancers. *Mol Cancer Ther* 2015; 14: 692–703.
59. Bergstrom, DA, Bodyak, N, Park, PU et al. XMT-1522 induces tumor regressions in pre-clinical models representing HER2-positive and HER2 low-expressing breast cancer. *Cancer Res* 2016; 76. http://cancerres.aacrjournals.org/content/76/4_Supplement/P4-14-28.
60. Taylor, VC, Buckley, CD, Douglas, M et al. The myeloid-specific sialic acid-binding receptor, CD33, associates with the protein-tyrosine phosphatases, SHP-1 and SHP-2. *J Biol Chem* 1999; 274: 11505–12.
61. Dinndorf, PA, Andrews, RG, Benjamin, D et al. Expression of normal myeloid-associated antigens by acute-leukemia cells. *Blood* 1986; 67: 1048–53.
62. Rowe, JM, Lowenberg, B. Gemtuzumab ozogamicin in acute myeloid leukemia: a remarkable saga about an active drug. *Blood* 2013; 121: 4838–41.
63. Kovtun, Y, Noordhuis, P, Whiteman, KR et al. IMGN779, a novel CD33-targeting antibody-drug conjugate with DNA alkylating activity, exhibits potent antitumor activity in models of AML. *Mol Cancer Ther* 2018; 17: 1271-1279.
64. Ribrag, V, Dupuis, J, Tilly, H et al. A dose-escalation study of SAR3419, an anti-CD19 antibody maytansinoid conjugate, administered by intravenous infusion once weekly in patients with relapsed/refractory B-cell non-Hodgkin lymphoma. *Clin Cancer Res* 2014; 20: 213–20.
65. Zammarchi, F, Corbett, S, Adams, L et al. ADCT-402, a PBD dimer-containing antibody drug conjugate targeting CD19-expressing malignancies. *Blood* 2018; 131: 1094–105.
66. Ricart, AD. Antibody-drug conjugates of calicheamicin derivative: gemtuzumab ozogamicin and inotuzumab ozogamicin. *Clin Cancer Res* 2011; 17: 6417–27.
67. Kantarjian, HM, DeAngelo, DJ, Advani, AS et al. Overall survival in relapsed/refractory B-cell acute lymphoblastic leukemia patients receiving inotuzumab ozogamicin vs standard care in the phase 3 Ino-Vate study. *Haematologica* 2016; 101: 339–9.
68. Chan, CH, Stanners, CP. Recent advances in the tumour biology of the GPI-anchored carcinoembryonic antigen family members CEACAM5 and CEACAM6. *Curr Oncol* 2007; 14: 70–3.
69. Cal, PM, Bernardes, GJ, Gois, PM. Cysteine-selective reactions for antibody conjugation. *Angew Chem Int Ed Engl* 2014; 53: 10585–7.
70. Rossin, R, van Duijnhoven, SM, Ten Hoeve, W et al. Triggered drug release from an antibody-drug conjugate using fast "click-to-release" chemistry in mice. *Bioconjug Chem* 2016; 27: 1697–706.
71. Nani, RR, Gorka, AP, Nagaya, T et al. In vivo activation of duocarmycin-antibody conjugates by near-infrared light. *ACS Cent Sci* 2017; 3: 329–37.
72. Sommer, A, Kopitz, C, Schatz, CA et al. Preclinical efficacy of the auristatin-based antibody-drug conjugate BAY 1187982 for the treatment of FGFR2-positive solid tumors. *Cancer Res.* 2016. 76: 6331–6339.
73. Beck, A, Goetsch, L, Dumontet, C et al. Strategies and challenges for the next generation of antibody drug conjugates. *Nat Rev Drug Discov* 2017; 16: 315–37.
74. Dreier, T, Baeuerle, PA, Fichtner, I et al. T cell costimulus-independent and very efficacious inhibition of tumor growth in mice bearing subcutaneous or leukemic human B cell lymphoma xenografts by a CD19-/CD3-bispecific single-chain antibody construct. *J Immunol* 2003; 170: 4397–402.
75. Bargou, R, Leo, E, Zugmaier, G et al. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. *Science* 2008; 321: 974–7.
76. Mazor, Y, Oganesyan, V, Yang, CN et al. Improving target cell specificity using a novel monovalent bispecific IgG design. *mAbs-Austin* 2015; 7: 377–89.
77. Hay, KA, Turtle, CJ. CD19-specific chimeric antigen receptor-modified (CAR)-T cell therapy for the treatment of chronic lymphocytic leukemia in the ibrutinib era. *Immunotherapy-Uk* 2018; 10: 251–4.
78. Gu, J, Ghayur, T. Rationale and development of multispecific antibody drugs. *Expert Rev Clin Pharmacol* 2010; 3: 491–508.
79. Govindan, SV, Sharkey, RM, Goldenberg, DM. Prospects and progress of antibody-drug conjugates in solid tumor therapies. *Expert Opin Biol Ther* 2016; 16: 883–93.

80. Challita-Eid, PM, Satpayev, D, Yang, P et al. Enfortumab vedotin antibody-drug conjugate targeting nectin-4 is a highly potent therapeutic agent in multiple preclinical cancer models. *Cancer Res* 2016; 76: 3003–13.
81. Nicolaou, KC, Erande, RD, Yin, J et al. Improved total synthesis of tubulysins and design, synthesis, and biological evaluation of new tubulysins with highly potent cytotoxicities against cancer cells as potential payloads for antibody-drug conjugates. *J Am Chem Soc* 2018; 140: 3690–711.
82. Toader, D, Harper, J, Lloyd, C et al. Abstract B170: discovery of tubulysin payloads for antibody drug conjugates with potent in vitro activity and X efficacy in solid tumor models. *Mol Cancer Ther* 2015; 14: B170–0.
83. Leverett, CA, Sukuru, SCK, Vetelino, BC et al. Design, synthesis, and cytotoxic evaluation of novel tubulysin analogues as ADC payloads. *ACS Med Chem Lett* 2016; 7: 999–1004.
84. Burke, PJ, Hamilton, JZ, Hunter, JH et al. Abstract 56: antibody-drug conjugates containing glucuronide-tubulysin payloads display activity in MDR+ and heterogeneous tumor models. *Cancer Res* 2017; 77: 56–6.
85. Aviles, P, Guillen, MJ, Dominguez, JM et al. Abstract A147: MI130004, a new ADC with a payload of marine origin shows outstanding activity against HER2-expressing tumors. *Mol Cancer Ther* 2015; 14: A147–7.
86. Brun, M-P, Bouchard, H, Clerc, F et al. Abstract LB-053: towards new cryptophycins as promising payloads for ADC. *Cancer Res* 2016; 76. http://cancerres.aacrjournals.org/content/76/14_Supplement/LB-053.
87. Kahler, J, Dougher, M, Xu, J et al. Abstract 3095: the development of CPI as a novel, next-generation DNA-targeting payload for ADCs. *Cancer Res* 2017; 77: 3095–5.
88. Sommer, A, Berndt, S, Lerchen, H-G et al. Abstract 46: preclinical activity of novel antibody-drug conjugates with pyrrole-based kinesin spindle protein inhibitors targeting different tumor antigens. *Cancer Res* 2017; 77: 46–6.
89. Moshnikova, A, Moshnikova, V, Andreev, OA et al. Antiproliferative effect of pHLIP-amanitin. *Biochemistry* 2013; 52: 1171–8.
90. Prota, AE, Bargsten, K, Diaz, JF et al. A new tubulin-binding site and pharmacophore for microtubule-destabilizing anticancer drugs. *Proc Natl Acad Sci U S A* 2014; 111: 13817–21.
91. Kaida, D, Motoyoshi, H, Tashiro, E et al. Spliceostatin A targets SF3b and inhibits both splicing and nuclear retention of pre-mRNA. *Nat Chem Biol* 2007; 3: 576–83.
92. Puthenveetil, S, Loganzo, F, He, H et al. Natural product splicing Inhibitors: a new class of antibody-drug conjugate (ADC) payloads. *Bioconjugate Chem* 2016; 27: 1880–8.
93. Yu, SF, Zheng, B, Go, M et al. A novel anti-CD22 anthracycline-based antibody-drug conjugate (ADC) that overcomes resistance to auristatin-based ADCs. *Clin Cancer Res* 2015; 21: 3298–306.
94. Weinstein, JN, Vanosdol, W. Early intervention in cancer using monoclonal-antibodies and other biological ligands—micropharmacology and the binding-site barrier. *Cancer Res* 1992; 52: S2747–51.
95. Vasalou, C, Helmlinger, G, Gomes, B. A mechanistic tumor penetration model to guide antibody drug conjugate design. *Plos One* 2015; 10, e0118977. 10.1371/journal.pone.0118977.eCollection2015.
96. Zhu, XD, Bidlingmaier, S, Hashizume, R et al. Identification of internalizing human single-chain antibodies targeting brain tumor sphere cells. *Mol Cancer Ther* 2010; 9: 2131–41.
97. Lee, JM, Lee, SH, Hwang, JW et al. Novel strategy for a bispecific antibody: induction of dual target internalization and degradation. *Oncogene* 2016; 35: 4437–46.
98. Polu, KR, Lowman, HB. Probody therapeutics for targeting antibodies to diseased tissue. *Expert Opin Biol Ther* 2014; 14: 1049–53.
99. Loganzo, F, Sung, M, Gerber, HP. Mechanisms of resistance to antibody-drug Conjugates. *Mol Cancer Ther* 2016; 15: 2825–34.
100. Seaman, S, Zhu, ZY, Saha, S et al. Eradication of tumors through simultaneous ablation of CD276/B7-H3-positive tumor cells and tumor vasculature. *Cancer Cell* 2017; 31: 501–5.