CHAPTER TWENTY-THREE

# Antibody–Drug Conjugates for Targeted Cancer Therapy

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## ABBREVIATIONS

**ADC** antibody–drug conjugate
**DM1 and DM4** thiol-containing maytansinoids
**FDA** U.S. Food and Drug Administration
**MMAE** monomethyl auristatin E
**MMAF** monomethyl auristatin F
**PSMA** prostate-specific membrane antigen
**vc** valine-citrulline dipeptide

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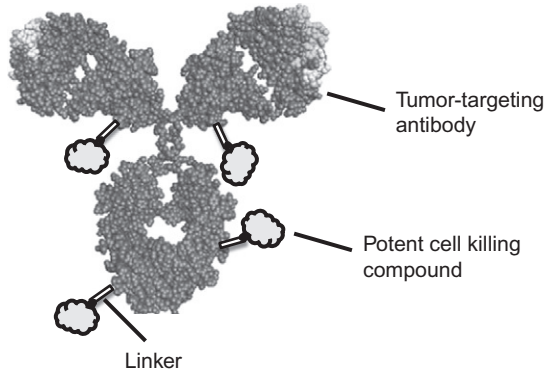
## 1. INTRODUCTION

An antibody–drug conjugate (ADC) for cancer therapy consists of a tumor-targeting antibody chemically attached (through a "linker") to a cytotoxic effector molecule (also called the "payload" or "drug").

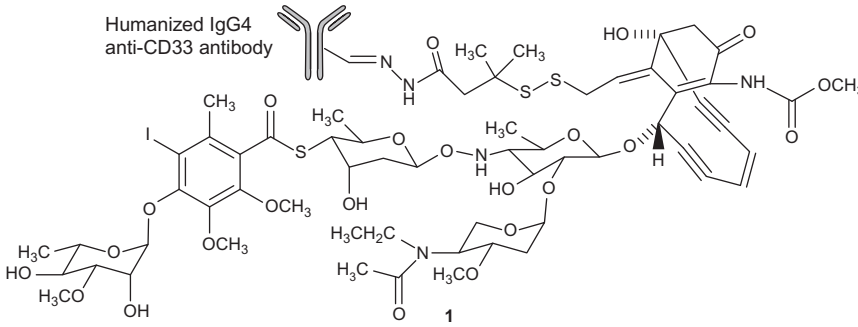



Mechanistically, an ADC acts by binding to the target antigen on the cell surface, followed by its internalization *via* antigen-mediated endocytosis, trafficking into the lysosome, and the release of the payload through the proteolytic degradation of the antibody moiety and/or cleavage of the linker.[1] The rationale for developing an ADC is that linking a cytotoxic agent to a tumor-targeting antibody will enable its selective targeting to cancer cells, leading to their eradication while sparing cells in normal tissues. Compelling clinical results with ADCs in both hematological malignancies and solid tumors has prompted renewed interest in the field and expanded efforts to exploit ADCs as a validated platform for developing new targeted anticancer compounds. ADCs represent a novel class of targeted therapy in oncology, with a distinct mode of action. Most targeted cancer therapies utilize small molecules or naked antibodies that inhibit key signal transduction molecules/pathways required for tumor cell growth or survival. Successful development of such agents requires intricate understanding of tumor cell biology, oncogenic driver mutations, and signaling pathways, which can vary widely even within a given tumor type. In the ADC approach, a potent cytotoxic agent is delivered selectively to cancer cells, exerting an antitumor mechanism of action that is not inherently dependent on particular oncogenic signaling pathways. ADC specificity is thus provided by the targeting

antibody rather than by blocking key signal transduction nodes uniquely required for tumor growth or survival.

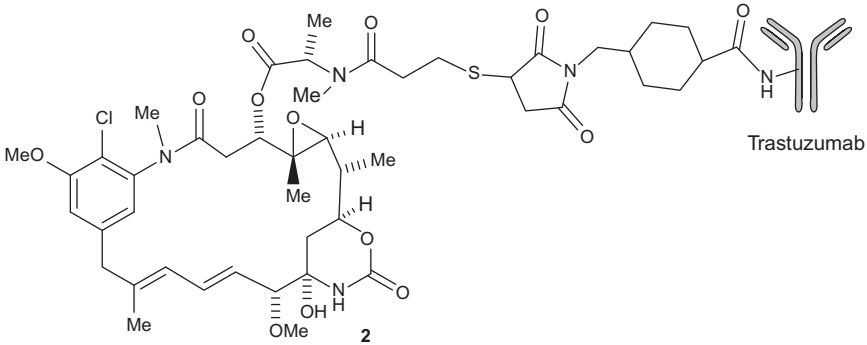
## 2. TARGET SELECTION

Selective expression of the target antigen on tumors relative to normal tissues provides the basis for ADC targeting to tumors and minimizes the potential for targeted toxicity in normal tissues. The abundance of the target antigen on the cell surface and its distribution in the tumor (homogeneous vs. heterogeneous expression) can be important determinants of ADC efficacy.[2,3] Attachment to secreted or shed antigen can alter the pharmacokinetics of an ADC and potentially limit effective targeting of tumors.[4] ADC effectiveness ultimately reflects a combination of target antigen properties including antigen density, internalization and intracellular processing, and sensitivity of the target cell to the cytotoxic payload. Most tumor-associated antigens are also expressed to some extent on normal tissues. Such antigens can be considered if expression is restricted to tissues that do not present a toxicity concern. For example, ADCs for prostate cancer have targeted PSMA or Prostate Stem Cell antigen (PSMA),[5,6] which are expressed on normal prostate tissue; gemtuzumab ozogamicin, **1**, targets CD33, which is expressed on both malignant and normal cells of myeloid lineage. Certain markers specific for B-cell malignancies targeted by ADCs, CD22, CD19, CD20, CD79b, and CD37,[5,7–9] are also expressed on normal B-cells, but their temporary depletion can be tolerated.




A subset of candidate ADC targets not only show differential expression in tumors compared to normal tissues but also play a biological role in the growth or survival of tumor cells. In trastuzumab emtansine (T-DM1, **2**), a conjugate of the anti-HER2 antibody, trastuzumab, with the cytotoxic maytansinoid, DM1, the antibody component, inhibits HER2-mediated

signal transduction and is used as naked antibody therapy for metastatic breast cancer.




All of the functional properties of trastuzumab are retained in **2**, including HER2 binding affinity, inhibition of HER2 signaling, and antibody-dependent cellular cytotoxicity.[10] Moreover, based on clinical experience, **2** can be dosed in patients at levels that approach those used for trastuzumab and other naked "functional" antibodies. This raises the attractive possibility of developing ADCs that combine intrinsic antitumor activity(s) from the antibody component with the potent cytotoxic activity provided by the payload agent. IMGN529 is an example of an ADC designed with this rationale, comprising an anti-CD37 antibody with direct antitumor activities (proapoptotic, complement-dependent cytotoxicity, and antibody-dependent cellular cytotoxicity), conjugated to DM1 using the same linker and conjugate design as in **2**.[8] IMGN529 exhibits potent activity against B-cell tumors in preclinical studies.[8]

## 3. CYTOTOXIC AGENTS AND LINKERS USED IN ADCs

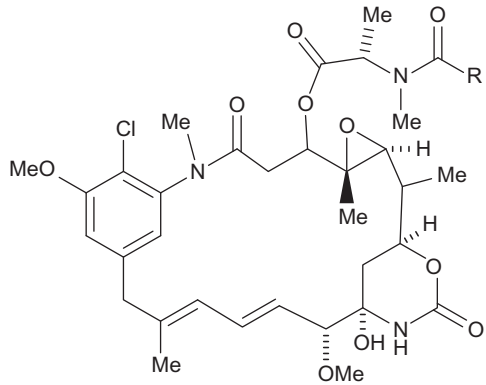
### 3.1. Considerations

Favorable parameters for the cytotoxic effectors include potency,[11] selectivity of impact on proliferating cells, low-molecular weight and the related lack of immunogenicity, reproducible and cost–effective production, attachability, and sufficient solubility and stability in water to facilitate preparation and storage of the ADC.[12] The cytotoxic molecules used in the ADCs currently in clinical testing are either microtubule-impacting agents or DNA-interacting agents. In addition, there are several ADCs with other cytotoxic agents in preclinical evaluation.[11,13,14] Delivery of the payload by an antibody requires that they are stably linked. However, once the antibody

has bound to the antigen on the surface of cancer cells and the antibody–antigen complex is internalized into the cell, the cytotoxic agent needs to be released to enable it to efficiently arrive at the target and to inactivate it.

## 3.2. Maytansinoids

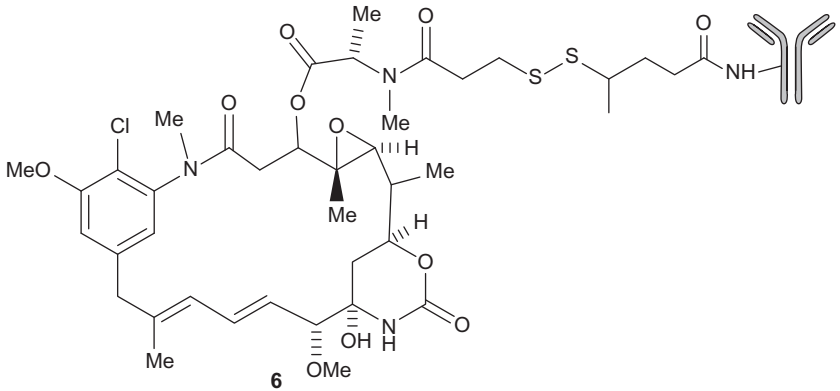
Maytansine **3** and its derivatives, maytansinoids, interfere with microtubule dynamics.[15]




**3**: R = $CH_3$
**4**: R = $CH_2CH_2SH$
**5**: R = $CH_2CH_2C(CH_3)_2SH$

Proliferating cells are more sensitive to maytansine than are non-proliferating cells.[16] Appropriately designed maytansinoid agents can have potent cytotoxic activity, and, at the same time, good aqueous stability and reasonable aqueous solubility.[17] Structure–activity relationship studies of maytansinoids showed that the C3 ester side chain is required for biological activity but that the structure of the side chain can be modified without a significant loss of activity. Incorporation of methyldisulfide substituents into the C3 ester side chain has resulted in proprietary maytansinoid derivatives that retained or exceeded the *in vitro* potency of the parent compound. Reduction of the methyldisulfide led to the formation of a thiol group that enables linkage to antibodies. Two of these thiol-containing maytansinoids, $N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine (DM1, **4**) and $N^{2'}$-deacetyl-$N^{2'}$-(4-mercapto-4-methyl-1-oxopentyl)-maytansine (DM4, **5**), were linked to antibodies *via* cleavable disulfide bonds to provide antibody-DM1 (**6**) and antibody-DM4, SAR3419, (**7**).[1,18] DM1 was also linked to antibodies *via* a noncleavable thioether linker as in **2**.[19] Newer

thiol-containing maytansinoids and linkers have been synthesized with one or more methyl groups (substituting hydrogen) on the carbon atom geminal to the sulfhydryl group, allowing for the generation of conjugates with varying degrees of steric hindrance around the disulfide bond, allowing a fine control of the rate of bond cleavage.[1,13]

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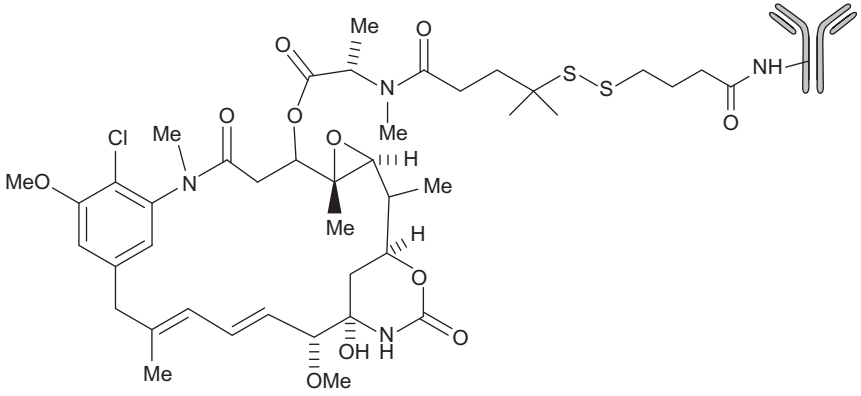
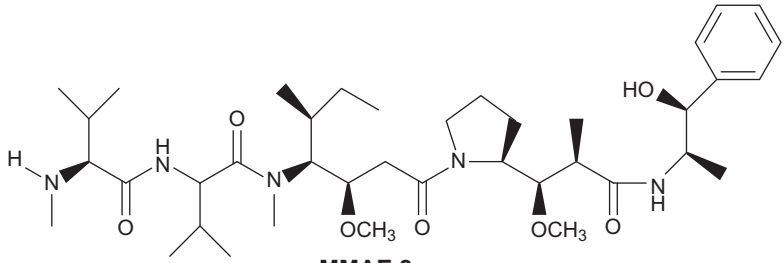



**7**

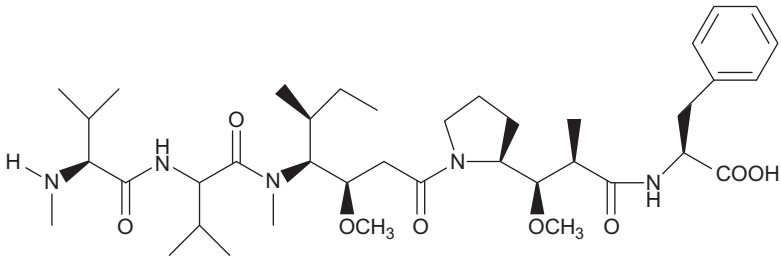
## 3.3. Auristatins

The auristatins, such as monomethyl auristatin E (MMAE) **8a** and monomethyl auristatin F (MMAF) **8b**, are analogs of dolastatin 10, a microtubule-impacting agent.[20] To prepare auristatin conjugates (MMAE conjugate **8c** shown), native disulfide bonds within the antibody are reduced to generate cysteine residues that are subsequently linked to a maleimido derivative of MMAE or MMAF, *via* a dipeptide linker.[20–22] New auristatin ADC linkers have been generated by

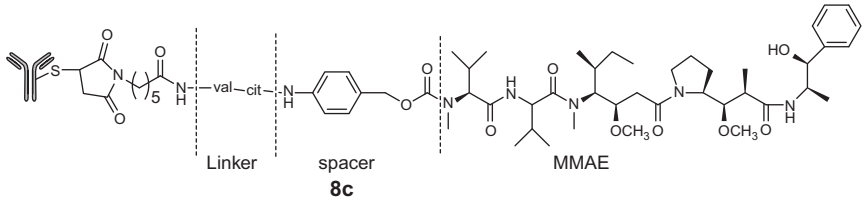
replacing the maleimide with a halo-acetamide and have shown improved *in vivo* stability in preclinical studies.[23] Novel dipeptide linkers and new auristatins linked through the C-terminus, and some of these new conjugates showed improved therapeutic windows over the original valine-citrulline (vc)-para-aminobenzyl carbamate–MMAF conjugate.[24]




**MMAE 8a**



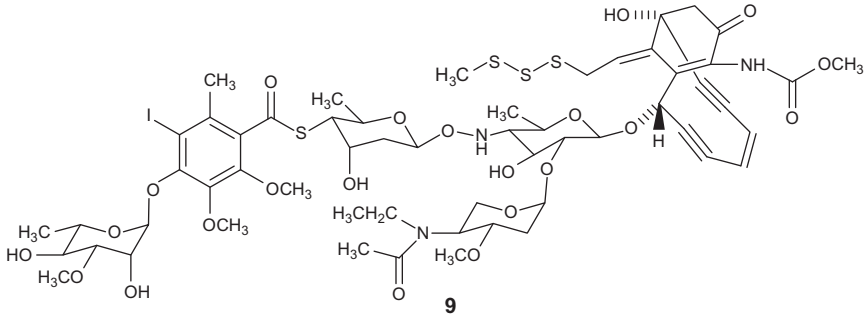

**MMAF 8b**




**8c**

## 3.4. Calicheamicins

Calicheamicins[25] induce DNA double-strand breaks. *N*-acetyl γ−calicheamicin, **9** was linked to lysine residues of antibodies through an acid-labile hydrazone bond or a noncleavable linker.[26] In gemtuzumab ozogamicin, *N*-acetyl-γ-calicheamicin dimethyl hydrazide is conjugated using a 4-(4′-acetylphenoxy) butanoic acid linker, which is stable at physiologic near-neutral pH, but hydrolyses at the acidic pH (∼4) of lysosomes.[27]



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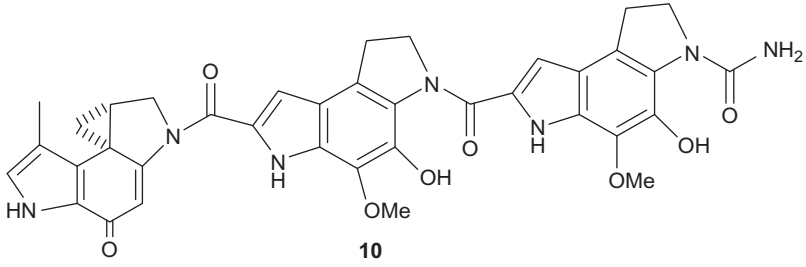
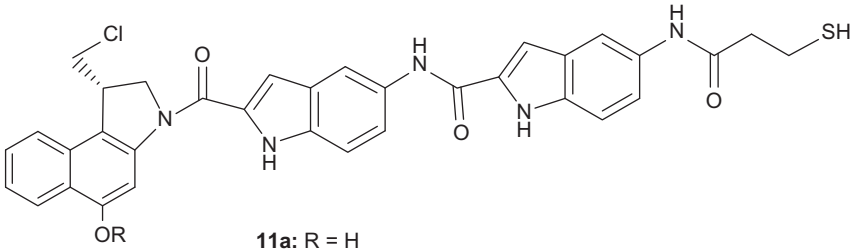
## 3.5. CC-1065 analogues

The parent compound CC-1065, **10**, bearing a cyclopropapyrroloindole pharmacophore alkylates *N*-3 guanine residues of DNA. Analogues containing another alkylating subunit, cyclopropabenzindole of CC-1065, proved chemically more stable, biologically more potent, and synthetically accessible.[28] A cyclopropabenzindole-based CC-1065 analogue, DC1, **11a** has been conjugated to antibodies *via* disulfide bonds. The resulting ADCs were highly cytotoxic in an antigen-specific manner but were not developed because of their instability in water and poor solubility. These problems were overcome by conversion of DC1 into the phenolic phosphate prodrug, DC4, **11b**.[29]

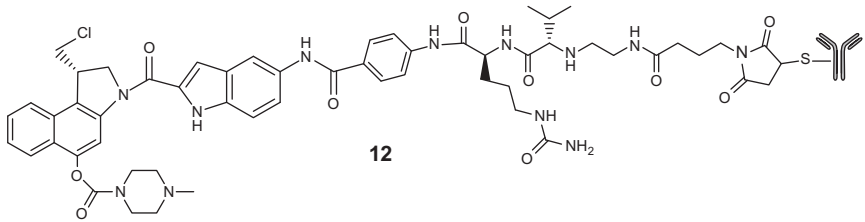


**10**



**11a:** R = H
**11b:** R = $PO_3H$

Duocarmycin is also structurally similar to CC-1065, except that it contains only one DNA-binding indolyl unit. In the MDX-1203 ADC **12**, a duocarmycin analogue was linked to an antibody *via* a dipeptide linker.[30]



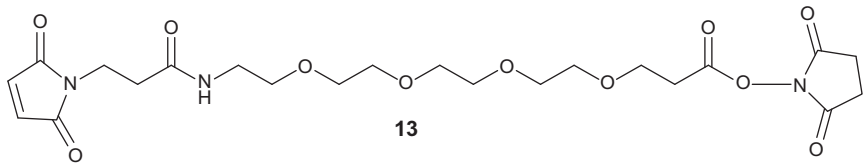
**12**

Conjugates currently in clinical testing (regardless of which cytotoxic compound, antibody, or linker was used) are mixtures of molecules with various drug-per-antibody ratio; most have, on average, 3–4 cytotoxic molecules per antibody molecule, randomly linked to lysine or cysteine residues on the antibody. Methods for site-specific conjugation are also being explored.[31]

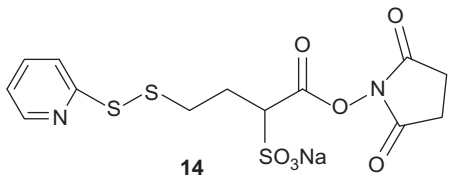
## 4. INTRACELLULAR CATABOLISM OF ADCs

Regardless of whether the linkage is cleavable or noncleavable, initial degradation of auristatin- and maytansinoid-based ADCs apparently takes place in lysosomes. Calicheamicin-based hydrazone-linked ADCs are possibly hydrolyzed in the low-pH endosomal compartment. MMAE-based ADC, such as **8c** releases **8a** in cells *via* protease-(probably, cathepsin)-mediated cleavage of the amide bond between the peptide and the aromatic amine, followed by elimination of the para-amino benzyl moiety and carbon dioxide.[20,23] An auristatin derivative with a negatively charged C-terminal phenylalanine residue, **8b**, is much less cytotoxic as an unconjugated compound than **8a** likely due its reduced plasma membrane permeability.[21] Nonetheless, antibody–MMAF conjugates, conjugated to the antibody through a noncleavable linker, are comparable in their potency with antibody–MMAE conjugates.[21] Maytansinoid conjugates are degraded in lysosomes yielding metabolites consisting of a lysine adduct of the maytansinoid and linker, and the efficacy of this process is important for ADC activity.[1] Metabolites derived from a disulfide-linked maytansinoid conjugate are processed further *via* reduction of the disulfide bond and subsequent *S*-methylation, to produce lipophilic *S*-methyl-maytansinoid metabolites, which have been found to be highly cytotoxic when added exogenously. These observations may help explain both the phenomenon

of target cell-activated killing of bystander cells and the superior efficacy of disulfide-linked conjugates over thioether-linked conjugates seen in some xenograft models.[32] The cytotoxic agents that are currently used in ADCs are substrates for at least one of the three multidrug-resistance transporters Multidrug Resistance Protein 1 (MDR1), Multidrug Resistance-associated Protein 1 (MRP1), or Breast Cancer Resistance Protein (BCRP).[18] Upon intracellular processing, conjugates are processed into cytotoxic metabolites,[1,21] which can be susceptible to the transporter-mediated efflux.[18] Hydrophilic linkers **13** and **14** that enable antibody–maytansinoid conjugates to overcome this MDR1-mediated resistance have been recently developed.[18,33]




**13**

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## 5. ADCs IN CLINICAL DEVELOPMENT

The ADCs that are in clinical development at the time of preparation of this review are listed in Table 23.1. One of those listed is gemtuzumab ozogamicin (Mylotarg), an anti-CD33-calicheamicin conjugate that was approved under an accelerated-approval process by U.S. Food and Drug Administration (FDA) in 2000 for treatment of acute myeloid leukemia, but withdrawn from the market in 2011 after an intended confirmatory trial showed no improvement in clinical benefit and an unfavorable toxicity. Brentuximab vedotin, **8c**, comprising an anti-CD30 antibody linked to MMAE, was granted conditional approval by FDA in 2011 for treating Hodgkin's lymphoma and ATCL. Several clinical trials are underway or in development to further evaluate the activity and safety of this ADC. Trastuzumab emtansine, described earlier in this review, exhibited robust antitumor activity and excellent tolerability in Phase I and Phase II trials,[34,35] suggesting that this agent may change the paradigm for the treatment of

**Table 23.1** ADCs in clinical development

| ADC | Target antigen | Linker-cytotoxic compound, class | Antibody | Tumor type(s) | Developer | Status |
|---|---|---|---|---|---|---|
| Gemtuzumab ozogamicin (Mylotarg®) | CD33 (Siglec-3) | Hydrazone, AcBu *N*-acetyl-γ calicheamicin Calicheamicin | hP67.6 Humanized IgG4 | Acute myeloid leukemia | Pfizer | US FDA conditional approval 5/2000. Withdrawn 8/2011 |
| Brentuximab vedotin (Adcetris®, SGN35) | CD30 | Dipeptide, vc-MMAE Auristatin | Brentuximab Chimeric IgG1 | Relapsed/refractory Hodgkin's lymphoma and systemic anaplastic large cell lymphoma | Seattle Genetics Millenium-Takeda | US FDA conditional approval 8/2011. Phase III and PhI/II combinations |
| Trastuzumab emtansine (T-DM1) | HER2 (ErbB2) | Thioether SMCC-DM1 Maytansinoid | Trastuzumab Humanized IgG1 | HER2-positive breast cancer | Genentech-Roche | Ph II and Ph III and Ph I/II combinations |
| Inotuzumab ozogamicin (CMC-544) | CD22 (Siglec-2) | Hydrazone, AcBu *N*-acetyl-γ calicheamicin Calicheamicin | G5/44 Humanized IgG4 | B-cell lymphomas | Pfizer | Ph I and Ph I/II Ph III combination |

*Continued*

**Table 23.1** ADCs in clinical development—cont'd

| ADC | Target antigen | Linker-cytotoxic compound, class | Antibody | Tumor type(s) | Developer | Status |
|---|---|---|---|---|---|---|
| Glembatumumab vedotin (CR011-vc-MMAE, CDX-011) | Glycoprotein NMB (osteoactivin) | Dipeptide, vc-MMAE Auristatin | Glembatumumab Fully human IgG1 | Metastatic breast cancer and melanoma | Celldex Therapeutics | Ph II |
| Lorvotuzumab mertansine (IMGN901, huN901-DM1, BB10901) | CD56 (NCAM) | Hindered disulfide SPP-DM1 Maytansinoid | Lorvotuzumab Humanized IgG1 | SCLC and other CD56-positive solid tumors, multiple myeloma | ImmunoGen | Ph I<br>Ph I/II combinations |
| SAR3419 (huB4-DM4) | CD19 | Highly hindered disulfide SPDB-DM4 Maytansinoid | huB4 Humanized IgG1 | B-cell malignancies | Sanofi | Ph II |
| BT-062 | CD138 Syndecan-1 | Highly hindered disulfide SPDB-DM4 Maytansinoid | Anti-CD138 chimeric IgG4 | Multiple myeloma | Biotest | Ph I/II |
| SAR566658 | CA6 | Highly hindered disulfide SPDB-DM4 Maytansinoid | DS6 Humanized IgG1 | CA6-positive solid tumors | Sanofi | Ph I |

| | | | | | | |
|---|---|---|---|---|---|---|
| BAY 94-9343 | Mesothelin | Highly hindered disulfide SPDB-DM4 Maytansinoid | Antimesothelin Fully human IgG1 | Mesothelin-positive solid tumors | Bayer Healthcare | Ph I |
| SGN-75 (h1F6-mcMMAF) | CD70 | Non-cleavable mcMMAF Auristatin | SGN-70 Humanized IgG1 | Non-Hodgkin's lymphoma and renal cell carcinoma | Seattle Genetics | Ph I |
| PSMA ADC | Prostate-specific membrane antigen | Dipeptide, vc-MMAE Auristatin | Anti-PSMA Fully human IgG1 | Metastatic, hormone-refractory prostate cancer | Progenics Pharmaceuticals | Ph I |
| ASG-5ME | SLC44A4 (AGS-5) | Dipeptide, vc-MMAE Auristatin | Anti-ASG-5 Fully human IgG2 | Pancreatic cancer and prostate cancer | Astellas (Agensys) Seattle Genetics | Ph I |
| ASG-22ME | AGS-22 Nectin-4 | Dipeptide, vc-MMAE Auristatin | Anti-Nectin Fully human IgG | Solid tumors | Astellas (Agensys) Seattle Genetics | Ph I |
| Anti-AGS-16 ADC (AGS-16M8F) | AGS-16 (ENPP3) | Auristatin | Anti-AGS-16 Fully human IgG2 | Renal cell carcinoma and liver cancer | Astellas (Agensys) | Ph I |
| RG7593 | CD22 | Auristatin | Anti-CD22 human IgG | B-cell lymphoma | Genentech-Roche | Ph I |

*Continued*

**Table 23.1** ADCs in clinical development—cont'd

| ADC | Target antigen | Linker-cytotoxic compound, class | Antibody | Tumor type(s) | Developer | Status |
|---|---|---|---|---|---|---|
| MDX-1203 | CD70 | Dipeptide (vc), prodrug of duocarmycin analog (DNA minor-groove binder and alkylator) | Anti-CD70 Fully human IgG | Non-Hodgkin's lymphoma and renal cell carcinoma | Bristol-Myers Squibb (Medarex) | Ph I |
| Milatuzumab-Doxorubicin hLL1-Dox | CD74 | Thioether (SMCC) Doxorubicin | IMMU-110 Humanized IgG1 | Multiple myeloma | Immunomedics | Ph I |
| IMGN529 | CD37 | Thioether SMCC-DM1 Maytansinoid | K7153A Humanized IgG1 | B-cell malignancies | ImmunoGen | IND active |
| 5 ADCs | Not identified | Auristatin | Not identified | Various solid and liquid cancers | Genentech-Roche | Ph I |
| 2 ADCs | Not identified | Maytansinoid | Not identified | Undisclosed cancers | Amgen | IND |

HER2-positive breast cancer, with promise for improved outcomes for patients. It is now being evaluated for treatment of HER2+ metastatic breast cancer in a number of Phase III trials. Besides **8c** and **2**, several other ADCs made with potent microtubule-impacting cytotoxic agents have entered phase II studies, or are in combination studies. Glembatumumab vedotin (see structure **8c** above, wherein the antibody is anti-NMB) combines an antiglycoprotein NMB fully human antibody with vc-MMAE.[36] The target, also known as osteoactivin, is highly expressed in melanoma[36] and breast cancer.[37] This ADC was active in preclinical xenograft models of melanoma.[36] Two Phase I/II trials were conducted in patients with advanced metastatic cancers: one in patients with melanoma, where antitumor activity was reported,[38] and the second in patients with metastatic breast cancer.[39] A Phase II trial in metastatic breast cancer is ongoing.

Lorvotuzumab mertansine, **6**, comprises a humanized version of the N901 antibody that targets CD56/NCAM, conjugated to DM1 *via* a hindered disulfide linker.[40] CD56 is expressed on a variety of cancers of hematopoietic and neuroendocrine origin, including multiple myeloma[40] and small-cell lung cancer.[41] Phase I trials showed encouraging evidence of antitumor activity coupled with an acceptable tolerability profile, in particular, a lack of clinically meaningful myelosuppression, both in CD56-positive solid tumors[42] and in multiple myeloma.[43] Clinical studies of **6** in combination with lenalidomide and dexamethasone in multiple myeloma, and in combination with carboplatin and etoposide in SCLC, have been initiated.[44]

SAR3419 comprises a humanized anti-CD19 antibody attached to DM4, linked *via* a highly hindered disulfide linker.[7,45] Phase I studies demonstrated good tolerability and promising activity in a variety of lymphoma subtypes, especially notable considering the heavy pretreatment of these patients and the mixed histology of those enrolled.[7,46,47] Three Phase II trials evaluating this ADC are underway in diffuse large B-cell lymphoma and acute lymphoblastic leukemia.

Inotuzumab ozogamicin, a conjugate of an anti-CD22 antibody with calicheamicin (see structure **1**, wherein the antibody is anti-CD22 instead of anti-CD33),[25,48] is being evaluated in a Phase III study in non-Hodgkin's lymphoma. The only other ADC with a DNA-acting payload is the duocarmycin conjugate **12**, wherein the antibody is anti-CD70; it is in a Phase I trial in CD70-positive renal cell cancer and non-Hodgkin's lymphoma.

There are several more ADCs in early clinical development (Table 23.1). Building upon the excitement around **8c** and **2**, nearly all of them utilize one of the two classes of potent tubulin-acting agents as payload.

## 6. CONCLUSION

The clinical development of ADCs, especially those employing potent microtubule-impacting agents, has changed the outlook for ADC technology. ADCs hold the promise of having an important role in cancer treatment, providing active therapeutics that reduces the severe toxicities associated with nontargeted cytotoxic chemotherapy.

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