Antibody-Drug Conjugates for Cancer Therapy

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Abstract: The antibody-drug conjugate (ADC) concept is to use an antibody to deliver a cytotoxic drug selectively to a target such as a tumor-associated antigen. Such conjugates represent a broadly applicable approach to enhance the antitumor activity of antibodies and improve the tumor-to-normal tissue selectivity of chemotherapy. Critical parameters for ADC development include target antigen selection, conjugate internalization by tumor cells, drug potency and stability of the linker between drug and antibody. Other important considerations include the conjugation methods, drug-to-antibody ratio, and the effects of drug conjugation on antibody properties. Highly potent drugs with more stable linkers have been attached to a new generation of antibodies to create conjugates with pronounced antitumor activities in preclinical studies and encouraging results in early stage clinical trials. This review details these advances, discusses some of the remaining challenges, and overviews ADCs currently in clinical trials for cancer therapy.

Key Words: antibody, immunoconjugate, targeted therapy

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Antibody-based therapeutics are of growing significance for cancer therapy as evidenced by 12 such drugs approved for oncologic indications since 1995, including 9 in the United States.[1] However, these antibody therapeutics are seldom curative, encouraging numerous approaches to enhance efficacy.[2–5] Two of the most promising strategies to enhance the antitumor activity of antibodies are antibody-drug conjugates (ADCs)—the focus of this review—and Fc optimization to augment secondary immune functions.[5]

ADCs comprise an antibody, usually in IgG format, conjugated to a cytotoxic drug via a chemical linker (Fig. 1). The therapeutic concept of ADCs is to use an antibody as a vehicle to deliver a cytotoxic drug to a tumor cell by means of binding to a target cell surface antigen (Fig. 1). ADCs are prodrugs requiring drug release for activation, commonly after ADC internalization into the target cell. Numerous preclinical efficacy studies show that ADCs have significant potential for enhancing the antitumor activity of "naked" antibodies and reducing the systemic toxicity of the conjugated drugs.[3,6] Moreover, clinical demonstration of the ADC concept has been provided by gemtuzumab ozogamicin (Mylotarg), a humanized anti-CD33 antibody conjugated to calicheamicin approved in the United States for the treatment of acute myeloid leukemia (AML).[7]

The concept of arming antibodies by conjugation to protein toxins dates back to 1970,[8] and was followed a few years later by antibody conjugates with cytotoxic drugs. Antibody therapeutics have come of age in the intervening decades with the advent of hybridoma technology to develop murine monoclonal antibodies (MAb),[9] chimerization and humanization to address the shortcomings of murine MAb as therapeutics, and more recently with direct routes to human antibodies using phage display or transgenic mice.[2] The clinical potential of ADCs has been greatly enhanced by improved choices of targets,[10] more potent drugs in conjunction with linkers of improved stability[6,11] and greatly expanded knowledge of ADC cell biology[32] and pharmacology.[13] ADCs are reviewed here from a design perspective from target selection, through ADC lead identification, optimization, and preclinical development to clinical experience (Fig. 2). Other payloading strategies for antibodies, as well as for alternative delivery vehicles, have been extensively reviewed elsewhere[3,11,12,14] and are outside the scope of this article. Of these, radioimmunoconjugates stand out, with the approval of 2 different anti-CD20 radioimmunoconjugates for the treatment of non-Hodgkin lymphoma: ibritumomab tiuxetan (Zevalin, ^{90}Y conjugate) and ^{131}I tositumomab (Bexxar).[15]

TARGET SELECTION

The de novo identification and validation of cell surface targets as candidates for ADCs is beyond the scope of this article and is reviewed elsewhere.[10] Target selection from a candidate pool is a critical first step in generating ADCs (Fig. 2). Expression profiling is a key aspect of target selection in that tumor-associated antigen expression is presumed to be one of the necessary criteria for successful targeting with an ADC, whereas little or no normal tissue expression is desirable and may be necessary to achieve an adequate therapeutic index.

Immunohistochemistry allows semi-quantitative analysis of protein expression in tissue samples and is often the most direct and consequently preferred method for evaluating potential ADC targets. The advent of tissue microarrays[16] facilitates high-throughput expression profiling—hundreds to thousands of samples—with greater standardization.[17] A limitation of immunohistochemistry is the high variability of data with different reagents, protocols and samples[17]—resolvable if concordant data are obtained using multiple antibodies. An additional challenge with immunohistochemistry is that data may be difficult to interpret if the antigen is

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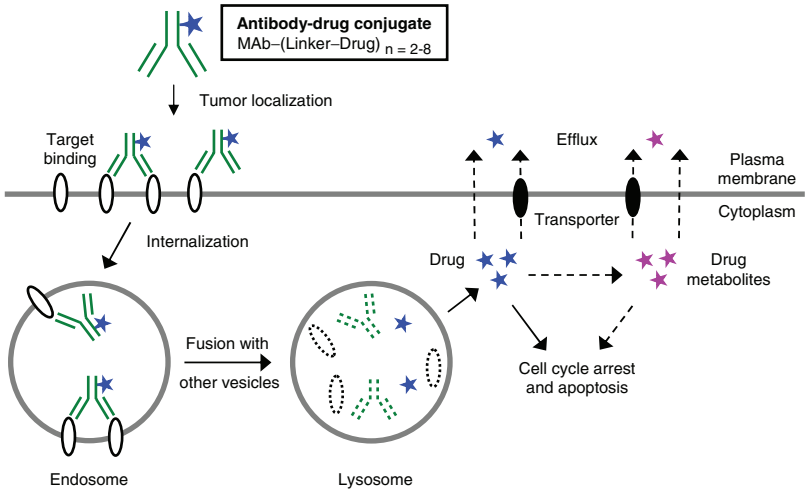

FIGURE 1. Antibody-drug conjugate concept. An ADC comprises an antibody conjugated via a linker to a cytotoxic drug. ADCs are commonly in IgG format with 2–8 drugs/antibody. Some portion of an ADC delivered intravenously localizes to a tumor and binds to a target antigen on the surface of the tumor cell. Internalization of the ADC commonly proceeds via a clathrin-coated pit mechanism[167] although other mechanisms such as calveolae[168] and pinocytosis sometimes occur.[32] Internalized vesicles are stripped of clathrin, fuse with other vesicles and enter the endosome-lysosome pathway. Proteases in the acidic environment of the endosomes digest the antibody, and potentially also the linker, to release free drug. The drug then crosses the membrane to enter the cytoplasm where it binds to its molecular target, commonly leading to cell cycle arrest and apoptosis. A portion of the drug is effluxed from the cell, either by passive diffusion, active transport, or leakage from dying cells. If the effluxed drug is cell permeable it may enter neighboring cells and give rise to so-called bystander cell killing.[25,131] In some cases, the drug is metabolized in the cell and these metabolites may differ from the parent drug in their ability to kill the tumor cells as well as in the propensity to be effluxed.[131]

located inside the cell as well as on the cell surface. Flow cytometry is also used for protein expression profiling, particularly for hematologic tumors. This method provides more quantitative information than immunohistochemistry and can demonstrate the accessibility of a target to an antibody. Limitations of flow cytometry include the limited availability of suitable patient samples, and the need to disaggregate solid tumor samples, that may potentially compromise antigen levels.

Expression profiling at the mRNA level is widely used for target assessment and can be a valuable, albeit indirect adjunct, to protein expression profiling.[10] It is important to verify the concordance of mRNA and protein levels, as demonstrated in at least a minority of cases.[18] Techniques available for mRNA expression profiling in clinical samples include microarrays, serial analysis of gene expression, quantitative real-time PCR, cDNA hybridizations using multiple tissue expression arrays/cancer profiling arrays, and RNA in situ hybridizations using frozen tissue samples.[10]

Tumor Expression of Antigen

ADCs to date have been used almost exclusively to target antigens located on the surface of tumor cells. These targets are almost always normal antigens either expressed at abnormal times in development, at aberrantly high levels on cell surfaces, or with such limited normal tissue expression as to render them potential suitable for targeted therapy. Beyond malignant cells, ADCs could potentially be used to target antigens on normal cells recruited to the tumor such as endothelial cell or tumor-associated neovasculature cells.[19,20] Indeed, at least 2 different ADCs have been developed to target prostate specific membrane antigen (PSMA),[21,22] an antigen associated with prostate cancer and more recently found to be expressed in the neovascular endothelium of a variety of solid tumors and essentially absent from normal vasculature.[19] The anti-PSMA ADC, MLN2704 (J591-DM1,[21]), has been evaluated in prostate cancer and showed antitumor activity at well-tolerated doses,[6] although clinical development of MLN2704 has subsequently been discontinued. Tumor-associated extracellular matrix protein variants such as the extradomain B of fibronectin domain and the long isoform of tenascin C have been successfully targeted with a panoply of experimental antibody-based drugs.[19] Targeting such extracellular matrix variants with ADCs would require drug and linker selections that facilitate extracellular drug release and drug entry into tumor cells.

High level and homogeneous antigen expression on the external surface of all tumor cells seems desirable to facilitate efficient targeting with ADCs. Elevated antigen expression can lead to greater ADC potency, as judged by in vitro and in vivo experiments with transfected cell lines.[127] However, such high level antigen expression is not a prerequisite for clinical benefit by ADCs as demonstrated by gemtuzumab ozogamicin approved for the treatment of AML with only approximately 5,000–10,000 copies of the cognate antigen, CD33, per cell.[23] Homogeneous antigen expression may be beneficial for targeting with ADCs, but is not a requirement, as demonstrated by in vivo xenograft studies with a heterogeneously expressed antigen, CanAg.[24] Successful targeting of heterogeneously expressed antigens may benefit from the

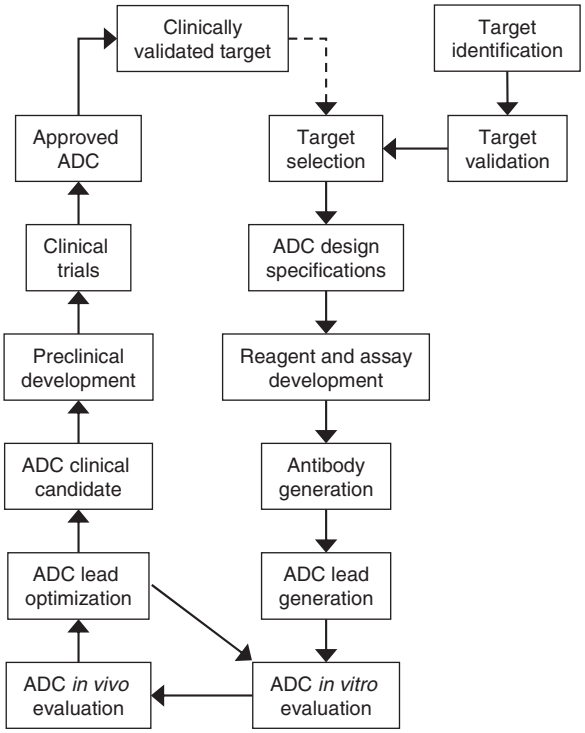

FIGURE 2. Antibody-drug conjugate development cycle. The development of ADCs, as for other antibody therapeutics,[2] can be conceptualized as a design-driven process that begins with target selection. The next step is to formulate the ADC design specification, which is a list of critical or desirable properties predicted to facilitate preclinical development of the ADC and to achieve the desired clinical outcome. The primary goal with an ADC is to deliver a cytotoxic drug selectively to a target within a tumor. An ADC can potentially be designed with additional mechanisms of antitumor activity, eg, by interfering with the biologic function of a target that is involved in oncogenesis, angiogenesis or metastasis. The design specification guides the method of antibody generation, plus the reagents and assays needed to identify ADC leads with the desired properties. Panels of ADCs are evaluated in vitro for cytotoxic activity against tumor cell lines and then in vivo for antitumor activity, pharmacokinetics and safety. Optimization of the antibody delivery vehicle and/or the drug-linker payload may be necessary or desirable, eg, to increase the therapeutic index of the ADC. The ADC clinical candidate is then subjected to further preclinical and, if warranted, clinical development. ADC clinical trial data—particularly if they validate the target through to drug approval—may provide the impetus to iterate the design cycle to create an improved ADC.

use of ADCs with bystander killing activity[25,26] or by combining an ADC with another therapeutic modality such as cytotoxic chemotherapy.[27]

Optimally, the target antigen is expressed in most, if not all, patients with a particular tumor type. If not, a diagnostic test for antigen expression may be required for patient selection as used for HER2 expression profiling before trastuzumab therapy, and for EGFR profiling before treatment with cetuximab or panitumumab.[17] Flow cytometry is used widely for identifying antigen-positive hematologic tumors.[28] In addition, the target antigen should be expressed throughout the course of disease. Minimally, the antigen should be expressed in late stage disease—ie, patient populations commonly available for evaluating experimental drugs.

Normal Tissue Expression of Antigen

The target antigen should ideally be absent from normal tissue, but this is seldom, if ever, achieved. Tumor-associated antigens are commonly selected on the basis of higher expression levels in tumors and limited normal tissue expression. Some normal tissue expression of antigen is anticipated and may be acceptable. Unfortunately, there is currently no reliable way to predict the significance of normal tissue expression of an antigen on ADC tolerability. Therefore, it is highly desirable to develop ADCs that cross-react to the corresponding antigen from other species—so called species cross-reactivity—to allow preclinical assessment of antigen-dependent toxicity, also known as "on-target" toxicity.

Antigen expression on normal tissue can lead to on-target toxicity with ADCs in patients,[29,30] but does not necessarily do so. For example, inefficient ADC localization to antigen-positive normal tissue is presumed to lower the risk of on-target toxicity. ADC localization may be particularly inefficient if the level of antigen expression is low, the antigenic sites are located in the central nervous system beyond the blood brain barrier or if the antigen is restricted to the apical surface of cells. Nonproliferating cells have reduced sensitivity to many cytotoxic drugs as compared with proliferating cells,[31] an attribute that that seems likely to apply to corresponding ADCs[32] and may reduce on-target toxicity. Extensive normal tissue expression of antigen may create a "sink" that soaks up the ADC and accelerate ADC clearance.[33]

Targeting may be possible for an antigen whose expression is restricted to a nonvital organ or cell population, even if the antigen is not differentially expressed between normal tissue and tumors. For example, CD20, CD33, and CD52 are cell surface markers for B cells, myeloid progenitors plus monocytes, and leukocytes, respectively—cell lineages whose elimination by antibody therapeutics can be tolerated on a temporary basis.

Antigen Shedding

Shedding of target antigen from the surface of tumor cells into circulation may increase the risk of toxicity because of immune complex formation and deposition in kidney, accelerated clearance of ADC and impaired tumor localization. Shed antigen can sometimes be problematic for ADCs as suggested by a phase II trial for an anti-MUC1-calicheamicin conjugate in ovarian cancer.[34] However, shed antigen can sometimes be tolerated as preliminary reports of a phase I study with an anti-HER2 ADC, trastuzumab-DM1, in metastatic breast cancer.[35,36]

Target Biology

The selection of target antigens that are causally involved in tumor pathogenesis, eg, HER2 and EGFR, is not a prerequisite for success with ADCs as suggested by targeting of CD33 with gemtuzumab ozogamicin. However the choice of such "functional antigens" may be advantageous for ADCs

by lowering the risk for tumor "escape" therapy by the reduction or elimination of antigen expression—down modulation. Additionally, the ADC may be screened for the ability to interfere with the function of the target, eg, by growth factor blockade for a growth factor receptor, to provide additional mechanisms of antitumor activity beyond delivery of a cytotoxic drug.

Antigen Internalization

ADC internalization (Fig. 1) is desirable and often necessary for efficient drug release, depending upon the drug and linker. Nevertheless, antigen internalization per se is not a useful criterion for target selection. This reflects the fact that antigen internalization may be impacted by many ADC attributes including choice of antibody epitope, as well as the drug and linker (see below). Moreover, inefficiently internalizing antigens such as CD20, a tetra-spanning membrane protein, have been successfully targeted with ADCs as judged by efficacy in xenograft experiments in vivo.[37,38] Antigen internalization can potentially be impacted by the presence of other membrane proteins, eg, CD21 expression was recently reported to impair the internalization of ADCs directed to its binding partner, CD19.[39]

ANTIBODY LEAD IDENTIFICATION

Lead generation for antibody therapeutics has evolved in recent years to the pursuit of large antibody panels—tens to hundreds of antibodies—to increase the likelihood of identifying leads that satisfy multiple design criteria.[2] This large-scale antibody screening is now being evaluated as a paradigm for ADC development. Important antibody attributes for use as conjugates include binding to the human antigen on tumor cells, cytotoxic activity as ADCs, and other desirable attributes such as cross-reactivity with species that can be used in toxicology. These important and desired antibody attributes for ADCs are discussed below, together with a comparison of the main alternative antibody generation technologies.

Antibodies that bind the target antigen on tumor cells can be conjugated to one or more specific drug-linkers (see ADC Optimization section) and screened for in vitro cytotoxic and/or antiproliferative activity against tumor cell line panels. ADCs provide the most direct assessment of antibodies as drug-delivery vehicles and are therefore recommended as an initial in vitro screening strategy. Anti-IgG (secondary) drug conjugates have also been used for antibody screening.[40] Internalization assays are not recommended for screening of ADCs or unconjugated antibodies as they are indirect and poorly predictive surrogates for targeted toxicity. The in vitro potency of ADCs is commonly compared for a panel of antigen positive cell lines versus antigen negative cell lines. The selectivity of ADCs for antigen-positive over antigen-negative lines is reflected in differences in IC_{50} values for cytotoxicity that are typically $\geq$100-fold. A highly desirable attribute of antibodies for ADCs is species cross-reactivity to the corresponding nonhuman primate, and ideally rodent antigens, to facilitate preclinical assessment of on-target toxicity of ADCs.

Large human antibody panels can be obtained from immunization of transgenic mice in conjunction with hybridoma technology[41] or by selection from phage display libraries.[42,43] Human antibodies can also be generated using emerging technologies such as yeast, ribosome or mRNA display.[42,43] Murine MAb identified using hybridoma technology,[9] can also provide a useful starting point for ADC lead generation. Murine MAb have several well-known shortcomings as therapeutics—immunogenicity, short terminal half-life, and commonly inefficient effector functions with human immune cells. However, these limitations of murine MAb have been largely overcome by chimerization and humanization.[2,44] These antibody technologies each have strengths and limitation for ADC lead generation as summarized below.

An attractive feature of phage and other display technologies is that they allow direct selection of species cross-reactive antibodies. Commonly, antibodies can be identified that cross-react with nonhuman primates, whereas cross-reactivity to rodents is less common. Phage display libraries also allow direct selection for efficiently internalizing antibodies,[45] that warrant exploration for use as ADCs. Phage-derived antibodies require reformatting of the displayed antibody fragment—commonly Fab or scFv—as an IgG for drug conjugation and functional comparison as ADCs. The advent of reformatting high-throughput methods has mitigated what was previously a significant bottleneck in deriving human IgG from phage display libraries.[42] It may be necessary or desirable to increase the antigen-binding affinity of phage-derived antibodies for therapeutic applications—affinity maturation. This suboptimal affinity issue has been addressed by larger and higher quality phage libraries that have facilitated the identification of higher affinity antibodies, as well by streamlined methods for affinity maturation using phage, yeast, ribosome, or mRNA display technologies.[42,43]

Murine hybridoma technology is more widely available than human hybridoma technology and provides a potentially more facile route to generating large antibody panels. However these advantages are offset by the need for subsequent humanization of murine MAb. A favorable attribute of hybridoma technologies is that the resultant IgG can be conjugated to cytotoxic drugs and rapidly screened for ADC activity. Species cross-reactive MAb can sometimes be obtained from hybridoma technology, including transgenic mice making human MAb. Immunization strategies that include antigen from different species and methods to break immunologic tolerance to self-antigens may be helpful. "Knockout" mice can be used to generate antibodies against virtually any self protein that is not essential for survival or immune system function.[46] Immune tolerance can also be broken by coupling the protein to an immunogenic carrier through cross-linking,[47] or expressing the self protein with a foreign antigen[48] or by inserting a T cell epitope.[49] The generation of species cross-reactive antibodies from hybridoma technology relies on screening rather than the more facile selection possible with display technologies. Surrogate antibodies that react to the antigen from the species used for toxicology offer an alternative, albeit less desirable strategy, than using a species cross-reactive antibody.

ANTIBODY LEAD OPTIMIZATION

The next step in ADC development is lead optimization, in which the antibody delivery vehicle and/or the drug payload are optimized. Permuting improvements in the antibody delivery vehicle with those in drug and linker payload is a potentially cumbersome and time-consuming task. ADC lead optimization can be streamlined in our experience by parallel optimization of the antibody and drug-linker and then combining the improvements obtained. Antibodies are commonly engineered to optimize their utility for specific therapeutic applications[2]—a paradigm that is now being extended to ADCs, as reviewed in this section.

IgG Isotype Choice for ADCs

A key issue for antibody therapeutics, and potentially also for ADCs, is that of isotype selection.[2,50,51] Most ADCs in clinical development use IgG1 (7/10), although IgG4 (2/10), and IgG2 (1/10) have also been used (Table 1). Isotype impacts the ability of IgG to support the secondary immune functions known as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC). For example, IgG1 can potentially bind to Fcγ receptors and support ADCC and ADCP. Additionally, IgG1 can potentially bind to the complement component, C1q, and support CDC. In contrast, IgG2 and IgG4 are typically inefficient in their effector functions.[52] For ADCs, effector functions could be advantageous by providing additional antitumor activities. Conversely, effector cell binding could impair ADC localization to tumors, ADC internalization by target cells or give rise to off-target toxicities. The impact of antibody isotype on ADC function is challenging to study preclinically because of numerous differences between human and mouse immune systems including Fcγ receptors and FcRn, as well the absence of endogenous IgG in immune-compromised mice typically used for xenograft studies.

IgG2 can form covalent dimers,[53] an intriguing observation whose generality and significance for IgG2-based therapeutics remain to be determined. Nevertheless, dimerization does not seem to be a major limitation for IgG2-based drugs as judged by one IgG2 that is approved for therapeutic use, namely panitumumab,[54] and encouraging progress with several other IgG2 in clinical development[51] including one IgG2-based ADC (Tables 1 and 2).

IgG3 have been largely avoided for therapeutic applications including as ADCs for reasons that are not entirely clear but presumably relate to the differences between IgG3 and other IgG isotypes: longer hinge and greater susceptibility to proteolysis, more extensive polymorphism (13 allotypes), potentially immunogenicity, and a shorter terminal half-life (7 days vs 21 days for other IgG isotypes[55]).[2,50,51]

IgG4 have been used successfully as ADCs as demonstrated by the FDA-approved drug, gemtuzumab ozogami-

TABLE 1. Antibody-Drug Conjugates in Clinical Development

Conjugate Names	Developer	Target Antigen Names	Antigen Class	Antibody	Drug Class, Drug	Linker Class, Linker	Mean Drug/ Antibody Ratio	References
BIIB015	Biogen-Idec	Cripto	EGF-CFC family	Hz IgG1	Maytansinoid, DM4	Sterically-hindered disulfide	NA	NA
SGN-35	Seattle Genetics	CD30 (TNFRSF8)	TNF receptor superfamily	Ch IgG1	Auristatin, MMAE	Dipeptide, valine-citrulline	4	26, 136
SAR3419 (huB4-DM4)	Sanofi-Aventis	CD19	IgG superfamily	Hz IgG1	Maytansinoid, DM4	Sterically-hindered disulfide	4	111
AVE9633 (huMy9-6-DM4)	Sanofi-Aventis	CD33 (Siglec-3)	Siglec	Hz IgG1	Maytansinoid, DM4	Sterically-hindered disulfide	3.5	112
CR011-vcMMAE	CuraGen	Glycoprotein NMB	Transmembrane glycoprotein	Hu IgG2	Auristatin, MMAE	Dipeptide, valine-citrulline	3.8	128, 154
HuC242-DM4 (IMGN242)	ImmunoGen	CanAg	Carbohydrate	Hz IgG1	Maytansinoid, DM4	Sterically-hindered disulfide	3.5	109, 131
Trastuzumab-DM1	Genentech	HER2 (ErbB2, HER2/*neu*)	Growth factor receptor, EGF receptor family	Hz IgG1	Maytansinoid, DM1	Thioether: SMCC	3.5	35, 36
huN901-DM1 (IMGN901, BB10901)	ImmunoGen	CD56 (NCAM)	Cellular adhesion molecule, IgG superfamily	Hz IgG1	Maytansinoid, DM1	Sterically-hindered disulfide	3.5	113
Inotuzumab ozogamicin (CMC-544)	Wyeth	CD22 (Siglec-2)	Siglec	Hz IgG4	Calicheamicin, *N*-acetyl-γ calicheamicin,	Hydrazone, AcBut	5–7	92, 93, 160, 169
Gemtuzumab ozogamicin (Mylotarg)	Wyeth	CD33 (Siglec-3)	Siglec	Hz IgG4	Calicheamicin, *N*-acetyl-γ calicheamicin	Hydrazone, AcBut	2–3*	95, 149, 152

*Gemtuzumab ozogamicin has a mean loading of 2–3 drugs/antibody and comprises a mixture of 50% ADC with 4–6 drugs/antibody and 50% unconjugated antibody.[95]

AcBut, 4-(4′-acetylphenoxy) butanoic acid; Ch, chimeric; EGF, epidermal growth factor; Hu, human; Hz, humanized; NA, not available; NCAM, neural cell adhesion molecule; siglec, sialic acid-binding immunoglobulin-like lectin; SMCC, *N*-succinimidyl-4-(*N*-maleidomethyl)cyclohexane-1-carboxylate and TNFRSF, tumor necrosis factor receptor superfamily.

TABLE 2. Preliminary Clinical Trial Data with Antibody-Drug Conjugates

Conjugate Names	Trial Phase	Indications	Patients Treated (*n*)	Responses (*n*)	ADC Dose Level*	ADC Dose Schedule (IV)	Dose-Limiting Toxicity	Reference
BIIB015	I	Cripto+ solid tumors	NA	NA	NA	NA	NA	NA
SGN-35	I	CD30+ hematologic malignancies	23	4 PR	0.1–1.8 mg/kg	Every 3 wk	Not reached	137
SAR3419 (huB4-DM4)	I	NHL	NA	NA	NA	NA	NA	NA
AVE9633 (huMy9–6-DM4)	I	AML	17	1 CRp, 1 PR	30–150 mg/m^2	2 consecutive weeks every 4 wk	2 of 3 patients at 150 mg/m^2; liver toxicity, keratitis	112
CR011-vcMMAE	I/II	Metastatic melanoma	25	0	0.03–1.88 mg/kg	Every 3 wk for 4 cycles	Not reached	155
HuC242-DM4 (IMGN242)	I	CanAg+ metastatic solid tumors	30	0	18–297 mg/m^2	Every 3 wk	2 of 6 patients at 223 mg/m^2, decreased visual acuity, corneal deposits and keratitis	163
	II	Gastric cancer	NA	NA	168 mg/m^2	Every 3 wk	NA	
Trastuzumab-DM1	I	HER2+ metastatic breast cancer	24	6 PR	0.3–4.8 mg/kg	Every 3 wk	2 of 3 patients at 4.8 mg/kg, thrombocytopenia	35, 36
	II	HER2+ metastatic breast cancer	NA	NA	NA	NA	NA	NA
huN901-DM1 (IMGN901, BB10901)	I	Multiple myeloma	12	0	40–90 mg/m^2	2 consecutive weeks every 3 wk	Not reached	166
	II	CD56+ small cell cancers	30	2 PR	60 mg/m^2	4 consecutive weeks every 6 wk	NA	165
Inotuzumab ozogamicin (CMC-544)	I	NHL	NA	NA	NA	NA	NA	NA
	I/II	NHL	NA	NA	NA	NA	NA	NA
	III	NHL	NA	NA	1.8 mg/m^2	Every 4 wk	NA	NA

Sources include company web sites and www.clinicaltrials.gov.

*Interconversion of surface area-based and weight-based dosing units requires knowledge of the patient weight and height, eg, a dose of 1.0 mg/kg for a 6 foot (1.83 m) tall patient is equivalent to 38 mg/m^2 using the dosing calculator on the FDA web site (www.fda.gov/cder/cancer/animalframe.htm).

AML, acute myeloid leukemia; CR, complete response: CRp, complete response with incomplete platelet recovery; NA, not available; NHL, non-Hodgkin lymphoma; NR, not reached; and PR, partial response.

cin.[7] Nevertheless, IgG4 molecules may be nonideal for drug delivery in that they can exchange Fab arms in vivo to become functionally monovalent, a hypothesis[56] now supported by significant experimental evidence.[57] This apparent limitation of IgG4s can be overcome by replacing the C_H3 domain with the corresponding domain from IgG1.[57] Preclinical studies in mice may underestimate the clinical potential of IgG4 ADCs in that IgG4 have shorter terminal half-lives than their IgG1 and IgG2 counterparts.[58] In contrast, IgG4 have similar terminal half-lives as IgG1 and IgG2 in man.[55]

ADC Binding to Fcγ Receptors and FcRn

Binding interactions between IgG Fc and Fcγ receptors have been determined structurally by x-ray crystallography[59] and functionally by mutational analysis.[68] Tailoring the interaction between IgG and Fcγ receptors by modifying the Fc glycan or protein sequence can be used to increase or decrease IgG effector functions.[2,5] Combining such IgG Fc modifications with ADCs warrants exploration as a strategy to enhance the clinical potential of ADCs.

The interaction between IgG and its salvage receptor, FcRn, is critical to the recycling and long serum persistence of IgG.[61] Proteins in circulation, including IgG, are subject to fluid phase uptake—pinocytosis—by some cells including vascular endothelia. IgG can then bind to FcRn in endosomes under slightly acidic conditions (pH 6.0–6.5) and recycle to the cell surface, where they are released under near neutral conditions (pH 7.0–7.4).[62,63] The binding interaction between IgG Fc and FcRn has been elucidated structurally by x-ray crystallography[64] and functionally by mutational analysis.[60,61,68] Tailoring the interaction between IgG and FcRn can be used to increase or decrease the terminal half-life of IgG.[2,61] Indeed, the terminal half-life of human IgG has been extended in rhesus[65,66] and cynomolgus[67] macaques. These data suggest the feasibility of extending the plasma half-life of antibody therapeutics by engineering the interaction of Fc with FcRn and are potentially applicable to ADCs.

Currently, there are limited data available regarding the impact of drug conjugation on IgG binding to either Fcγ receptors or FcRn. Drugs conjugated through solvent accessible cysteine residues are distant from the binding sites for Fcγ receptors[59,68] and FcRn[68,69] and unlikely to provide a major steric impediment to receptor binding. However, dis-

ruption of both hinge disulfides in a chimeric IgG1 by Cys→Ser mutations can greatly diminish ADCC activity, presumably via impaired binding to Fcγ receptors.[70] In contrast, maintaining at least one of the interheavy chain hinge disulfide bonds in a humanized IgG1 preserves efficient binding to FcγRIIIA as judged by Cys→Ser mutations.[71] For lysine conjugation chemistry, as exemplified by huN901-DM1, the drug is distributed among ~40 different sites.[72] Thus, interference with binding to FcRn and Fcγ receptors is likely minimized by the low occupancy of individual lysine sites in the population of lysine-conjugated ADCs.

Antigen-Binding Affinity

One of the most intensively studied properties of antibodies is that of antigen-binding affinity.[2] Antibody conjugation to drugs is undertaken so as to have little or no impact on antigen-binding affinity.[73] This is accomplished by carefully controlling the number of drugs attached to each antibody, as discussed below. Limited data are available on the relationship between antigen-binding affinity and antitumor activity of ADCs. Important and as yet unanswered questions are whether or not there is an optimal affinity for ADC activity in vitro and in vivo, and if the antigen binding affinity impacts the therapeutic index.

ADC OPTIMIZATION

Many different antibodies, drugs, and linkers have been combined to create ADCs. Cytotoxic drugs being explored for ADCs generally have potencies several orders of magnitude greater than for conventional chemotherapeutics, making them too potent for systemic delivery. Linkers used to attach the drugs to the antibody delivery vehicles have been designed to exploit intracellular conditions for drug release including the acidic environment of endosomes (pH 5.5–6.2) and lysosomes (pH 4.5–5.0), high thiol concentrations in the cytosol, and proteolytic enzymes in lysosomes. These linkers have provided half-lives of drug retention on circulating ADCs in clinical studies of ~43 hours for the hydrazone in BR96-doxorubicin,[74] and ~41 hours for the sterically-hindered disulfide in huC242-DM1.[75] Peptide linkers offer the promise of even greater stability as judged by half-lives of 7–10 days in preclinical in vivo studies.[76]

Drug Potency and ADC Activity

Early ADCs used clinically approved chemotherapeutic drugs because of their ready availability, amenability to chemical manipulation and their well known toxicological properties.[77] Much about the strengths and pitfalls of antibody-mediated drug delivery was learned from BR96-doxorubicin (Fig. 3), an ADC directed against the Lewis^Y tetra-saccharide antigen on human carcinomas.[78,79] Doxorubicin was attached to a chimeric version of the BR96 antibody, through an acid-labile hydrazone linker. Upon binding to the antigen, the antibody was internalized and doxorubicin released within acidic endosomal and lysosomal vesicles. Treatment of tumor-bearing mice and rats with BR96-doxorubicin led to immunologically specific tumor cures, albeit at high (>100 mg/kg) doses, reflecting the low potency of the targeted drug.[79] In a phase I clinical trial, the maximum-tolerated dose of BR96-doxorubicin was ~700 mg/m^2, with dose-limiting gastrointestinal toxicities.[74] In a subsequent phase II trial, the toxicities were attributed to normal gut expression of Lewis^Y.[30] In both trials, limited antitumor activity was obtained. Thus, the limitations of BR96-doxorubicin include low molar potency of the cytotoxic drug and antigen expression on sensitive normal tissue. Additionally, the hydrazone linker had a half-life of ~43 hours that was ~7-fold lower than the pharmacokinetic terminal half-life of the BR96 antibody.[74]

Several groups[24,80–83] hypothesized that it may be necessary to construct ADCs with drugs of much higher molar potencies than conventional chemotherapeutics to achieve therapeutically effective levels of active drug within tumors in patients. A major advance in ADC technology came with the utilization of such highly potent drugs as calicheamicins[84], maytansinoids,[85] auristatins,[11] and CC1065 analogs.[80,86] This progress reflects an appreciation that the localization of antibodies to tumors is inefficient in man: typically 0.0003%–0.08% injected dose per gram of tumor.[87]

Calicheamicins are a family of enediyne antibiotics derived from the soil microorganisim, *Micromonspora echinospora* ssp. *calichensis*.[88] These drugs bind to the minor groove of DNA and induce double-stranded DNA breaks, resulting in cell death with >100-fold the potency of most standard chemotherapeutics.[84] As with BR96-doxorubicin, an acid-labile hydrazone linker was used to link calicheamicin to antibody delivery vehicles (Fig. 3), with a similar half-life for drug release from the ADC of ~48 hours.[89] Pronounced antitumor activities have been obtained with calicheamicin-containing ADCs targeting MUC1.[82,90] This calicheamicin ADC technology has also been successfully applied to antibodies recognizing a range of tumor antigens including: CD22,[92–94] CD33,[91,95] Lewis^Y[96] and oncofetal protein, 5T4.[97] ADCs utilizing a related enediyene, calicheamicin θ^I_1, have been used successfully to target tumors expressing ganglioside GD2[98] and γ-glutamyltransferase.[99] The doses required for activity were much lower than that required for a doxorubicin ADC, reflecting the greatly increased potency of the targeted drug. The sole clinically approved ADC to date is the calicheamicin-containing conjugate, gemtuzumab ozogamicin.

The effects of calicheamicin ADCs are in many cases antigen-specific.[90–97,100] In contrast, gemtuzumab ozogamicin can elicit potent antitumor activity in an antigen-independent manner on solid tumors in vivo.[101] These effects were attributed to passive targeting, and required the use of an acid-labile hydrazone linker. Nonspecific drug release through linker hydrolysis may contribute to the activity of gemtuzumab ozogamicin in these models. Passive targeting offers a potential explanation of the activity of gemtuzumab ozogamicin in some AML patients where the cognate antigen, CD33, was not detected[101,102] and is potentially extendable to other tumor types.

Maytansinoids[85] and auristatins[11] represent other classes of highly potent drugs that have been widely used for ADC development. These chemically unrelated drugs (Fig. 3) are 100–1,000 fold more cytotoxic than most cancer chemotherapeutics.[85,103] They act by binding to tubulin at the same site

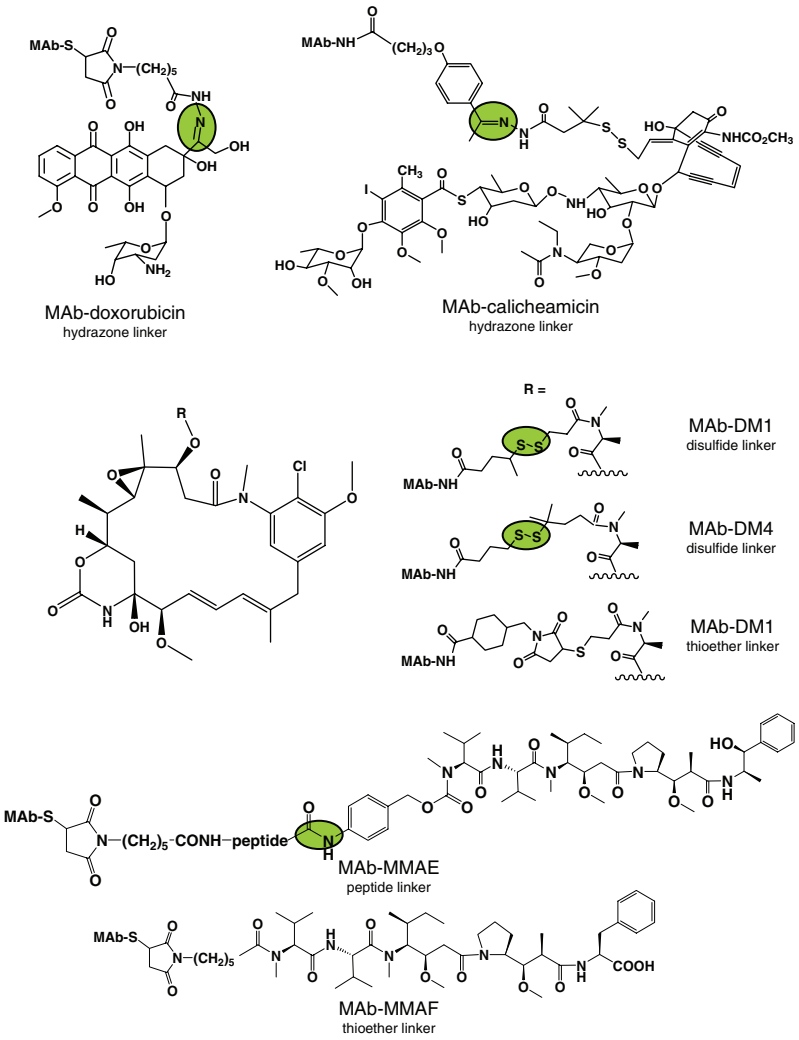

FIGURE 3. Drugs and linkers commonly used in ADCs. Conventional chemotherapeutics such as doxorubicin were once used for ADCs but have been superseded for this purpose by more potent drugs including calicheamicins, maytansinoids (DM1 and DM4) and auristatins (MMAE and MMAF). Commonly used linkers in ADCs include hydrazones, disulfides, and peptides that are cleaved (highlighted region) when exposed to acidic conditions, reducing agents or proteases, respectively. Thioether linkers have also been used for ADCs and drug release likely requires antibody proteolysis within lysosomes.

as the vinca alkaloids and inhibiting tubulin polymerization. A variety of maytansinoids have been derived semi-synthetically from ansamitocin isolated from the microorganism, *Actinosynema pretiosum* spp. *pretiosum*.[104] In contrast, auristatins[103,105] are fully synthetic analogs of the natural product dolastatin 10, which was originally isolated from the sea mollusk, *Dolabela auricularia*.[106]

One of the first maytansinoid-containing ADCs was C242-DM1, which targets CanAg, a tumor-selective carbohydrate epitope.[24] C242-DM1 was generated by modifying antibody lysine residues with a bifunctional cross-linking reagent, followed by the addition of the thiol-containing maytansinoid, DM1 (Fig. 3). The resultant ADC, C242-DM1, contained ~4 drugs/antibody and had potent and immunologically specific antitumor activity. Indeed, C242-DM1 eradicated established tumors in one xenograft model and gave complete responses in 2 other models with heterogeneous antigen expression.[24] The clinical potential of C242-DM1 was enhanced by humanization of the antibody component to create, huC242-DM1 (cantuzumab mertansine). The huC242-DM1 conjugate, like C242-DM1, had robust activity against tumors with heterogeneous antigen expression, reflecting its ability to kill bystander antigen negative tumor cells.[25] In vivo studies revealed that conjugation of DM1 to huC242 had no significant impact upon the biodistribution of the antibody in tumor-bearing mice.[17] The antibody component of huC242-DM1 has a terminal half-life of ~100 hours in mice, whereas terminal half-life of DM1 was about 4-fold shorter,[24] suggesting slow release of DM1 from the ADC in circulation. Similarly, in a subsequent phase I clinical study, the terminal half-lives of the huC242-DM1 ADC and DM1 were ~100 hours and ~24 hours, respectively.[75] The most likely mechanism for DM1 release from huC242-DM1 is by disulfide exchange with other sulfhydryls.[171] Analysis of human plasma indicates the presence of up to ~500 μM free sulfhydryls that are almost entirely from albumin.[107] The huC242-DM1 conjugate was generally well tolerated in 2 phase I clinical trials with evidence for antitumor activity.[75,108]

A series of maytansinoid-disulfide linker derivatives with varying degrees of steric hindrance were subsequently developed and leads such as DM4 selected for antibody attachment.[109] The huC242-DM4 conjugate displays improved linker stability over huC242-DM1 resulting from increased steric hindrance around the disulfide bond[109] (Fig. 3). Additionally, huC242-DM4 shows improved efficacy over huC242-DM1 in

some xenograft models.[85,109] The huC242-DM4 conjugate has now superseded huC242-DM1 in clinical development (Table 2). Similarly, an anti-integrin-α_V-DM4 ADC showed superior antitumor activity in vivo to its DM1 counterpart.[110] Beyond the examples discussed above, maytansinoid ADC technology has been successfully applied to antibodies recognizing a wide range of tumor antigens, including CD19,[111] CD33,[112] CD56,[113] CD79,[114] CD138,[115] HER2,[116] PSCA,[117] and PSMA.[21]

Use of peptide-based linker technologies may present significant advantages over those that are hydrolytically or reductively labile, since hydrolysis is enzymatic, and enzymes can be selected for preferential expression within tumors. ADCs comprises drugs such as doxorubicin,[118] mitomycin C,[119] camptothecin,[120] tallisomycin,[121] and auristatins[103,105,122] have been prepared using peptide linkers for intracellular drug release. Auristatins are of particular interest, because they are highly potent, synthetic, stable, and amenable to chemical modification for linker attachment.

The auristatin, monomethyl auristatin E (MMAE), was modified with dipeptide linkers, and the resulting drug derivatives were linked to solvent-accessible antibody thiol groups,[105] generating conjugates of uniform composition (Fig. 3). The conjugates target CD30 on Hodgkin lymphoma and Lewis^Y on carcinomas. In vitro studies demonstrated that the MMAE peptide-linked ADCs were highly potent with 10–100-fold greater immunologically dependent cell kill compared with corresponding hydrazone-based ADCs. The half-lives of drug release from ADCs in vivo were 6 and 10 days in mice and cynomolgus macaques, respectively,[76] longer than previously described linkers. Furthermore, the peptide-linked MMAE ADCs were less toxic than corresponding hydrazone-linked ADCs.[103]

As shown first for antibodies targeting CD30 and Lewis^Y, auristatin-based ADCs with peptide linkers had pronounced antitumor activity in xenograft models, leading to cures of established tumors at very small fractions of the maximum tolerated doses.[103] Subsequently, auristatin ADC technology has been successfully applied to antibodies recognizing a wide range of tumor antigens, including BCMA,[123] CD19,[124] CD20,[37] CD70,[125] CD79,[114] E selectin,[126] EphB2,[127] glycoprotein NMB,[128] melanotransferrin/p97,[168] MUC16,[129] PSMA,[22] and TMEFF2.[130]

Because auristatins are synthetic, integral structural modifications can be made that significantly alter the properties of the drug. One such auristatin, MMAF, terminates with phenylalanine, a negatively charged residue that impairs cell membrane permeability.[105] Consequently, ADCs containing MMAF that facilitate drug uptake by antigen-positive cells are >2,000-fold more potent than the free drug itself. MMAF linked to an anti-CD70 antibody through a peptide linker was active against renal cell carcinoma xenografts and also circumvented multidrug resistance mechanisms.[125]

A surprising finding with MMAF ADCs was that the cleavable peptide linker could be eliminated, and highly active ADCs were obtained by direct attachment of the drug to anti-CD30 and anti-Lewis^Y antibodies through thioether adducts (Fig. 3).[105] Mass spectrometry showed that the released drug was a cysteine-adduct of the linker-MMAF derivative, presumably resulting from antibody degradation within lysosomes. The closely related auristatin, MMAE, was not active when attached in this manner, indicating that ADCs requiring antibody degradation for drug release are highly dependent on the nature of the drug for activity. MMAF can sustain significant modification to the N-terminal position and still remain active, while most other drugs are inactivated when modified in such an extensive manner. Similar results to that of MMAF were obtained with a thioether adduct of DM1, which was appended to lysine residues on the humanized antibody, huC242, (Fig. 3). In this case, the released drug upon cell incubation was a lysine-DM1 derivative in which the lysine residue was the attachment residue on the antibody delivery vehicle.[131] The requirements for effective cell kill using linkers that require antibody degradation for drug release remain to be elucidated. Although there was little potency loss with thioether adducts of MMAF in hematologic malignancies targeted with an anti-CD30 antibody (cAC10), a thioether adducts of DM1 had approximately one hundredth the in vitro potency of a corresponding disulfide-linked ADC[81] together with greatly diminished in vivo efficacy and potency.[131] The efficacy of ADCs requiring antibody degradation for drug release are likely to be antibody, drug and tumor dependent, in contrast to ADCs with cleavable linkers.

In a mouse preclinical model, an anti-CD70 (h1F6) ADC with a thioether linkage to MMAF had a half-life of 7 days for drug release,[132] which is similar to the 6–10 day half-life for drug release from the corresponding peptide-linked ADC.[76] Furthermore, an ADC with acetamide-thiol adduct in place of the maleimide-thioether (Fig. 3) did not lose any detectable drug from circulating ADC over 2 weeks in mice. The tolerability, efficacy, and intratumoral drug concentrations of the maleimide and acetamide-linked ADCs were similar.[132] Thus, extending the half-life of the ADCs beyond that of 6–10 days did not lead to activity improvements in preclinical models.

Conjugation Technologies

ADCs described thus far were formed by reacting drugs or chemical crosslinking reagents with solvent accessible reactive amino acids, such as lysine and cysteine. The process used to covalently attach the drug to the antibody delivery vehicle can sometimes adversely affect activity, eg, linker technologies have been developed to attach as many as 16 doxorubicin molecules/antibody, but many of the resulting ADCs were largely aggregated and had impaired antigen binding.[133] Some drugs are prone to self-associate when attached to antibodies and may cause aggregation. Such aggregation of gemtuzumab ozogamicin was minimized by limiting the loading to a mean of 2–3 drugs/antibody: 50% ADC with 4–6 drugs/antibody and 50% unconjugated antibody.[95] The heterogeneity of calicheamicin containing ADCs has recently been reduced by additives in the conjugation process.[134] Conjugation technology is a critical aspect in generating effective ADCs, and optimization strategies can vary with the drug, linker, and the antibody used.

The first auristatin-containing ADCs were generated by reducing all of the interchain disulfide bonds in the antibody,

and then attaching the drugs to the resulting cysteine residues.[103,122] These ADCs were close to homogeneous with 8 drugs/antibody, attached at the predetermined cysteine residues. The binding properties of the antibody were unaffected, because the drug was conjugated in the hinge region, which is distant from the antigen binding sites. However, the pharmacokinetic properties of the antibody delivery vehicle were adversely affected, and the 8-loaded ADC cleared much more rapidly than the parent antibody.[135] This prompted investigation of anti-CD30 ADCs (cAC10-MMAE) with varied drug/antibody ratios to assess the impact of drug stoichiometry on therapeutic potential. An ADC with a mean loading of 4 drugs/antibody was fractionated by hydrophobic interaction chromatography to yield purified ADCs with 2, 4, or 8 MMAE drugs/antibody, designated E2, E4, and E8, respectively.[135] ADC potency in vitro correlated with drug loading in that the order of potency was E8 > E4 > E2. In contrast, the in vivo antitumor activity of E4 was comparable with E8 at equal antibody doses, although the E4 contained only half the amount of drug per antibody. These efficacy differences correlated with increased exposure to the E4 conjugate, since slower clearance resulted in a greater pharmacokinetic area-under-the-curve. E2 also had antitumor activity in vivo, albeit less potent than for E4. The maximum-tolerated dose increased inversely with respect to drug loading. Thus, the drug/antibody ratio is an important parameter in the design of ADCs as evidenced by ≥2-fold increase in therapeutic index by reducing the loading from 8 to 4 drugs/antibody.[135] An optimized anti-CD30 ADC with a mean loading of 4 MMAE drugs/antibody, now known as SGN-35,[26,136] is well tolerated and has elicited multiple partial responses in a phase I clinical trial (Tables 1 and 2,[137]).

Antibodies can be engineered for site-specific drug conjugation to enable the production of homogeneous ADCs with defined sites and stoichiometries of drug attachment, eg, a panel of anti-CD30 antibodies was generated in which solvent accessible cysteines were systemically replaced with serines. These Cys→Ser antibody variants were used to create homogeneous ADCs with 2 or 4 MMAE drugs/antibody[73] that were compared with those derived from the parent anti-CD30 IgG1. Surprisingly, the most homogenous ADCs with 4 drugs/antibody at defined sites had comparable, rather than improved, in vitro and in vivo properties as the most heterogeneous conjugate having 0–8 drugs/antibody with a mean of 4 drugs/antibody and variable sites of drug attachment. Thus, the mean drug/antibody ratio seems to be a more significant determinant of ADC potency and tolerability than conjugate homogeneity or sites of drug attachment.[73,135,138]

Antibody engineering involving the addition of accessible cysteines has also been used for site-specific drug conjugation[139,140] or to create novel intermolecular[141,142] or intramolecular[143] disulfide bonds. Such site-specific conjugation to IgG was first demonstrated by Lyons et al who engineered an extra cysteine residue at various locations within the C_H1 domain of an IgG4.[139] Subsequently, Stimmel et al engineered a cysteine residue into the C_H3 domain of an IgG4.[140] Although the engineered cysteine was blocked, mild and selective reduction conditions were devised thereby site-specific labeling with a radionuclide chelator. Recently, the efficiency of site-specific conjugation to IgG has been improved by better choices of cysteine replacement[144]—"thiomabs"—combined with refinements in conjugation methodology that reduce and then restore native interchain disulfide bonds.[170] A thiomab-drug conjugate with 1.6 auristatin drugs/antibody was compared with a conventional ADC with 3.1 auristatin drugs/antibody. The thiomab-drug conjugate showed comparable efficacy in a xenograft study, improved tolerability in rats and nonhuman primates and thus an increased therapeutic index (Junutula et al, unpublished). This improved therapeutic index may be due to thiomabs per se and/or reduction in the drug/antibody ratio.[135] The potential broad applicability of thiomab-drug conjugates provides a strong impetus to assess the reproducibility and transferability of the increased therapeutic index between different xenograft models, antibodies, drugs, and linkers.

CLINICAL EXPERIENCE WITH GEMTUZUMAB OZOGAMICIN

Gemtuzumab ozogamicin represents a major milestone as the first, and currently sole, ADC approved for clinical use. Here we review the clinical data that led to the approval of gemtuzumab ozogamicin for AML, whereas subsequent clinical trials with this drug are reviewed elsewhere.[145–147] Gemtuzumab ozogamicin comprises a humanized anti-CD33 IgG4 antibody conjugated to *N*-acetyl-γ-calicheamicin dimethyl hydrazide via the acid labile hydrazone, 4-(4′-acetylphenoxy) butanoic acid, known as "AcBut."[95] CD33 (SIGLEC-3) is a sialic-acid dependent adhesion protein expressed on the surface of ~90% of leukemic blasts in AML[148] and immature normal cells of the myelomonocytic lineage, but absent from normal hematopoietic stem cells.

Gemtuzumab ozogamicin was approved by the US Food and Drug Administration in 2000 for the treatment of CD33 positive AML in first relapse in patients of at least 60 years of age and considered unsuitable candidates for cytotoxic chemotherapy.[7] Gemtuzumab ozogamicin approval was based upon interim data analysis of 3 open label single-arm phase II studies in AML at first relapse. Patients received intravenous gemtuzumab ozogamicin at 9 mg/m^2 at 2 weekly intervals for 2 doses. Thirty percent of the patients (42 of 142) achieved a complete remission: ≤5% blasts in the marrow, recovery of neutrophils to ≥1,500/μL and erythrocyte and platelet transfusion independence. Final analysis of these 3 gemtuzumab ozogamicin phase II studies with a larger number of patients (n = 277) demonstrated a 26% remission rate and a median overall survival of 4.9 months.[149]

Gemtuzumab ozogamicin induced severe myelosuppression in all patients including grade 3 or 4 thrombocytopenia (99%), neutropenia (98%), and anemia (52%), as anticipated from the CD33 expression on normal hematopoietic precursors.[149] The myelosuppression was reversible as expected, since hematopoietic stem cells are CD33 negative and presumably spared. Gemtuzumab ozogamicin, as for other antibody-based therapeutics, can also give rise to acute infusion-related adverse events. Grade 3 or 4 infusion-related adverse events were much more common for the first dose

(30%) than the second dose (10%) and included chills (8%), fever (6%), hypotension (4%), nausea (3%), and hypertension (2%). The incidence of hepatotoxicity associated with gemtuzumab ozogamicin included grade 3 or 4 hyperbilirubinemia (29%), elevations of aspartate aminotransferase (18%) or alanine aminotransferase (9%) that were generally transient and reversible without medical intervention. None of the patients developed an antibody response to either the antibody or drug-linker components of gemtuzumab ozogamicin. Additional grade 3 and 4 adverse events associated with gemtuzumab ozogamicin include sepsis and dyspnea.

Severe veno-occlusive disease (sinusoidal obstruction) has been associated with the use of gemtuzumab ozogamicin at incidences that vary greatly with the clinical setting.[149–152] A low incidence (~1%) of veno-occlusive disease was observed following gemtuzumab ozogamicin monotherapy in patients without prior or subsequent hematopoietic stem cell transplants.[149] In contrast, a higher incidence (22%) of veno-occlusive disease, sometimes fatal, was observed in patients who had received hematopoeitic stem cell transplantation before gemtuzumab ozogamicin. An intermediate rate (15%) of veno-occlusive disease was observed in patients transplanted after gemtuzumab ozogamicin therapy.

In contrast to the US Food and Drug Administration, the European Medicines Agency denied marketing authorization for gemtuzumab ozogamicin in Europe in 2007, citing concerns that the benefits of gemtuzumab ozogamicin in the treatment of AML had not been established and did not outweigh its risks. Several randomized and controlled trials are in progress to assess the benefit of gemtuzumab ozogamicin. Moreover, preliminary analysis of one such randomized clinical trial, MRC AML 15 (n = 1,113), revealed that the addition of gemtuzumab ozogamicin to induction chemotherapy for AML improves disease free survival without extra toxicity.[153]

ADCs in Clinical Development

There are a growing number of ADCs in clinical development as discussed below—10 as of April 2008 including gemtuzumab ozogamicin (Tables 1 and 2). Below is a brief description of many of these ADCs and their performance in early stage clinical trials.

SGN-35

SGN-35 is a CD30-targeting ADC with a protease-cleavable linker (valine-citrulline) between the antibody and the auristatin drug, MMAE, with a mean loading of ~4 drugs/antibody.[26,136] CD30 is a member of the TNF receptor family with highly restricted normal tissue expression that includes activated, but not resting, T and B cells. CD30 expression by malignant Reed-Sternberg cells is a defining feature of Hodgkin lymphoma. CD30 is also expressed in other hematologic malignancies including diffuse large B cell, anaplastic large cell, and cutaneous T cell lymphomas as well as Kaposi sarcoma.

A phase I trial of SGN-35 is ongoing in CD30 positive hematologic malignancies (Table 2). Preliminary data have been reported on the first 23 patients, including 21 with Hodgkin lymphoma, who received escalating doses of SGN-35 from 0.1 to 1.8 mg/kg administered intravenously every 3 weeks.[137] Tumor reduction was observed in over 75% of the patients treated, including 4 partial responses. SGN-35 was reasonably well tolerated, with most observed adverse events of grade 1 and 2.

CR011-vcMMAE

CR011-vcMMAE is an ADC targeting glycoprotein NMB that, like SGN-35, includes a valine-citrulline protease cleavable linker between the antibody and the auristatin drug, MMAE, with a mean loading of ~3.8 drugs/antibody.[128,154] Glycoprotein NMB is a 560 amino acid glycoprotein of unknown function that is highly expressed in melanoma tissue, albeit predominantly intracellularly with a small cell surface component. Glycoprotein NMB expression has also been associated with glioma, liver carcinoma, squamous cell lung cancer, and soft tissue tumors, whereas normal tissue expression is restricted.[128]

A phase I/II trial of CR011-vcMMAE has been initiated in patients with unresectable stage III or IV melanoma (Table 2). Preliminary data have been reported on 25 patients who received escalating doses of CR011-vcMMAE from 0.03 to 1.88 mg/kg administered intravenously every 3 weeks for 4 cycles.[155] No objective responses were observed, although 7 of 22 evaluable patients demonstrated stable disease. Overall, CR011-vcMMAE has been well tolerated with no dose-limiting toxicities. Most drug-related adverse events were grade 1 and 2, with grade 3 or 4 neutropenia and leukopenia reported for 2 patients.

Trastuzumab-DM1

The ADC, trastuzumab-DM1,[35,36,116] contains the humanized anti-HER2 IgG1 antibody, trastuzumab (formerly known as humAb4D5–8,[156]) conjugated to the maytansinoid, DM1, via a thioether linkage.[25,131] HER2 is a member of the EGFR family of growth factor receptors. HER2 is overexpressed as a result of gene amplification in ~25% of primary breast cancers and is associated with poor prognosis.[157] HER2 is expressed at lower levels in several normal tissues including gastrointestinal tract epithelia, tonsil, kidney tubules, and endometrium as described in the package insert for the diagnostic, HercepTest™. Trastuzumab-DM1 is unique among the ADCs in clinical development in that the parent antibody, trastuzumab (Herceptin) is a clinically and commercially successful therapeutic in its own right. Indeed, trastuzumab in combination with cytotoxic chemotherapy prolongs overall survival in patients with HER2-positive breast cancer in both the metastatic[158] and adjuvant[159] settings.

In a phase I study in HER2-positive metastatic breast cancer trastuzumab-DM1 was administered at 6 dose levels of 0.3–4.8 mg/kg every 3 weeks.[35,36] Partial responses were observed in 6 of 24 patients treated at or at below the maximum-tolerated dose of 3.6 mg/kg. Drug-related adverse events of grade 2 or higher have been infrequent and manageable. Dose-limiting grade 4 thrombocytopenia was observed in 2 of 3 patients treated at 4.8 mg/kg. Platelet nadirs were observed on about day 8 with recovery by day 15. No cardiotoxicity has been observed with trastuzumab-DM1, in contrast to trastuzumab used in conjunction with anthracy-

clines.[158] A phase II trial of trastuzumab-DM1 has been initiated in metastatic breast cancer.

AVE9633

AVE9633 is a humanized antibody-maytansinoid (DM4) conjugate[112] that targets CD33—the tumor antigen validated by gemtuzumab ozogamicin. A phase I study has been initiated with AVE9633 in patients with relapsed or refractory CD33-positive AML (Table 2). AVE9633 was administered intravenously at dose levels of 30–150 mg/m^2 on 2 consecutive weeks every 4 weeks.[112] One complete response with incomplete platelet recovery and one partial response were observed from the first 17 patients treated. Two dose-limiting toxicities were seen at the dose of 150 mg/m^2 × 2 consisting of liver toxicity and keratitis. The most frequent grade 1 or 2 adverse events were infusion reactions—fevers, chills, hypotension, and musculoskeletal pain. Saturation and down-regulation of CD33 on the surface of blood and bone marrow blasts was observed from the 50 mg/m^2 dose. No human antibody response has been detected to either the ADC or DM4.

Inotuzumab Ozogamicin

Inotuzumab ozogamicin is an ADC targeting CD22 that shares some attributes of gemtuzumab ozogamicin: humanized IgG4 linked to *N*-acetyl-γ-calicheamicin dimethyl hydrazide via an acid labile, AcBut, linker.[92] CD22 (SIGLEC-2) is a sialic-acid dependent adhesion protein of the immunoglobulin superfamily that is expressed in B cell malignancies such as non-Hodgkin B cell lymphoma, and acute lymphoblastic leukemia. Normal tissue expression of CD22 is highly restricted to mature B cells and it is absent from other hematopoietic cells including hematopoietic stem cells.

Interim data from on-going phase I and I/II trials with inotuzumab ozogamicin are not readily available, but presumably were sufficiently favorable to provide a rationale for the recent initiation of a pivotal trial in follicular non-Hodgkin lymphoma. Specifically, a 2-arm, randomized, open-label phase III trial was initiated in late 2007 to compare rituximab in combination with inotuzumab ozogamicin to rituximab in combination with the investigator's choice of cytotoxic chemotherapy. Preclinically, the antitumor activity of inotuzumab ozogamicin in combination with rituximab against non-Hodgkin B-cell lymphoma was superior to either inotuzumab ozogamicin or rituximab alone.[160]

HuC242-DM4

As previously described, huC242-DM4 is a humanized antibody-maytansinoid conjugate targeting CanAg,[109,131] a tumor-selective carbohydrate epitope. CanAg is highly expressed in most pancreatic, biliary and colorectal cancers as well as a significant proportion of gastric (55%), uterine (45%), nonsmall cell lung cancer (40%), and bladder cancers,[161,162] whereas only minimal immunostaining of CanAg in normal tissue has been reported.[162]

A phase I trial of HuC242-DM4 has been initiated in patients with metastatic or inoperable colorectal, pancreatic, and other CanAg positive cancers (Table 2). HuC242-DM4 was administered by intravenous infusion at dose levels of 18–297 mg/m^2—every 3 weeks.[163] No objective responses have been reported from the first 30 patients treated. Dose-limiting toxicity of decreased visual acuity, corneal deposits, and keratitis were experienced by 2 of 6 patients treated at the 223 mg/m^2 dose level. Both patients responded favorably to treatment with lubricating eye drops. HuC242-DM4 was well tolerated at the 168 mg/m^2 with 1 of the 8 patients experiencing a dose-limiting toxicity consisting of grade 3 diarrhea and dehydration that improved with intravenous fluids. Preliminary pharmacokinetic data revealed that huC242-DM4 had a half-life of ~5 days in patients with low plasma levels of antigen. Clearance of huC242-DM4 was increased in patients with high levels of shed antigen (>900 units/mL). No clinically significant myelosuppression has been observed, and antibody generation to the humanized antibody or DM4 has not been detected.

HuN901-DM1

HuN901-DM1 is a humanized antibody-maytansinoid conjugate targeting CD56,[113] the neuronal cell adhesion molecule that is expressed in virtually all small cell lung carcinomas, neuroblastomas, gliomas, astrocytomas, and rhabdomyosarcomas, as well as ~78% of multiple myelomas and ~53% of AML.[164] As for normal tissue, CD56 is expressed on natural killer cells, neuroendocrine glands, the central and peripheral nervous systems and also cardiomyocytes.[164]

In a phase II study, patients with relapsed small-cell lung cancer and other CD56-positive small cell cancers were treated with huN901-DM1 intravenously at 60 mg/m^2/wk for 4 consecutive weeks every 6 weeks (Table 2). Two partial responses and 5 stable diseases were observed in the first 30 patients treated.[165] Transient severe headaches were observed in a few patients, but did not occur in subsequent patients premedicated with steroids. HuN901-DM1 was generally well tolerated, with no evidence of clinically significant myelosuppression and no serious infusion reactions. No human antibody response has been detected to either the antibody or drug components of the ADC. Additionally, a phase I study of huN901-DM1 is underway in patients with relapsed or refractory CD56-positive multiple myeloma (Table 2). Patients received escalating doses of huN901-DM1 of 40–90 mg/m^2/wk by intravenous infusion on 2 consecutive weeks every 3 weeks.[166] One minimal objective response has been observed for the first 12 patients treated. HuN901-DM1 has been well tolerated with no serious drug-related adverse events and no dose-limiting toxicity observed for the first 12 patients treated. Immunohistochemistry performed on bone marrow aspirates 24 hours after huN901-DM1 infusion at 40 mg/m^2 confirmed localization of ADC to myeloma cells. Preliminary pharmacokinetic analysis revealed a ~20 hour terminal half-life for huN901-DM1.

CONCLUSIONS AND PERSPECTIVES

Significant advances have been made with ADCs over the last 3 decades. Building on the success of gemtuzumab ozogamicin, highly potent drugs have been attached to antibodies recognizing numerous different antigens expressed on a broad array of hematologic and solid tumors. The activities of these new ADCs underscore the importance of good target

selection as well as drug potency and high stability linkers for drug attachment. Early ADC linkers were primarily hydrazones or minimally hindered disulfides, whereas newer linkers include more hindered disulfides, thioethers, and peptides that have achieved greater in vivo stability.

As for the antibody delivery vehicle, much remains to be learned about the importance of antigen binding affinity, antibody isotype, binding to Fcγ receptors and FcRn, and pharmacokinetics in optimizing ADCs for cancer therapy. In addition, the roles that antigen density, internalization rates and recycling play in ADC activity remain to be elucidated. Two major paradigms established with antibody therapeutics[2] are now being explored in the context of ADCs: large panels of antibodies are being developed to increase the likelihood of identifying leads that satisfy multiple design criteria, and antibodies are being engineered to optimize their utility as ADCs.

Insights from many early ADCs have led to a promising new generation of experimental drugs. These include SGN-35[26,136,137] and trastuzumab-DM1,[35,36,116] which are demonstrating pronounced antitumor responses in CD30-positive hematologic malignancies and HER2-positive metastatic breast cancer, respectively. The technologies developed for these ADCs are being broadly applied to other antibody delivery vehicles, to generate potent ADCs that are, or soon will be, in clinical trials. ADCs now seemed poised to realize their potential to play a significant role in cancer therapy.

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