Antibody Conjugate Therapeutics: Challenges and Potential

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Abstract

Antibody conjugates are a diverse class of therapeutics consisting of a cytotoxic agent linked covalently to an antibody or antibody fragment directed toward a specific cell surface target expressed by tumor cells. The notion that antibodies directed toward targets on the surface of malignant cells could be used for drug delivery is not new. The history of antibody conjugates is marked by hurdles that have been identified and overcome. Early conjugates used mouse antibodies; cytotoxic agents that were immunogenic (proteins), too toxic, or not sufficiently potent; and linkers that were not sufficiently stable in circulation. Investigators have explored 4 main avenues using antibodies to target cytotoxic agents to malignant cells: antibody-protein toxin (or antibody fragment–protein toxin fusion) conjugates, antibody-chelated radionuclide conjugates, antibody–small-molecule drug conjugates, and antibody-enzyme conjugates administered along with small-molecule prodrugs that require metabolism by the conjugated enzyme to release the activated species. Only antibody-radionuclide conjugates and antibody-drug conjugates have reached the regulatory approval stage, and nearly 20 antibody conjugates are currently in clinical trials. The time may have come for this technology to become a major contributor to improving treatment for cancer patients. *Clin Cancer Res; 17(20); 6389–97. ©2011 AACR.*

Introduction

The challenges posed by the discovery of therapeutically effective antibody conjugates are as formidable as those encountered in the discovery and development of small-molecule drugs. Over the past 20 years, many cell surface proteins that have selective aberrant expression on malignant cells or are aberrantly highly expressed on the surface of malignant cells have been identified. In some cases, specific antibodies that bind tightly to such proteins were developed. Unfortunately, not infrequently, exposing the tumor cells in culture or treating human tumor xenograft-bearing mice with these antibodies did not alter tumor growth. Antibody conjugates provide an opportunity to make use of antibodies that are specific to cell surface proteins and thus offer some important advantages over current therapeutics, such as improved target specificity and potency. However, this approach has some limitations, notably for the treatment of solid tumors, such as the difficulty of delivering a macromolecule to solid tumors, heterogeneity of antigen expression on the tumor surface, and expression of the antigen by normal tissues (Table 1). Hematological malignancies composed of leukemia or lymphoma cells may be more amenable to treatment with antibody conjugates in view of the ready accessibility of these cells. Typically, antigen expression is specific and homogeneous, although the actual number of antigens on the cell surface may be lower than that found on solid tumors. This issue of *CCR Focus* offers insight into some of the key criteria to consider in the development of antibody conjugates.

The notion that antibodies directed toward targets on the surface of malignant cells could be used for drug, radionuclide, or cytotoxic protein delivery is not new. In the late 1980s, after great efforts, a few mouse antibodies that went into clinical trials were found to be inactive and also rapidly neutralized by the immune system of patients. Subsequently, the idea of using these same antibodies to deliver powerful tumoricidal agents in a single or limited number of doses emerged. Over the next several years, investigators explored 4 main avenues using antibodies to target cytotoxic species to malignant cells: antibody-protein toxin conjugates (or antibody-protein toxin fusion proteins), antibody-radionuclide conjugate, antibody–small-molecule drug conjugates, and antibody-enzyme conjugates administered along with small-molecule prodrugs [also called antibody-directed enzyme prodrug therapy (ADEPT)], which require metabolism by the conjugated enzyme to release the active drug (1–5). ADEPT will not be further discussed in this *CCR Focus* because this technology has not advanced due to drawbacks, such as the immunogenicity of the bacterial enzyme component and the short half-life of the conjugates.

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Table 1. Antibody conjugate advantages and disadvantages

Advantages
Targeted therapeutic binding specifically to the target antigen
Highly potent agents can be delivered selectively to tumor cells
Wide therapeutic index
Prolonged circulation half-life; conjugate remains stable in circulation
Decreased adverse effects
Disadvantages
Requires that the tumor be tested for expression of the antigen
Molecular target may have some normal tissue expression, potentially leading to toxicity
Toxic payload may have some premature release
Antibody conjugate may not reach the target cells in sufficient concentration to be lethal
Antigen expression could be heterogeneous, especially in solid tumors

Antibody-Protein Toxin Conjugates (Immunotoxins)

The first tumoricidal agents to be linked to antibodies were potent, plant-derived protein toxins, such as gelonin, ricin, abrin, and pokeweed antiviral protein, and bacterial toxins such, as *Pseudomonas* exotoxin and Diphtheria toxin (6–8). Some of these immunotoxins were tested in the clinic with little success, and interest in this approach waned. The identified shortcomings included immunogenicity of the murine antibody and the protein toxin, rapid clearance from the blood stream, and systemic toxicity at low doses. In addition, these early immunotoxins were composed of intact IgGs linked to full-length toxins by chemical coupling methods and thus were large in size, potentially limiting penetration into solid tumors, and chemically heterogeneous (9). The lessons learned during these explorations have led to improvements in the design of immunotoxins. The second-generation immunotoxins were made with the use of recombinant techniques whereby the DNA sequences encoding only the antigen-binding site of the antibody (the Fv portion engineered as a single chain) were fused to DNA sequences encoding the toxin, and thus were much smaller in size and homogeneous. In a further refinement applied to *Pseudomonas* exotoxin, a truncated form lacking the cell surface binding domain was fused to the scFv portion of the antibody. Two different versions of anti-CD22-*Pseudomonas* exotoxin conjugate targeting B-cell malignancies are currently under clinical evaluation (10). The first version, called BL22 [RFB4-(dsFv)-PE38], showed significant activity in a phase II trial in patients with hairy cell leukemia ($n = 36$), with an overall response rate of 50%. Because the activity of BL22 was much lower in other B-cell malignancies [i.e., chronic lymphocytic leukemia, acute lymphoblastic leukemia (ALL), and non-Hodgkin's lymphoma], an improved version of BL22, called moxetumomab pasudotox, with a higher binding affinity for CD22 and greater *in vitro* potency, was developed. In a phase I trial conducted in patients with hairy cell leukemia ($n = 32$), moxetumomab pasudotox showed a slightly better complete response rate than its predecessor, BL22 (31% vs. 25%, respectively). Clinical trials in other hematological malignancies are ongoing (10).

Although this class of immunotoxins may have meaningful activity in isolated disease settings, particularly in certain hematologic malignancies, the fundamental problem of immunogenicity and fast clearance will continue to limit their therapeutic activity. In addition, the maximum tolerated dose achievable with such immunotoxins is very low (~0.05 mg/kg). The low dose coupled with fast clearance will likely limit localization to solid tumors: even for an intact IgG with a long half-life, the amount of antibody that gets to the tumor is <0.01% of the injected dose per gram of tumor (11). Thus, it is unlikely that a therapeutic concentration of such an immunotoxin can be delivered to solid tumors. Indeed, most therapeutic monoclonal antibodies in clinical use for solid tumors (e.g., trastuzumab and cetuximab) are used at a 40- to 200-fold higher dose (2 to 10 mg/kg weekly).

Antibody-Radionuclide Conjugates

The second strategy investigators employed in developing antibodies as targeted therapeutics was to conjugate an antibody to a radionuclide. The goal of radiotherapy in cancer is to deliver a sufficiently high dose of radiation locally to eradicate the tumor while sparing the surrounding normal tissue. Radioimmunotherapy, which exploits the specificity of an antibody to deliver a radionuclide, affords some potential benefits over conventional radiotherapy, including the ability to (i) more precisely deliver radiation to the tumor, (ii) deliver radiation to metastatic sites, and (iii) affect tumors that express the antigen heterogeneously (as radiation can damage cells in proximity to those that are not directly hit). Antibody-radionuclide conjugates have been successfully developed for the treatment of non-Hodgkin's lymphoma, resulting in the approval of 2 CD20-targeted agents: ^{131}I-tositumomab (Bexxar) and ^{90}Y-ibritumomab tiuxetan (Zevalin), which can produce response rates of 50 to 85% in a variety of lymphomas (12). Although radioimmunotherapy has been successful in the treatment of hematological malignancies, clinical experience in solid tumors has been disappointing, presumably due to the poorer radiosensitivity of these cell types (13, 14). It is generally accepted that radiation doses of at least 60 Gy are required to eradicate solid tumors (15). However, the highest dose of radiation delivered via an immunoconjugate has been estimated to be in the subtherapeutic range (typically 1 to 20 Gy). For example, no responses were observed in a phase II clinical trial of ^{131}I-labeled CC49 antibody in colorectal cancer, exemplifying the lack of

therapeutic activity in solid tumors. Despite the high affinity for CC49 antigen, the dose delivered to the tumor was only in the range of 0.2 to 6.7 Gy (16). Thus, the effectiveness of radioimmunotherapy may be limited to radiosensitive diseases such as lymphomas.

Antibody-Drug Conjugates

The concept of antibody-drug conjugates (ADC) evolved from the hope that targeted delivery with monoclonal antibodies would confer a degree of tumor selectivity to approved anticancer drugs and thus improve their therapeutic index. Early ADCs were composed of tumor-specific murine monoclonal antibodies covalently linked to anticancer drugs, such as doxorubicin, vinblastine, and methotrexate. These early conjugates were evaluated in human clinical trials but had limited success due to immunogenicity, lack of potency, and insufficient selectivity for tumor versus normal tissue. The lessons learned from these early explorations led to improvements in essentially all aspects of antibody conjugate therapeutics and hence to renewed interest in ADC technology (17). Immunogenicity was overcome by replacing murine antibodies with humanized or fully human antibodies, potency was improved by using drugs that were 100- to 1,000-fold more cytotoxic than previously used drugs, and selectivity was addressed by more careful target and antibody selection.

As the result of such improvements, in 2000, gemtuzumab ozogamicin (Mylotarg) became the first ADC to be approved by the U.S. Food and Drug Administration (FDA) for the treatment of acute myelogenous leukemia (AML). However, this ADC was withdrawn from the market in 2010 because in postmarketing follow-up clinical trials, it failed to meet prospective efficacy targets that were required as a condition of its accelerated approval by the FDA. However, 2 other ADCs, trastuzumab emtansine (T-DM1) and brentuximab vedotin (SGN-35), are showing promising activity and are now in advanced stages of clinical evaluation (a U.S. marketing application was recently filed for the latter). Nearly 20 additional ADC constructs are in earlier-stage clinical trials. As this issue of *CCR* focus was going to press, the US FDA granted accelerated approval of brentuximab vedotin for the treatment of patients with refractory Hodgkins lymphoma and systemic anaplastic large cell lymphoma.

When selecting cell surface protein targets, whether on malignant cells or malignant disease–associated cells (e.g., tumor endothelial cells), it is important to ensure that the antigen expression is abundant on the target cells and very limited on all other cells (18–26). With antibody-conjugate therapeutics, the patient whose tumor expresses high levels of the target antigen is most likely to benefit from treatment. Although these agents are targeted, they are potent cytotoxic agents. Most of the proteins being targeted with antibody conjugates are normal proteins, as opposed to mutant proteins; therefore, some expression on normal cells is possible and even likely. Technologies for antibody discovery, development, and engineering are now well established. Varied phage display libraries and humanized mice

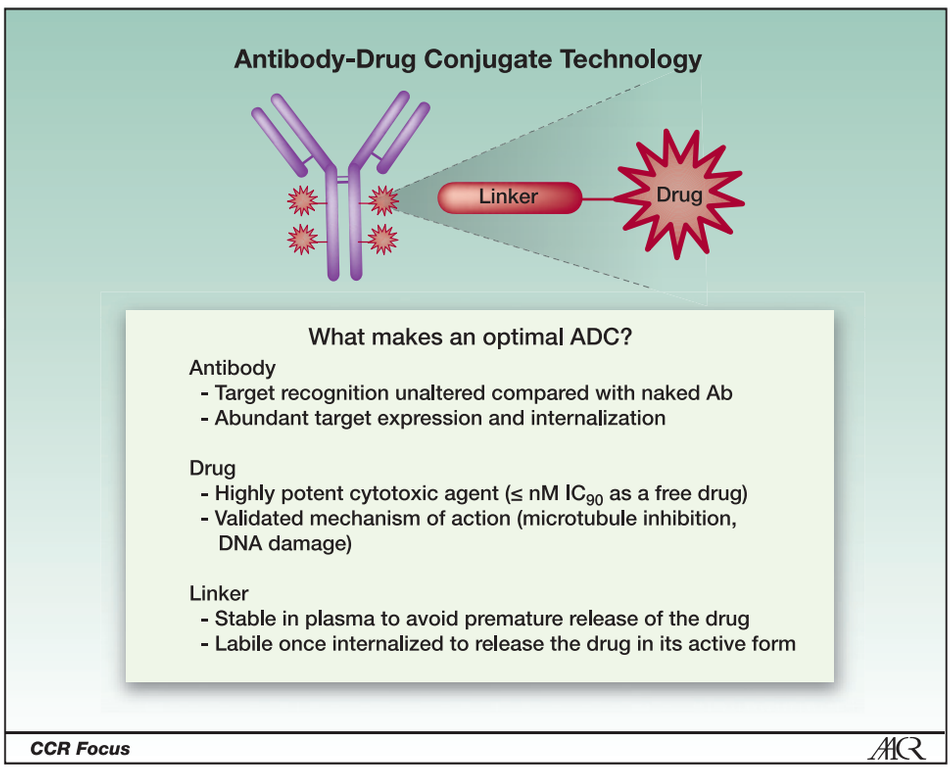

Figure 1. Schematic illustrating an ADC (adapted from ref. 18).

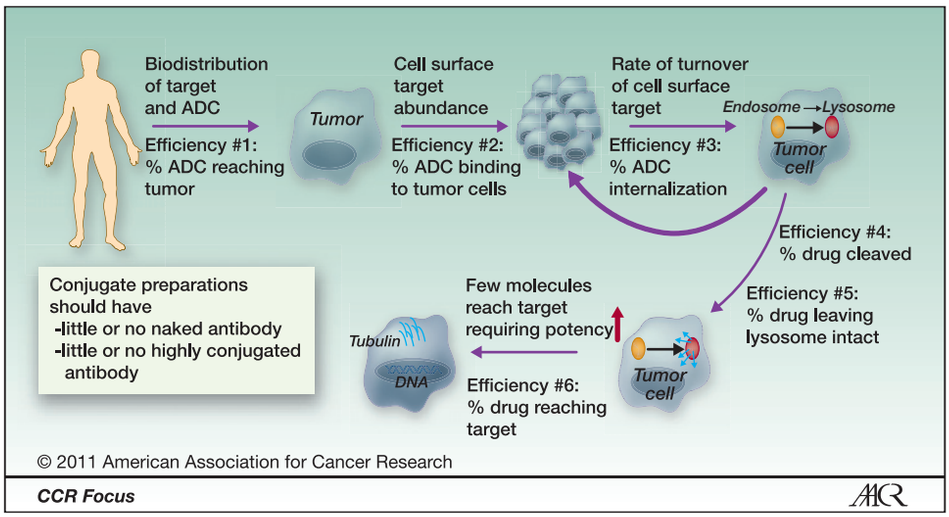

Figure 2. Schematic illustrating the several steps from administration of the antibody conjugate to the patient to release of the toxic agent in the tumor cells. If the efficiency of each step is 50%, only 1.56% of the administered dose will reach the intracellular target.

can produce fully human antibodies, and humanization of mouse antibodies can lead to highly specific nonimmunogenic antibodies (Fig. 1). In most cases, the selection of the most appropriate antibody for use in antibody-conjugate therapeutics requires that the antibody-antigen target complex internalize into the target cells where the small-molecule drug can be released.

The small-molecule drugs that have been widely applied to ADCs target tubulin or DNA. These compounds are uniformly extremely potent cytotoxic agents against cultured cancer cells, with IC_{50} values in the picomolar range. As illustrated in Fig. 2, in the best case, ADCs are among the most tumor-selective anticancer therapeutics developed to date; however, even with this high degree of selectivity, only a small percentage of the linked cytotoxic agent can be expected to be delivered to the tumor. If each of the 6 steps shown in Fig. 2 is associated with an efficiency of 50%, only 1.56% of the administered dose of the small-molecule drug will reach the intracellular target. Thus, the concentration of the cytotoxic drug delivered to the intracellular target via an ADC will be very low. The maytansinoids and dolastatin analogs target tubulin, and both suppress microtubule dynamics (27–29). The duocarmycins and calicheamicins target the minor groove of DNA. These molecules have in common an extreme potency and lack of selectivity, which limit their use as small-molecule drugs in the clinic. Dolastatin 10, the parent molecule of the auristatins, underwent clinical trials in the 1990s (30). The development of dolastatin 10 was terminated in 1995 when it failed to demonstrate efficacy in a phase II trial in prostate cancer patients. The maytansinoids are exquisitely potent cytotoxic agents (31). Maytansinoids are 19-membered macrocyclic lactams that are related to ansamycin antibiotics. Maytansine was developed and assessed in early clinical trials in the early 1980s. The phase II clinical trials were disappointing, with very little evidence of response (32). Duocarmycins are members of a small family of antibiotics that also includes yatakemycin and CC-1065 (33). This class of compounds binds to and alkylates DNA in the A-T–rich regions of the double-helix minor groove. Several semisynthetic derivatives of CC-1065 and duocarmycin, including adozelesin, carzelesin, bizelesin, and KW2189, were evaluated in early clinical trials (34–36). In each case, dose-limiting toxicities to critical normal tissues occurred at doses too low to achieve antitumor activity. The calicheamicins bind in the minor grove of DNA in a sequence-specific manner and induce double-strand breaks, causing cell death (37). Because of its narrow therapeutic index and late-emerging toxicities, the development of calicheamicin as a single-agent therapeutic was not pursued. Gemtuzumab ozogamicin, the only ADC approved by the FDA to date, incorporates calicheamicin, an enediyne antibiotic, as the potent cytotoxic drug (38, 39).

The use of antibody conjugates is an effective method to increase the therapeutic index of these highly potent cytotoxic agents. For application of the highly potent cytotoxic compounds in antibody conjugates, the analogs used must have sufficient water solubility and prolonged stability in aqueous formulations and in plasma, because antibody conjugates may be in circulation for several days. In addition, these compounds must have a functional group that is suitable for conjugation with a linker and must not be readily susceptible to lysosomal enzyme degradation. Consistent with the potent nature of the drug component of ADCs, these agents are often scheduled like cytotoxic chemotherapy in clinical regimens, with dosing once every 3 weeks (40, 41).

Linkers that are short spacers that covalently couple the drug to the antibody protein must be stable in circulation (Fig. 1). Inside of the cell, most linkers are labile; however, some are stable, requiring degradation of the antibody and linker to release the cytotoxic agent. Thus, linkers are a key component of antibody-conjugate structures (42–45). Currently used linkers most frequently react with lysine side chains or sulfhydryls in the hinge regions of the antibody. Linkers in clinical use include acid-labile hydrazone linkers that are degraded under the low pH conditions found in lysosomes. Disulfide-based linkers are selectively cleaved in

the cytosol in the reductive intracellular milieu (46). Noncleavable thioether linkers release the small-molecule drug after degradation of the antibody in the lysosome, and peptide linkers, such as citrulline-valine, are stable in circulation and degraded by lysosomal proteases in cells. More recently, linkers with polyethylene glycol spacers have been developed in an effort to increase the solubility of the conjugate (47, 48). Linkers can influence the circulating half-life and safety of conjugates by minimizing the release of the drug molecule in circulation and optimizing the delivery of the conjugate to the target tissue. Often during the drug development process, investigators will test several linkers in safety and efficacy assays to select the best candidate conjugate.

Drug-loading stoichiometry and homogeneity are also important determinants of the safety and efficacy of antibody conjugates (Fig. 1). The goal of efforts to synthesize antibody conjugates is to produce nearly homogeneous preparations (i.e., single chemical species). It is important to avoid both underconjugated antibodies, which decrease the potency of the antibody conjugate, and highly conjugated species, which can have markedly decreased circulating half-lives and impaired binding to the target protein, thus decreasing the potency and efficacy of the antibody conjugate (49). Thus, for most ADCs, linkage of an average of 3 to 4 drug molecules per antibody molecule seems to be optimal because it minimizes the percentage of unconjugated antibody, maintains the circulating half-life near that of the naked antibody, preserves antibody binding to the target protein, and delivers sufficient numbers of cytotoxic molecules to the target cell to be lethal. Site-specific conjugation approaches are being explored in an effort to improve the homogeneity of drug loading. The chemistry manufacturing control (CMC) for ADCs requires specialized facilities to handle proteins and very potent cytotoxic drugs and to provide quality assurance regarding stability and batch-to-batch consistency. These processes have in large part been worked out.

Targets

ADCs currently in clinical trials are listed in Table 2. CD33 is a 67-kD transmembrane cell-surface glycoprotein of the sialo adhesion family that is expressed by mature and immature myeloid cells and erythroid, megakaryocyte, and multipotent progenitor cells (50). Approximately 90% of AML patients are CD33-positive, although the actual level of CD33 expression is low. AML samples have the highest number of CD33 molecules per cell, with a mean of ~10,000, followed by myelodysplastic syndrome with a mean of 7,000 and myeloid leukemia with a mean of 4,000. Gemtuzumab ozogamicin (Mylotarg), a first-generation antibody drug conjugate, is a humanized IgG4 anti-CD33 monoclonal antibody conjugated to the antitumor antibiotic calicheamicin (50). Upon binding of anti-CD33 to the antigen, the complex is rapidly internalized. Intracellularly released calicheamicin binds in the minor groove of DNA and causes double-strand breaks at oligopyridimidine-oligopurine tracts. Gemtuzumab ozogamicin was studied in the clinic for over 10 years. In 2010, gemtuzumab ozogamicin was voluntarily withdrawn from the market (Table 2).

CD22 is a B-lymphoid, lineage-specific differentiation antigen that is expressed on both normal and malignant B cells. Approximately 85% of ALL cases arise from the B-cell lineage (pre-B-cell or B-ALL). CMC-544 (inotuzumab ozogamicin) is a CD22-targeted anti-CD22-calicheamicin conjugate that is currently being evaluated in B-cell non-Hodgkin's lymphoma patients (50, 51). The anti-CD22 antibody in CMC-544 is a humanized IgG4. Exposure to CMC-544 does not interfere with the antibody-dependent cellular cytotoxicity of rituximab (anti-CD20). Preclinical *in vivo* studies explored CMC-544 activity as a single agent and in combination with rituximab (52). Inotuzumab ozogamicin is in phase III clinical testing (Table 2; ref. 50). Although CD22 expression is generally lower on B-ALL cell lines than on B-cell lymphoma cell lines, CMC-544 was shown to be a potent cytotoxin toward ALL cells in the same concentration range observed for CD22-positive B-cell lymphoma cells (53).

CD30 (also known as TNFRSF8), a member of the tumor necrosis factor receptor (TNFR) superfamily, was originally described as a marker of Hodgkin's and Reed-Sternberg cells in Hodgkin's lymphoma. CD30 is highly expressed on Hodgkin's lymphoma and anaplastic large cell lymphoma. Soluble CD30, the extracellular domain of CD30 that is shed, can reduce the effects of CD30-targeting agents by competitive binding. The anti-CD30 antibody designated SGN-30 has potent antitumor activity *in vivo*, possibly as a mediator of antibody-dependent cellular phagocytosis. SGN30 has limited antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity (54). The efficacy of SGN-35, an anti-CD30-monomethyl auristatin E (MMAE) conjugate, in Hodgkin's lymphoma and anaplastic large cell lymphoma xenograft models as a single agent and in combination with chemotherapeutic regimen is marked (55, 56). SGN-35 (brentuximab vedotin) is currently under FDA review (Table 2; ref. 56).

CD340 is HER2/*neu* (ErbB-2, ERBB2, p185), a member of the epidermal growth factor receptor (EGFR) ErbB protein family of cell surface transmembrane receptor tyrosine kinases. *HER2/neu* gene amplification and/or HER2/*neu* protein overexpression occurs in 15 to 25% of breast cancers, as well as in ovarian cancer, stomach cancer, and aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma. Breast cancers are routinely checked for overexpression of HER2/*neu* as a diagnostic tool to select appropriate patients for treatment with trastuzumab, a humanized HER2-targeted antibody. The anticancer mechanisms attributed to trastuzumab include antibody-dependent cellular cytotoxicity and blockade of HER2 signal transduction, resulting in cell cycle arrest and ultimately cell death. In one study, the efficacy, pharmacokinetics, and toxicity of trastuzumab-maytansinoid conjugates varied with the linker used (57). Trastuzumab linked to the maytansinoid DM1 showed similar efficacy whether the linker was a nonreducible thioether or a disulfide linker.

Table 2. ADCs in clinical trial

ADC	Target; indications	Clinical stage	Company
Gemtuzumab ozogamicin Gemtuzumab-hydrazone-calicheamicin (Mylotarg)	CD33; myeloid leukemia	FDA approved 2000, withdrawn 2010	Wyeth
Brentuximab vedotin Brentuximab-MC-VC-MMAE (SGN-35)	CD30; hematologic malignancies, Hodgkin's lymphoma	FDA approved	Seattle Genetics
Inotuzumab ozogamicin Inotuzumab-hydrazone-calicheamicin (CMC-544)	CD22; non-Hodgkin's lymphoma, lymphocytic leukemia	Phase III	Wyeth
Trastuzumab emtansine Trastuzumab-MCC-DM1 (T-DM1)	HER2/neu; HER2+ breast cancer	Phase III	Genentech/Roche/ImmunoGen
Lorvotuzumab mertansine HuN901-SPP-DM1 (IMGN901)	CD56; Merkel cell cancer, small cell lung cancer, multiple myeloma ovarian cancer	Phase II	ImmunoGen
Glembatumumab vedotin CDX-011-MC-VC-MMAE (CDX-011)	GPNMB; melanoma, breast cancer	Phase II	Celldex Therapeutics/ Seattle Genetics
SAR3419 huB4-SPDB-DM4 (huB4-DM4)	CD19; B-cell lymphoma	Phase I	Sanofi/ImmunoGen
IMGN388 Antibody-SPDB-DM4	Integrin; antivascular/ solid tumors	Phase I	Centocor (JnJ)/ImmunoGen
BIIB-015 Antibody-SPDB-DM4	Cripto; solid tumors	Phase I	Biogen-IDEC/ImmunoGen
BT-062 Anti-CD138-SPDB-DM4	CD138; multiple myeloma	Phase I/II	Biotest/ImmunoGen
3ee9-MMAE (BAY 79-4620)	CAIX (MN); solid tumors	Phase I	Bayer/Seattle Genetics
MDX-1203-MC-VC-MGBA (duocarmycin)	CD70; renal cell carcinoma, non-Hodgkin's lymphoma	Phase I	Bristol-Myers Squibb
1 C1- MC-MMAF (MEDI-547)	EphA2; ovarian cancer, solid tumors	Phase I	AstraZeneca MedImmune/ Seattle Genetics
SGN-70- MC-VC-MMAF (SGN-75)	CD70; renal cell carcinoma, non-Hodgkin's lymphoma	Phase I	Seattle Genetics
SAR566658 huDS6-DM4	CA6 ovarian, cervical, breast	Phase I	Sanofi/ImmunoGen
PSMA-ADC anti-PSMA-MMAE	PSMA	Phase I	Progenics/Seattle Genetics

In trastuzumab-DM1, the noncleavable linker was selected based on the improved *in vivo* tolerability of the resulting conjugate. Trastuzumab-DM1 was shown to be an effective anticancer agent even in models refractory to treatment with trastuzumab, and it is currently in phase III clinical trials (Table 2; refs. 58 and 59).

CD19 binds with CD21 to form a receptor on B cells and various B-cell lymphomas. CD19 has wider expression on both normal B cells and non-Hodgkin's lymphoma cells than does CD20, the molecular target of rituximab. Some antibodies directed toward CD19 internalize. Cells with low or no expression of the coreceptor CD21 were shown to have the most rapid internalization of anti-CD19-CD19 complexes (60). Anti-CD19 drug conjugates with several small-molecule drugs, including DNA minor groove-binding alkylating agent duocarmycin analogs, and tubulin fragmenting auristatin and maytansine analogs, have been reported. SAR3419 (huB4-DM4), a conjugate of the huB4 antibody linked to the maytansinoid DM4 (61) via the hindered disulfide linker SPDB, is being evaluated in phase I clinical trials in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma, and it is moving toward phase II trials (Table 2; refs. 62 and 63).

CD56 (neuronal cell adhesion protein NCAM) is a membrane glycoprotein that belongs to the immunoglobulin superfamily. CD56 is expressed by a variety of cancers, including hematopoietic tumors, neuroendocrine tumors (e.g., small cell lung cancer), multiple myeloma, neuroblastoma, and ovarian cancer (64). Lorvotuzumab mertansine is a conjugate of the humanized monoclonal antibody huN901 and the maytansine derivative DM1 (65, 66). It is currently in phase I/II

clinical trials in patients with relapsed small cell lung cancer, and in phase I clinical trial in patients with refractory multiple myeloma (Table 2).

GPNMB [glycoprotein (transmembrane) nmb protein] is a type I transmembrane glycoprotein with homology to the pMEL17 precursor, a melanocyte-specific protein. A fully human monoclonal antibody to GPNMB designated CR011 was conjugated to MMAE via a valine-citrulline (vc) linkage (67, 68). CR011-vc-auristatin antitumor activity was dose-dependent but not strongly schedule-dependent. CR011-vc-auristatin is in phase II clinical trial in patients with unresectable stage III/IV melanoma (Table 2).

Some of the other ADCs in phase I clinical trials are listed in Table 1, and they target diverse antigens: Cripto is in the EGF-Cripto-FRL-Criptic (EGF-CFC) family; however, Cripto does not bind to EGF receptors. Cripto is overexpressed in carcinomas, including breast, ovary, stomach, lung, and pancreatic cancers, and it is absent in normal adult tissues (69, 70). CD138 (syndecan1) is an integral cell surface proteoglycan and an extracellular matrix receptor. It is expressed by differentiated plasma cells and is a primary diagnostic marker of multiple myeloma. CD138 is also expressed by Hodgkin's lymphomas with classic Reed-Sternberg cells (71–73). Carbonic anhydrase IX (CAIX, CA9) is a transmembrane protein and the only tumor-associated carbonic anhydrase isoenzyme. CA9 is overexpressed in a range of tumor types, including gastric, non–small cell lung, pancreatic, and colorectal cancers. CAIX expression is predictive of poor prognosis in several cancers (74–76). Expression of CD70 (tumor necrosis superfamily member TNFSF7) is restricted to activated T and B lymphocytes and mature dendritic cells. CD70 is expressed on hematological malignancies and on carcinomas, including nasopharyngeal, thymic, and renal cell cancers, and glioblastoma (77). Interaction between CD70 and CD27, its receptor, contributes to robust immune responses through costimulation of T and B lymphocyte maturation into effector and memory cells (78–80). The EphA2 receptor tyrosine kinase is selectively expressed on the surface of many human malignant cells. EphA2 protein overexpression is observed in carcinomas and glioblastoma multiforme (81, 82). In ovarian, esophageal, and renal cancer patients, increased EphA2 expression correlates with decreased survival, suggesting that EphA2 receptor expression may have functional significance. CA6 antigen, an *O*-linked tumor-associated sialoglycotope on muc1, is overexpressed in ovarian, breast, cervical, lung, and pancreatic carcinomas (83). Prostate-specific membrane antigen (PSMA) is a membrane glycoprotein that is expressed in prostate tumors (84). Finally, αv integrin is found on several types of solid tumors, including lung, breast, and prostate cancers (85). It is also found on vascular cells in the process of forming new blood vessels, a process that needs to occur for any solid tumor to grow.

Conclusions

Varied and interesting ADC cohorts are currently directed toward targets on liquid and solid tumors in clinical trials, and more are nearing clinical trial. The next hurdle is demonstration of clinical activity worthy of regulatory body approval. With the understanding that ADCs are chemotherapeutics that will be used in combination treatment regimens, the time may have come for this technology to become a major contributor to improving treatment for cancer patients.

Disclosure of Potential Conflicts of Interest

R.V.J. Chari, employee and shareholder, ImmunoGen. B.A. Teicher disclosed no potential conflicts of interest.

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