Treatment of Hematologic Malignancies with Immunotoxins and Antibody-Drug Conjugates

David J. FitzGerald¹, Alan S. Wayne², Robert J. Kreitman¹, and Ira Pastan¹

Abstract
To enable antibodies to function as cytotoxic anticancer agents, they are modified either via attachment to protein toxins or highly potent, low-molecular-weight drugs. Such molecules, termed immunotoxins and antibody-drug conjugates, respectively, represent a second revolution in antibody-mediated cancer therapy. Thus, highly toxic compounds are delivered to the interior of cancer cells based on antibody specificity for cell-surface target antigens. Cancer Res; 71(20): 6300–9. ©2011 AACR.

Introduction
We are approaching the end of the first phase of the antibody revolution. Several monoclonal antibodies (mAb) are approved for the treatment of hematologic malignancies, providing effective clinical options for the management of these malignancies and extending the life of many patients (1). New mAbs and constructs designed to improve potency may eventually be shown to be clinically effective in some leukemias and lymphomas. However, current evidence suggests that unconjugated mAbs have limited utility in many subtypes of hematologic malignancies. Thus, in the next phase of this revolution, mAbs will be used to deliver cytotoxic substances to cells. On the basis of their chemical properties, cytotoxic agents can be divided into different categories: low-molecular-weight agents, high-molecular-weight protein toxins, and radioisotopes. Also, the variable fragments (Fv) of antibodies are used to direct immune effector cells, such as cytotoxic T cells, to antigens on cancer cells via chimeric antigen receptors (2) and to create bispecific T-cell–engaging antibodies (3).

In this review, we focus on mAbs or fragments of mAbs that are attached to cytotoxic agents produced by bacteria or plants, including high-molecular-weight protein toxins and low-molecular-weight chemical entities, such as calicheamicin, mytansinoids, and auristatin. (Radioimmunotherapy is discussed elsewhere; refs. 4, 5.) Initial efforts using antibodies to deliver cytotoxic compounds to cancer cells were not successful for several reasons, including lack of specificity of the antibody, low activity of the cytotoxic conjugate, and side effects due to the toxic moiety. Over the past several years, many of these problems have been recognized and overcome. This review covers advances that have been reported over the past 5 years, showing that immunotoxins and antibody-drug conjugates (ADC) have efficacy and will likely play an increasingly important role in cancer treatment.

General Features of Immunotoxins and Antibody-Drug Conjugates
Immunotoxins and ADCs are assembled in a number of different ways. Antibody fragments or whole antibodies are combined with either protein toxins or low-molecular-weight toxic drugs. Linkage options include gene fusions (peptide bonds), disulfide bonds, and thioether bonds. Design goals dictate that immunotoxins and ADCs remain intact while in systemic circulation but disassemble inside the target cell, releasing the toxic "payload." Uncoupling the toxin or drug from the antibody is accomplished by protease degradation, disulfide bond reduction, or hydrolysis of an acid-labile bond. Toxin or drug attachment to the antibody must not interfere with antigen binding.

Antibodies
As with all cancer therapeutics, the goal of antibody-mediated killing is to eliminate the malignant cells. The choice of antibody will depend on the disease target. Generally, differentiation antigens or receptors that are expressed on malignant cells are appropriate targets, provided they are not expressed on normal vital tissues. Antigens and receptors should be internalized after antibody binding; this ensures that the toxin or drug is transported to the cell interior, where it separates from the antibody and kills the cell.

Protein and Chemical Cytotoxics
Protein toxins are chosen for their potency as enzymes, with the rationale being that only a small number of molecules...
need to be delivered to the site of action, usually the cell cytosol. Once delivered, the turnover rate of the enzyme will allow many substrate molecules to be modified per toxin molecule. Likewise, nonenzymatic toxic products are also selected because of their potency. Protein toxins have several positive attributes: They can be attached directly to antibodies via peptide bonds (see below), and they can be modified easily with engineered modifications of toxin genes. The latter is particularly useful in the design of improved versions. However, because toxins are "foreign" proteins and can induce antibody formation, immunogenicity is a drawback, although solutions to this problem by removing B- or T-cell epitopes may occur in the near future (6, 7). Nonprotein cytotoxics are attached chemically to antibodies via "mild" reactions, such as disulfide exchange or lysine modification (8). Examples of clinically relevant antibody-linked cytotoxics that have undergone or are currently undergoing study in humans are provided in Table 1.

The majority of the protein toxins in clinical development are different types of enzymatic inhibitors of protein synthesis. Diptheria toxin (DT) and Pseudomonas exotoxin A (PE) catalyze the ADP ribosylation of elongation factor 2 (EF2), halting the elongation of growing peptide chains. Plant toxins inactivate ribosomes via glycosidase activity. Specifically, ricin hydrolyses the N-glycosidic bond of the adenine residue at position 4324. Ricin-like toxins exhibit similar activities. Low-molecular-weight chemical cytotoxics include auristatin and maytansine, which target tubulin and calicheamicin and cause double-strand breaks in DNA. Enzymatic turnover rates indicate that only a few molecules of a protein toxin need be delivered to the cytosol to inactivate protein synthesis. In addition, although no direct comparisons have been made, biochemical principles dictate that greater numbers of nonenzymatic cytotoxics need to be delivered to their targets to be equally effective as the enzymatic toxins.

Attachment of Toxins to Antibodies

By using genetic engineering, truncated DT and PE genes are fused with cDNAs encoding antibody Fv's or antibody-binding fragments (Fab) to make recombinant immunotoxins (9). To accomplish this, the native binding domain of the toxin is replaced with the antibody gene sequence. To preserve essential toxin functions, the Fv or Fab is inserted in the same location as the toxin-binding domain (e.g., at the N-terminus of PE and C-terminus of DT). The preferred construct of DT includes 388 amino acids followed by a cell-binding moiety. For PE-based immunotoxins, the Fv is followed by a 38-kDa toxin fragment that includes a putative translocation domain, followed by its ADP-ribosylating domain. For PE but not DT, a C-terminal sequence that binds the KDEL receptor is needed for cytoxic action. For antibody-drug conjugates, attachment is via chemical linkage. Auristatin is coupled to antibody cysteines via a thioether bond. Cytoxic auristatin is further modified to include a dipeptide that is susceptible to lysosomal protease. Linkage of maytansine is achieved via coupling to lysine residues using either disulfide or thioether linkages (10). Calicheamicin can be attached to either carbohydrate or lysine residues. However, lysine modification is preferred, to avoid possible antibody damage via periodate oxidation.

How Toxins and Drugs Enter Cells and Are Released

The intracellular fate of immunotoxins and ADCs is a 2-part story: The first part takes place while the cytotoxic is still attached to the antibody, and the second, after release. The antibody must internalize the cytotoxic to a release site, and the released compound must complete the journey to the cytosol. Endosomes, lysosomes, and the endoplasmic reticulum play critical roles in the fate of these molecules. Cytotoxins are generally more active when targeted to antigens that are efficiently internalized. However, protein toxins must stay intact and avoid lysosomal degradation, whereas drug cytotoxins must be released from antibodies and may benefit from the action of lysosomal proteases. DT translocates to the cytosol from acidic endosomes, whereas PE requires a KDEL-like sequence to traffic to the endoplasmic reticulum, from which it can then enter the cytosol (Fig. 1).

Cytotoxins are released from antibodies in several ways: by proteases, by disulfide bond reduction, or by exposure to an acidic environment. Protein toxins joined to antibodies via peptide bonds or drugs like auristatin linked through dipeptides require proteolytic cleavage. Toxins or drugs attached chemically by disulfide bonds require reduction. Some acid-labile linkers favor release of the cytotoxic in endosomes or lysosomes.

In living cells, delivery to the appropriate intracellular location for release of the cytotoxic seems to be a key factor in determining the effectiveness of the ADC or immunotoxin. Polson and colleagues found that cleavable cross-linkers mediate toxicity when targeted to any one of several antigens on non-Hodgkin lymphomas (NHL), but noncleavable linkers were effective only when targeted to CD22 and CD79b, suggesting that those 2 antigens internalized efficiently (10). This result emphasizes the point that even when cells have the factors needed for toxin release, poor delivery to a specific location will result in little or no killing. Similarly, immunotoxins directed to CD22 are more potent than those directed to CD19, despite the fact that the number of cell-associated immunotoxin molecules is greater for CD19 (see below and ref. 11).

Release of auristatin is claimed to require the action of lysosomal proteases (12). A feature of this release relates to the hydrophobic nature of the drug and the fact that the authors present evidence for bystander effects when the drug is released in close proximity to antigen-negative cells (see below).

Release of DT- and PE-related proteins depends on a furin-like cleavage followed by the reduction of a key disulfide bond. Although the intracellular locations for cleavage and reduction have not been established, there is some evidence that release of protein toxins is antigen specific. Immunotoxins directed to CD22 were potently active, whereas similar immu-
notoxins to CD19 were less potent, despite the fact that both were internalized. This differential effect could be due to different rates of internalization (11).

**Bystander Effects**

Protein toxins released from dead or dying cells are not thought to be active against bystander cells, but this may not be true of all cytotoxins. Auristatins are hydrophobic compounds that are membrane permeable and, once released from their antibody carriers, are free to diffuse to neighboring cells, regardless of whether they display the target antigen. Okeley and colleagues propose that this diffusion could enhance clinical outcomes if lesions consist of mixtures of cells with variable target antigen expression (12). Released drug from strongly antigen-positive cells could kill dimly expressing low-uptake cells. Although this cell killing has been noted in model tissue culture systems, it is not clear if this is operational against tumors in animals.

**Immunogenicity and Antidrug Antibodies**

Typically, protein toxins are foreign proteins and induce antibody formation when injected into patients with intact immune systems. Over 90% of individuals with epithelial cell cancers treated with protein immunotoxins make antitoxin antibodies after 1 or 2 cycles of treatment. However, when immunotoxins are given to patients with hematologic cancers, the incidence of antitoxin or antidrug antibodies is low, and even if they develop, it is commonly after several cycles of therapy. Protein toxins are likely to contain many epitopes, whereas low-molecular-weight cytotoxins will have few or no epitopes. The recent study with the auristatin conjugate SGN-35 revealed that only 2 of 40 patients made antidrug antibodies (13). Notably, subjects on that trial were likely immunosuppressed by the nature of their disease [93% Hodgkin lymphoma (HL)] and prior therapy [median, 3 prior regimens and 73% post-autologous stem cell transplant (ASCT)]. For protein toxins to be useful in multicycle protocols in patients with normal immune systems, immunogenicity needs to be reduced or suppressed. This reduction can be accomplished in several ways: mutagenesis to remove immunodominant B- (7) or T-cell epitopes or the coadministration of immunosuppressive drugs. At the current time, immunotoxins are given predominantly to patients who have been heavily pretreated with bone marrow–damaging and immune-depleting therapies, so antibody formation is suppressed. However, as immunotoxin and ADC treatment regimens become more successful, immunogenicity may emerge as an issue that will need to be addressed.

**Immunotoxins in Clinical Trials**

**Targeting the interleukin-2 receptor**

Denileukin diftitox (DD) is a fusion protein composed of interleukin (IL)–2 fused to the first 388 amino acids of DT. Although it has IL-2 instead of an Fv, it targets and kills cells in the same manner as does an immunotoxin, and is thus described here in this context. DD, like IL-2, binds tightly to the IL-2 receptor 3-chain complex (alpha, beta, gamma), but it binds much less tightly (Kd 10^{-8} versus 10^{-11}) to the alpha subunit, which commonly greatly outnumber other subunits on B- and T-cell malignancies. DD is approved for the treatment of cutaneous T-cell lymphoma (CTCL) in adults (14). In early studies in which DD alone was given to CTCL patients, the objective response rate (ORR) was 38% to 49%. Efficacy was enhanced to an ORR of 67% in patients who also received the retinoid bexarotene, which raises IL-2 receptor levels (15). DD also has activity in other hematologic malignancies. In 27 patients with refractory T-cell NHL, it had an ORR of 48%, with 22% complete responses (CR; ref. 16). In patients with B-cell chronic lymphocytic leukemia (CLL), in which the levels of IL-2 receptor are low, it produced partial responses (PR) in only 2 of 18 (11%) patients (17). Several trials were carried out in IL-2–receptor–expressing B-cell NHL. When DD was given alone, it had low activity, but activity was increased when it was combined with rituximab, with an ORR of 32% with 16% CRs (18).

LMB2 [anti-Tac(Fv)–PE38] is a fusion protein in which the Fv portion of an antibody to CD25, the alpha chain of the IL-2 receptor, is fused to a 38-kDa truncated PE (PE38). LMB2 was originally evaluated in a phase I trial in which a maximum tolerated dose (MTD) of 50 meg/kg given every other day × 3 doses per cycle was established, and an ORR of 23%, including 4 of 4 patients with hairy cell leukemia (HCL), was seen (19). LMB2 is now being evaluated in the treatment of adult T-cell leukemia and/or lymphoma (ATLL), in combination with cyclophosphamide and fludarabine to try to decrease antibody formation and reduce tumor bulk.

**Targeting CD22**

CD22 is a lineage-restricted differentiation antigen expressed on B cells and most B-cell malignancies. Because it is rapidly internalized following immunotoxin or antibody binding, it is an attractive target for immunotoxins and ADCs (11).

RFB4–deglycosylated ricin–A chain (dgA) is an immunotoxin composed of a dgA chemically attached to the RFB4 anti-CD22 antibody that was developed by Messmann and colleagues (20). This agent has activity in animal models and has been tested in adults alone and in combination with an anti-CD19 immunotoxin (21). Capillary leak syndrome (CLS) was a major side effect observed in phase I trials.

Our laboratory produced a recombinant immunotoxin targeting CD22, in which the Fv of the anti-CD22 antibody RFB4 was fused to PE38 (22). It was named BL22 and later CAT-3888. Following studies in which it showed excellent cell-killing activity against patient cells (23) and against tumor xenografts (24), phase I and II trials were carried out at the National Cancer Institute (NCI). The agent was given intravenously every other day × 3 to adults and every other day × 3 (or × 6) to children, with cycles repeated every 21 to 28 days. In the phase I trial of 46 adults, 31 patients with drug-resistant HCL showed an ORR of 81% with 61% CRs (25). The dose-limiting toxicity (DLT) was a completely reversible hemolytic uremic syndrome (HUS). In a phase II study in HCL, the high response...
<table>
<thead>
<tr>
<th>Antigen</th>
<th>Target malignancy</th>
<th>Agent</th>
<th>Cytotoxic</th>
<th>ClinicalTrials.gov (January 2011)</th>
<th>References (2006–2010)</th>
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<td>CD3</td>
<td>T-lineage ALL, NHL</td>
<td>A-dmDT390-bisFv (UCHT1)</td>
<td>DT A</td>
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<td>(35,59,60)</td>
<td>Fever, chills, nausea, hepatic, hypoalbuminemia, lymphopenia, viral reactivation</td>
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<td>SAR3419</td>
<td>Maytansinoid DM4</td>
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<td>(61)</td>
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<td>Ricin-blocked</td>
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<td>CD19 and CD22</td>
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<td>Combotox (mixture of HD37-dgA and RFB4-dgA)</td>
<td>Ricin-dgA</td>
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<td>DD</td>
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<td>HuM195/rgel</td>
<td>Gelonin</td>
<td>+</td>
<td>(74)</td>
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(Continued on the following page)
rate was confirmed with an ORR of 72% and 47% CRs (26). Because BL22 had low activity in other B-cell malignancies in which the number of CD22 molecules on the cell surface was much fewer (CLL) or in which the cells grew very rapidly (acute lymphoblastic leukemia; ALL; ref. 27), we stopped developing BL22 and are now developing a new agent with higher affinity and activity (described below).

HA22 (moxetumomab pasudotox) is an improved form of BL22 in which 3 amino acids in CDR3 of the heavy chain of the Fv are mutated from SSY to THW (28). This mutation results in a 14-fold increase in affinity and a significant increase in cell-killing activity. A phase I trial in HCL has been completed, with additional patients currently being treated at the MTD (29). In addition, phase I trials in adults with CLL and NHL and in children with ALL are ongoing. MedImmune, LLC, has assumed the clinical development of moxetumomab pasudotox.

Clinical trial results of HA22 (moxetumomab pasudotox)

**Hairy cell leukemia.** A total of 32 adults with HCL refractory to therapy or relapsed after at least 2 prior courses of purine analogues received moxetumomab pasudotox (29). It was administered as 30-minute i.v. infusion every other day, with cycles repeated every 4 weeks at doses between 5 and 50 mcg/kg. DLT was not achieved, and dose escalation was stopped because responses were observed at all dose levels and did not seem to correlate with dose escalation. Major responses were seen at all dose levels, including CRs at all dose levels beginning at 10 mcg/kg, and the overall CR rate was 31%. Neutralizing antibodies eventually developed in 14 (44%) of subjects. A phase II study of HA22 for HCL is in development.

**Acute lymphoblastic leukemia.** CD22 is expressed in almost all cases of ALL in children, making it an excellent target for antibody-based therapies (27). BL22 was tested in a phase I trial in children with ALL and NHL. Although there were no objective responses on that trial, transient clinical activity was seen in 16 of 23 (70%) subjects (27). This trial was stopped because of the availability of moxetumomab pasudotox, which was much more active in preclinical models. A pediatric phase I trial for CD22+ ALL and NHL is in progress, and interim analyses reported clinical activity in 8 of 12 patients (67%) including 3 (25%) CRs (30, 31). Because 2 of 7 patients treated at 30 mcg/kg experienced grades 3 and 4 CLS, the trial was amended to include prophylactic corticosteroids during the first cycle of therapy. Notably, in comparison with adult trials, a more intensive dosing schedule has been developed for children with ALL (every other day × 6 every 21 days). Phase II trials of HA22 for pediatric ALL are planned. Note that preclinical studies indicate that moxetumomab pasudotox is synergistic with standard chemotherapy against childhood ALL blasts (32).

**Targeting CD19**

CD19, like CD22, is expressed in most B-cell malignancies and is internalized sufficiently well to bring cytotoxic compounds into the cell. A number of immunotoxins that target
CD19 are being developed for human testing. Results have recently been reported of a trial of the dgA immunotoxin HD37-dgA in combination with an anti-CD22 immunotoxin (see below).

**Targeting CD19 and CD22**

The combination of HD37-dgA and RFB4-dgA (Combotox) has been studied in adults and children with B-lineage hematologic malignancies. In a recently reported trial in children with ALL, 17 patients were treated, and a MTD of 5 mg/m² established. Three (18%) CRs were observed, and hematologic activity was noted in other patients. Incidence of severe adverse events was high; 2 of 11 patients (18%) developed antidrug antibodies (33). An alternative approach to target these 2 antigens has been to make a bivalent recombinant immunotoxin (DT2219), in which 1 Fv binds to CD19 and the other to CD22. DT2219 has shown excellent activity in preclinical models and is now in phase I testing (34).

**Targeting CD3**

CD3 is widely expressed in T-cell malignancies. An anti-CD3 recombinant immunotoxin AdmDT390-bisFv (UCHT1) is constructed of a divalent molecule consisting of 2 single-chain antibody fragments reactive with the extracellular domain of CD3ε, fused to the catalytic and translocation domains of DT (35). This agent is now in clinical trials for adults with T-cell malignancies. An interim report of an ongoing phase I trial noted 2 PRs in 5 evaluable adults with CTCL (35).

**Immunotoxin improvements**

Although immunotoxins have shown efficacy in several types of hematologic malignancies, a few undesirable properties have been identified in clinical trials. In some patients, neutralizing antibodies develop after several cycles of treatment. In the case of immunotoxins made from PE, we have been able to identify and remove the B-cell epitopes recognized by the mouse immune system, resulting in an...

*Figure 1. Pathways of binding, internalization, and processing by immunotoxins and ADCs leading to the killing of target cells.*

1. PE-derived immunotoxin binds CD22, is internalized via clathrin-coated pits, is processed within the cell, and traffics through the endoplasmic reticulum to the cytosol, where an enzymatically active fragment ADP ribosylates EF2, which inhibits protein synthesis and leads to cell death.

2. A DT-based toxin, targeted to the IL-2 receptor, is internalized to endosomes where the A chain of the toxin is released to the cytosol: it also ADP ribosylates EF2, inhibiting protein synthesis and leading to cell death.

3. ADCs bind target receptors, are internalized, and traffic to lysosomes. In the lysosome, drugs are cleaved from the carrier antibody and then released to the cytosol to target tubulin (for auristatin and maytansine) or DNA (for calicheamicin). The pathway for ricin-based immunotoxins (not shown) resembles that of PE, except its cytosolic target is ribosomal RNA. Ricin-mediated depurination of rRNA results in inhibition of protein synthesis.
active immunotoxin that can be given repeatedly to mice without inducing antibody formation (7). However, this immunotoxin also has many human epitopes removed and needs further study to determine whether it will be less immunogenic in humans.

A second problem identified in clinical trials is the development of CLS. In the case of ricin-based immunotoxins, this side effect has been attributed to carbohydrate residues on the toxin binding to endothelial cells. It has been diminished but not eliminated by using forms of ricin that do not contain carbohydrate modifications. In the case of PE-based immunotoxins, CLS has only rarely been dose limiting, but is nonetheless a significant side effect. Weldon and colleagues have reported on an immunotoxin that has a deletion of a large portion of domain II of PE that is fully cytotoxic, yet its nonspecific toxicities in mice, which may reflect the ability of these immunotoxins to cause CLS in patients, are greatly diminished (36).

A third problem concerns the half-life in the circulation of immunotoxins. DD has a short half-life with an alpha of 70 to 80 minutes. BL22, moxetumomab, and LMB2 have longer half-lives in the range of 2 to 3 hours in adults and 0.5 to 4 hours in children. Recombinant immunotoxins were originally designed to be small, with the goal of enhancing penetration into solid tumors masses (37). Although small size may be useful, it also causes these agents to be rapidly removed from the circulation. It is possible to make recombinant immunotoxins of larger sizes by fusing the toxin to Fabs or even entire antibody molecules and producing these in *Escherichia coli* (38, 39).

**Antibody-Drug Conjugates**

**Targeting CD22**

Inotuzumab ozogamicin (CMC-544) is a humanized anti-CD22 mAb attached to calicheamicin. Results of a phase I trial in adults were recently reported (40), and a MTD of 1.8 mg/m² every 4 weeks was established. ORRs of 68% and 15% were observed in patients with follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL), respectively, treated at the MTD. A phase II trial was carried out in 43 patients (41). The ORR was 53%, including 66% for FL and 19% for DLBCL. In a recent trial, CMC-544 was administered with rituximab prior to high-dose therapy and ASCT. The ORR in 19 patients with DLBCL was 21%, with 2 CRs and 2 PRs (42). In a large study reported preliminarily in 2009, 119 patients with relapsed FL and DLBCL were enrolled in a combination study in which rituximab was administered 1 day prior to CMC-544. The ORR was 87% in FL and 80% in DLBCL. Of these patients, 25 were previously refractory to rituximab, and the ORR was only 20% for this subgroup (43).

**Targeting CD30**

CD30 is expressed on several types of hematologic tumors, particularly HL and anaplastic large-cell lymphoma (ALCL). The anti-CD30 mAb cAC10 was conjugated to monomethyl auristatin E (MMAE) through a valine-citrulline peptide linker to make SGN-35 or brentuximab vedotin. Brentuximab vedotin kills cells by depolymerizing tubulin, causing phase growth arrest at G2 and M and apoptotic cell death. Modifications in the linker chemistry improved the therapeutic index in preclinical models using a monomethyl auristatin F (MMAF) derivative (44). A phase I trial of SGN-35 enrolled 45 patients with HL (n = 42), ALCL (n = 2), and angioimmunoblastic T-cell lymphoma (n = 1; ref. 13). The MTD is 1.8 mg/kg administered as a single i.v. infusion every 3 weeks. Of 45 patients, 17 (38%) responded, with 11 (24%) CRs (13). The dose response was clear, with 10 out of 25 (45%) patients achieving CR at ≥1.8 mg/kg versus only 1 out of 20 (5%) patients achieving CR at lower dose levels. ORR at the MTD (n = 12) was 50%, including 4 (33%) CRs and 2 (17%) PRs. Of 42 evaluable patients, 36 (86%) had tumor regression, and 13 (31%) patients with tumor-related symptoms at baseline became symptom free. Median response duration was 17.3 months, and median progression-free survival was 5.9 months.

Additional clinical reports of SGN-35 were presented in late 2010 at the annual meeting of the American Society of Hematology. In a trial of 58 patients with ALCL, 30 evaluable patients with a median of 2 prior therapies were reported to have an ORR of 87% with 57% CRs (45). A pivotal phase II trial of SGN-35 in HL was reported to have enrolled 102 patients, all with prior ASCT and a median of 4 prior therapies (46). A median of 9 cycles of SGN-35 was administered at 1.8 mg/kg every 3 weeks. Tumor regression was reported in 95%, and B symptoms were resolved in 83% of the 35 patients with baseline B symptoms (46).

**Targeting CD33**

CD33 is expressed on the surface of early multilineage hematopoietic progenitors, myelomonocytic precursors, cells of the monocyte-macrophage system, some lymphoid cells, 80% to 90% of cases of acute myeloid leukemia (AML), and some cases of B-cell precursor and T-cell ALL. Gemtuzumab ozogamicin is a humanized IgG4 anti-CD33 antibody linked to calicheamicin (47). On the basis of the results of 3 phase II trials in 142 adults with relapsed AML, the U.S. Food and Drug Administration (FDA) granted marketing approval of gemtuzumab ozogamicin under the accelerated approval regulations in 2000. The CR rate with full blood count recovery was 16%. When patients with CR and incomplete platelet recovery (CRp) were included, the overall response rate was 30%. In patients over 60 years of age, the overall response rate was 26% (48). Similar response rates were also seen in a phase I trial in pediatric patients with AML, in which CR was achieved in 8 of 29 (28%) patients, 4 of these with incomplete platelet count recovery (49). However, on the basis of the lack of shown benefit in randomized phase III studies (50), gemtuzumab ozogamicin was voluntarily withdrawn by its manufacturer in 2010 at the request of the FDA. A randomized phase III trial designed to assess the efficacy of gemtuzumab ozogamicin in combination with standard chemotherapy in newly diagnosed pediatric patients with AML is being conducted by the Children’s Oncology Group (COG; AAML0531; ref. 51). However, accrual to this trial was discontinued in 2010,
and gemtuzumab ozogamicin is no longer available for patients who remain on the study. Final analysis of patients who completed randomized therapy awaits longer follow-up.

Additional anti-CD33 conjugates undergoing study include AVE9633, an ADC composed of the humanized mAb huMy9-6 and maytansinoid DM4 (52). Additionally, immunotoxins composed of deglycosylated gelonin linked to lintuzumab (HuM195; ref. 53) and one constructed using truncated PE linked to a single-chain Fv directed to CD33 (54) have been described.

**Targeting CD123**

Myeloid leukemic progenitors express the IL-3 receptor (CD123), and after binding IL-3, the complex undergoes receptor-mediated endocytosis. Frankel and colleagues developed an immunotoxin composed of the catalytic and translocation domains of DT (DT388) linked to human IL-3 (55). An MTD of 12.5 mg/kg was shown in a phase I trial in adults with AML and myelodysplastic syndrome (MDS). Responses were observed in 3 of 39 (8%) patients with AML (1 CR, 2 PRs) and 1 of 3 with MDS (1 PR). A total of 90% of patients had baseline antibody likely related to prior diphtheria vaccine, and 23 of 30 (77%) evaluable patients developed increased antibody titers after treatment (56).

**Targeting CD56**

CD56 is a neural cell adhesion molecule that is expressed by natural killer (NK) cells, a subset of T cells, and by a variety of malignancies including multiple myeloma (MM) and certain solid tumors. IMGN901 (huN901-DM1) is an ADC composed of the maytansine DM1 conjugated to the anti-CD56 antibody huN901. An MTD of 112 mg/m² was shown in a phase I trial in adults with MM treated with this agent, and 2 of 28 (7%) PRs were observed (57, 58).

**Targeting CD138**

BT062 is an antibody-maytansinoid (DM4) conjugate that targets CD138 (or Syndecan1), a proteoglycan expressed on the surface of plasma cells; a number of other normal tissues; and the majority of cases of MM. Clinical activity was observed in a phase I trial in adults with MM treated with this agent (58).

**What Do We Expect in the Next 5 Years?**

After many years of preclinical development, the number of clinical trials using antibodies or antibody fragments to target potent cytotoxic molecules to cancer cells has recently increased. Several of these trials have shown impressive clinical responses, indicating that we are at the beginning of a new and exciting phase of cancer treatment. Additional studies are now required to define the optimal dose, schedule, and combinations for specific malignancies. Also, several problems have been identified. One is immunogenicity, which may be solved by removing B- and T-cell epitopes. Another problem is likely to be drug and toxin resistance. Nevertheless, we expect this new approach will probably have a major impact in cancer treatment.

**Disclosure of Potential Conflicts of Interest**

D.J. Fitzgerald, A.S. Wayne, R.J. Kreitman, and I. Pastan are inventors for some of the PE-based immunotoxins described in this report. Patent rights to these technologies are held by the NIH.

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David J. FitzGerald, Alan S. Wayne, Robert J. Kreitman, et al.


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