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Treatment of Hematologic Malignancies with Immunotoxins and Antibody-drug Conjugates

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Abstract

To enable antibodies to function as cytotoxic anticancer agents, they are modified either via attachment to protein toxins or highly potent small 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.

Introduction

We are approaching the end of the first phase of the antibody revolution. Several monoclonal antibodies 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 monoclonal antibodies 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 monoclonal antibodies have limited utility in many subtypes of hematologic malignancies. Thus, in the next phase of this revolution monoclonal antibodies will be used to deliver cytotoxic substances to cells. On the basis of their chemical properties cytotoxic agents can be divided into different categories: small molecular weight agents, high molecular weight protein toxins and radioisotopes. Also the variable fragments (Fvs) 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 bi-specific T cell-engaging antibodies (3).

In this review we focus on monoclonal antibodies (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 (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

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Disclosure of Potential Conflicts of Interest

Drs. FitzGerald, Wayne, Kreitman and Pastan are all inventors of immunotoxins that are described in this report. All patents are held by the NIH.

Terminology: protein toxins are called ‘toxins’; small molecular weight toxic compounds are called ‘drugs’ and, generically, toxic compounds are referred to as ‘cytotoxics.’

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 demonstrating that immunotoxins and antibody drug conjugates (ADCs) 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 small 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 either 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, non-enzymatic 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 when designing 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). Non-protein 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. *Diphtheria* 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. Small molecular weight chemical cytotoxics include auristatin and maytansine, which target tubulin and calicheamicin, which causes 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. And while no direct comparisons have been made, biochemical principles dictate that greater numbers of non-enzymatic cytotoxics need to be delivered to their targets to be equally effective as the enzymatic toxins.

Attachment of toxins to antibodies

Using gene engineering techniques truncated DT and PE genes are fused with cDNAs encoding antibody Fvs or antibody binding fragments (Fabs) to make recombinant immunotoxins (9). To accomplish this, the toxin's native binding domain is deleted and 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 kD 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 cytotoxic action. For antibody-drug conjugates, attachment is via chemical linkage. Auristatin is coupled to antibody cysteines via a thioether bond. Cytotoxic 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 either to carbohydrate residues 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 two-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. Cytotoxics are generally more active when targeted to antigens that are efficiently internalized. However, protein toxins must stay intact and avoid lysosomal degradation, while drug cytotoxics must be released from antibodies and may benefit from the action of lysosomal proteases. DT translocates to the cytosol from acidic endosomes while PE requires a KDEL-like sequence to traffic to the endoplasmic reticulum, from which it can then enter the cytosol (Fig. 1).

Cytotoxics 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 appears to be a key factor in determining the effectiveness of the ADC or immunotoxin. Polson et al found that cleavable cross linkers mediate toxicity when targeted to any one of several antigens on non-Hodgkin's lymphomas, but non-cleavable linkers were effective only when targeted to CD22 and CD79b, suggesting that those two 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 to CD19 despite the fact that the number of cell-associated immunotoxin molecules is greater for CD19 (see below and reference (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 drug is released in close proximity to antigen-negative cells (see below).

Release of DT and PE related proteins depend on a furin-like cleavage followed by the reduction of a key disulfide bond. While 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 while similar immunotoxins 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 true of all cytotoxics. 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 et al. propose that this could enhance clinical outcomes if lesions consisted of mixtures of cells with variable target antigen expression (12). Released drug from strongly antigen-positive cells could kill dimly expressing low-uptake cells. While this has been noted in model tissue culture systems, it is not clear if this is operational against tumors in animals.

Immunogenicity and anti-drug antibodies

Typically, protein toxins are foreign proteins and induce antibody formation when injected into patients with intact immune systems. Greater than 90% of individuals with epithelial cell cancers treated with protein immunotoxins make anti-toxin antibodies after 1 or 2 cycles of treatment. However, when immunotoxins are given to patients with hematologic cancers, the incidence of anti-toxin or anti-drug antibodies is low, and even if they develop it is commonly after several cycles of therapy. Protein toxins are likely to contain many epitopes while small-molecular weight cytotoxics will have few or no epitopes. The recent study with the auristatin conjugate SGN-35 revealed that only 2 of 40 patients made anti-drug antibodies (13). Notably, subjects on that trial were likely immunosuppressed by the nature of their disease (93% Hodgkin lymphoma) and prior therapy (median, 3 prior regimens and 73% post-autologous stem cell transplant [ASCT]). For protein toxins to be useful in multi-cycle protocols in patients with normal immune systems, immunogenicity needs to be reduced or suppressed. This can be accomplished in several ways: mutagenesis to remove immunodominant B (7) or T cell epitopes or the co-administration of immunosuppressive drugs. At the current time, immunotoxins are given predominantly to patients who have been heavily pre-treated with bone marrow damaging and immune-depleting therapies so antibody formation is suppressed. However as immunotoxin and ADCs 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 IL2 instead of an Fv, it targets and kills cells like an immunotoxin and will be described with them. DD, like IL2, binds tightly to the IL2 receptor three chain complex (alpha, beta, gamma), but binds much less tightly (Kd 10^{-8} vs 10^{-11}) to the alpha subunit, which most commonly greatly outnumbers 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 there was an objective response rate (ORR) of 38-49%. Efficacy was enhanced to an ORR of 67% in patients who also received the retinoid bexarotene, which raises IL2 receptor levels (15).

DD also has activity in other hematologic malignancies. In 27 patients with refractory T cell non-Hodgkin lymphoma (NHL), it had an ORR of 48% with 22% complete responses (CRs) (16). In patients with B cell chronic lymphocytic leukemia (CLL), where the levels of IL2 receptor are low, it produced PRs in only 2 of 18 (11%) patients (17). Several trials were carried out on in IL2-receptor expressing B cell NHL. When 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 potion of an antibody to CD25, the alpha chain of the IL2 receptor, is fused to a 38kd truncated PE (PE38). LMB2 was originally evaluated in a phase I trial where a maximum tolerated dose (MTD) of 50 mcg/kg given every other day (QOD) x 3 doses per cycle was established and an ORR of 23% including 4 of 4 with hairy cell leukemia (HCL) was seen (19). LMB2 is now being evaluated in the treatment of adult T-cell leukemia/lymphoma (ATLL) in combination with cyclophosphamide and fludarabine to try and 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-dgA is an immunotoxin composed of a deglycosylated A chain of ricin chemically attached to the RFB4 anti-CD22 antibody that was developed by Vitetta 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 lab has produced a recombinant immunotoxin targeting CD22 in which the Fv of the anti-CD22 antibody RFB4 is 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 phase II trials were carried out at the NCI. The agent was given intravenously (IV) QOD x3 to adults and QOD x3 or x6 to children with cycles repeated every 21-28 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 rate was confirmed with an ORR of 72% and 47% CRs (26). Because BL22 had low activity in other B cell malignancies, where 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) (27), we stopped developing BL22 and are now developing a new agent with higher affinity and activity that is described below.

HA22 (moxetumomab pasudotox) is an improved form of BL22 in which three 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)

HCL—A total of 32 adults with HCL refractory to therapy or relapsed after at least 2 prior courses of purine analogs received moxetumomab pasudotox (29). It was administered as 30-minute IV infusions QOD x 3 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 ug/Kg x3, and the overall CR rate was 31%. Neutralizing antibodies eventually developed in 14 (44%). A phase II study of HA22 for HCL is in development.

ALL—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 due to 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 two of 7 patients treated at 30 mcg/kg experienced Grade 3 and Grade 4 CLS, the trial was amended to include prophylactic corticosteroids during the first cycle of therapy. Notably, in comparison to adult trials, a more intensive dosing schedule has been developed for children with ALL (QOD x 6 every 21 days). Phase II trials of HA22 for pediatric ALL are planned. It is interesting to 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 deglycosylated ricin A chain 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 5mg/m^2 established. Three (18%) CRs were observed and hematologic activity noted in other patients. There was a high incidence of severe adverse events and 2 of 11 (18%) developed anti-drug antibodies (33). An alternative approach to target these 2 antigens has been to make a bivalent recombinant immunotoxin (DT2219) in which one 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 two 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 demonstrated 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 active immunotoxin that can be given repeatedly to mice without inducing antibody formation (7). This immunotoxin also has many human epitopes removed and needs further study to determine if 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 and 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 none-theless a significant side effect. Weldon et al have reported on an immunotoxin that has a deletion of a large portion of domain II of PE that is fully cytotoxic yet its non-specific 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 70-80 minutes. BL22, moxetumomab and LMB2 have longer half-lives in the range of 2-3 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 tumor masses (37). While 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 E. 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 an MTD of 1.8 mg/m^2 every 4 weeks was established. ORRs of 68% and 15% were observed in patients with follicular lymphoma (FL) and diffuse large B cell lymphoma respectively treated at the MTD. A phase II trial was performed 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 where 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 Hodgkin's lymphoma (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/M and apoptotic cell death. Modifications in the linker chemistry have improved the therapeutic index in preclinical

models using a 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 (13). The MTD is 1.8 mg/kg administered as a single intravenous infusion every 3 weeks. Seventeen out of 45 patients (38%) responded with 11 (24%) CRs (13). There was a clear dose-response, with 10 out of 25 (45%) of patients achieving CR at ≥ 1.8 mg/kg vs. only 1 out of 20 (5%) at lower dose levels. ORR at the MTD (n=12) was 50%, including 4 (33%) CRs and 2 (17%) PRs. Thirty-six (86%) of 42 evaluable patients had tumor regression and 13 (81%) of patients with tumor-related symptoms at baseline became symptom-free. Median response duration was 17.3 months and median progression free survival (PFS) 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 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, 80-90% of cases of acute myeloid leukemia (AML) and some cases of B-cell precursor and T-ALL. Gemtuzumab ozogamicin (Mylotarg) is a humanized IgG4 anti-CD33 antibody linked to calicheamicin (47). Based on results of three Phase II trials in 142 adults with relapsed AML, the 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, where CR was achieved in 8 of 29 (28%) patients, 4 of these with incomplete platelet count recovery (49). However, based on the lack of demonstrated benefit in randomized phase III studies (50), at the request of the FDA, gemtuzumab ozogamicin was voluntarily withdrawn by its manufacturer in 2010. 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 COG (AAML0531) (51). However, accrual to this trial was discontinued in 2010 and gemtuzumab ozogamicin is no longer available for patients who remain on 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) (53) and one constructed using truncated PE linked to a scFv directed to CD33 (54) have been described.

Targeting CD123

Myeloid leukemic progenitors express the IL3 receptor (CD123) and after binding IL3 the complex undergoes receptor-mediated endocytosis. Frankel et al developed an immunotoxin composed of the catalytic and translocation domains of DT (DT388) linked to human IL3 (55). An MTD of 12.5 μg/kg was demonstrated in a phase I trial in adults with AML and myelodysplastic syndrome. Responses were observed in 3 of 39 (8%) patients with AML (1 CR, 2 PRs) and 1 of 3 with MDS (1 PR). 90% of patients had baseline anti-drug antibodies

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 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/m^2 was demonstrated 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, which is 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 pre-clinical development, there has been a recent burst in the number of clinical trials using antibodies or antibody fragments to target potent cytotoxic molecules to cancer cells. 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 of these is immunogenicity, which may be solved by removing B and T cell epitopes. Another is likely to be drug and toxin resistance. Never the less we expect this new approach is likely to have a major impact in cancer treatment.

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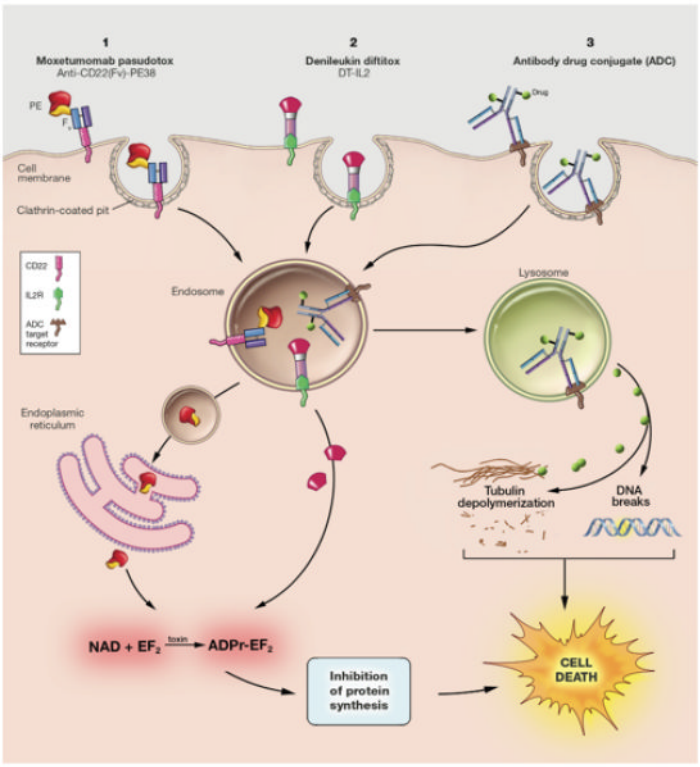

Figure 1.

Pathways of binding, internalization and processing by immunotoxins and ADCs leading to the killing of target cells. 1. A PE-derived immunotoxin binds CD22, is internalized via clathrin-coated pits, is processed within the cell, trafficks through the ER to the cytosol where an enzymatically active fragment ADP-ribosylates EF2, which inhibits protein synthesis leading to cell death. 2. A DT-based toxin, targeted to the IL2 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 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 the ribosomal RNA. Ricin-mediated depurination of rRNA results in inhibition of protein synthesis.

Table 1

Immunotoxin and antibody drug conjugates for hematologic malignancies*

Antigen	***Target Malignancy***	***Agent***	***Cytotoxic***	***Trials in Clinical Trials. Gov (1/2011)***	***References (2006-2010)***	***Toxicities****
CD3	T-lineage ALL, NHL	A-dmDT390-bisFv (UCHT1)	*Diphtheria* toxin A	+	(35,59,60)	Fever, chills, nausea, hepatic, hypoalbuminemia, lymphopenia, viral reactivation
CD19	B-lineage ALL, CLL, NHL, MM	SAR3419	Maytansinoid DM4	+	(61)	Blurred vision
		Anti-B4-BR	Ricin-blocked		(62)	Hepatic, myelosuppression, fever, anorexia, nausea, vomiting, myalgia, CLS
CD19 & CD22	B-lineage ALL, CLL, NHL	Combotox (mixture of HD37-dgA and RFB4-dgA)	Ricin-dgA	+	(33)	Pancreatitis, anaphylaxis, graft-vs-host disease exacerbation, hypoalbuminemia, CLS
		DT2219ARL (bispecific)	*diphtheria* toxin	+		Unavailable
CD22	B-lineage ALL, CLL, HCL, NHL	Moxetumomab pasudotox, (HA22, CAT-8015)	*Pseudomonas* exotoxin A	+	(29,63-65)	Hypoalbuminemia, peripheral edema, fever, hepatic, CLS, HUS
		BL22 (CAT-3888)	*Pseudomonas* exotoxin A		(26,31,66)	Hypoalbuminemia, hepatic, HUS
		Inotuzumab ozogamicin (CMC-544)	Calicheamicin	+	(40-42,67)	Thrombocytopenia, asthenia, nausea, neutropenia, hepatic, anorexia
CD25	ALL, ATLL, CTCL, HL, NHL	LMB-2	*Pseudomonas* exotoxin A	+	(19,68)	Hepatic, hypoalbuminemia, fever, cardiomyopathy
		Denileukin difititox (Ontak)	IL-2 conjugated to *diphtheria* exotoxin	+	(14,69)	Rigors, fever, nausea, CLS

Antigen	*Target Malignancy*	*Agent*	*Cytotoxic*	*Trials in Clinical Trials. Gov (1/2011)*	*References (2006-2010)*	*Toxicities*[*]
CD30	HL, NHL	Brentuximab vedotin (SGN-35)	Monomethyl auristatin E (MMAE)	+	(12,13)	Neutropenia, thrombocytopenia, lymphopenia, hyperglycemia, fatigue, fever, nausea, diarrhea, abdominal pain, peripheral neuropathy, electrolyte abnormalities
CD33	AML, CML, MDS	Gemtuzumab ozogamicin (Mylotarg)	Calicheamicin	+	(70-72)	Myelosuppression, hepatic, veno-occlusive disease
		AVE9633	Maytansinoid DM4		(73)	Hepatic
		HuM195/rgel	Gelonin	+	(74)	Allergy, fever
CD56	MM, NK/T cell leukemias and NHL	Lorvotuzumab mertansine (huN901-DM1, (IMGN901)	Maytansinoid N(2′)-deacetyl-N(2′)-(3-mercapto-1-oxopropyl)-maytansine (DM1)		(57,75)	Fatigue, weakness, neuropathy
CD123	AML, MDS, HCL	$DT_{388}IL3$	*Diphtheria* toxin A		(56)	Hepatic, fever, chills, hypoalbuminemia, hypocalcemia, hypotension, CLS
CD138	MM	BT062	Maytansinoid (DM4)	+	(58,75-77)	Mucositis, hand/foot syndrome, dry eye, blurred vision

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; ATLL: adult T-cell leukemia/lymphoma; CLL: chronic lymphocytic leukemia; CLS: capillary leak syndrome; CTCL: cutaneous T-cell lymphoma; DCs: dendritic cells; dgA: deglycosylated ricin A chain; HL: Hodgkin lymphoma; HUS: hemolytic uremic syndrome; MCL: mantle cell lymphoma; MDS: myelodysplastic syndromes; MM: multiple myeloma; NHL: non-Hodgkin lymphoma; RNase: human ribonuclease; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand

[*] Clinical trials noted in publications and abstracts from 2006-2010 and active clinical trials registered in ClinicalTrials.Gov as of 1/2011 are included. Toxicity summary includes common or severe adverse events associated with the specific agent based on previous reports.