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New Drug Development and Clinical Pharmacology

Advances in Anticancer Immunotoxin Therapy

Christine Alewine,[a] Raffit Hassan,[b] Ira Pastan[a]

[a]Laboratory of Molecular Biology and [b]Thoracic and Gastrointestinal Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Antibody conjugate • Recombinant immunotoxin • Vascular leak syndrome • Antidrug antibody

Abstract

Immunotoxins are a novel class of antibody-conjugated therapeutics currently in clinical development for a variety of malignancies. They consist of an antibody-based targeting domain fused to a bacterial toxin payload for cell killing. Immunotoxins kill cells by inhibiting protein synthesis, a unique mechanism of action that is toxic to both dividing and nondividing cells. Recent advances in the design and administration of immunotoxins are overcoming historical challenges in the field, leading to renewed interest in these therapeutics. ***The Oncologist*** 2015;20:176–185

Implications for Practice: Immunotoxins are a novel class of antibody-based therapeutics currently in clinical development. A review of the field will help physicians better inform patients about the potential benefits and toxicities of these experimental treatments.

Introduction

Immunotoxins combine the specificity of antibody therapeutics with the cell-killing power of some of nature's most toxic proteins. They are chimeric molecules that consist of a protein toxin fused to a targeting moiety. The targeting domain is most commonly the antigen-binding fragment of a monoclonal antibody. The antibody brings the toxin to a cancer cell, then the toxin enters and kills the cell. Structures of several modern immunotoxins are depicted in Figure 1. These molecules are synthesized by recombinant DNA techniques and contain only those portions of the antibody and toxin needed to kill a target cell. The native receptor domain of the toxin is replaced by the antibody. The two are recombinantly fused by a peptide bond. Portions of the toxin that are not essential for cytotoxic activity or processing are deleted from the sequence. Point mutations can be created in the native toxins to improve activity, reduce off-target toxicity, or limit immunogenicity. The modular nature of immunotoxins allows for extensive recombinant tailoring.

The first immunotoxins were made in the early 1980s when monoclonal antibodies reacting with cancer cells became widely available. Protein toxins from a variety of plants and several bacteria were investigated. These areas have been extensively reviewed elsewhere [1]. We will focus on agents that have properties suitable for clinical development or that are already in clinical use. Our group has focused on the use of *Pseudomonas* exotoxin A (PE) to make immunotoxins. We have previously reported that immunotoxins targeting CD22 can cause complete remissions in patients with refractory hairy cell leukemia (HCL) [2]. In addition, we recently found that recombinant immunotoxins targeting the protein mesothelin produced major tumor regressions in some patients with advanced chemotherapy-resistant mesothelioma [3]. In this review, we summarize the current state of the immunotoxin field, analyze the advantages and disadvantages of immunotoxins compared with antibody-drug conjugates (ADCs) and radioimmunotherapies, and discuss future directions.

Immunotoxin Mechanism of Action

The plant and bacterial toxins used in immunotoxins kill cells by halting cellular protein synthesis. Intracellular delivery to the cytosol is required for antitumor activity. After the immunotoxin targeting moiety binds to the cancer cell surface, the molecule is internalized to the endocytic compartment. As shown in Figure 2, processing and trafficking of these molecules is target and toxin specific but ultimately results in delivery of the enzymatically active portion of the toxin to the cytosol. The bacterial toxins diphtheria toxin (DT) and PE irreversibly modify and inactivate eukaryotic elongation factor 2 (eEF2), a critical component of the protein synthesis machinery [4, 5]. Plant toxins such as gelonin and ricin also arrest protein synthesis but do so by inactivating the ribosome instead of eEF2 [6, 7]. These toxin-mediated modifications stimulate the apoptotic pathway, leading to cell death.

Correspondence: Ira Pastan, M.D., Laboratory of Molecular Biology, National Cancer Institute, 37 Convent Drive, Room 5106, Bethesda, Maryland 20892-4264, USA. Telephone: 301-496-4797; E-Mail: pastani@mail.nih.gov Received September 17, 2014; accepted for publication November 25, 2014; first published online in ***The Oncologist*** *Express* on January 5, 2015. ©AlphaMed Press 1083-7159/2015/$20.00/0 http://dx.doi.org/10.1634/theoncologist.2014-0358

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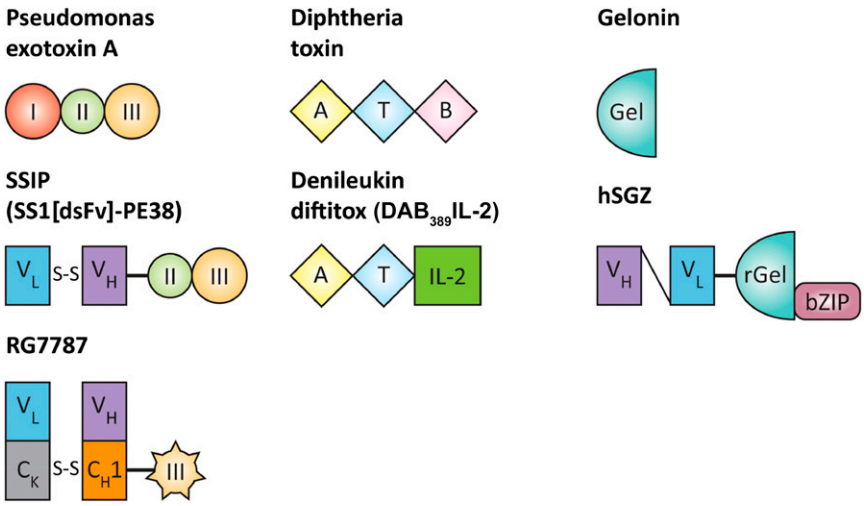

Figure 1. Structure of select toxins and immunotoxins. Native *Pseudomonas* exotoxin A contains three domains: domain I (binding), domain II (unknown function), and domain III (catalytic domain). In the SS1P immunotoxin, domain I is replaced by a double-stranded Fv (V_L and V_H) that targets mesothelin. The engineered disulfide bond links V_L and V_H. The RG7787 immunotoxin uses a humanized Fab fragment and lacks PE domain II. Diphtheria toxin also contains three domains: catalytic, transmembrane, and binding. In denileukin diftitox, the B domain is replaced by human IL-2 to permit binding to cells bearing the IL-2 receptor. Gelonin is a plant toxin with N-glycosidase activity that inhibits ribosomal activity to halt protein synthesis. It consists of a single domain. In the hSGZ immunotoxin, recombinant gelonin is fused to a single-chain Fv (V_L and V_H) that binds fibroblast growth factor receptor 14-kDa protein (Fn14), and a bZIP domain that increases activity of the immunotoxin by allowing dimerization.

Abbreviations: A, catalytic domain; B, binding domain; Gel, gelonin; IL-2, interleukin-2; rGel, recombinant gelonin; T, transmembrane domain.

Comparison With Antibody-Drug Conjugates

ADCs consist of a monoclonal antibody chemically attached to a highly toxic chemotherapy agent with an insufficient therapeutic window for use in traditional systemic therapy. Antibody targeting localizes the drug to the tumor but limits its deposition elsewhere, increasing antitumor activity and decreasing systemic toxicity [8, 9]. Both ADCs currently approved by the U.S. Food and Drug Administration (FDA) and most of those in development use antitubulin chemotherapeutics. Once inside the cell, these drugs disrupt microtubule organization, leading to mitotic arrest and cell death (Fig. 2). Clinical development of ADCs has been vigorous over the past few years because of their decreased toxicity compared with standard chemotherapy and their effectiveness against some refractory tumors.

Immunotoxins have several favorable properties not shared by ADCs. First, the novel immunotoxin mechanism of action translates into a nonoverlapping toxicity profile, allowing for easy combination with standard of care agents. Second, unlike traditional chemotherapeutics and those used in ADCs, immunotoxins can effectively kill quiescent, nondividing cells. In addition, immunotoxins appear to have little cross-resistance with other agents and have demonstrated activity in chemorefractory patients. Finally, although ADCs can cause off-target toxicity due to inappropriate payload dissociation from the chemical linker that joins the chemotherapy to the antibody, modern recombinant immunotoxins do not have this issue. The recombinant peptide linkers that join toxin to antibody in immunotoxins require the action of specific intracellular proteases to unlink.

The same general principles apply when selecting targets for ADCs and for immunotoxins. First, antigens must be expressed on the cancer cell surface for good antitumor efficacy. The antibody must trigger internalization of the molecule with suitable kinetics. Second, for both therapeutic types, the antigens must have strong differential expression between normal and tumor cells to limit on-target off-tumor toxicity. This requirement is more stringent for immunotoxins than for ADCs because immunotoxins kill even quiescent, antigen-expressing cells. Many targets suitable for ADCs are not suitable for immunotoxin development. The very successful anti-Her2 monoclonal antibody trastuzumab, for example, and the subsequent ADC ado-trastuzumab maytansine rarely cause hepatotoxicity. In contrast, development of the Her-2-targeted immunotoxin erb-38 was halted early in phase I testing because of marked hepatotoxicity in all of the first six patients [10]. Further investigations identified very low-level Her-2 expression on the surface of hepatocytes that had not been appreciated in prior studies. Although the exquisite sensitivity of hepatocytes to immunotoxins that is highlighted by this example suggests that this class of therapeutics could be highly effective against naturally chemoresistant hepatocellular carcinoma, it also illustrates how low-level antigen expression in vital organs can affect the safety profile of an immunotoxin.

Comparison With Radioimmunotherapy Agents

Monoclonal antibodies can also be tagged with radioactive agents for use as anticancer therapeutics. This strategy concentrates therapeutic radioactivity at sites of disease; however, because the radioactive cargo does not require cell internalization for activity, these agents produce significant toxic bystander effects on neighboring non-neoplastic, radiosensitive tissues. Two radiolabeled anti-CD20 monoclonal antibodies have been approved by the FDA for

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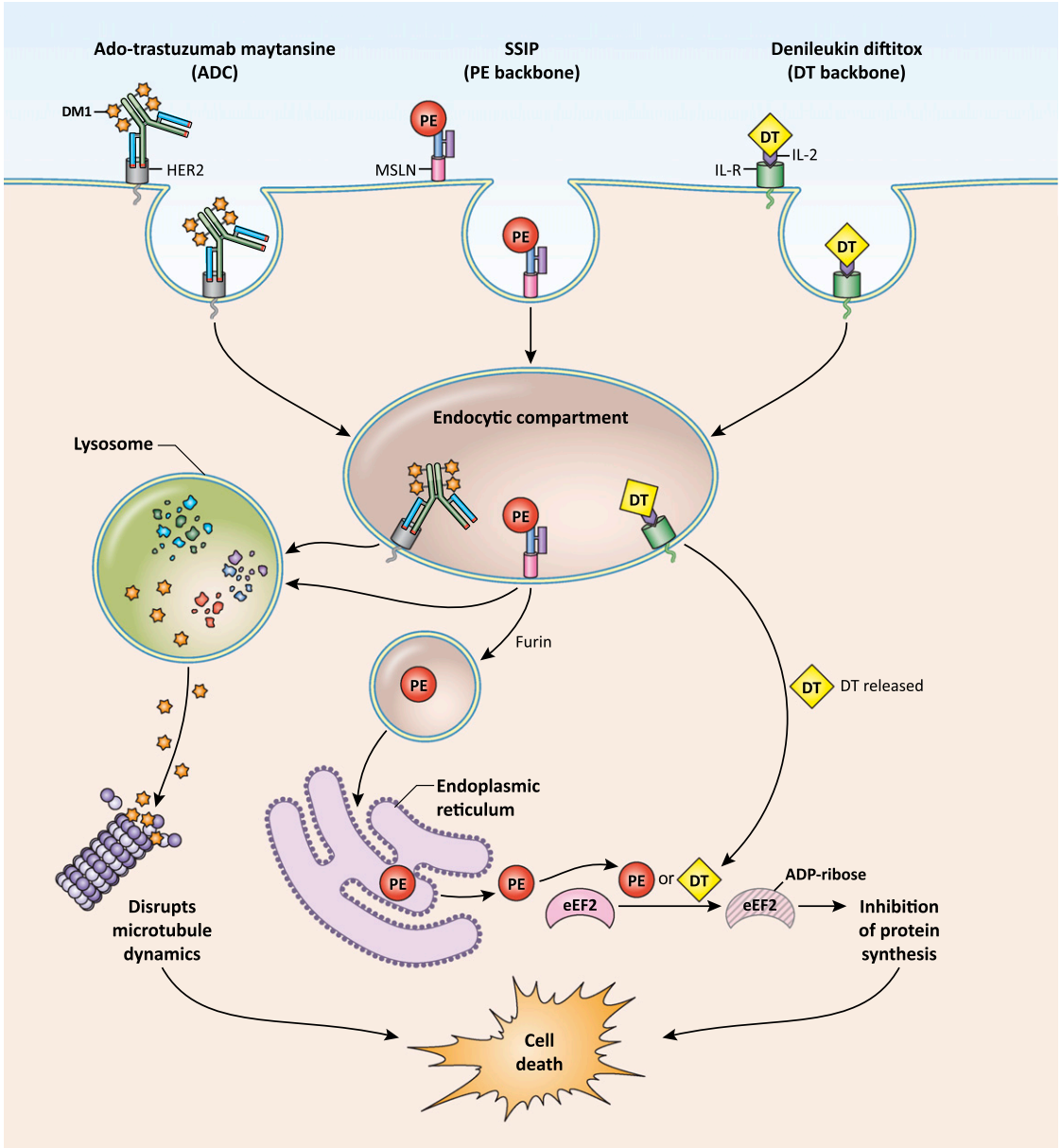

Figure 2. Delivery of ADCs and immunotoxins. ADCs and immunotoxins bind to partners (HER-2, MSLN, or IL-R) on the cell surface and are internalized into an endocytic compartment. The ADC ado-trastuzumab maytansine traffics to lysosomes, where the maytansine chemotherapeutic is released from the antibody structure, allowing drug penetration into the cytosol, disruption of microtubule dynamics, and cell death (left). In endosomes, the modified PE toxin is cleaved from SS1P by the furin protease (middle). PE then undergoes retrograde transport through the Golgi to the endoplasmic reticulum. The method for egress from the endoplasmic reticulum to the cytosol is unknown. The toxin catalyzes irreversible ADP ribosylation of eEF2, leading to global inhibition of protein synthesis and cell death. The T domain (Fig. 1) of DT forms a pore in the membrane of the endosome, allowing transit of DT into the cytoplasm (right). Like PE, DT also catalyzes inhibitory modification of eEF2.

Abbreviations: ADC, antibody-drug conjugate; ADP-ribose, adensodine diphosphate ribose; DT, diphtheria toxin; eEF2, eukaryotic elongation factor 2; IL-2, interleukin-2; IL-R, interleukin-2 receptor; MSLN, mesothelin; PE, *Pseudomonas* exotoxin A.

treatment of refractory non-Hodgkin lymphoma [11, 12]. Both increase the depth and length of clinical response but produce profound myelosuppression that can persist for months. This myelosuppressive toxicity overlaps with that of standard chemotherapy agents used to treat this disease, making combination difficult, unlike the situation with immunotoxins. No successful radioimmunotherapy agents have been developed for treating solid tumors. These tumors are generally much less sensitive to radiation than lymphocytes, and current targeting technology delivers insufficient radioisotope to solid tumors for adequate antitumor effect [13].

Overcoming Historical Obstacles to Clinical Use of Immunotoxins

The first successful in vivo use of an immunotoxin was described in *Nature* in 1981 [14]. The technology developed rapidly, and the first clinical trials in solid tumor patients followed [15, 16]. Unfortunately, these early immunotoxins

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lacked a sufficient therapeutic window to merit further clinical development. The dose-limiting toxicity for these agents was a vascular leak syndrome (VLS) characterized by generalized edema, weight gain, hypoalbuminemia, and orthostatic hypotension. No clinical responses were observed, even at dose levels that caused life-threatening VLS. In addition, only a few doses could be given successfully because all patients on study rapidly developed antidrug antibodies. Additional trials of other immunotoxins in various patient populations produced similar results, and interest in immunotoxins waned. Over the past few years, significant strides have been made to overcome immunotoxin-induced vascular leak syndrome and immunogenicity such that immunotoxins are again being considered as promising anticancer therapeutics.

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Vascular Leak Syndrome

Immunotoxin-mediated damage to endothelial cells is presumed to be responsible for VLS because induction of VLS requires an enzymatically active toxin molecule. Mutant toxins that lack enzymatic activity or that fail to traffic to the cytosol do not cause VLS, suggesting that VLS is an off-target effect of the active molecule [17]. One study suggested that toxins contain short amino acid motifs that bind endothelial cells and demonstrated that the ricin toxin could bind to endothelial cells using these motifs [18]. Subsequent studies showed that modification or deletion of these sequences reduced toxin-induced VLS [19, 20]. Most recently, we reported that deletion of the near entirety of PE domain II can prevent VLS while preserving on-target cytotoxicity. This deletion does not affect two of the three putative endothelial binding motifs previously identified [21]. Further studies will be required to determine the minimal sequence responsible for PE-induced VLS.

Clinically, severity of VLS depends on the immunotoxin administered and varies among patients. Multiple clinical trials with ricin-based toxins have reported severe and even fatal cases of VLS [22–25], whereas studies with PE-based immunotoxins over the past 15 years have reported only mild VLS [2, 3, 10, 26, 27]. Clinical factors may also be important. In a retrospective study of Hodgkin's lymphoma patients who had received ricin-based immunotoxin therapy, VLS was found to be more frequent and more severe in patients with a history of prior radiation therapy [28]. No similar studies have been reported for solid tumor patients or for patients administered immunotoxins with PE or DT backbones.

For our immunotoxin clinical trials using PE-based agents, we have found that simple supportive measures can significantly lessen VLS. All patients on our studies receive premedication with dexamethasone, which has been shown to diminish severity of VLS [29, 30]. In addition, bolus intravenous fluids are administered before each immunotoxin dose to transiently increase intravascular volume. Hypotension was not observed when these supportive measures were applied. Hypoalbuminemia accompanied by dependent edema still occurs in most patients; however, rapid spontaneous normalization of fluid status occurs in the majority following each treatment cycle. A few patients require short-term treatment with a loop diuretic to normalize fluid status. This mild, self-limited VLS has not proven a barrier to clinical development.

Immunogenicity

Immunotoxins are immunogenic molecules. Host anti-mouse antibodies against the antibody portion of the immunotoxin can be avoided by humanizing this portion of the molecule. Unsurprisingly, the toxin domain is even more immunogenic. Patients with hematologic malignancies have impaired immune reactions secondary to their tumor and generally develop antidrug antibodies later in treatment or not at all [2]. In contrast, almost all solid tumor patients rapidly develop anti-immunotoxin antibodies after treatment administration. Several immunosuppressive regimens have been tested in this population. Administration of a single intravenous dose of cyclophosphamide prior to immunotoxin therapy was found to be ineffective [31]. Similarly, pretreatment with oral cyclosporine A did not reduce anti-immunotoxin antibody formation [32]. Suppression of antibody response by rituximab was also examined. All patients had undetectable peripheral B cells when immunotoxin was administered but developed anti-immunotoxin antibodies despite this [33].

Recently, a novel lymphocyte-depleting regimen consisting of pentostatin plus cyclophosphamide was shown to be effective in delaying formation of neutralizing anti-immunotoxin antibodies in solid tumor patients. The pentostatin plus cyclophosphamide regimen was originally designed to combat host-versus-graft reactivity in major histocompatibility complex-mismatched allogeneic bone marrow transplant patients. This regimen depletes T and B cells while largely sparing myeloid cells. It also causes marked durable suppression of T-cell effector function disproportionate to what one would expect based on numerical depletion alone [34]. The combination was tested as a preparative regimen preceding immunotoxin treatment in preclinical studies with mice [35] and then in patients with malignant mesothelioma [3]. In the clinical pilot study, 8 of 10 patients could receive repeated cycles of immunotoxin before development of anti-immunotoxin neutralizing antibodies. This breakthrough allows repetitive administration of immunotoxins to solid tumor patients for the first time.

Immunotoxins in the Clinic

A number of immunotoxins that have been investigated in recent or ongoing clinical trials are listed in Table 1. Others shown in Table 2 have shown promise in the preclinical setting and may shortly begin testing. Additional information about these agents is provided below.

Immunotoxins for Hematologic Malignancies

Denileukin Diftitox (ONTAK, DAB_{389}IL-2)

The FDA granted initial approval of denileukin diftitox in 2001 for treatment of cutaneous T-cell lymphoma (CTCL). This immunotoxin contains a traditional DT backbone; however,

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Table 1. Immunotoxins in the clinic

Immunotoxin	Target	Payload	Regimen	Patient population	Phase	Trial identifier
Approved						
Denileukin diftitox	IL-2R	DT	IV, single agent	Cutaneous T-cell lymphoma	NA	NA
Open trials						
Moxetumomab pasudotox	CD22	PE	IV, single agent	Hairy cell leukemia	III	NCT01829711
			IV, single agent	Adult ALL	I/II	NCT01891981
			IV, single agent	Childhood ALL or non-Hodgkin's lymphoma	I	NCT00659425
LMB-2	CD25	PE	IV, single agent	Hairy cell leukemia	II	NCT00321555
			IV with fludarabine/ cyclophosphamide	Adult T-cell leukemia	II	NCT00924170
A-dmDT390-bisFv(UCHT1)	CD3ε	DT	IV, single agent	Cutaneous T-cell lymphoma	I/II	NCT00611208
DT2219ARL	DT19/CD22	DT	IV, single agent	B-cell lineage leukemia or lymphoma	I	NCT00889408
SS1P	MSLN	PE	IV with pentostatin/ cyclophosphamide	Mesothelioma	II	NCT01362790
Completed trials						
MOC31PE	EpCAM	PE		Epithelial carcinomas	I	NCT01061645
Oportuzumab monatox	EpCAM	PE	Intravesicular, single agent	Bladder cancer in situ		Not listed
HuM195-gelonin	CD33	Gelonin	IV, single agent	AML, CML, MDS	I	NCT00038051

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; DT, diphtheria toxin; IL-2R, interleukin-2 receptor; IV, intravenous; MDS, myelodysplastic syndrome; MSLN, mesothelin; NA, not applicable; PE, *Pseudomonas* exotoxin A.

Table 2. Immunotoxins in preclinical testing

Immunotoxin	Target	Payload	Disease targets	Advance	Reference
RG7787	MSLN	PE	Mesothelioma, pancreatic, cholangiocarcinoma, gastric, TNBC, ovarian	Decreased immunogenicity and toxicity	[58–60]
VB6-845	EpCAM	deBouganin	Epithelial carcinomas	Less immunogenic	[71]
hSGZ	Fn14	Gelonin	Lung, melanoma, breast	New target	[65]
D2C7-(scdsFv)-PE38KDEL	EGFR and EGFRvIII	PE	Glioblastoma multiforme	Binds both EGFR and EGFRvIII	[77]

Abbreviations: EGFR, endothelial growth factor receptor; EGFRvIII, endothelial growth factor receptor variant III; MSLN, mesothelin; PE, *Pseudomonas* exotoxin A; TNBC, triple-negative breast cancer.

the targeting domain of denileukin diftitox does not contain an antibody. Instead, recombinant human interleukin-2 (IL-2) is fused to the C-terminus of the toxin. The ligand targets the molecule to cells that express the IL-2 receptor (IL-2R). IL-2R is transiently expressed on activated T cells but is constitutively present in a number of hematologic malignancies, including CTCL, making it a good therapeutic target. Testing of denileukin diftitox in a single-arm phase III trial of patients with recurrent IL-2R-positive CTCL demonstrated a 30% response rate with a median duration of response of 6.9 months [36]. A later randomized, placebo-controlled trial confirmed improved the response rate with this agent [37]. In practice, denileukin diftitox is used infrequently because of poor tolerability. Side effects include flu-like symptoms (e.g., fever, fatigue, rigors, diarrhea, and nausea), infusion-related events, disruption of color vision, VLS with hypotension, and frequent grade 3–4 hypoalbuminemia. Supportive measures that can be used to ameliorate side effects have been described previously [38].

LMB-2 (anti-Tac[Fv]-PE38)

LMB-2 also targets IL-2R. The high-affinity IL-2R consists of three subunits. The antibody fragment in LMB-2 selectively binds the α-subunit, called CD25 or Tac. This is fused to PE38, a modified PE toxin developed by our laboratory. LMB-2 was tested in patients with relapsed CD25-positive hematologic malignancies in a phase I clinical trial. Partial responses were observed in patients with HCL, CTCL, chronic lymphocytic leukemia (CLL), adult T-cell leukemia (ATL), and Hodgkin's disease. One patient with HCL developed a durable complete response [39, 40]. Currently, LMB-2 is being investigated in combination with fludarabine and cyclophosphamide for patients with relapsed ATL. Complete responses have been observed in otherwise treatment-refractory disease [27].

Moxetumomab Pasudotox

CD22 is a B-cell differentiation antigen expressed on mature B cells and by many B-cell malignancies. Moxetumomab pasudotox consists of a high-affinity anti-CD22 Fv fused to

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PE38. In phase I testing, moxetumomab pasudotox was studied in 28 patients with chemotherapy-refractory HCL. No dose-limiting toxicity was observed up to 50 μg/kg, the maximum dose tested. The overall response rate was 86%, and 46% of patients achieved complete remission. These responses have proven durable in all but one patient [2]. Based on these results, a single-arm phase III registration trial investigating the efficacy of moxetumomab pasudotox for relapsed and refractory HCL recently opened (ClinicalTrials.gov identifier NCT01829711). This immunotoxin is also under evaluation for childhood and adult acute lymphoblastic leukemia. A 24% complete response rate was reported in treatment-refractory pediatric patients, with the molecule demonstrating activity in a total of 67% of patients [41]. No results are available yet from the adult trial.

A-dmDT390-bisFv(UCHT1)

The mature T-cell receptor is a heterodimer composed of CD3 chains $\gamma, \delta, \varepsilon$, and ζ [42]. A-dmDT390-bisFv(UCHT1) is a bivalent immunotoxin that consists of a DT fragment bound to two single-chain anti-CD3ε Fv fragments. It targets T cells. It was tested in patients with CTCL (11 patients) and 1 patient with peripheral T-cell lymphoma in a phase I study. Two patients achieved complete remissions lasting $>$15 months, and 4 patient experienced partial remissions [43]. A phase II trial is currently accruing for patients with previously treated CTCL. Preference is being given to patients with stage IB and IIB disease because the responses were observed in this subgroup.

DT2219ARL

CD19, like CD22 described above, is a B-cell differentiation antigen and a validated target for antibody-based therapies, given the clinical success of rituximab. DT2219ARL is a DT-based molecule that binds both CD19 and CD22 through a complex single-chain design that includes two oppositely oriented Fv fragments linked in series. This allows effective application to more tumor types compared with molecules binding either target alone [44]. A phase I study in patients with relapsed or refractory B-lineage leukemia or lymphoma is ongoing. The trial was temporarily halted after a dose-limiting lower extremity weakness was observed but has since reopened. No data are available on outcomes in the initial cohort of patients.

HuM195-Gelonin

HuM195 is a humanized monoclonal antibody that targets CD33. CD33 was originally thought to be a myeloid-specific marker; however, some natural killer and T-cell subsets also express this leukocyte-differentiation antigen [45]. Importantly, many acute myelogenous leukemias (AMLs) have strong CD33 surface expression [46]. The HuM195-gelonin immunotoxin was tested in a phase I study in patients with relapsed or refractory AML, chronic myelogenous leukemia in accelerated blast phase, and defined subgroups of myelodysplastic syndrome. No vascular leak syndrome and little immunogenicity were observed, but there were no responses. This study is the first published using a gelonin-based immunotoxin. Although efficacy was limited with this particular construct, the safety results suggest that gelonin has a more favorable toxicity profile than the related plant toxin ricin and warrants further clinical investigation.

Immunotoxins for Solid Tumors

SS1P

SS1P was developed in our laboratory. It is the only immunotoxin currently being clinically tested as a systemic agent in solid tumor patients. SS1P consists of the same PE38 fragment found in moxetumomab pasudotox and LMB-2 but uses the SS1 anti-mesothelin (anti-MSLN) antibody for targeting. MSLN is a cell-surface glycoprotein normally expressed only in mesothelial cells that line the pleura, pericardium, and peritoneum. The protein is not critical because mesothelin knockout mice develop normally and have no discernible phenotype [47]. Mesothelin is also robustly expressed by many solid tumors including mesothelioma [48], pancreatic adenocarcinoma [49, 50], nonmucinous ovarian cancer [51], gastric cancer [52, 53], cholangiocarcinoma [54], lung adenocarcinoma [55], cervical cancer [56], and triple-negative-type breast cancer [57]. These properties make MSLN one of the few antigens with sufficient differential expression to allow safe targeting of solid tumor malignancies.

Two phase I trials of single-agent SS1P were performed, each examining a different administration schedule. Efficacy and toxicity profile were similar [26, 58]. The large majority of patients developed antidrug antibodies by the end of their first cycle, resulting in nontherapeutic drug levels if any additional cycles were given. Dose-limiting toxicities included a self-limited on-target pleuritis, presumably from damage to the normal pleura caused by targeting MSLN. Later experience demonstrated that this toxicity could be managed effectively by premedication with steroids and prompt administration of narcotic pain medications at the first signs of onset. A self-limited mild vascular leak syndrome and fatigue were also noted. Efficacy was limited; one radiographic response was documented, and one patient had improvement in ascites. These studies again demonstrated the need for an effective means to suppress the host immune reaction to the therapeutic.

SS1P was next tested in patients with newly diagnosed malignant mesothelioma in combination with standard cisplatin and pemetrexed [59]. Toxicity of the combination in this phase I study was similar to that observed with the individual agents. No reduction from the established single-agent dose of SS1P was required for safe combination. Fatigue was the dose-limiting toxicity. Overall, 60% of patients (12 of 20) achieved a partial response, although the hematologic suppression caused by the chemotherapy failed to delay development of neutralizing antidrug antibodies. This compares favorably to the response rate (41.3%) reported for cisplatin and pemetrexed alone in this patient population [60]. Randomized studies would be required to determine whether addition of a single cycle of SS1P to standard chemotherapy improves patient outcomes.

Most recently, SS1P was tested in a pilot study in combination with the pentostatin plus cyclophosphamide immunosuppressive regimen [3]. As described above, lymphocyte depletion allowed patients to receive more than one cycle of SS1P before development of neutralizing antibodies. In this study, 3 of 10 patients with advanced, treatment-refractory mesothelioma

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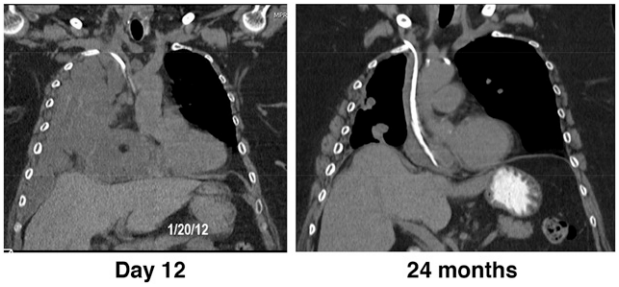

Figure 3. Durable response with SS1P plus lymphocyte-depleting regimen. Coronal computed tomography images of a responding patient 12 days after initiation of therapy on an SS1P, pentostatin, and cyclophosphamide clinical trial with durable response continuing at 24 months, more than 16 months after the last treatment.

experienced major responses that persisted for >18 months (Fig. 3). Interestingly, one of these patients experienced a delayed response that was first evident months after treatment. This was preceded by a dramatic increase in fluorodeoxyglucose uptake on positron emission tomography scan with no concordant tumor growth seen on simultaneous computed tomography, consistent with infiltration by activated immune cells. Moreover, several additional patients who had previously progressed on chemotherapy developed partial responses to the same chemotherapy when it was administered after the immunotoxin regimen. It is interesting to speculate that these observations may be the result of regimen activation of a previously tumor-tolerant immune system. Understanding the mechanism of these responses is an active area of investigation in our laboratory. This regimen has been advanced into phase II studies and is currently accruing patients with pleural and peritoneal mesothelioma. Phase II pancreatic and lung adenocarcinoma cohorts are also planned.

RG7787

This mesothelin-targeted immunotoxin is being developed collaboratively between our laboratory and Roche (Basel, Switzerland, http://www.roche.com) with the goal of engineering a molecule that can be given repeatedly to solid tumor patients without coadministration of immunosuppressive agents. It contains a humanized Fab version of the SS1 antibody and a newly developed PE fragment that has been modified to reduce immunogenicity. Fv RNA of B cells from study patients who had developed neutralizing antibodies following treatment with PE-based therapy were used to pan for antigens in the catalytic domain of PE by a phage-display assay. Reactive phages were then tested in competitive assays for binding to a series of mutated PE fragments bearing alanine substitutions at residues that contain large polar amino acids in the native protein [61]. These bulky residues were known to be important in B-cell recognition and generation of immune response in mice [62]. Using this strategy, six reactive epitopes were identified in the catalytic domain. We also identified seven alanine point mutations that could ablate these B-cell epitopes without destroying PE catalytic activity. Two additional epitopes were removed by deletion of the near entirety of noncatalytic domain II. This modification also improved the therapeutic window of the molecule because PE fragments bearing this modification do not cause vascular leak syndrome in animal models and thus can be given at much higher doses than previous-generation PE molecules, like SS1P. As a single agent, RG7787 shrinks tumors in xenograft models of MSLN-expressing lung [63], gastric, and triple-negative breast cancers [64]. In addition, combination with paclitaxel resulted in marked in vivo synergy and induced complete responses in a xenograft model of pancreatic cancer [65]. Clinical testing of RG7787 is scheduled to begin in early 2015.

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hSGZ-fibroblast growth factor receptor 14-kDa protein (Fn14) is a highly-inducible cell surface receptor for the tumor necrosis factor-related TWEAK ligand. It is frequently upregulated following injury and in disease states. Many solid tumor malignancies express Fn14 [66–68]. The hSGZ immunotoxin consists of a single-chain humanized anti-Fn14 Fab fused to recombinantly modified gelonin, a ribosome inhibiting plant toxin (Fig. 1) [69]. In mouse xenograft models of melanoma and in Her-2-positive breast cancer, hSGZ delayed tumor progression [69, 70]. No major toxicities were observed in these studies, although Fn14 is known to be expressed at low levels in several vital organs [71, 72]. Fn14 could prove to be an interesting target.

Oportuzumab Monatox by Intravesicular Administration

The immunotoxin oportuzumab monatox uses a single-chain anti-EpCAM antibody fused to PE. Intravesicular administration was tested in patients with urothelial carcinoma in situ who were intolerant or refractory to bacillus Calmette-Guérin. This delivery method limits systemic toxicity by delivering the immunotoxin directly to the site of the tumor and avoids issues with immunogenicity of the circulating molecule [73]. No dose-limiting toxicity was observed in a phase I study [74]. The immunotoxin was advanced into phase II testing in which 44% of patients achieved a complete response and 16% continued to maintain this response at 1 year [75]. Successful therapy in this setting has the potential to delay or spare these patients from cystectomy and the decreased quality of life that goes with it.

VB6-845

This is an anti-EpCAM immunotoxin intended for use in solid tumor malignancies. The backbone consists of deBouganin plant toxin fused to a humanized Fab. Preclinical studies have suggested that this recombinant toxin is significantly less immunogenic than other payloads. Studies in mice demonstrated efficacy against EpCAM-positive solid tumors. VB6-845 caused severe vascular leak syndrome in rats at high doses but appeared to be better tolerated in cynomolgus monkeys [76]. A phase I study was opened in 2007 but closed for nonmedical reasons.

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Convection-Enhanced Drug Delivery to Central Nervous System Tumors

Local delivery of immunotoxins to malignant brain tumors has shown potential in early clinical trials. In these trials, convection-enhanced drug delivery (CED) is typically used. This technique uses interstitial microinfusion through surgically placed catheters to deliver immunotoxin to the tumor bed and surrounding affected tissue. Immunotoxins targeting tumor-associated antigens or surface proteins particularly enriched in the tumor versus the normal brain tissue are used. These targets have included transferrin receptor (found in Tf-CRM107) [77], IL-13 receptor (in cintredekin besudotox) [78], epidermal growth factor receptor (EGFR; in TP-38) [79], and the mutant EGFR variant EGFRvIII (in MR1-1). Toxicities observed with local treatment are generally neurologic and low grade. Promising responses, including a few complete responses in glioblastoma, have been seen in a minority of patients. Variability in effectiveness has been attributed to difficulty in consistently achieving effective catheter placement, resulting in variable immunotoxin delivery to the tumor site. In addition, heterogeneity of immunotoxin target expression in patient tumors may also play a role. A recent review has examined this literature in depth [80].

D2C7-(scdsFv)-PE38KDEL

The majority of glioblastomas multiforme overexpress EGFR and a constitutively active mutant form called variant III (EGFRvIII) [81]. D2C7 is a new antibody that reacts with both forms. A PE toxin payload is used. In orthotopic mouse models of glioblastoma, treatment with D2C7-(scdsFv)-PE38KDEL resulted in dramatic increases in survival time compared with previously developed immunotoxins that could bind only EGFR or EGFRvIII [82]. This immunotoxin is a promising candidate for clinical development using CED.

Conclusion

Immunotoxins are exciting therapeutics that are once again receiving attention as potential antineoplastic agents now that previous problems with toxicity and immunogenicity are being solved. Recent studies of immunotoxins have produced promising results in patients with relapsed and refractory malignancies. Immunotoxins have a unique mechanism of action and nonoverlapping toxicity compared with standard chemotherapies and could be ideal molecules for combination therapy.

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Conception/Design: Christine Alewine, Raffit Hassan, Ira Pastan
Manuscript writing: Christine Alewine, Raffit Hassan, Ira Pastan
Final approval of manuscript: Christine Alewine, Raffit Hassan, Ira Pastan

Disclosures

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