Immunotoxin Treatment of Cancer*

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Annu. Rev. Med. 2007. 58:221–37

First published online as a Review in Advance on October 23, 2006

The *Annual Review of Medicine* is online at http://med.annualreviews.org

This article's doi: 10.1146/annurev.med.58.070605.115320

0066-4219/07/0218-0221$20.00

Key Words

Pseudomonas toxin, diphtheria toxin, monoclonal antibody, recombinant immunotoxin

Abstract

Immunotoxins are proteins used to treat cancer that are composed of an antibody fragment linked to a toxin. The immunotoxin binds to a surface antigen on a cancer cell, enters the cell by endocytosis, and kills it. The most potent immunotoxins are made from bacterial and plant toxins. Refinements over many years have produced recombinant immunotoxins; these therapeutic proteins are made using protein engineering. Individual immunotoxins are designed to treat specific cancers. To date, most success has been achieved treating hematologic tumors. Obstacles to successful treatment of solid tumors include poor penetration into tumor masses and the immune response to the toxin component of the immunotoxin, which limits the number of cycles that can be given. Strategies to overcome these limitations are being pursued.

Immunotoxin: antibody-toxin hybrid molecule; the antibody binds cells and the toxin kills them

Recombinant: made using cloned DNA to produce homogenous protein

mAb: monoclonal antibody

Disulfide bond: a sulfur-sulfur bond usually between two cysteine amino acids

VLS: vascular leak syndrome

HISTORY

Paul Ehrlich, who first reported on the differential staining of various tissues in 1877, argued that one could rationally develop agents specifically to kill cancer cells (1). The discovery of monoclonal antibodies (mAbs) and the ability to produce large amounts of mAbs that react with specific antigens on cancer cells reawakened an interest in using toxins to kill cancer cells. Bacterial and plant protein toxins are among the most potent cytotoxic agents in nature. By attaching these toxins to mAbs, novel agents were designed to kill tumor cells. These agents were named immunotoxins because they use a major product of the immune system (antibody) to direct the toxin to the tumor cells.

CHARACTERISTICS OF PROTEIN TOXINS USED TO MAKE IMMUNOTOXINS

The toxins that have been used to make immunotoxins are made by microorganisms, plants, insects, and vertebrates and have diverse biological functions. Because only small amounts of toxin are delivered by these organisms, the toxins must be very potent; undoubtedly toxin potency has been increased by natural selection over millions of years.

Toxins that have been successfully used to make immunotoxins consist of several domains. Typically there is a cell recognition (binding) domain, which concentrates the toxin on the surface of the target cell; a translocation domain, which enables the toxin to cross a membrane to reach the cytosol, where essential cell machinery is located; and a death (activity) domain, which inactivates some vital cellular process and kills the cell (**Figure 1**). To make an immunotoxin, the cell recognition domain is replaced with a new recognition or targeting moiety.

Many protein toxins have been used to make immunotoxins, and excellent reviews are available (2–4). In this review, we discuss some important principles learned from those studies. The principles of how toxins and immunotoxins kill cells are simple, but the details, as discussed below, are complex and greatly affect the result.

Advances in Immunotoxin Design: From Chemical Conjugates to Recombinant Immunotoxins

The first immunotoxins were produced by chemically coupling native toxins to antibodies with reagents that form disulfide bonds connecting the toxin to the antibody (**Figure 2*a***). (Basic antibody structure and the location of functional domains are provided in **Figure 3**.) These first-generation immunotoxins had several drawbacks, including lack of specificity, poor stability, and heterogeneous composition.

Since these first-generation immunotoxins were produced, many biochemical and crystallographic studies have provided essential information on the structure and function of toxins. In particular, the knowledge that toxins are made up of discrete domains with different functions and the elucidation of their crystal structures have enabled significant improvements in immunotoxin design.

Second-generation immunotoxins, like their predecessors, were made by chemical coupling methods. Using the new knowledge of the structure and function of toxins, cell-binding domains were removed, and the resulting toxin fragment that no longer could bind to normal cells was coupled to an antibody (**Figure 2*b***). This approach increased the amount of immunotoxin that could be safely given to experimental animals and humans, but problems of heterogeneity persisted. Moreover, some immunotoxins still bound weakly to normal cells and produced an undesirable side effect called vascular leak syndrome (VLS). VLS is caused by damage to endothelial cells and results in leakage of fluid from the circulation into the tissues, edema, a fall in serum proteins, hypotension, and, at worst, vascular collapse (5). Because

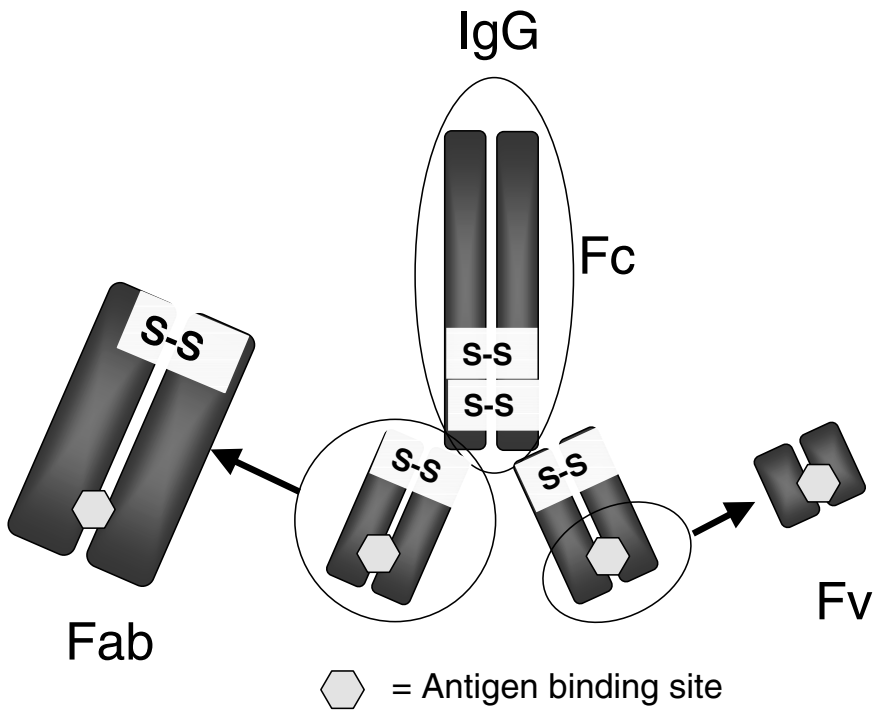

Figure 3

Antibody structure. IgG molecules are divided into functional domains. The main divisions are Fc and Fab. The Fc interacts with cellular Fc receptors and complement; the Fab fragment contains the antigen binding site. The Fab fragment is further divided into the Fv fragment, the smallest fragment that retains antigen binding via contacts with both the heavy and light chains. The two chains of the Fab fragment are held together by a naturally occurring disulfide bond. No such covalent bond exists for Fv. The two chains of Fv are held together either by a flexible peptide linker or by a novel disulfide bond.

immunotoxins are administered to patients intravenously, so that the circulatory system will carry them to the tumor cells, endothelial cells are exposed to high levels of immunotoxin. Sufficient amounts can enter endothelial cells to damage them and allow leakage of serum proteins.

Third-generation immunotoxins and related growth factor and cytokine fusion proteins are made by recombinant DNA techniques and as a group are called recombinant toxins; those containing Fv portions of antibodies are called recombinant immunotoxins (5a). Recombinant immunotoxins are homogeneous and relatively inexpensive to produce. **Figure 2*c*** illustrates the structure of some molecules made by replacing the cell-binding domain of the toxin with the Fv portion of an antibody or a growth factor. Because toxins kill animal cells, they must be produced in a microorganism, and *Escherichia coli* is used for this purpose. Most of the recombinant immunotoxins currently in clinical trials use either diphtheria toxin (DT) or *Pseudomonas* exotoxin A (PE) because these bacterial toxins are more easily produced in *E. coli* than plant toxins and have shown more activity and fewer side effects in humans.

Antibody Fv: variable fragment of an antibody responsible for antigen binding

DT: diphtheria toxin

PE: *Pseudomonas* exotoxin A

scFv: single-chain Fv

Design and Production of Recombinant Immunotoxins

Initially, recombinant immunotoxins utilized single-chain Fvs (scFvs) to target the

toxin. These are made up of the light and heavy chains of an antibody connected by a 15–amino acid peptide (5a). An example is immunotoxin LMB-2 (anti-TacFv-PE38), which contains the Fv of an antibody directed at the alpha subunit of the interleukin-2 receptor (IL2R). Although LMB-2 and a few other immunotoxins are sufficiently stable for clinical use, many single-chain immunotoxins are not, because the disulfide bond that normally stabilizes the Fv region lies in the portion of the Fab that is removed to make the scFv (**Figure 3**). As a consequence, the light and heavy chains of many scFvs can dissociate and bind to other dissociated Fvs, leading to aggregation and loss of activity.

This instability was overcome by designing a very stable Fv in which the peptide linker of the scFv is replaced by a disulfide bond inserted in the framework region of the Fv. Using the crystal structures of various antibodies, B.K. Lee and colleagues determined which two amino acid residues in the light and heavy chains of the Fv are in a location that allows them to be replaced with cysteine residues that combine to form a stable cystine connecting the two chains of the Fv (6). It is also possible to use protein engineering to make stable single-chain immunotoxins by mutating residues that lie in the region where the light and heavy chains of the Fv are in contact (7, 8).

Many immunotoxins with disulfide-stabilized Fvs (dsFvs) have been constructed, and several have been evaluated in clinical trials. The first to enter the clinic was directed at Her2/neu expressed on breast cancer cells (9). Another is BL22, which has produced many complete remissions in hairy-cell leukemia (HCL) (10). Cytokine-toxin fusion proteins such as DT388-IL2, TGF-alpha-PE38 (**Figure 2*c***), and related proteins are produced in a similar manner and are stable because they consist of two intrinsically stable protein moieties: the toxin and a cytokine.

IL2R: interleukin-2 receptor

dsFv: disulfide-stabilized Fv

HCL: hairy-cell leukemia

Endocytosis: the process of bringing extracellular material to compartments in the cell's interior

Endosomes: vesicles derived from invaginations of the cell surface that transport molecules into the cell

EF2: elongation factor 2

ER: endoplasmic reticulum

ADP ribosylation: the covalent transfer of ADP-ribose from NAD to a protein

Mechanism of Toxin- and Immunotoxin-Induced Cell Death

Immunotoxins are designed to bind to specific antigens on the surface of cancer cells and enter cells by endocytosis (**Figure 4**). The affinity of immunotoxin for antigen determines how much is bound and how long it stays associated with the cell surface antigen. Therefore the higher the affinity the more active the immunotoxin is in killing target cells. However, high local concentrations of immunotoxin may allow nonadsorptive or pinocytic uptake into nontarget cells such as endothelial cells, producing undesirable side effects. Endocytosis carries the toxin to an acidic compartment. Here, toxins such as DT and PE are processed via furin cleavage, producing two fragments that are initially held together via a disulfide bond (11, 12). For DT immunotoxins, the acidic environment of the endosome provokes a structural change that allows translocation across the endocytic membrane, where the A chain ADP-ribosylates elongation factor 2 (EF2). ADP-r-modified EF2 can no longer make new protein and the cell dies via apoptosis. For PE immunotoxins, the cleaved C-terminal portion of the toxin proceeds in a retrograde fashion through the Golgi to the endoplasmic reticulum (ER), where it translocates to the cytosol and ADP-ribosylates EF2. For ricin A immunotoxins the A chain is separated from the antibody via reduction of the disulfide bond linking the two. The translocation of ricin, like that of PE, is thought to involve the ER compartment. From there the A chain translocates to the cytosol, cleaves ribosomal RNA, and thereby inhibits protein synthesis.

Behavior of Immunotoxins in Vivo

Immunotoxin molecules escape from the circulation slowly because of their large size. Consequently, leukemia cells in the blood are exposed to high immunotoxin levels, but cells within solid tumors are not. Several

factors restrict the entry of proteins into tumor masses composed of cancerous epithelial cells, or even into many enlarged lymphoma masses or dense masses of cells in the bone marrow. These include the normal slow rate of release of proteins into the extravascular space, the abnormal blood vessels in the tumor, and the architecture of tumors. In normal tissues, proteins such as transferrin, which delivers iron to the cell, exit the capillaries and are carried by convective flow to their target cells in various organs. Convective flow is effective only if there are lymphatic vessels that can return the convected fluid to the circulatory system and prevent the buildup of interstitial pressure. Unfortunately tumors do not have functional lymphatics, (13) and immunotoxins reach cells in solid tumors by the slow and inefficient process of diffusion, which is greatly affected by the size of the protein (**Figure 5*a***). An undesirable consequence of this difference in physiology is that normal cells are rapidly exposed to higher immunotoxin levels than cancer cells and are rapidly killed if they contain the same antigen as the cancer cell (**Figure 5*b***). So it is important to make immunotoxins from antibodies that do not bind to essential normal cells.

Preclinical Evaluation of Immunotoxin Activity and Safety

Initial evaluation of immunotoxins involves testing for specific cytotoxic activity on cancer cell lines by incubating them with target cells for 1–3 days. Because most immunotoxins directly arrest protein synthesis by inactivating EF2 or damaging ribosomes, the incorporation of ^{3}H-leucine into protein is decreased in a dose-dependent manner in <24 h. To avoid radioactive methods, dye-based assays are now frequently used, but these require an incubation of 2–3 days to demonstrate significant cell death.

Tumor Models in Mice

To replicate how immunotoxins will target tumor cells in humans, cell lines from human cancers are used to make tumors in immunodeficient mice. The cells are usually injected subcutaneously to produce solid tumors but are also given intravenously to produce micro- or macrometastases, or injected into the peritoneal cavity to mimic ovarian cancer or mesothelioma. The most difficult tumors to treat are subcutaneous solid tumors because of the immunotoxin's poor penetration into solid tumor masses. Mouse models can also provide toxicity data, but these data are generally of limited value. Monkey models can be very useful to predict toxicities if the antibody used to make the immunotoxin has a similar reactivity with monkey and human antigenic targets.

HD: Hodgkin's disease

CTCL: cutaneous T cell lymphoma

ATL: adult T cell leukemia

CLINICAL TRIALS OF IMMUNOTOXINS FOR TREATMENT OF HEMATOLOGIC MALIGNANCIES

A wide variety of hematologic malignancies have been targeted with immunotoxins—both those with peripheral blood involvement, as in leukemias, and those with malignant cells predominantly outside the vasculature, as in Hodgkin's disease (HD), other lymphomas, and multiple myeloma. Clinical studies in hematologic malignancies are summarized in **Table 1** and only some major features of key or recent trials are discussed below.

Targeting IL2 Receptor with Denileukin Difitox (Ontak)

IL2Rs are present in many different hematologic malignancies, including HD, cutaneous T cell lymphoma (CTCL), adult T cell leukemia (ATL), and other B- and T cell leukemias and lymphomas (14–17). IL2Rs, also present on normal T cells, probably have a role in various autoimmune diseases (18).

To target IL2R-expressing cells, a truncated DT, containing amino acids 1–389, was fused to IL2 to make DAB$_{389}$IL2, also called denileukin diftitox or Ontak (19, 20)

Table 1 Immunotoxins used against hematologic malignancies

Immunotoxin	Toxin used	Target antigen	Tumor type	Clinical trial phase	No. of patients	Response	References
$DAB_{486}IL2$	DAB_{486}	IL2R	Ly, Le	I/II	112	3 CR, 8 PR	86
Denileukin diftitox	DAB_{389}	IL2R	CTCL, NHL	I	73	6 CR, 10 PR	21
Denileukin diftitox	DAB_{389}	IL2R	CTCL	Pivotal trial	71	7 CR, 14 PR	87, 23
LMB-2	PE38	CD25	Ly, Le	I	35	1 CR, 7 PR	88, 34
RFT5-dgA	dgA	CD25	HD	I/II	18	2 PR	38
RFB4-dgA	dgA	CD22	Ly, Le	I	41	2 CR, 10 PR	44, 45
BL22	PE38	CD22	Ly, Le	I	46	19 CR, 7 PR	51, 10
DTGM	DT_{388}	GM-CSFR	AML	I	31	1 CR, 2 PR	55
Anti-B4-bR	bRicin	CD19	NHL	II	16	None	61
HD37-dgA + RFB4-dgA	dgA	CD19 +CD22	NHL	I	22	2 PR	67
Ki-4-dgA	dgA	CD30	HD, NHL	I	15	1 PR	68

Abbreviations: bRicin, blocked ricin; dgA, deglycosylated ricin A chain; DT, diphtheria toxin; DAB or DT, truncated DT; PE, *Pseudomonas* exotoxin A; PE38, truncated PE; Ly, lymphoma; Le, leukemia; CTCL, cutaneous T cell lymphoma; NHL, non-Hodgkin's lymphoma; AML, acute myelogenous leukemia; CR, complete response; PR, partial response.

NHL: non-Hodgkin's lymphoma

CR: complete remission

CLL: chronic lymphocytic leukemia

CD: cluster determinant; e.g., the surface protein CD22 is cluster determinant #22

(structure as in **Figure 2*c***). In phase I testing, denileukin diftitox produced a 37% response rate in CTCL and an 18% response rate in patients with other non-Hodgkin's lymphomas (NHLs) (21). Severe fatigue was dose-limiting. In the pivotal phase III trial in 71 CTCL patients, a 30% response rate was achieved, including 10% complete remissions (CRs), and most patients had objective skin improvement (22, 23). Antibodies to denileukin diftitox were assessed by ELISA and increased from a 32% baseline to nearly 100% after one treatment cycle, but retreatment was sometimes effective, indicating that antitoxin antibodies detected by ELISA were not always neutralizing. Pharmacokinetic studies showed that mean terminal half-lives were 43–82 min (9).

After approval for CTCL, denileukin diftitox was tested in panniculitic lymphoma (24), B cell chronic lymphocytic leukemia (CLL) (25), B-NHL (26), and psoriasis (27). Responses were observed in all these trials, although not at the high percentage observed with CTCL. One limitation in the treatment of CTCL, CLL, or NHL is the absence of high-affinity IL2Rs in a large percentage of cases, usually due to lack of CD122. Bexarotene, which has been reported to increase CD25 and CD122 expression, was combined with denileukin diftitox, and the combination produced a relatively high response rate (58%) in 14 CTCL patients in a phase I trial (28).

Targeting the CD25 Subunit of the IL2R with Anti-TacFv-PE38 (LMB-2)

LMB-2 is a single-chain immunotoxin in which the Fv of an antibody to the CD25 subunit of the IL2 receptor is fused to PE38 (**Figure 2*c***). LMB-2 is selectively cytotoxic toward both $CD25^+$ cell lines and leukemic cells freshly obtained from patients (29–33), and it caused complete regressions in mice bearing $CD25^+$ xenografts (30, 32). Primary ATL and HCL cells are much more sensitive than primary CLL cells to LMB-2 owing to lower CD25 expression in CLL (33).

In a phase I trial, LMB-2 was administered to 35 patients with chemotherapy-resistant leukemia, lymphoma, and HD, resulting in a response rate of 40% in the 20 patients receiving upper dose levels (**Table 1**). All four patients with HCL responded, including a

complete remission with long-term (>7-year) resolution of transfusion dependence. In addition to HCL, responses were observed in patients with CLL, ATL, CTCL, and HD (34). Common toxicities were transaminase elevations associated with fever, probably mediated by released cytokines (35, 36). Some (17%) of the patients made neutralizing antibodies after one cycle and could not be retreated. However, none of the eight CLL patients made neutralizing antibodies after a total of 16 cycles. Phase II trials have now been initiated in CLL, CTCL, and HCL. LMB-2 is also being evaluated in patients with melanoma, to determine if it can improve the response to a melanoma vaccine by selectively killing $CD4^+CD25^+$ regulatory T cells (37).

Targeting $CD25^+$ Malignancies with RFT5-dgA

Treatment with RFT5-dgA, which contains the anti-CD25 RFT5 mAb chemically conjugated to deglycosylated ricin A-chain (dgA), resulted in a response rate of 11% in HD (**Table 1**) (38, 39). RFT5-dgA was also used in an attempt to prevent graft-versus-host disease (GVHD) in patients undergoing allogeneic stem cell transplantation but unexpectedly produced a higher incidence of grade III/IV GVHD than historical controls (40). It is possible that $CD4^+CD25^+$ regulatory T cells were somehow selectively killed by this immunotoxin, allowing enhanced activity of cells responsible for the GVHD.

Immunotoxins Targeting CD22

Several chemical conjugates targeting CD22 on B cell malignancies have been evaluated in clinical trials. The first of these contained mAbs H6 or RFB4 conjugated to dgA (41, 42), and mAbs HD6 and HD39 conjugated to Saporin (43). RFB4-dgA resulted in responses in 29% of patients with B cell malignancies over several phase I trials. The dose was limited by VLS (**Table 1**) (44, 45). In an effort to prevent VLS, a ricin A-chain mutant was made in which an alanine residue replaced asparagine at position 87 of ricin. RFB4-N87A produced less VLS in mice but has not been tested in humans (46, 47).

dgA: deglycosylated ricin A-chain

The first anti-CD22 immunotoxin containing PE was composed of the mAb LL2 conjugated to truncated PE. It induced complete regressions of human tumors in mice but was not developed for clinical use (48). Instead, recombinant immunotoxins were developed targeting this antigen.

Clinical Development of Recombinant Immunotoxin BL22 in Patients with B Cell Malignancies

The RFB4 antibody that was previously used to make chemical conjugates with the ricin A-chain was also used to make BL22, a recombinant immunotoxin targeting CD22. BL22 is stable disulfide-linked immunotoxin in which the Fv of the RFB4 antibody is fused to PE38 (**Figure 2*c***). Preclinical studies showed BL22 produced complete regressions in mice of human $CD22^+$ B cell lymphoma xenografts at plasma levels, which were safely tolerated in *Cynomolgus* monkeys (49). Leukemic cells from patients were also sensitive to BL22 (50).

BL22 was administered to 46 patients, including 31 patients with HCL, 11 with CLL, and 4 with NHL (51, 10). All patients had failed prior chemotherapy. The response rate in 31 HCL patients was very high (81%), including 19 (61%) CRs (**Table 1**). Three of the treated patients had HCLv, a variant of HCL that responds poorly to chemotherapy (52, 53), and all three went into CR. Most of those with CR achieved CR after the first cycle, but other patients required from 2 to 14 cycles for CR. Levels of neutralizing antibodies rose from 0% before treatment to 24% after three cycles of therapy. The median half-life of BL22 was about 3 h at the 30–50 μg/kg dose level, somewhat longer than that of denileukin diftitox. Non-dose-limiting toxicities were mild or moderate hypoalbuminemia, transaminase elevations, fatigue, and edema. The most serious toxicity was

hemolytic uremic syndrome (HUS), observed in one patient with NHL and four with HCL. HUS completely resolved in all four HCL patients in the phase I trial and in another patient treated by special exemption, and all five HCL patients still had a normal creatinine 34–75 (median 57) months later. A phase II trial in HCL has begun, and the incidence of HUS is much lower owing to a change in dosing schedule.

BL22 is the first agent since purine analogs reported to induce CR in the majority of patients with HCL, and it is the only agent reported to do this in chemotherapy-resistant HCL patients. The activity of BL22 in CLL was less pronounced, probably because of the lower expression of CD22 on such cells (50). An immunotoxin with a tenfold higher affinity for CD22 has been produced, which has high cytotoxic activity toward CLL cells (54). Phase I clinical trials will open in 2007.

Hemolytic uremic syndrome (HUS): syndrome of renal insufficiency from glomerular thrombosis, thrombocytopenia, and microangiopathic hemolytic anemia

GM-CSFR: granulocyte-macrophage colony stimulating factor receptor

AML: acute myelogenous leukemia

DTGM: DT388-GM-CSF

PR: partial response

Targeting GM-CSFR with DT388-GM-CSF

To target the granulocyte-macrophage colony stimulating factor receptor (GM-CSFR) expressed in acute myelogenous leukemia (AML), human GM-CSF was fused to DT388 to make DT388-GM-CSF (DTGM). In a phase I trial in patients with AML, DTGM induced responses in 10% of patients. The major toxicity was cytokine release syndrome causing hepatotoxicity (**Table 1**) (55). This toxicity is thought to be due to binding of DTGM to GM-CSFR$^+$ monocytes, macrophages, and Kupffer cells in the liver. To target AML and thus avoid cytokine release syndrome, IL3, which does not bind to macrophages, was used instead of GM-CSF. A clinical trial is ongoing using DT-IL3 (56, 57).

Targeting CD19 or Both CD19 and CD22 with Immunotoxins

The anti-CD19 immunotoxin anti-B4-blocked ricin showed some activity in phase I trials (58–61) but appeared less active in a follow-up phase II trial in patients with bulky disease (61). Subsequent trials used anti-B4-blocked ricin in the setting of minimal residual disease or in combination with chemotherapy, and no activity was observed (62–66). The combination of anti-CD19 HD37-dgA and anti-CD22 RFB4-dgA showed some efficacy in patients with B cell malignancies (67) and was safe in patients with circulating tumor cells, but it caused dose-limiting HUS and VLS in patients without circulating tumor cells.

Immunotoxins Targeting CD30

An anti-CD30 immunotoxin composed of mAb Ki-4 conjugated to dgA induced one partial response (PR) out of 15 patients with NHL and HD (68). This antibody was then used to make a single-chain immunotoxin containing Ki-4(scFv) and a truncated form of PE (ETA′) containing amino acids 252–613. Ki-4(scFv)-ETA′ displayed antitumor activity in mice with disseminated human HD but was not developed for clinical use (69, 70). Stable disulfide-linked immunotoxins targeting CD30 were also developed using Fvs of antibodies fused to PE38. These immunotoxins have been shown to kill CD30-expressing tumor cell lines and to cause regressions of tumors in mice, but they have not yet been developed for clinical use (71, 72).

Clinical Trials of Immunotoxins for Treatment of Solid Tumors

Phase I and phase II trials have evaluated immunotoxins made with various forms of ricin or PE attached to murine mAbs or mAb fragments in patients with solid tumors, and their findings are summarized in **Table 2**. These immunotoxins target cell surface antigens that are highly expressed on the tumor cells but are also expressed on normal tissues, usually at a lower level than on the tumor.

Table 2 Immunotoxins used against solid tumors

Immunotoxin	Toxin	Target antigen	Cancer type	Clinical trial phase	No. of patients	Comments	References
260F9-rRTA	ricin	55 kDa	breast	I	9	dose-limiting neuropathies	89, 90
OVB3-PE	PE	Ovary	ovarian	I	23	CNS toxicity	91
XMMME-001-RTA	ricin	High M.W. antigen	melanoma	I II with CTX I/II with cyclosporine	20 20 9	dose-limiting fatigue, myalgias, and arthralgias. cyclophosphamide did not abrogate antibodies to immunotoxin	92–94
Xomazyme-791	ricin	72 kDa	colorectal	I	29	no responses	95, 96
Anti-CEA-bR	bRicin	CEA	colorectal	I/II	27	no responses	97
N901-bR	bRicin	CD56	small cell lung	I	20	one PR. VLS dose-limiting	98, 99
				II	9		
LMB-1	PE38	Lewis Y	adenoca	I	38	one CR, one PR. VLS toxicity	74
LMB-7	PE38	Lewis Y	adenoca	I		no responses, renal and GI toxicity	unpublished
LMB-9	PE38	Lewis Y	adenoca	I		no responses, renal toxicity	unpublished
BR96sFv-PE40	PE40	Lewis Y	adenoca	I	46	no responses, GI toxicity	75
NBI-3001	PE38	IL4R	solid tumors	I	14	liver toxicity	100
Erb-38	PE38	erbB2	breast, ovarian	I	6	liver toxicity	9
scFv(FRP5)-ETA	PE40	erbB2	breast		11	regression of nodules	76, 77
				I	18	no responses, liver toxicity	
SS1P	PE38	mesothelin	mesothelioma, ovarian	I (continuous infusion and bolus)	58	one PR, evidence of antitumor activity in 10 other patients	80

Abbreviations: bRicin, blocked ricin; CEA, carcinoembryonic antigen; CNS, central nervous system; PR, partial response; CR, complete response; VLS, vascular leakage syndrome; GI, gastrointestinal.

Anti–Lewis Y Immunotoxins for Treatment of Gastrointestinal and Breast Cancer

The first evidence of clinical activity of PE-based immunotoxins was observed in a phase I clinical trial of LMB-1. This immunotoxin consists of the mAb B3, which reacts with the Lewis Y carbohydrate present on the glycoproteins of many adenocarcinomas (73), attached to PE38 (**Figure 2*b***). In this study of 38 patients, one CR and one PR were observed (74). A dose-limiting toxicity was VLS ascribed to endothelial damage, possibly accentuated by small amounts of the Lewis Y antigen present on endothelial cells. The

development of LMB-1 was not pursued; instead, recombinant immunotoxins (LMB-7, LMB-9, BR96-Fv-PE40) that targeted the Lewis Y antigen were produced and evaluated in clinical trials of patients with Lewis Y–expressing malignancies (75; I. Pastan, R. Hassan, D.J. FitzGerald, R.J. Kreitman, unpublished data). No significant antitumor activity was observed in these clinical trials. Renal toxicity was dose-limiting and is likely due to the relatively rapid clearance of these small proteins by the kidney and their binding to Lewis Y antigen present in renal tubular cells. Renal toxicity is not observed with whole-antibody conjugates, which are large and therefore are not filtered by the kidney.

Immunotoxins Targeting Her2/neu for Cancer Therapy

Because of the high expression of Her2/neu on many breast cancers, Her2/neu seemed an excellent candidate for immunotoxin therapy. Therefore erb38, a disulfide-linked immunotoxin that targets the Her/2neu receptor, was produced. Six patients (five with breast and one with esophageal cancer) received erbB38 at 1.0 and 2.0 μg/kg. Even at this very low dose level, hepatotoxicity was observed in all patients (9). Subsequent immunohistochemistry studies showed erbB2 expression on hepatocytes accounting for this targeted toxicity. Recently von Minckwitz and colleagues reported a phase I trial in 18 patients with Her2/neu-expressing cancers of a single-chain immunotoxin [scFv(frp5)-ETA] that also targets Her2/neu. They also observed dose-limiting liver toxicity with no objective responses, although direct intratumoral injections of this immunotoxin did cause tumor shrinkage (76, 77).

Anti-Mesothelin Immunotoxin, SS1P, for Treatment of Mesothelioma and Ovarian and Pancreatic Cancer

Mesothelin is a differentiation antigen expressed on normal mesothelial cells and highly expressed in mesothelioma, as well as in ovarian and pancreatic cancers (78). SS1P, a high-affinity immunotoxin targeting mesothelin, was produced by a combination of antibody phage display and Fv mutagenesis (79) (**Figure 2*c***). Because this immunotoxin produced CRs of mesothelin-bearing tumors in mice and was well tolerated by monkeys (whose cells bind SS1P), two phase I clinical trials were carried out at the National Cancer Institute (80). In one trial SS1P was given by 30-min infusion every two days for 3–6 doses; in the other trial SS1P was given by continuous infusion over 10 days to try to saturate the tumor. Using either method of administration, several minor but significant antitumor responses were observed. Phase II studies of SS1P in mesothelioma and ovarian cancer will begin in 2007. In these trials, SS1P will be combined with chemotherapy because recent studies in nude mice bearing mesothelin-expressing tumor xenografts reported striking synergy when SS1P was combined with Taxol or other anticancer drugs (81).

Other Toxins and Approaches

To avoid the immunogenicity associated with bacterial or plant toxins, several groups have used human cytotoxic proteins such as granzyme B or a ribonuclease to target endothelial cells in tumors or tumor cells (82, 83). Also, the expression of cancer-related proteases provides the opportunity to convert toxins into precursor toxins by replacing the furin cleavage site with a protease expressed in cancer cells. For example, aerolysin and anthrax toxin are not active until cleaved by furin, so the furin site can be replaced by a site cleaved by prostate-specific antigen for prostate cancer targeting, or by urokinase, which is produced by many types of tumors (84). Several single-chain ribosome-inactivating proteins, such as bryodin, gelonin, and saporin, have also been used to make immunotoxins (85).

SUMMARY AND CONCLUSIONS

Immunotoxins are hybrid proteins that take advantage of the specificity of antibodies and the potent cytotoxic activity of protein toxins. Clinical trials have been carried out in hematologic malignancies, where the immunotoxins can rapidly reach the tumor cells, and in solid tumors, where tumor entry is limited. Significant antitumor activity has been observed with immunotoxins targeting the IL2 receptor and CD22; one of these, denileukin diftitox, has been approved by the U.S. Food and Drug Administration for the treatment of CTCL. The other BL22 has produced many complete remissions in drug resistant HCL now in phase II trials. Sufficient activity of an immunotoxin directed against solid tumors expressing mesothelin has been observed to also warrant phase II trials. Because immunotoxins contain bacterial or plant proteins, antibody formation prevents retreatment of patients with solid tumors where the immune system is intact. In many hematologic malignancies, the immune system has been damaged, and many cycles of immunotoxin therapy can be given without antibody formation. Further progress will depend on the identification of new antigenic targets on tumors and the production of less immunogenic immunotoxins so patients can receive several treatment cycles.

DISCLOSURE STATEMENT

IP and DJF are named inventors of patents and patent applications related to immunotoxin development. RJK is a coinventor on an NIH patent for BL22.

LITERATURE CITED

1. Gensini GF, Conti AA, Lippi D. 2006. The 150th anniversary of the birth of Paul Ehrlich, chemotherapy pioneer. *J. Infect.* In press
2. FitzGerald DJ, Kreitman R, Wilson W, et al. 2004. Recombinant immunotoxins for treating cancer. *Int. J. Med. Microbiol.* 293:577–82
3. Foss F. 2006. Clinical experience with denileukin diftitox (ONTAK). *Semin. Oncol.* 33:S11–16
4. Ghetie V, Vitetta ES. 2001. Chemical construction of immunotoxins. *Mol. Biotechnol.* 18:251–68
5. Vitetta ES. 2000. Immunotoxins and vascular leak syndrome. *Cancer J.* 6:S218–24

5a. Chaudhary VK, Xu YH, FitzGerald D, et al. 1989. A recombinant immunotoxin consisting of two antibody variable domains fused to *Pseudomonas* exotoxin. *Nature* 339:394–97

6. Brinkmann U, Pai LH, FitzGerald DJ, et al. 1991. B3(Fv)-PE38KDEL, a single chain immunotoxin that causes complete regression of a human carcinoma in mice. *Proc. Natl. Acad. Sci. USA* 88:8616–20

7. Benhar I, Pastan I. 1995. Identification of residues that stabilize the single-chain Fv of monoclonal antibodies B3. *J. Biol. Chem.* 270:23373–80
8. Zhu Z, Presta LG, Zapata G, et al. 1997. Remodeling domain interfaces to enhance heterodimer formation. *Protein Sci.* 6:781–88
9. Pai-Scherf LH, Villa J, Pearson D, et al. 1999. Hepatotoxicity in cancer patients receiving erb-38, a recombinant immunotoxin that targets the erbB2 receptor. *Clin. Cancer Res.* 5:2311–15

10. Kreitman RJ, Wilson WH, Bergeron K, et al. 2001. Efficacy of the anti-CD22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. *N. Engl. J. Med.* 345:241–47

6. First description of method to stabilize Fv portion of recombinant immunotoxin by replacing peptide linker with disulfide bond.

10. High CR rate in chemoresistant HCL by BL22, the first successful systemic therapy for HCL since purine analogs.

11. Chiron MF, Fryling CM, FitzGerald DJ. 1994. Cleavage of *Pseudomonas* exotoxin and diphtheria toxin by a furin-like enzyme prepared from beef liver. *J. Biol. Chem.* 269:18167–76
12. Williams DP, Wen Z, Watson RS, et al. 1990. Cellular processing of the interleukin-2 fusion toxin DAB486-IL-2 and efficient delivery of diphtheria fragment A to the cytosol of target cells requires Arg194. *J. Biol. Chem.* 265:20673–77
13. **Jain RK. 1996. Delivery of molecular medicine to solid tumors. *Science* 271:1079–80**
14. Kodaka T, Uchiyama T, Ishikawa T, et al. 1990. Interleukin-2 receptor b-chain (p70–75) expressed on leukemic cells from adult T cell leukemia patients. *Jpn. J. Cancer Res.* 81:902–1008
15. Kreitman RJ, Pastan I. 1994. Recombinant single-chain immunotoxins against T and B cell leukemias. *Leuk. Lymphoma* 13:1–10
16. Strauchen JA, Breakstone BA. 1987. IL-2 receptor expression in human lymphoid lesions. *Am. J. Pathol.* 126:506–12
17. Yagura H, Tamaki T, Furitsu T, et al. 1990. Demonstration of high-affinity interleukin-2 receptors on B-chronic lymphocytic leukemia cells: functional and structural characterization. *Blut* 60:181–86
18. Robb RJ, Greene WC, Rusk CM. 1984. Low and high affinity cellular receptors for interleukin 2. *J. Exp. Med.* 160:1126–46
19. Bacha P, Forte S, Kassam S, et al. 1990. Pharmacokinetics of the recombinant fusion protein DAB486IL-2 in animal models. *Cancer Chemother. Pharmacol.* 26:409–14
20. Bacha P, Williams DP, Waters C, et al. 1988. Interleukin 2 receptor-targeted cytotoxicity: interleukin 2 receptor-mediated action of a diphtheria toxin-related interleukin 2 fusion protein. *J. Exp. Med.* 167:612–22
21. LeMaistre CF, Saleh MN, Kuzel TM, et al. 1998. Phase I trial of a ligand fusion-protein (DAB389IL-2) in lymphomas expressing the receptor for interleukin-2. *Blood* 91:399–405
22. Duvic M, Kuzel TM, Olsen E, et al. 2002. Quality-of-life improvements in cutaneous T-cell lymphoma patients treated with denileukin diftitox (ONTAK). *Clin. Lymphoma* 2:222–28
23. **Olsen E, Duvic M, Frankel A, et al. 2001. Pivotal phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma. *J. Clin. Oncol.* 19:376–88**
24. McGinnis KS, Shapiro M, Junkins-Hopkins JM, et al. 2002. Denileukin diftitox for the treatment of panniculitic lymphoma. *Arch. Dermatol.* 138:740–42
25. Frankel AE, Fleming DR, Hall PD, et al. 2003. A phase II study of DT fusion protein denileukin diftitox in patients with fludarabine-refractory chronic lymphocytic leukemia. *Clin. Cancer Res.* 9:3555–61
26. Dang NH, Hagemeister FB, Pro B, et al. 2004. Phase II study of denileukin diftitox for relapsed/refractory B-cell non-Hodgkin's lymphoma. *J. Clin. Oncol.* 22:4095–102
27. Martin A, Gutierrez E, Muglia J, et al. 2001. A multicenter dose-escalation trial with denileukin diftitox (ONTAK, DAB(389)IL-2) in patients with severe psoriasis. *J. Am. Acad. Dermatol.* 45:871–81
28. Foss F, Demierre MF, DiVenuti G. 2005. A phase-1 trial of bexarotene and denileukin diftitox in patients with relapsed or refractory cutaneous T-cell lymphoma. *Blood* 106:454–57
29. Kobayashi H, Kao CK, Kreitman RJ, et al. 2000. Pharmacokinetics of In-111- and I-125-labeled antiTac single-chain Fv recombinant immunotoxin. *J. Nucl. Med.* 41:755–62

13. Discussion of factors that inhibit the entry of antibodies and other large proteins into solid tumors.

23. IL2-toxin trial in CTCL randomizing between 2 dose levels, leading to FDA approval in this disease.

30. Kreitman RJ, Bailon P, Chaudhary VK, et al. 1994. Recombinant immunotoxins containing anti-Tac(Fv) and derivatives of *Pseudomonas* exotoxin produce complete regression in mice of an interleukin-2 receptor-expressing human carcinoma. *Blood* 83:426–34
31. Kreitman RJ, Pastan I. 1995. Targeting *Pseudomonas* exotoxin to hematologic malignancies. *Semin. Cancer Biol.* 6:297–306
32. Kreitman RJ, Pastan I. 1998. Accumulation of a recombinant immunotoxin in a tumor in vivo: fewer than 1000 molecules per cell are sufficient for complete responses. *Cancer Res.* 58:968–75
33. Robbins DH, Margulies I, Stetler-Stevenson M, et al. 2000. Hairy cell leukemia, a B-cell neoplasm which is particularly sensitive to the cytotoxic effect of anti-Tac(Fv)-PE38 (LMB-2). *Clin. Cancer Res.* 6:693–700
34. Kreitman RJ, Wilson WH, White JD, et al. 2000. Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. *J. Clin. Oncol.* 18:1614–36
35. Onda M, Kreitman RJ, Vasmatzis G, et al. 1999. Reduction of the nonspecific toxicity of anti-Tac(Fv)-PE38 by mutations in the framework regions of the Fv which lower the isoelectric point. *J. Immunol.* 163:6072–77
36. Onda M, Willingham M, Wang Q, et al. 2000. Inhibition of TNF alpha produced by Kupffer cells protects against the nonspecific liver toxicity of immunotoxin anti-Tac(Fv)-PE38, LMB-2. *J. Immunol.* 165:7150–56
37. Attia P, Powell DJJ, Maker AV, et al. 2006. Selective elimination of human regulatory T lymphocytes in vitro with the recombinant immunotoxin, LMB-2. *J. Immunother.* 29:208–14
38. Schnell R, Borchmann P, Staak JO, et al. 2003. Clinical evaluation of ricin A-chain immunotoxins in patients with Hodgkin's lymphoma. *Ann. Oncol.* 14:729–36
39. Schnell R, Vitetta E, Schindler J, et al. 2000. Treatment of refractory Hodgkin's lymphoma patients with an anti-CD25 ricin A-chain immunotoxin. *Leukemia* 14:129–35
40. Martin PJ, Pei J, Gooley T, et al. 2004. Evaluation of a CD25-specific immunotoxin for prevention of graft-versus-host disease after unrelated marrow transplantation. *Biol. Blood Marrow Transplant.* 10:552–60
41. Ghetie MA, May RD, Till M, et al. 1988. Evaluation of ricin A chain-containing immunotoxins directed against CD19 and CD22 antigens on normal and malignant human B-cells as potential reagents for in vivo therapy. *Cancer Res.* 48:2610–17
42. Ghetie MA, Richardson J, Tucker T, et al. 1991. Antitumor activity of Fab' and IgG-anti-CD22 immunotoxins in disseminated human B lymphoma grown in mice with severe combined immunodeficiency disease: effect on tumor cells in extranodal sites. *Cancer Res.* 51:5876–80
43. Bregni M, Siena S, Formosa A, et al. 1989. B-cell restricted saporin immunotoxins: activity against B-cell lines and chronic lymphocytic leukemia cells. *Blood* 73:753–62
44. Amlot PL, Stone MJ, Cunningham D, et al. 1993. A phase I study of an anti-CD22-deglycosylated ricin A chain immunotoxin in the treatment of B-cell lymphomas resistant to conventional therapy. *Blood* 82:2624–33
45. Sausville EA, Headlee D, Stetler-Stevenson M, et al. 1995. Continuous infusion of the anti-CD22 immunotoxin IgG-RFB4-SMPT-dgA in patients with B-cell lymphoma: a phase I study. *Blood* 85:3457–65
46. Kreitman RJ. 2003. Taming ricin toxin. *Nat. Biotechnol.* 21:372–74
47. Smallshaw JE, Ghetie V, Rizo J, et al. 2003. Genetic engineering of an immunotoxin to eliminate pulmonary vascular leak in mice. *Nat. Biotechnol.* 21:387–91

32. Measured how many immunotoxin molecules must enter tumor and bind to each cell to obtain complete regression of tumor.

34. Dose-finding study of LMB-2, reporting responses in HCL, CLL, ATL, HD, and CTCL.

44. Bolus phase I trial of RFB4-dgA, reporting responses in patients with B-cell malignancies.

48. Kreitman RJ, Hansen HJ, Jones AL, et al. 1993. *Pseudomonas* exotoxin-based immunotoxins containing the antibody LL2 or LL2-Fab' induce regression of subcutaneous human B-cell lymphoma in mice. *Cancer Res.* 53:819–25
49. Kreitman RJ, Wang QC, FitzGerald DJP, et al. 1999. Complete regression of human B-cell lymphoma xenografts in mice treated with recombinant anti-CD22 immunotoxin RFB4(dsFv)-PE38 at doses tolerated by Cynomolgus monkeys. *Int. J. Cancer* 81:148–55
50. Kreitman RJ, Margulies I, Stetler-Stevenson M, et al. 2000. Cytotoxic activity of disulfide-stabilized recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) towards fresh malignant cells from patients with B-cell leukemias. *Clin. Cancer Res.* 6:1476–87
51. Kreitman RJ, Squires DR, Stetler-Stevenson M, et al. 2005. Phase I trial of recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) in patients with B-cell malignancies. *J. Clin. Oncol.* 23:6719–29
52. Blasinska-Morawiec M, Robak T, Krykowski E, et al. 1997. Hairy cell leukemia-variant treated with 2-chlorodeoxyadenosine—a report of three cases. *Leuk. Lymphoma* 25:381–85
53. Matutes E, Wotherspoon A, Brito-Babapulle V, et al. 2001. The natural history and clinico-pathological features of the variant form of hairy cell leukemia. *Leukemia* 15:184–86
54. **Salvatore G, Beers R, Margulies I, et al. 2002. Improved cytotoxic activity towards cell lines and fresh leukemia cells of a mutant anti-CD22 immunotoxin obtained by antibody phage display. *Clin. Cancer Res.* 8:995–1002**
55. Frankel AE, Powell BL, Hall PD, et al. 2002. Phase I trial of a novel diphtheria toxin/granulocyte macrophage colony-stimulating factor fusion protein (DT388GMCSF) for refractory or relapsed acute myeloid leukemia. *Clin. Cancer Res.* 8:1004–13
56. Frankel A, McCubrey J, Miller MS, et al. 2000. Diphtheria toxin fused to human interleukin-3 is toxic to blasts from patients with acute phase chronic myeloid leukemia. *Leukemia* 14:576–85
57. Urieto JO, Liu T, Black JH, et al. 2004. Expression and purification of the recombinant diphtheria fusion toxin DT388IL3 for phase I clinical trials. *Protein Expr. Purif.* 33:123–33
58. Grossbard ML, Fidias P, Kinsella J, et al. 1998. Anti-B4-blocked ricin: a phase II trial of 7 day continuous infusion in patients with multiple myeloma. *Br. J. Haematol.* 102:509–15
59. Grossbard ML, Freedman AS, Ritz J, et al. 1992. Serotherapy of B-cell neoplasms with anti-B4-blocked ricin: a phase I trial of daily bolus infusion. *Blood* 79:576–85
60. Grossbard ML, Lambert JM, Goldmacher VS, et al. 1993. Anti-B4-blocked ricin: a phase I trial of 7-day continuous infusion in patients with B-cell neoplasms. *J. Clin. Oncol.* 11:726–37
61. Multani PS, O'Day S, Nadler LM, et al. 1998. Phase II clinical trial of bolus infusion anti-B4 blocked ricin immunoconjugate in patients with relapsed B-cell non-Hodgkin's lymphoma. *Clin. Cancer Res.* 4:2599–604
62. Grossbard ML, Multani PS, Freedman AS, et al. 1999. A phase II study of adjuvant therapy with anti-B4-blocked ricin after autologous bone marrow transplantation for patients with relapsed B-cell non-Hodgkin's lymphoma. *Clin. Cancer Res.* 5:2392–98
63. Grossbard ML, Gribben JG, Freedman AS, et al. 1993. Adjuvant immunotoxin therapy with anti-B4-blocked ricin after autologous bone marrow transplantation for patients with B-cell non-Hodgkin's lymphoma. *Blood* 81:2263–71
64. Longo DL, Duffey PL, Gribben JG, et al. 2000. Combination chemotherapy followed by an immunotoxin (anti-B4-blocked ricin) in patients with indolent lymphoma: results of a phase II study. *Cancer J. Sci. Am.* 6:146–50

54. Construction of highly active immunotoxin against CD22-expressing cells by mutagenesis of antigen-binding region of Fv yielding 10-fold increase in affinity.

65. Scadden DT, Schenkein DP, Bernstein Z, et al. 1998. Immunotoxin combined with chemotherapy for patients with AIDS-related non-Hodgkin's lymphoma. *Cancer* 83:2580–87
66. Szatrowski TP, Dodge RK, Reynolds C, et al. 2003. Lineage specific treatment of adult patients with acute lymphoblastic leukemia in first remission with anti-B4-blocked ricin or high-dose cytarabine: cancer and leukemia group B study 9311. *Cancer* 97:1471–80
67. Messmann RA, Vitetta ES, Headlee D, et al. 2000. A phase I study of combination therapy with immunotoxins IgG-HD37-deglycosylated ricin A chain (dgA) and IgG-RFB4-dgA (Combotox) in patients with refractory CD19(+), CD22(+) B cell lymphoma. *Clin. Cancer Res.* 6:1302–13
68. Schnell R, Staak O, Borchmann P, et al. 2002. A phase I study with an anti-CD30 ricin A-chain immunotoxin (Ki-4.dgA) in patients with refractory CD30+ Hodgkin's and non-Hodgkin's lymphoma. *Clin. Cancer Res.* 8:1779–86
69. Barth S, Huhn M, Matthey B, et al. 2000. Ki-4(scFv)-ETA', a new recombinant anti-CD30 immunotoxin with highly specific cytotoxic activity against disseminated Hodgkin tumors in SCID mice. *Blood* 95:3909–14
70. Klimka A, Barth S, Matthey B, et al. 1999. An anti-CD30 single-chain Fv selected by phage display and fused to *Pseudomonas* exotoxin A (Ki-4(scFv)-ETA') is a potent immunotoxin against a Hodgkin-derived cell line. *Br. J. Cancer* 80:1214–22
71. Nagata S, Onda M, Numata Y, et al. 2002. Novel anti-CD30 recombinant immunotoxins containing disulfide-stabilized Fv fragments. *Clin. Cancer Res.* 8:2345–55
72. Rozemuller H, Chowdhury PS, Pastan I, et al. 2001. Isolation of new anti-CD30 scFvs from DNA immunized mice by phage display and biologic activity of recombinant immunotoxins produced by fusion with truncated *Pseudomonas* exotoxin. *Int. J. Cancer* 92:861–70
73. Pastan I, Lovelace ET, Gallo MG, et al. 1991. Characterization of monoclonal antibodies B1 and B3 that react with mucinous adenocarcinomas. *Cancer Res.* 51:3781–87
74. Pai LH, Wittes R, Setser A, et al. 1996. Treatment of advanced solid tumors with immunotoxin LMB-1: an antibody linked to *Pseudomonas* exotoxin. *Nat. Med.* 2:350–53
75. Posey JA, Khazaeli MB, Bookman MA, et al. 2002. A phase I trial of the single-chain immunotoxin SGN-10 (BR96 sFv-PE40) in patients with advanced solid tumors. *Clin. Cancer Res.* 8:3092–99
76. Azemar M, Djahansouzi S, Jager E, et al. 2003. Regression of cutaneous tumor lesions in patients intratumorally injected with a recombinant single-chain antibody-toxin targeted to ErbB2/HER2. *Breast Cancer Res. Treat.* 82:155–64
77. von Minckwitz G, Harder S, Hovelmann S, et al. 2005. Phase I clinical study of the recombinant antibody toxin scFv(FRP5)-ETA specific for the ErbB2/HER2 receptor in patients with advanced solid malignomas. *Breast Cancer Res.* 7:R617–26
78. Hassan R, Bera T, Pastan I. 2004. Mesothelin: a new target for immunotherapy. *Clin. Cancer Res.* 10:3937–42
79. Chowdhury PS, Pastan I. 1999. Improving antibody affinity by mimicking somatic hypermutation in vitro. *Nat. Biotechnol.* 17:568–72
80. Hassan R, Bullock S, Kindler H, et al. 2004. Updated results of the phase I study of SS1(dsFv)PE38 for targeted therapy of mesothelin expressing cancers. *Eur. J. Cancer* 2(Suppl.):280a (Abstr.)
81. Zhang Y, Hassan R, Paik CH, et al. 2006. Synergistic antitumor activity of taxol and immunotoxin SS1P in tumor bearing mice. *Clin. Cancer Res.* 12:4695–4701

74. Evidence that an immunotoxin can cause regression of epithelial cancers.

79. Novel method to improve affinities of Fvs by identifying and targeting natural mutational hot spots.

82. Dalken B, Giesubel U, Knauer SK, et al. 2006. Targeted induction of apoptosis by chimeric granzyme B fusion proteins carrying antibody and growth factor domains for cell recognition. *Cell Death Differ.* 13:576–85
83. Hursey M, Newton DL, Hansen HJ, et al. 2002. Specifically targeting the CD22 receptor of human B-cell lymphomas with RNA damaging agents: a new generation of therapeutics. *Leuk. Lymphoma* 43:953–59
84. Abi-Habib RJ, Liu S, Bugge TH, et al. 2004. A urokinase-activated recombinant diphtheria toxin targeting the granulocyte-macrophage colony-stimulating factor receptor is selectively cytotoxic to human acute myeloid leukemia blasts. *Blood* 104:2143–48
85. Bolognesi A, Tazzari PL, Tassi C, et al. 1992. A comparison of antilymphocyte immunotoxins containing different ribosome-inactivating proteins and antibodies. *Clin. Exp. Immunol.* 89:341–46
86. Foss FM. 2001. Interleukin-2 fusion toxin: targeted therapy for cutaneous T cell lymphoma. *Ann. NY Acad. Sci.* 941:166–76
87. Duvic M, Kuzel T, Olsen E, Martin AG. 2002. Quality-of-life improvements in cutaneous T-cell lymphoma patients treated with denileukin diftitox (ONTAK). *Clin. Lymphoma* 2:222–28
88. Kreitman RJ, Wilson WH, Robbins D, et al. 1999. Responses in refractory hairy cell leukemia to a recombinant immunotoxin. *Blood* 94:3340–48
89. Gould BJ, Borowitz MJ, Groves ES, et al. 1989. Phase I study of an antibreast cancer immunotoxin by continuous infusion: report of a targeted toxic effect not predicted by animal studies. *J. Natl. Cancer Inst.* 81:775–81
90. Weiner LM, O'Dwyer J, Kitson J, et al. 1989. Phase I evaluation of an antibreast carcinoma monoclonal antibody 260F9-recombinant ricin A chain immunoconjugate. *Cancer Res.* 49:4062–67
91. Pai LH, Bookman MA, Ozols RF, et al. 1991. Clinical evaluation of intraperitoneal *Pseudomonas* exotoxin immunoconjugate OVB3-PE in patients with ovarian cancer. *J. Clin. Oncol.* 9:2095–103
92. Gonzalez R, Salem P, Bunn PAJ, et al. 1991. Single-dose murine monoclonal antibody ricin A chain immunotoxin in the treatment of metastatic melanoma: a phase I trial. *Mol. Biother.* 3:192–96
93. Oratz R, Speyer JL, Wernz JC, et al. 1990. Antimelanoma monoclonal antibody-ricin A chain immunoconjugate (XMMME-001-RTA) plus cyclophosphamide in the treatment of metastatic malignant melanoma: results of a phase II trial. *J. Biol. Response Mod.* 9:345–54
94. Selvaggi K, Saria EA, Schwartz R, et al. 1993. Phase I/II study of murine monoclonal antibody-ricin A chain (XOMAZYME-Mel) immunoconjugate plus cyclosporine A in patients with metastatic melanoma. *J. Immunother.* 13:201–7
95. Byers VS, Rodvien R, Grant K, et al. 1989. Phase I study of monoclonal antibody-ricin A chain immunotoxin XomaZyme-791 in patients with metastatic colon cancer. *Cancer Res.* 49:6153–60
96. LoRusso PM, Lomen PL, Redman BG, et al. 1995. Phase I study of monoclonal antibody-ricin A chain immunoconjugate Xomazyme-791 in patients with metastatic colon cancer. *Am. J. Clin. Oncol.* 18:307–12
97. Zalcberg JR, Pietersz G, Toohey B, et al. 1994. A phase I/II study of the intralesional injection of ricin-monoclonal antibody conjugates in patients with hepatic metastases. *Eur. J. Cancer* 30A:1227–31
98. Fidias P, Grossbard M, Lynch TJJ. 2002. A phase II study of the immunotoxin N901-blocked ricin in small-cell lung cancer. *Clin. Lung Cancer* 3:219–22

99. Lynch TJ, Lambert JM, Coral F, et al. 1997. Immunotoxin therapy of small-cell lung cancer: a phase I study of N901-blocked ricin. *J. Clin. Oncol.* 15:723–34
100. Garland L, Gitlitz B, Ebbinghaus S, et al. 2005. Phase I trial of intravenous IL-4 *Pseudomonas* exotoxin protein (NBI-3001) in patients with advanced solid tumors that express the IL-4 receptor. *J. Immunother.* 28:376–81

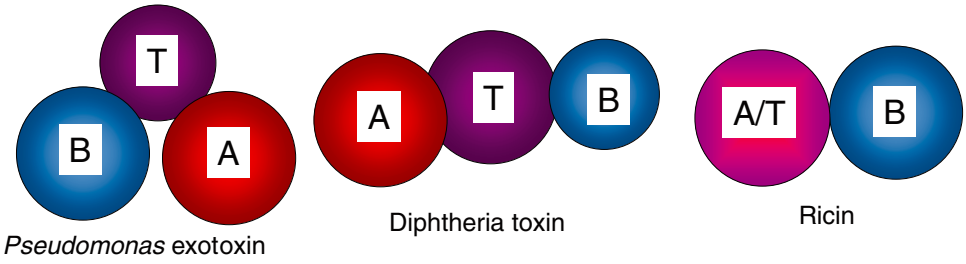

Figure 1

Toxins with discrete functional domains are well suited for immunotoxin construction. Abbreviations: B, binding domain; T, translocation domain; A, activity domain. The existence of a separate binding domain for diphtheria toxin (DT), *Pseudomonas* exotoxin A (PE), and ricin allows the modification of these toxins via elimination of the binding domain and retention of the translocating and activity domains. In DT, the activity domain is at the N terminus and the binding domain is at the C terminus (ATB). In PE, this orientation is reversed (BTA). For DT and PE, there are separate A and T domains. For the A chain of ricin, the A and T domains are not clearly separable (reflected in the blended color).

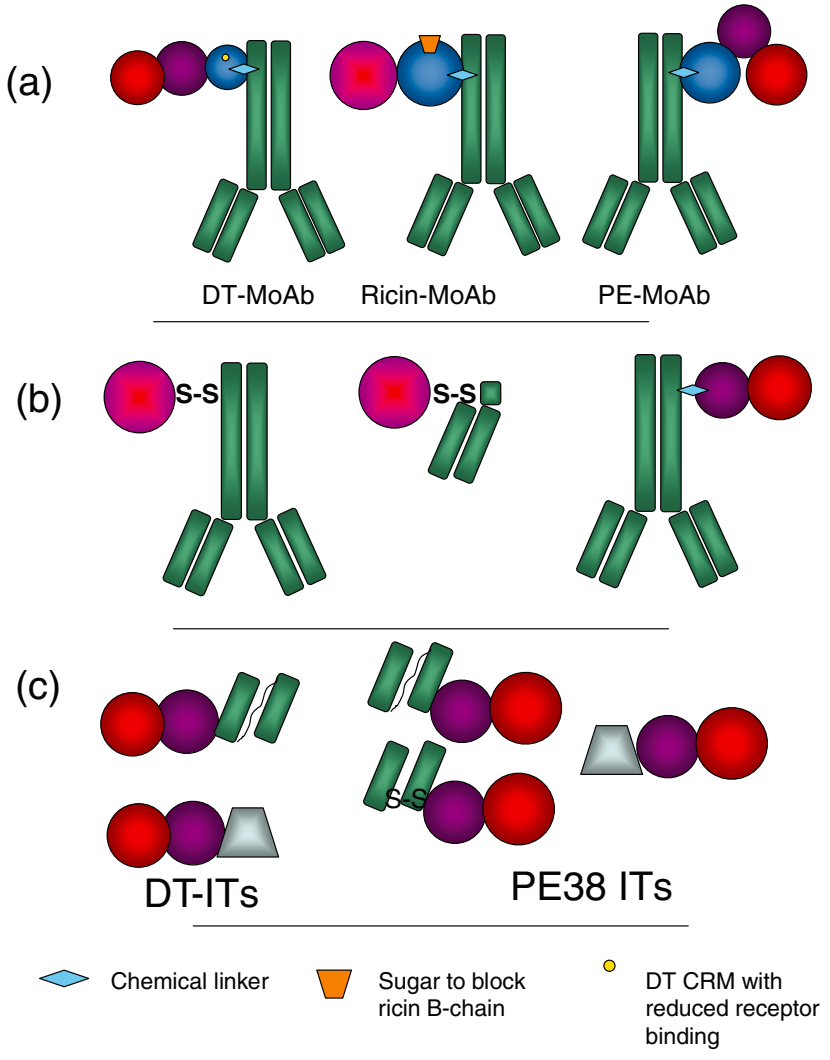

Figure 2

(*a*) The first-generation immunotoxins were constructed via chemical linkage (usually a disulfide bond) between intact toxin molecules and monoclonal antibodies. For DT, the availability of cross-reacting material (CRM) mutants reduced the danger of nonspecific toxicity for normal cells. Sugars were used to block the binding activity of the ricin B chain. (*b*) The second-generation immunotoxins were constructed with toxins that lack binding domains. Two versions of second-generation ricin immunotoxins were made routinely: one conjugate with intact IgG and one with a Fab fragment. The Fab construct was smaller and potentially enabled greater tumor penetration and a more uniform final product. For PE38 immunotoxins, conjugation was usually linked via a thioether bond. To help make homogeneous conjugates, an additional lysine residue was inserted near the beginning of PE38. (*c*) The third-generation immunotoxins are wholly recombinant molecules. Single-chain immunotoxins and ligand toxin constructs are transcribed from a single plasmid, usually from an *E. coli* host such as BL21. DT immunotoxins (*left*) are typified by DT-IL2, where IL2 replaces the toxin's binding domain. For PE38 immunotoxins, single-chain and disulfide stabilized Fvs are shown along with ligand fusions typified by TGFα and IL13. The dsFv-PE38 immunotoxin is transcribed from two plasmids: one for the variable portion of the light chain and one for the heavy-chain variable region fused to PE38.

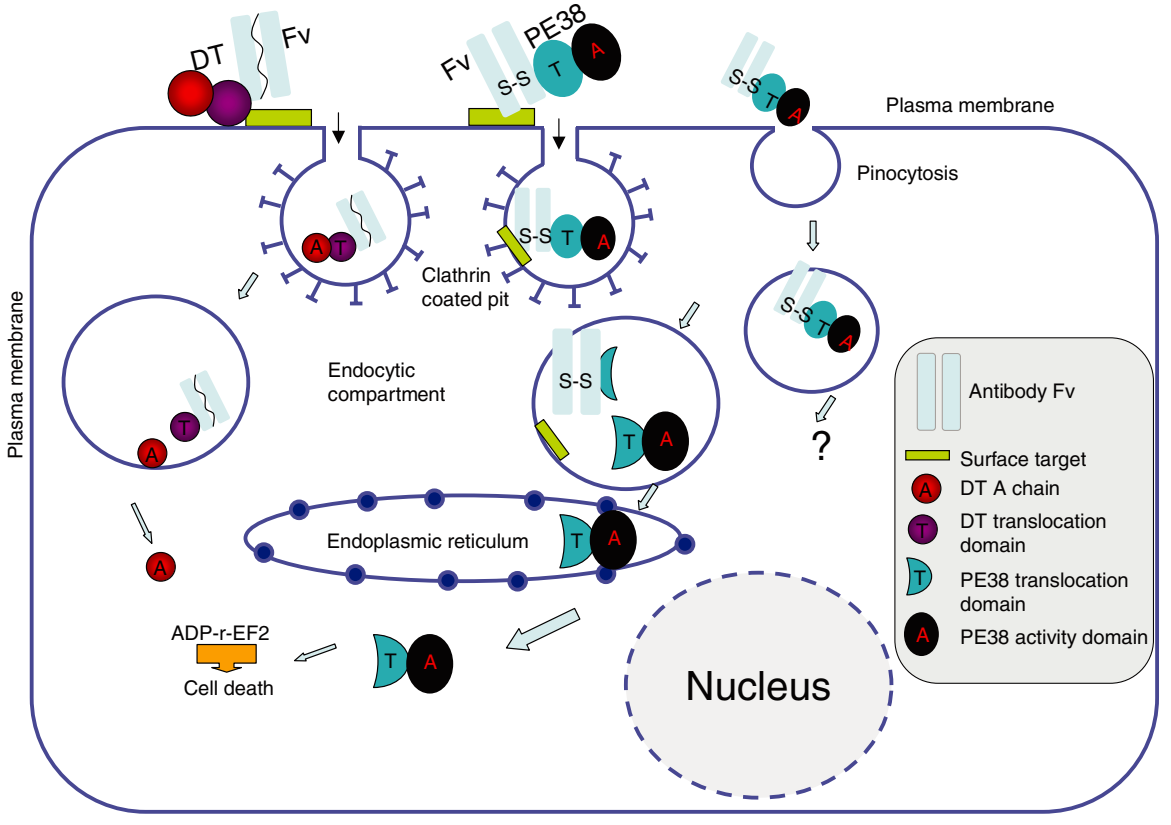

Figure 4

Immunotoxin endocytosis and trafficking within mammalian cells. Recombinant immunotoxins are targeted to bind antigens on the surface of cancer cells. This is the primary entry pathway of the toxin into cells.

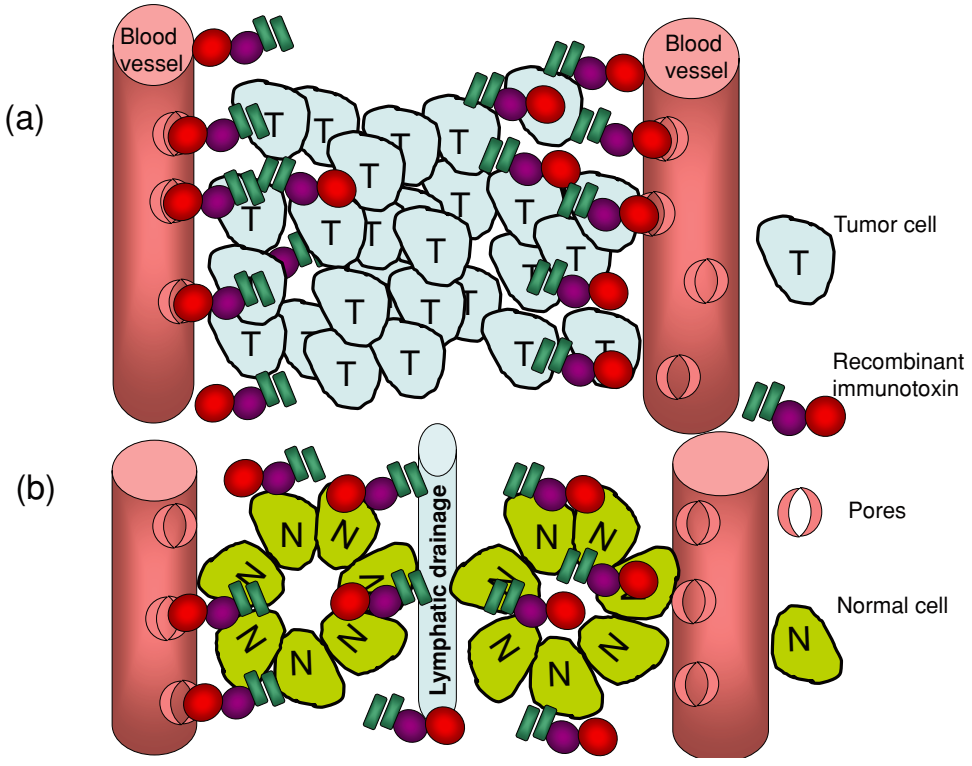

Figure 5

Poor access of immunotoxins to cells within solid tumors limits efficacy. (*a*) Diffusion of immunotoxin molecules from vessels into a tumor mass. Immunotoxin concentration is high close to the site of egress of the immunotoxin and low in the middle of the mass. (*b*) Entry of immunotoxins into normal tissue.

Annual Review of Medicine

Volume 58, 2007

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Errata

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