Immunotoxin Treatment of Cancer*

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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.
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 2a). (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 2b). 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
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 immunotoxins (5a). Recombinant immunotoxins are homogeneous and relatively inexpensive to produce. Figure 2c 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.

**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.
**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  

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-ribosylated EF2 can no longer make new protein and the cell dies by 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.
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 5a). 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 5b). 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 3H-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.

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 Diftitox (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 DAB389IL2, also called denileukin diftitox or Ontak (19, 20)
### Table 1  Immunotoxins used against hematologic malignancies

<table>
<thead>
<tr>
<th>Immunotoxin</th>
<th>Toxin used</th>
<th>Target antigen</th>
<th>Tumor type</th>
<th>Clinical trial phase</th>
<th>No. of patients</th>
<th>Response</th>
<th>References</th>
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<tr>
<td>DAB486-IL2</td>
<td>DAB486</td>
<td>IL2R</td>
<td>Ly, Le</td>
<td>I/II</td>
<td>112</td>
<td>3 CR, 8 PR</td>
<td>86</td>
</tr>
<tr>
<td>Denileukin diftitox</td>
<td>DAB389</td>
<td>IL2R</td>
<td>CTCL, NHL</td>
<td>I</td>
<td>73</td>
<td>6 CR, 10 PR</td>
<td>21</td>
</tr>
<tr>
<td>Denileukin diftitox</td>
<td>DAB389</td>
<td>IL2R</td>
<td>CTCL</td>
<td>Pivotal trial</td>
<td>71</td>
<td>7 CR, 14 PR</td>
<td>87, 23</td>
</tr>
<tr>
<td>LMB-2</td>
<td>PE38</td>
<td>CD25</td>
<td>Ly, Le</td>
<td>I</td>
<td>35</td>
<td>1 CR, 7 PR</td>
<td>88, 34</td>
</tr>
<tr>
<td>RFT5-dgA</td>
<td>dgA</td>
<td>CD25</td>
<td>HD</td>
<td>I</td>
<td>18</td>
<td>2 PR</td>
<td>38</td>
</tr>
<tr>
<td>RFB4-dgA</td>
<td>dgA</td>
<td>CD22</td>
<td>Ly, Le</td>
<td>I</td>
<td>41</td>
<td>2 CR, 10 PR</td>
<td>44, 45</td>
</tr>
<tr>
<td>BL22</td>
<td>PE38</td>
<td>CD22</td>
<td>Ly, Le</td>
<td>I</td>
<td>46</td>
<td>19 CR, 7 PR</td>
<td>51, 10</td>
</tr>
<tr>
<td>DTGM</td>
<td>DT388</td>
<td>GM-CSFR</td>
<td>AML</td>
<td>I</td>
<td>31</td>
<td>1 CR, 2 PR</td>
<td>55</td>
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<td>Anti-B4-bR</td>
<td>bRicin</td>
<td>CD19</td>
<td>NHL</td>
<td>II</td>
<td>16</td>
<td>None</td>
<td>61</td>
</tr>
<tr>
<td>HD37-dgA + RFB4-dgA</td>
<td>dgA</td>
<td>CD19 + CD22</td>
<td>NHL</td>
<td>I</td>
<td>22</td>
<td>2 PR</td>
<td>67</td>
</tr>
<tr>
<td>Ki-4-dgA</td>
<td>dgA</td>
<td>CD30</td>
<td>HD, NHL</td>
<td>I</td>
<td>15</td>
<td>1 PR</td>
<td>68</td>
</tr>
</tbody>
</table>

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.

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 2c). 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<sup>+</sup>CD25<sup>+</sup> regulatory T cells (37).

**Targeting CD25<sup>+</sup> 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<sup>+</sup>CD25<sup>+</sup> 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).

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 2c). Preclinical studies showed BL22 produced complete regressions in mice of human CD22<sup>+</sup> 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): 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

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.

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

<table>
<thead>
<tr>
<th>Immunotoxin</th>
<th>Toxin</th>
<th>Target antigen</th>
<th>Cancer type</th>
<th>Clinical trial phase</th>
<th>No. of patients</th>
<th>Comments</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>260P9-rRTA</td>
<td>ricin</td>
<td>55 kDa</td>
<td>breast</td>
<td>I</td>
<td>9</td>
<td>dose-limiting neuropathies</td>
<td>89, 90</td>
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<tr>
<td>OVB3-PE</td>
<td>PE</td>
<td>Ovary</td>
<td>ovarian</td>
<td>I</td>
<td>23</td>
<td>CNS toxicity</td>
<td>91</td>
</tr>
<tr>
<td>XMMME-001-RTA</td>
<td>ricin</td>
<td>High M.W. antigen</td>
<td>melanoma</td>
<td>I II with CTX I/II with cyclosporine</td>
<td>20 20 9</td>
<td>dose-limiting fatigue, myalgias, and arthralgias. cyclophosphamide did not abrogate antibodies to immunotoxin</td>
<td>92–94</td>
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<tr>
<td>Xomazine-791</td>
<td>ricin</td>
<td>72 kDa</td>
<td>colorectal</td>
<td>I</td>
<td>29</td>
<td>no responses</td>
<td>95, 96</td>
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<td>Anti-CEA-bR</td>
<td>bRicin</td>
<td>CEA</td>
<td>colorectal</td>
<td>I/II</td>
<td>27</td>
<td>no responses</td>
<td>97</td>
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<td>N901-bR</td>
<td>bRicin</td>
<td>CD56</td>
<td>small cell lung</td>
<td>I</td>
<td>20</td>
<td>one PR, VLS dose-limiting</td>
<td>98, 99</td>
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<td>LMB-1</td>
<td>PE38</td>
<td>Lewis Y</td>
<td>adenocarcinoma</td>
<td>I</td>
<td>38</td>
<td>one CR, one PR, VLS toxicity</td>
<td>74</td>
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<td>LMB-7</td>
<td>PE38</td>
<td>Lewis Y</td>
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<td>Lewis Y</td>
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<td>BR96sFv-PE40</td>
<td>PE40</td>
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<td>46</td>
<td>no responses, GI toxicity</td>
<td>75</td>
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<td>NBI-3001</td>
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<td>breast, ovarian</td>
<td>I</td>
<td>6</td>
<td>liver toxicity</td>
<td>9</td>
</tr>
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<td>scFv(FRP5)-ETA</td>
<td>PE40</td>
<td>erbB2</td>
<td>breast</td>
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<td>11</td>
<td>regression of nodules</td>
<td>76, 77</td>
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<tr>
<td>SS1P</td>
<td>PE38</td>
<td>mesothelin</td>
<td>mesothelioma, ovarian</td>
<td>I (continuous infusion and bolus)</td>
<td>58</td>
<td>one PR, evidence of antitumor activity in 10 other patients</td>
<td>80</td>
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</table>

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 2b). 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 Her2/neu receptor, was produced. Six patients (five with breast and one with esophageal cancer) received erbB38 at 1.0 and 2.0 \( \mu \)g/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(frap5)-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 2c). 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 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


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


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.
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Errata

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