

Themed Issue: Drug Delivery Systems for Targeted Drug Delivery  
Guest Editors - Victor C. Yang, Joseph P. Balthasar, Yoon Jeong Park and Jun Feng Liang

## Immunotoxins for Targeted Cancer Therapy

Submitted: January 24, 2006; Accepted: June 14, 2006; Published: August 18, 2006

Robert J. Kreitman<sup>1</sup>

<sup>1</sup>Clinical Immunotherapy Section, Laboratory of Molecular Biology, Centers for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

### ABSTRACT

Immunotoxins are proteins that contain a toxin along with an antibody or growth factor that binds specifically to target cells. Nearly all protein toxins work by enzymatically inhibiting protein synthesis. For the immunotoxin to work, it must bind to and be internalized by the target cells, and the enzymatic fragment of the toxin must translocate to the cytosol. Once in the cytosol, 1 molecule is capable of killing a cell, making immunotoxins some of the most potent killing agents. Various plant and bacterial toxins have been genetically fused or chemically conjugated to ligands that bind to cancer cells. Among the most active clinically are those that bind to hematologic tumors. At present, only 1 agent, which contains human interleukin-2 and truncated diphtheria toxin, is approved for use in cutaneous T-cell lymphoma. Another, containing an anti-CD22 Fv and truncated *Pseudomonas* exotoxin, has induced complete remissions in a high proportion of cases of hairy-cell leukemia. Refinement of existing immunotoxins and development of new immunotoxins are underway to improve the treatment of cancer.

**KEYWORDS:** Monoclonal antibody, CD22, CD25, interleukin, *Pseudomonas*, diphtheria

### INTRODUCTION

#### *Definition of Immunotoxins*

Immunotoxins are protein toxins connected to a cell binding ligand of immunologic interest. Classically, beginning 35 years ago, immunotoxins were created by chemically conjugating an antibody to a whole protein toxin, or, for more selective activity, by using a protein toxin devoid of

its natural binding domain.<sup>1,2</sup> Immunologic proteins that are smaller than monoclonal antibodies (MAbs), like growth factors and cytokines, have also been chemically conjugated and genetically fused to protein toxins.<sup>3</sup> While some do not consider growth factor toxin fusions or conjugates to be immunotoxins, these newer immunotoxins, like classical immunotoxins, bind to target cells and contain a toxin that kills cells. This review will consider antibody and growth factor toxins directed to cancer cells and focus on those that have been tested clinically or developed preclinically in the past several years.

#### *Immunotoxins Compared With Other Surface-Targeted Therapies*

One type of surface-targeted biologic therapy is unlabeled MAbs. Examples include rituximab<sup>4</sup> and alemtuzumab,<sup>5</sup> which kill cells after binding. Humanized MAbs are effective clinically in up to half of patients via mechanisms of apoptosis induction, antibody-dependent cytotoxicity, and complement-dependent cytotoxicity. Patients with malignant cells resistant to apoptosis, and patients whose immune systems will not perform antibody- or complement-dependent cytotoxicity, may be resistant. To kill cells directly without relying on these mechanisms, a second type of surface-targeted therapy is used, one in which MAbs are conjugated to radionuclides. These agents, considered radioimmunotherapy, induce responses in patients who are resistant to unlabeled MAbs.<sup>6</sup> However, radioimmunotherapy is limited by the potency of the radionuclide and the small number of radionuclide molecules that can be added to each MAb molecule. Patients will often incur dose-limiting toxicity to the bone marrow because of nonspecific uptake of the MAb and have an incomplete response in the tumor. A third type of surface-targeted therapy involves conjugating chemotherapy molecules to MAbs, which in many cases are more potent and cause less nonspecific damage than radionuclides. Examples include gemtuzumab ozogamicin, a conjugate of an anti-CD33 MAb and calicheamicin,<sup>7</sup> which is approved for acute myelogenous leukemia (AML), and the anti-CD30-monomethyl auristatin E conjugate cAC10-vcMMAE, under development for Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL).<sup>8</sup> In the case of gemtuzumab ozogamicin, cells that

---

**Corresponding Author:** Robert J. Kreitman, Clinical Immunotherapy Section, Laboratory of Molecular Biology, Centers for Cancer Research, National Cancer Institute, National Institutes of Health, 9000 Rockville Pike, Building 37, Room 5124B, Bethesda, MD 20892-4255. Tel: (301) 496-6947; Fax: (301) 576-3920; E-mail: kreitmar@mail.nih.gov

are multidrug resistant are, as would be expected, resistant to the target chemotherapeutic.<sup>9</sup> Currently under development is a fourth type of surface-targeted therapy, which employs ribonucleases conjugated to MAbs.<sup>10</sup> Immunotoxins which are distinct from these approaches, target the surface of cancer cells with considerable potency, using protein toxins capable of killing a cell with a single molecule.<sup>11,12</sup> These potent proteins include plant toxins like ricin, saporin, and pokeweed antiviral protein (PAP), which inactivate ribosomes; and single-chain bacterial toxins such as diphtheria toxin (DT) and *Pseudomonas* exotoxin (PE), which inhibit protein synthesis by adenosine diphosphate (ADP) ribosylating elongation factor 2.<sup>13</sup>

### Mechanism of Action of Plant Toxins

Plant holotoxins (also referred to as class II ribosome-inactivating proteins) include ricin, abrin, mistletoe lectin, and modeccin. Hemitoxins, or class I ribosome-inactivating proteins, include PAP, saporin, bryodin 1, bouganin, and gelonin.<sup>14</sup> As shown in Figure 1, holotoxins contain both

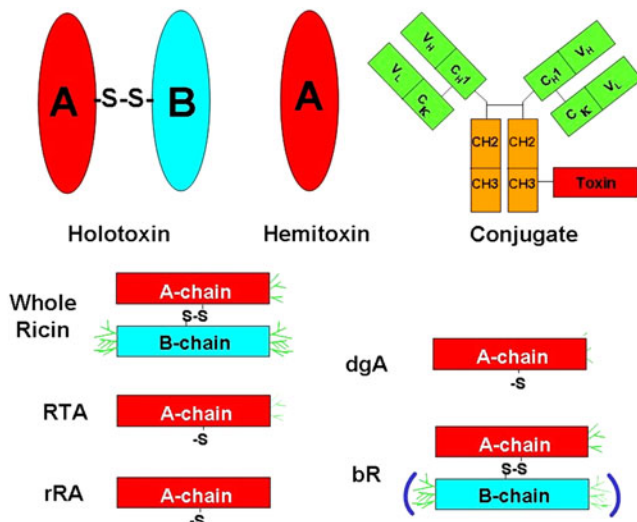
binding and catalytic domains, whereas hemitoxins contain only catalytic domains. Plant toxins have been shown to prevent the association of elongation factor-1 and -2 (EF-1 and EF-2) with the 60s ribosomal subunit by removing the base of A<sup>4324</sup> in 28s rRNA.<sup>15</sup> Ricin also removes the neighboring base G<sup>4323</sup>.<sup>15</sup> Apoptosis has been shown to be involved in cell death induced by plant toxins.<sup>16-18</sup> Only the enzymatic domain of both holo- and hemitoxins translocates to the cytosol, so the binding domains of holotoxins must be removed by reduction of the disulfide bond prior to translocation. Exactly how plant toxins move from the cell surface to the cytosol is unknown; the process probably differs for each plant toxin. The intracellular transport of ricin is dependent on sorting receptors that cycle between the endoplasmic reticulum (ER) and the terminal compartments of the Golgi.<sup>19</sup> It has been shown that glycolipids that bind ricin may be transported from endosomes to the Golgi and that the Lysine-aspartic acid-glutamic acid-Leucine (KDEL) ER retention sequence, if added to ricin, enhances the delivery of this plant toxin to the cytosol.<sup>20</sup>

### Mutant Plant Toxins for Connecting to Ligands

Originally, antibodies were chemically conjugated through a disulfide bond to the catalytic subunits of holotoxins such as ricin or abrin, each of which had been removed from its binding domain by reduction.<sup>2</sup> Even without its binding domain, however, ricin A chain (RTA) was taken up nonspecifically by macrophages and hepatic nonparenchymal Kupffer cells.<sup>21</sup> This uptake was due to glycosylated side residues of RTA (Figure 1) binding to mannose receptors on the liver.<sup>22</sup> The most successful technique for reducing nonspecific uptake of RTA was through chemical deglycosylation. Deglycosylated ricin A chain (dgA) immunotoxins had significantly prolonged lifetimes in mice, leading to an improved therapeutic index.<sup>21,23</sup> Half-lives improved further when the disulfide bond between the MAb and the toxin was formed in a hindered fashion using the derivatizing agent 4-succinimidylloxycarbonyl-a-methyl-a(2-pyridyldithio)toluene (SMPT).<sup>24</sup> Because the ricin B chain facilitates the cytotoxicity of RTA-containing immunotoxins,<sup>25</sup> whole ricin has been targeted after blocking its oligosaccharide binding sites to prevent normal cell binding (Figure 1). These sites on ricin were blocked with ligands prepared by chemical modification of glycopeptides containing triantennary N-linked oligosaccharides.<sup>26</sup> The resulting blocked ricin (bR) was then chemically conjugated to antibodies to make immunotoxins.

### Attempts to Construct Fusion Toxins Using Plant Toxins

The cytotoxicity of both plant and bacterial toxins is optimal when the catalytic domain alone translocates to the cytosol.<sup>27</sup> A binding domain can be translocated to the cytosol if



**Figure 1.** Plant toxins and chemical conjugation. Holotoxins such as ricin and abrin contain activity (A) and binding (B) domains disulfide-bonded together, while hemitoxins contain only activity (A) domains. Whole ricin contains carbohydrate groups and multiple residues in both A and B chains that bind to liver and other normal tissues. Reduction of the disulfide bond results in ricin A chain (RTA), which has reduced but still measurable binding to normal tissues. Options to reduce normal tissue binding further include making recombinant RTA in *Escherichia coli* (rRA), chemically deglycosylating RTA (dgA), and chemically blocking carbohydrates on whole ricin (blocked ricin, bR). An immunotoxin chemical conjugate contains a toxin chemically linked to a monoclonal antibody, optimally at a point removed from the antigen binding (V<sub>L</sub> or V<sub>H</sub>) domains. Generally, the toxin-ligand junction and the ratio of toxin to ligand is not constant within the conjugate mixture.

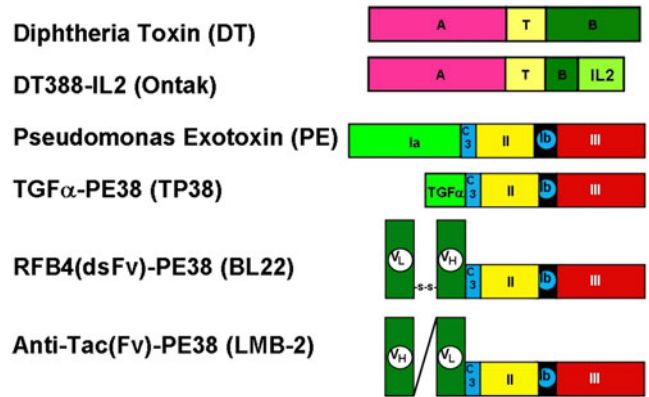
placed within the catalytic domain, but cytotoxic activity is significantly reduced.<sup>28</sup> In an attempt to construct a fusion toxin containing RTA from which free A-chain could be generated, interleukin-2 (IL-2) was fused to recombinant RTA through a linker that contained a proteolytic cleavage site for DT or clotting factor Xa.<sup>29</sup> Although the recombinant toxin could be cleaved extracellularly, it could not selectively target cells since the ligand and toxin were no longer connected. Later, IL-2 was fused to a mutant of PAP, but the fusion toxin was not purified and was not very cytotoxic.<sup>30</sup> Ligands fused to plant toxins have produced recombinant toxins with significant cytotoxic activity, including 1 containing a CD40 single-chain antibody and bryodin 1,<sup>31</sup> 1 containing urokinase binding domain and saporin,<sup>32</sup> and 1 containing human fibroblast growth factor and saporin.<sup>33</sup> For these molecules, it is not known whether (1) the recombinant toxin entered the cytosol of target cells intact, or (2) the ligand was unstable after internalization, permitting the catalytic domain alone to translocate to the cytosol. The ability of even stable ligands to predictably separate from the catalytic domain is an important feature of recombinant toxins<sup>34</sup> and a unique feature among all toxins provided by the bacterial toxins PE and DT.

### Mechanism of Action of Bacterial Toxins

Both PE and DT enzymatically ADP-ribosylate EF-2 in the cytosol.<sup>13</sup> They each catalyze the ADP-ribosylation of histidine-699 of EF-2, which is posttranslationally modified to a diphthimide residue.<sup>35</sup> Despite their similar action, PE and DT differ greatly in their amino acid sequence, and in fact PE's enzymatic domain is near the carboxyl terminus, while DT's is near the amino terminus. Conversely, PE's binding domain is near its amino terminus, and DT's is near its carboxyl terminus.

### Mechanism of Intoxication of PE

Full-length 613-amino-acid PE, as shown in Figure 2, is a single-chain protein containing 3 functional domains.<sup>36,37</sup> Domain Ia (amino acids 1-252) is the binding domain, domain II (amino acids 253-364) is responsible for translocating the toxin to the cytosol, and domain III (amino acids 400-613) contains the ADP-ribosylating enzyme that inactivates EF-2 in the cytosol. The catalytic process of ADP ribosylation has been shown to involve residues His440 and Glu553.<sup>42</sup> His440 binds nicotinamide adenine dinucleotide (NAD) via Adenosine monophosphate (AMP) ribose. The carboxyl group of the Glu553 side chain, through a water-mediated hydrogen bond with Tyr481 and Glu546, allows Tyr481 to bind NAD through a ring-stacking mechanism. The function of domain Ib (amino acids 365-399) is unknown. Thus, a current model of how PE kills cells contains the following steps: (1) The C-terminal residue



**Figure 2.** Schematic structure of bacterial toxins and recombinant toxins. PE is a single-chain 613-amino-acid protein containing 3 functional domains.<sup>36,37</sup> Domain Ia (amino acids 1-252) is the binding domain, domain II (amino acids 253-364) is the translocating domain, and domain III (amino acids 400-613) contains the adenosine diphosphate ribosylating enzyme that inactivates elongation factor 2 in the cytosol, resulting in cell death.<sup>38</sup> Domain Ib separates domains II and III and contains amino acids 365 to 399. DT is 535 amino acids in length and is composed of the enzymatic A domain (amino acids 1-193)<sup>39</sup> and the binding B domain (amino acids 482-535).<sup>40</sup> The translocation or transmembrane (T) domain is located in between.<sup>41</sup> PE38 is a 38 kDa truncated form of PE containing amino acids 253 to 364 and 381 to 613. The truncated form of DT used in recombinant toxins is DT388 in DT388-GM-CSF (DTGM) or DAB<sub>389</sub> in denileukin diftotox (shown), each of which contain methionine followed by the first 388 amino acids of DT. The single-chain recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) contains the variable heavy domain (V<sub>H</sub>) of the anti-Tac monoclonal antibody (MAb) fused via the peptide linker (G<sub>4</sub>S)<sub>3</sub> to the variable light domain (V<sub>L</sub>), which in turn is fused to PE38. The recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) is composed of the V<sub>L</sub> from the MAb RFB4 disulfide bonded to a fusion of V<sub>H</sub> with PE38. The disulfide bond connecting V<sub>H</sub> and V<sub>L</sub> is formed between 2 cysteine residues replacing Arg44 of V<sub>H</sub> and Gly100 of V<sub>L</sub>. For single-chain recombinant toxins containing DT, the ligand is at the carboxyl rather than at the amino terminus of the toxin.

(Lys613) is removed by a carboxypeptidase in the plasma or culture medium.<sup>43</sup> (2) Domain Ia binds to the  $\alpha$ 2 macroglobulin receptor that is present on animal cells and is internalized via endosomes to the transreticular Golgi.<sup>44</sup> (3) After internalization, the protease furin cleaves domain II between amino acids 279 and 280.<sup>45</sup> (4) The disulfide bond between cysteines 265 and 287, which joins the 2 fragments generated by proteolysis, is reduced.<sup>46</sup> (5) Amino acids 609 to 612 Arginine-glutamic acid-aspartic acid-leucine (REDL) bind to an intracellular sorting receptor that transports the 37 kDa carboxy terminal fragment from the transreticular Golgi apparatus to the ER.<sup>47,48</sup> (6) Amino acids 280 to 313 mediate translocation of the toxin to the cytosol.<sup>49,50</sup> (7) The ADP-ribosylating enzyme within amino acids 400 to 602

inactivates EF-2.<sup>13</sup> (8) While inhibition of protein synthesis is sufficient to induce cell death eventually, recent experiments indicate that cell death from toxins is facilitated by apoptosis.<sup>51,52</sup>

### ***Mechanism of Intoxication of DT***

DT is a single-chain protein 535 amino acids in length. It is composed of an enzymatic A domain (amino acids 1-193) and a binding B domain (amino acids 482-535).<sup>40</sup> A third domain, which is the translocation or transmembrane (T) domain, is located in the center of the molecule.<sup>53</sup> Based on DT's 3-dimensional structure in the presence and absence of NAD,<sup>54</sup> DT is thought to undergo these steps to kill cells: (1) DT is proteolytically cleaved outside the cell between Arg193 and Ser194,<sup>55</sup> which is within a disulfide loop formed by Cys186 and Cys201. (2) DT binds on the cell surface via residues 482 to 535 to a complex of heparin-binding Epidermal growth factor (EGF)-like growth factor precursor and CD9.<sup>40</sup> (3) DT internalizes into an endosome and unfolds at low pH,<sup>56</sup> and the disulfide bond linking amino acids 186 and 201 is reduced. (4) The TH8 (amino acids 326-347) and TH9 (amino acids 358-376) domains form a hairpin, which inserts into the membrane of the endosome and forms a channel through which the enzymatic fragment translocates to the cytosol,<sup>41</sup> probably from early endosomes.<sup>57</sup> (5) In the cytosol, NAD binds to the active-site cleft of DT (amino acids 34-52), and the ADP ribose of NAD is transferred to EF-2.<sup>58,59</sup> (6) As with PE, cell death is facilitated by apoptosis.<sup>52</sup>

### ***Mutated Bacterial Toxins for Fusing to Ligands***

The structures of mutated and truncated forms of DT and PE are shown in Figure 2. To improve specificity, toxins for labeling MAbs are mutated to prevent their binding to normal cells. DT is mutated by converting Leu390 and Ser525 each to phenylalanine, resulting in CRM107.<sup>60</sup> Truncated forms of PE and DT include PE40, containing amino acids 253 to 613 of PE, and Diphtheria toxin A and B domains (DAB<sub>486</sub>), containing the first 485 amino acids of DT.<sup>36,61,62</sup> Shorter versions more recently used include PE38, composed of amino acids 253 to 364 and 381 to 613 of PE, and DT388 or DAB<sub>389</sub>, containing the first 388 amino acids of DT.<sup>63-66</sup> To allow the ADP-ribosylating domain to translocate to the cytosol without the ligand, the ligand is placed at the amino terminus of PE and at the carboxyl terminus of DT. Another form of PE has an altered carboxyl terminus from the Arginine-glutamic acid-aspartic acid-leucine-lysine (REDLK) to the KDEL sequence, which binds with higher affinity to the KDEL receptor and results in increased cytotoxicity.<sup>48</sup> Immunotoxins containing mutants of PE ending in KDEL (ie, PE38KDEL or PE40KDEL) are more cytotoxic than comparable immunotoxins where the PE mutant

ends in the native sequence REDLK.<sup>48,64,67</sup> The translocated fragment of PE38 is 35 kDa in length beginning with Gly280, and since methionine in this position does not alter activity, the new mutant PE35 is produced; it begins with a methionine at position 280 and contains amino acids 281 to 364 and 381 to 613.<sup>68</sup> This molecule would not be appropriate for fusing to ligands, but since it contains a single disulfide bond, it is ideal for chemically conjugating to ligands.

### ***Production of Immunotoxins***

Chemical conjugates of growth factor and toxin generally involve either reducible disulfide (S-S) or nonreducible thioether (S-C) bonds.<sup>69</sup> A thioether bond is appropriate if the ligand is conjugated to a bacterial toxin in the part that does not translocate to the cytosol, such as the binding domain.<sup>70</sup> Otherwise, a disulfide bond is commonly used. Derivatization of the toxin requires only reduction in the case of RTA and its mutants, and also in the case of PE35, since both contain only 1 cysteine each. Derivatization of the ligand requires care to produce sulfhydryls without harming the molecule, unless the ligand also has a single cysteine. Once the ligand and toxin are derivatized, they must be purified and conjugated, and then the conjugate of correct toxin-ligand ratio must be repurified. The difficulty and cost of these multiple steps have pushed development of recombinant toxins, which may be produced in *Escherichia coli* transformed with a plasmid encoding the recombinant toxin. A common method of producing material for clinical trials is harvesting recombinant protein from insoluble bacterial inclusion bodies.<sup>71-73</sup> The insoluble protein can be washed extensively with detergent to remove endotoxin, solubilized, denatured, and reduced in guanidine-dithioerythritol solution. The recombinant protein is then renatured by rapid dilution into refolding redox buffer containing arginine and glutathione, and the dialyzed renatured protein purified by anion exchange and sizing chromatography. Other published methods of producing recombinant toxins from *E. coli* involve harvesting the protein from cytoplasm or cell lysate<sup>74</sup> and then using an affinity column to capture the dilute protein. Reverse-phase chromatography followed by sizing chromatography has also been used. Eukaryotic expression systems normally fail with recombinant toxins since eukaryotic EF-2 is highly susceptible to the toxin. However, insect and plant cells have been produced that are resistant to toxin and can produce active toxin.<sup>75,76</sup>

### ***Testing Immunotoxins in Preclinical Models***

Immunotoxins are typically first tested on a cell line that contains the receptor or antigen that attracts the binding domain. To determine whether the immunotoxin might be effective *in vivo*, murine models are produced in which mice contain human xenografts of tumor cell lines. In this

regard, solid tumors have been found more difficult to treat than disseminated leukemia of the same cell line.<sup>77</sup> Once antitumor activity is found in vivo, it is still not clear that the agent would result in responses in patients. One reason for this is that cell lines may grossly overestimate the number of antigen-binding sites/cell in patients. Thus, primary tumor cells freshly isolated from patients are often tested ex vivo to determine sensitivity to the immunotoxin. Another problem with murine models is that patients may have much more unwanted toxicity than mice since the murine receptor or antigen may not even bind the immunotoxin as the human antigen would. For this reason, nonhuman primates that display the antigen on their normal cells are used for toxicity experiments. Even so, expensive experiments of this type are often not predictive of human toxicity. The remainder of this review will focus on immunotoxins tested in patients or being developed for clinical testing.

## IMMUNOTOXINS TARGETING HEMATOLOGIC TUMOR ANTIGENS

### *Antigens Targeted by Immunotoxins in Hematologic Tumor Clinical Trials*

Hematologic malignancies are optimal for treating with immunotoxins, since malignant cells are often intravascular and accessible to intravenously administered drug, and since patients often lack sufficient immunity to make antibodies against the toxin. That said, only a handful of antigens have been used to target immunotoxins to hematologic malignancies in patients. These are summarized in Table 1.

### *Targeting DT to the IL-2 Receptor*

The IL-2 receptor (IL2R) binds IL-2 with high affinity ( $K_d \sim 10^{-11}M$ ) and is composed of a complex of alpha (CD25), beta (CD122), and gamma (CD132) subunits.<sup>117</sup> The complex of CD122 and CD132 bind IL-2 with intermediate affinity ( $K_d \sim 10^{-9}M$ ), and CD25 alone binds IL-2 with low affinity ( $K_d \sim 10^{-8}M$ ). IL2Rs of some type are present on a wide variety of hematologic malignancies, including cutaneous T-cell lymphoma (CTCL), adult T-cell leukemia (ATL), HD, and other B- and T-cell leukemias and lymphomas.<sup>118-121</sup> IL2Rs are also displayed by normal T cells and these T-cells can mediate graft rejection and graft vs host disease (GVHD). Only a small percentage of T cells are ordinarily IL2R+.<sup>122</sup> To target the IL2R, human IL-2 was fused to truncated DT, originally a fragment of DT containing methionine plus the first 485 amino acids of DT.<sup>62,123</sup> Clinical trials showed some efficacy with DAB<sub>486</sub>IL-2 in hematologic malignancies with dose-limiting transaminase elevations.<sup>124-126</sup> A new fusion toxin was created by removing amino acids 389 to 485, and it was found that DAB<sub>389</sub>IL-2, also called denileukin diftitox or Ontak (Figure 2), had improved half-

life, cytotoxicity, and tolerance in animals.<sup>65</sup> In phase I testing there were 5 complete remissions (CRs) and 8 partial responses (PRs) in 35 patients with CTCL, and 1 CR and 2 PRs out of 17 patients with NHL.<sup>127</sup> The maximum tolerated dose (MTD) was 27  $\mu g/kg$  daily (QD)  $\times$  5, and the dose-limiting toxicity was asthenia (fatigue). Common toxicities included transaminase elevations (62%), hypoalbuminemia (86%), rashes (32%), and hypotension (55%).<sup>65</sup> In the pivotal phase III CTCL trial, 7 CRs and 14 PRs were achieved in 71 patients, but most patients had objective skin improvement.<sup>128,129</sup> Two dose levels were tested (9  $\mu g/kg$  and 18  $\mu g/kg$  QD  $\times$  5), and patients with more advanced disease benefited from the higher dose. Vascular leak syndrome (VLS), attributed to cytokine release after the killing of perivascular T cells in the dermis, was usually without pulmonary edema and could be prevented with steroid prophylaxis.<sup>95,128,130</sup> Immunogenicity toward anti-DAB<sub>389</sub>IL-2 increased from 32% baseline to nearly 100% after 1 cycle, but retreatment was sometimes effective, indicating that antitoxin antibodies were not always neutralizing.

### *Postapproval Testing of Denileukin Diftitox*

Denileukin diftitox was approved by the Food and Drug Administration for the treatment of advanced CTCL. Other treatments like bexarotene are indicated for early CTCL.<sup>131</sup> Denileukin diftitox has also shown activity in clinical trials of other tumors and in autoimmune disease, including peripheral T-cell lymphoma,<sup>132</sup> panniculitic lymphoma,<sup>133</sup> B-Chronic lymphocytic leukemia (CLL),<sup>96</sup> B-NHL,<sup>97</sup> and psoriasis.<sup>134</sup> Out of 18 patients with B-CLL with an average of 4.5 prior treatments/patient, 12 received at least 3 cycles at 9 or 18  $\mu g/kg$  QD  $\times$  5, and 6 (50%) of 12 had 95% to 99% reductions of circulating malignant cells. Four (33%) out of 12 patients had 29% to 80% reductions in lymph nodes, with 2 qualifying for PR, lasting 14 and >19 months. Out of 45 evaluable patients with NHL treated in a phase II trial, there were 3 (7%) CRs and 8 (18%) PRs. Durability of response was somewhat limited, with a 7-month median time to treatment failure in responding patients.<sup>97</sup> Thus, denileukin diftitox, the only targeted protein toxin so far approved for use, is effective in several hematologic malignancies. One limitation for CTCL, CLL, and NHL is the lack of high-affinity IL2Rs in a large percentage of cases, usually because of lack of CD122. Several types of agents, including rexinoids<sup>135,136</sup> and arginine butyrate,<sup>136</sup> can upregulate CD25 and/or CD122 and could potentially expand the clinical utility of this recombinant toxin.

### *Targeting PE to CD25, Preclinical Studies*

To target IL2R+ disorders expressing CD25 regardless of the presence of other subunits of the IL2R, the anti-CD25 MAb anti-Tac was used as a ligand instead of IL-2. The

**Table 1.** Immunotoxins Tested Clinically in Recent Years\*

<b>Chemical Conjugates</b>						
<b>Agent</b>	<b>Antigen</b>	<b>Ligand</b>	<b>Truncated Toxin</b>	<b>Basic Toxin</b>	<b>Diseases</b>	<b>References</b>
RFT5-dgA	CD25	MAb	dgA	Ricin	HD	78,79
RFB4-dgA	CD22	MAb	dgA	Ricin	B-NHL, CLL	80,81
RFB4-Fab'-dgA	CD22	Fab'	dgA	Ricin	B-NHL	82
HD37-dgA	CD19	MAb	dgA	Ricin	B-NHL	83
Anti-CD7-dgA	CD7	MAb	dgA	Ricin	T-NHL	84
K <sub>i</sub> -4.dgA	CD30	MAb	dgA	Ricin	HD	85
LMB-1	Le <sup>y</sup>	MAb	Lys-PE38	PE	Carcinoma	70
TF-CRM107	TFR	Tf	CRM107	DT	Glioma	86
B43-PAP	CD19	MAb	PAP	PAP	ALL	87
Anti-B4-bRicin	CD19	MAb	bR	Ricin	B-NHL	88-90
Ber-H2-Sap6	CD30	MAb	Sap6	Saporin	HD	91
Anti-My9-bRicin	CD33	MAb	bR	Ricin	AML	90
454A12-rRA	TFR	MAb	rRA	Ricin	CSF cancer	92
N901-bR	CD56	MAb	bR	Ricin	SCLC	90,93,94
<b>Recombinant toxins</b>						
<b>Agent</b>	<b>Antigen</b>	<b>Ligand</b>	<b>Truncated Toxin</b>	<b>Basic Toxin</b>	<b>Diseases</b>	<b>References</b>
Ontak	IL2R	IL-2	DAB <sub>389</sub>	DT	CTCL, CLL, NHL	95-97
BL22	CD22	dsFv	PE38	PE	HCL, CLL, NHL	98
LMB-2	CD25	scFv	PE38	PE	NHL, leukemias	99,100
DT388-GM-CSF	GM-CSF	GM-CSF	DT388	DT	AML	101
B3(Fv)-PE38	Le <sup>y</sup>	scFv	PE38	PE	Carcinoma	102
B3(dsFv)-PE38	Le <sup>y</sup>	dsFv	PE38	PE	Carcinoma	103
TP40	EGFR	TGF $\alpha$	PE40 <sup>4a</sup>	PE	Bladder cancer, CIS	104
TP38	EGFR	TGF $\alpha$	PE38	PE	Glioblastoma	105
BR96(scFv)-PE40	Le <sup>y</sup>	scFv	PE40	PE	Carcinoma	106-108
erb38	erbB2	dsFv	PE38	PE	Breast cancer	109
NBI-3001	IL4R	IL-4(38-37)	PE38KDEL	PE	Glioma	110,111
IL13-PE38QQR	IL13R	IL-13	PE38QQR	PE	Renal cell	112-114
SS1(dsFv)-PE38	Mesothelin	dsFv	PE38	PE	Mesothelioma	115
DAB <sub>389</sub> EGF	EGFR	EGF	DAB <sub>389</sub>	DT	Carcinoma	116

\*Toxins, several of which are shown schematically in Figure 1, include recombinant ricin A chain (rRA), blocked ricin (bR), deglycosylated ricin A chain (dgA), pokeweed antiviral protein (PAP), truncated diphtheria toxin (DT388 or DAB<sub>389</sub>), truncated *Pseudomonas* exotoxin (PE38 or PE40), and mutated diphtheria toxin (CRM107). Non-monoclonal antibody (MAb) ligands include interleukin-2, -4, and -13 (IL-2, IL-4, and IL-13); granulocyte-macrophage colony stimulating factor (GM-CSF); epidermal growth factor (EGF); transforming growth factor (TGF $\alpha$ ); and transferrin (Tf). PE40<sup>4a</sup> is PE40 with alanine substituted for cysteine at positions 265, 287, 372, and 379. PE38QQR is PE38 with 2 glutamine residues and 1 arginine replacing the 3 lysine residues of PE38 at positions 590, 606, and 613. Diseases include non-Hodgkin's lymphoma (NHL, B- or T-cell), cutaneous T-cell lymphoma (CTCL), Hodgkin's disease (HD), chronic lymphocytic leukemia (CLL), carcinoma in situ (CIS), acute myelogenous leukemia (AML), metastatic tumor involving the cerebrospinal fluid (CSF cancer), renal cell carcinoma (renal cell), small cell lung cancer (SCLC), Acute lymphoblastic leukemia (ALL) and hairy cell leukemia (HCL).

rationale is based on the higher binding of CD25 alone to anti-Tac ( $K_d \sim 10^{-10}M$ ) than to IL-2 ( $K_d = 10^{-8}M$ ).<sup>137</sup> CD25 greatly outnumbers CD122 and CD132 on most malignant cell types.<sup>118,119</sup> Although early studies indicated CD25 alone would not internalize anti-Tac,<sup>138</sup> CD25 alone does internalize bound recombinant toxin.<sup>64,139,140</sup> A recombinant single-chain Fv<sup>141,142</sup> was constructed containing the vari-

able heavy domain ( $V_H$ ) fused to the variable light domain ( $V_L$ ) via the peptide linker (G<sub>4</sub>S)<sub>3</sub>, and  $V_L$  was fused to truncated PE.<sup>143</sup> The resulting recombinant immunotoxin anti-Tac(Fv)-PE40 and its slightly shorter derivative anti-Tac(Fv)-PE38 (called LMB-2) were selectively cytotoxic toward CD25+ malignant cell lines and toward leukemic cells freshly obtained from patients.<sup>64,140,144-147</sup> Antitumor



studies in mice bearing CD25+ xenografts showed complete regressions, and biodistribution studies showed a concentration of LMB-2 in such tumors *in vivo*.<sup>140,145</sup> Primary ATL and hairy cell leukemia (HCL) cells were much more sensitive than primary CLL cells, probably because of lower CD25 expression in the latter, and CLL cells have been shown to upregulate CD25 by phosphorothioate oligodeoxynucleotides.<sup>148</sup> Cyclosporine has been reported to increase the sensitivity of ATL cells toward anti-Tac(Fv) toxin, but such cells were already very sensitive.<sup>149</sup>

### ***Clinical Development of LMB-2 in CD25+ Hematologic Malignancies***

LMB-2 was administered to 35 patients with chemotherapy-resistant leukemia, lymphoma, and HD. There were 7 PRs and 1 CR, all in the 20 patients receiving a total dose of >60 µg/kg/cycle. All 4 patients with HCL responded, with 1 CR and 3 PRs.<sup>99</sup> CR was associated with resolution of severe pancytopenia and eradication of circulating malignant cells. Patients with CLL, ATL, CTCL, and HD achieved PR.<sup>100</sup> The most common toxicities included transaminase elevations that were associated with fever and thus appeared to be mediated by cytokines.<sup>150,151</sup> Immunogenicity resulted in 6 out of 35 patients being excluded from further treatment after the first cycle. The 8 CLL patients did not make neutralizing antibodies after a total of 16 cycles. In HD, high levels of neutralizing antibodies were observed in 3 out of 11 patients after 1 cycle and after 2 to 3 cycles in 2 additional patients. Phase II trials are currently underway in CD25+ CLL and CTCL. HCL patients are being treated now with the anti-CD22 recombinant immunotoxin BL22 (see below) instead of LMB-2.

### ***Development of RFT5-dgA for CD25+ Malignancies***

The anti-CD25 MAb RFT5 was isolated and chemically conjugated to dgA, and the resulting immunotoxin RFT5-dgA induced 2 PRs out of 18 patients with HD treated at the optimal dose level.<sup>78,79,152,153</sup> RFT5-dgA was also tested in the prevention of GVHD in patients undergoing allotransplantation.<sup>154</sup> It was reported that patients receiving RFT5-dgA had a higher incidence of grade III/IV GVHD than historical controls, suggesting that CD4+/CD25+ T-regulatory cells were targeted and that activated T cells may have been spared because of reduction of CD25 expression by cyclosporine.<sup>155</sup>

### ***Preclinical Development of Other Recombinant Toxins Targeting CD25***

Production of a more stable form of LMB-2 was accomplished by replacing the peptide linker between the variable domains with a disulfide bond via cysteine residues engi-

neered into the framework region; the resulting disulfide-stabilized recombinant immunotoxin had improved stability, but binding, cytotoxicity, and antitumor activity were not compromised.<sup>156,157</sup> Mik-β1(Fv)-PE40, a recombinant immunotoxin targeting CD122, was produced and shown to cointernalize with LMB-2 into cells expressing both CD25 and CD122 subunits of the IL2R.<sup>158</sup> This agent was cytotoxic toward natural killer leukemia cells, which express more CD122 than CD25, but has not been developed further. The MAb RFT5 was converted to the recombinant immunotoxin RFT5(scFv)-ETA'. This recombinant immunotoxin showed antitumor activity in Severe combined immunodeficiency (SCID) mice bearing disseminated human HD.<sup>159-161</sup>

### ***Targeting CD22 With Immunotoxins***

Chemical conjugates were previously constructed to target CD22 on B-cell malignancies, including the MAbs H6 or RFB4 conjugated to dgA,<sup>162,163</sup> and the MAbs HD6 and HD39 linked to saporin.<sup>164</sup> RFB4-dgA resulted in 2 CRs and 10 PRs out of 41 patients with B-cell lymphoma/leukemia (combining both bolus and continuous infusion trials), and patients had dose-limiting VLS.<sup>80,81,165</sup> To avoid VLS, a derivative of recombinant RTA was prepared containing an N87A mutation. RFB4-N87A led to significantly less VLS in mice, suggesting that this new immunotoxin may be useful in patients.<sup>166,167</sup> The first anti-CD22 immunotoxin with PE contained the MAb LL2 and induced complete regression in human xenograft models.<sup>68,168</sup> However, LL2 as a single-chain Fv was unstable, and an active recombinant immunotoxin could not be made. Therefore, the variable domains from RFB4 were cloned and a stable recombinant immunotoxin RFB4(Fv)-PE38 was made and shown to be cytotoxic toward CD22+ cell lines.<sup>169</sup>

### ***Preclinical Development of BL22***

To improve the stability of RFB4(Fv)-PE38, the variable domains were connected by a disulfide bond instead of a peptide linker, and V<sub>H</sub> was fused to PE38, resulting in BL22.<sup>170</sup> The disulfide bond is between cysteine residues replacing framework residues Arg44 of V<sub>H</sub> and Gly100 of V<sub>L</sub>. This technology had been used for stabilizing Fvs of a variety of different MAbs, including anti-Tac.<sup>156</sup> The double-chain immunotoxin, termed RFB4(dsFv)-PE38 or BL22, is considered fully recombinant since the disulfide bond between V<sub>L</sub> and V<sub>H</sub>-PE38 forms naturally during *in vitro* renaturation of the 2 fragments and chemical conjugation is not needed. BL22 induced complete regressions in mice of human CD22+ B-cell lymphoma xenografts at plasma levels that could be tolerated in cynomolgus monkeys.<sup>171</sup> Leukemic cells freshly obtained from patients with CLL and NHL were found to be sensitive to BL22.<sup>172</sup> This study, which showed specific killing of such cells, was important

for preclinical development because malignant cells freshly obtained from patients typically display far fewer CD22 sites/cell than do cell lines. Much greater activity toward the CLL cell was observed with the mutant HA22, which has higher affinity for CD22 because of THW replacing amino acids SSY at positions 100, 100a, and 100b of V<sub>H</sub>.<sup>173</sup>

### ***Phase I Testing of BL22 in Patients with B-Cell Malignancies***

In one study, BL22 was administered to 46 patients with HCL B-cell lymphomas and leukemias.<sup>98,174</sup> A total of 265 cycles of BL22 were administered to 16 patients, with up to 33 cycles/patient. All patients were pretreated with 1 to 6 separate courses of cladribine. A total of 19 out of 31 patients (61%) had CR, and 6 patients had PR (19%). Seven patients had marginal responses, with up to 99.5% reductions in circulating HCL counts but less than 50% decreases in lymph node masses. CR was achieved in all 3 patients with the poor-prognosis variant HCLv.<sup>175</sup> Eleven had CR after cycle 1, and 8 had CR after cycles 2 to 9. Only 1 out of 19 CRs had minimal residual disease in the bone marrow biopsy by immunohistochemistry, which is reported to be a risk factor for early relapse.<sup>176</sup> Cytopenias resolved in all responders. Within the follow-up time of 5 to 67 (median 36) months, 7 patients were still in CR. High levels of neutralizing antibodies were observed in 11 patients after cycles 1 to 5. Plasma levels in patients with high disease burden were much greater on subsequent cycles after patients responded, compared with cycle 1. In the HCL patients, dose-limiting toxicity included a cytokine release syndrome in 1 patient with fever, hypotension, bone pain, and weight gain (VLS) without pulmonary edema; this resolved within 3 days. Also, 4 patients with HCL had completely reversible hemolytic uremic syndrome (HUS), confirmed by renal biopsy. HUS presented clinically with hematuria and hemoglobinuria by day 8 of cycle 2 in each case. These patients required 6 to 10 days of plasmapheresis but not dialysis for complete resolution of renal function and correction of thrombocytopenia and anemia. Three of these 4 HCL patients achieved CR, and in all 4, there was resolution of preexisting cytopenias as well as those related to HUS. BL22 is the first agent since purine analogs reported to induce CR in the majority of patients with HCL. Its success in chemoresistant patients is clearly related to the fact that CD22 is highly conserved at high density on HCL cells despite purine analog resistance.

### ***Targeting the Granulocyte-Macrophage Colony Stimulating Factor Receptor With DT388-GM-CSF***

To target the granulocyte-macrophage colony stimulating factor receptor (GM-CSFR), which is expressed in AML cells from most patients, human GM-CSF was fused to truncated bacterial toxins. DT388-GM-CSF (DTGM) was found

to be more cytotoxic than GM-CSF-PE38KDEL.<sup>177</sup> DTGM was tested in 31 patients with relapsed or refractory AML, all of whom were resistant to chemotherapy.<sup>101</sup> One CR and 2 PRs were observed, and the major toxicity was cytokine release syndrome. Preexisting antibodies to DT were observed in 28 of 31 patients.<sup>101,178</sup> Cytotoxic plasma levels of DTGM could be detected in 14/20 patients with anti-DT antibody concentrations <2.2 µg/mL and in 2/11 patients with anti-DT antibody concentrations >2.2 µg/mL.<sup>101</sup>

### ***Targeting CD19 or Both CD19 and CD22 With Immunotoxins***

The anti-CD19 immunotoxin anti-B4-blocked ricin (anti-B4-bR) had previously been tested in phase I trials, which showed responses, including CRs, but later trials showed more limited activity, possibly because of limited tumor penetration.<sup>88</sup> Subsequent trials used anti-B4-bR in the setting of minimal residual disease or in combination with chemotherapy. In these nonrandomized trials, the most recent of which was in patients with acute lymphoblastic leukemia in first CR, no obvious activity was observed.<sup>89,179-183</sup> The combination of anti-CD19 HD37-dgA and anti-CD22 RFB4-dgA was tested in an animal model<sup>184</sup> and had some efficacy in patients with B-cell malignancies,<sup>185</sup> but safety could be established in only patients with circulating tumor cells.

### ***Targeting CD30 With Immunotoxins***

The anti-CD30 MAb K<sub>i</sub>-4 conjugated to dgA was tested in 15 patients with NHL and HD, achieving 1 PR.<sup>186</sup> Preclinical work with a related immunotoxin, K<sub>i</sub>-3-dgA, showed that inhibition of metalloproteinases enhanced internalization and cytotoxicity.<sup>187</sup> K<sub>i</sub>-4 was converted to a recombinant immunotoxin, termed K<sub>i</sub>-4(scFv)-ETA', which displayed antitumor activity in mice with disseminated human HD.<sup>188,189</sup> Without a hybridoma being obtained, anti-CD30 single-chain Fvs were obtained by immunizing mice with DNA encoding human CD30, harvesting the spleens, and constructing an scFv phage display library. Several anti-CD30 recombinant immunotoxins were obtained with selective cytotoxicity toward cell lines, and anti-CD30-CL2(Fv)-PE38KDEL had antitumor activity in a CD30+ human solid tumor mouse xenograft model.<sup>190</sup> For higher-affinity recombinant immunotoxins targeting CD30, mice were DNA-immunized and the hybridomas isolated prior to scFv construction. Two molecules, T25(dsFv)-PE38 and T6(dsFv)-PE38, were found to have potent cytotoxicity toward CD30+ cell lines.<sup>191</sup>

### ***Preclinical Studies With Other Immunotoxins Targeted to Hematologic Tumors***

To target AML cells with a molecule that would not cross-react with monocytes and macrophages and hence would



not cause cytokine release syndrome, a DT containing human IL-3 was produced. DT388-IL3 was found to be cytotoxic toward AML cell lines<sup>192</sup> and primary AML or Chronic myelogenous leukemia (CML) cells<sup>193,194</sup> but not normal hematopoietic progenitors.<sup>195</sup> DT388-IL3 prolonged survival in tumor-bearing mice<sup>196</sup> and has been produced for phase I clinical testing.<sup>197</sup> To target multiple myeloma and T-cell leukemias, CD38 was targeted with anti-CD38 saporin and improved cytotoxicity was observed in combination with anti-CD7 saporin.<sup>198</sup> Retinoic acid was found to induce CD38 expression and enhance cytotoxicity to anti-CD38 gelonin.<sup>199</sup> A recombinant anti-CD7 immunotoxin was found to kill T-cell acute lymphoblastic leukemia (T-ALL) cells by apoptosis.<sup>200</sup> To target CD20+ B cells, rituximab was conjugated to saporin-S6 and synergy was found with fludarabine.<sup>201</sup> CD64 was targeted using an RTA MAb conjugate,<sup>202</sup> and a recombinant anti-CD64 immunotoxin was produced and found to be cytotoxic toward AML cells.<sup>203</sup> Anti-CD80 and anti-CD86 were used as conjugates with gelonin to target HD cells, and safety was established in monkeys.<sup>204</sup> The antigen JL1 on leukemias was targeted using an MAb-gelonin conjugate.<sup>205</sup> Finally, CTLA4 was targeted using 2 different Fvs, each conjugated to saporin-S6, and activity against both lymphoid and myeloid leukemias was observed.<sup>206</sup>

## IMMUNOTOXINS TARGETING SOLID TUMOR ANTIGENS

Targeting solid tumors with immunotoxins is much more difficult than targeting hematologic tumors. Not only are the cellular junctions tighter and the tumor cells more tightly packed, but the patients are less immunosuppressed and more likely to make neutralizing antibodies to the toxin. Below, recent published information regarding solid tumor immunotoxin trials, along with recent preclinical development, is discussed. The findings are summarized in Table 1.

### *Immunotoxins Targeting the Epidermal Growth Factor Receptor*

Some of the earliest chemical conjugates and recombinant fusion toxins contained either epidermal growth factor (EGF) or transforming growth factor  $\alpha$  (TGF $\alpha$ ), both ligands for the EGF receptor (EGFR), and Pseudomonas exotoxin.<sup>28,207-210</sup> Antitumor activity in tumor-bearing mice was demonstrated,<sup>211</sup> but tolerated doses were low because of expression of EGFR by the liver. Thus, TGF $\alpha$  toxins were tested nonsystemically, either by intravesical treatment of bladder cancer<sup>104</sup> or by intracerebral injection of patients with glioblastoma multiforme.<sup>105</sup> In the latter trial, several patients responded, including 1 with long-term CR. EGF fused to diphtheria was also developed for targeting EGFR-bearing tumors.<sup>212</sup> DAB<sub>389</sub>EGF was administered systemi-

cally in phase I trials to patients with prostate, gastrointestinal, head and neck, renal, lung, and breast cancer,<sup>116</sup> and the dose-response rate was limited by renal tubular acidosis and immunogenicity. More recently, DAB<sub>389</sub>EGF and anti-EGFR immunotoxins have been developed for local treatment of brain and pancreatic tumors.<sup>213-215</sup> One method for improving specificity for EGFR+ malignant cells is to target mutant versions of the EGFR with recombinant immunotoxins.<sup>216,217</sup> Another is to target the heparin binding form of the EGFR, which can be modulated by heparin.<sup>218-220</sup>

### *Targeting the Le<sup>y</sup> Antigen on Solid Tumors*

To target the carbohydrate antigen Le<sup>y</sup>,<sup>221</sup> a chemical conjugate of B3 with PE38, termed LMB-1, was produced, developed preclinically,<sup>222-224</sup> and tested in 38 patients with Le<sup>y</sup>-expressing carcinomas of breast, ovarian, and gastrointestinal origin.<sup>70</sup> One CR and 1 PR were achieved, the first major responses to immunotoxins for metastatic breast and colon cancer, respectively. The dose-limiting toxicity was due to VLS. Experiments with human umbilical vein endothelial cells indicated that the MAb B3 rather than PE38 was binding to the Le<sup>y</sup> antigen on endothelial cells.<sup>225</sup> To target Le<sup>y</sup>-expressing tumors with a smaller immunotoxin that would leave the vasculature quickly before causing VLS, the Fv of B3 was cloned and fused to PE38.<sup>226</sup> B3(Fv)-PE38 (LMB-7) and B3(dsFv)-PE38 (LMB-9) are 2 recombinant immunotoxins that have recently undergone clinical testing, the former having a single-chain structure like LMB-2 and the latter having a disulfide-stabilized structure like BL22. LMB-9 is more appropriate for administration by continuous infusion, because of its extreme stability at 37°C.<sup>103</sup> The recently published clinical results of the anti-Le<sup>y</sup> recombinant immunotoxin BR96(sFv)-PE40 indicated that the molecule was reasonably stable and the dose was limited by gastrointestinal toxicity rather than by VLS.<sup>106</sup>

### *Recombinant Toxins Targeting erbB2*

To target the erbB2 antigen expressed in poor-prognosis breast cancer and other carcinomas,<sup>227</sup> several MAbs that vary in affinity have been cloned to produce recombinant single-chain or disulfide-stabilized immunotoxins.<sup>228-230</sup> One of these, erb-38, containing a disulfide-stabilized Fv fused to PE38, was tested in patients with carcinomas, mostly breast cancer.<sup>109</sup> Erb-38 bound to normal liver tissue, resulting in dose-limiting toxicity at a low dose level. This supports the important principle that targeting any antigen expressed even at very low levels on an organ that is already sensitive to a toxin's effects may prevent selective tumor targeting. Thus, antitumor activity in animals that do not express the targeted antigen on normal cells may not translate into response in humans.<sup>231</sup> Further preclinical development of such immunotoxins is proceeding using the

intratumoral injection route.<sup>232</sup> In a clinical trial of ScFv(FRP5)-ETA, 6 of 10 patients achieved tumor regression of cutaneous metastases of colon and breast cancers.<sup>233</sup>

### ***Recombinant Toxins Targeting the IL-4 Receptor***

The IL-4 receptor (IL4R) is widely expressed by solid tumors and hematologic malignancies.<sup>234,235</sup> Early IL4-PE fusions had limited binding because the toxin interfered with the IL4-IL4R binding site. To optimize binding, the circularly permuted mutant IL-4 toxins were made, containing IL-4 amino acids 38 to 129 connected through the peptide linker GGNGG to IL-4 amino acids 1 to 37, which were in turn fused to the toxin.<sup>236-238</sup> This resulted in enhanced cytotoxicity and antitumor activity.<sup>238-240</sup> Because IL4(38-37)-PE38KDEL was highly toxic to the liver at low doses, it was developed for intratumoral therapy of glioblastoma multiforme.<sup>240,241</sup> Of the first 9 patients treated in this fashion, 1 multiply relapsed patient had extensive tumor necrosis followed by a long-term (>18-month) CR.<sup>110</sup> Toxicity was usually related to the edema associated with high infusion volumes and rates. In some patients requiring reoperation, toxicity to normal brain tissue caused by the toxin was excluded histologically. In a phase I/II trial of 31 patients, tumor necrosis was observed in 71%, and 1 patient experienced long-term survival.<sup>111</sup>

### ***Targeting the IL-13 Receptor on Solid Tumors***

The IL13 receptor, which is related to the IL4R, is also expressed in a variety of solid tumors.<sup>242,243</sup> The recombinant fusion toxin IL13-PE38QQR is cytotoxic and showed antitumor efficacy toward a variety of tumor cell lines.<sup>112,113</sup> This molecule is currently undergoing phase I clinical testing in patients with metastatic renal cell carcinoma. In an interim report, out of 46 patients across 3 trials, histopathologic tumor effect was seen at drug concentrations of 0.5 to 2 µg/mL.<sup>114</sup>

### ***Targeting the Transferrin Receptor by Compartmental Administration***

Transferrin receptors are present on all normal cells that are actively taking up iron, particularly in the liver. Using a chemical conjugate containing human transferrin and a mutant form of DT,<sup>60</sup> Tf-CRM107 was infused directly into the tumors of 18 patients using catheters placed stereotactically. Two CRs and 7 PRs were documented in 15 evaluable patients.<sup>86</sup> In 6 of 9 patients who responded and in some of the nonresponders, tumors exhibited early central necrosis. There was evidence that the chimeric toxin escaped from the central nervous system, resulting in transient transaminase elevations, hypoalbuminemia, and an increase in anti-DT titer. At doses at or above 1 µg/mL, peritumoral brain

toxicity was observed, consisting of thrombosed cortical vessels, attributed to the presence of Transferrin receptor (TFR) on endothelial cells. In a phase II trial, Tf-CRM107 resulted in a 35% response rate<sup>244</sup> at the maximum tolerated dose (MTD), 0.66 µg/mL. One strategy for improving the safety of Tf-CRM107 is to coadminister chloroquine intravenously, which blocks the toxicity of DT toward endothelial cells that express TFR.<sup>245</sup> The use of this strategy showed promising results in an animal model.

### ***Targeting the Mesothelin Antigen on Solid Tumors***

Phage display technology was used to generate new Fvs binding to mesothelin, an antigen on mesotheliomas, ovarian and pancreatic carcinomas, and other tumors.<sup>115,246,247</sup> A recombinant immunotoxin was obtained that underwent affinity improvement, and the recombinant immunotoxin generated, SS1(dsFv)-PE38 (SS1P), was developed for systemic therapy of patients.<sup>248-252</sup> SS1P is now undergoing clinical testing.

### ***Targeting the N901 Antigen on Solid Tumors***

Over 10 years ago, N901-bR, targeting the small cell lung cancer (SCLC) antigen NCAM (also named CD56) was tested in patients with SCLC and found to induce 1 PR out of 19 patients and dose-limiting VLS.<sup>93,253</sup> The complete phase I report included 1 PR in 21 patients.<sup>94</sup> In a phase II trial of N901-bR after CR or near-CR from chemotherapy, 9 patients were treated, 1 with a long-term (>6-year) survival, but the trial was closed because of toxicity (VLS).<sup>254</sup>

### ***Recent Preclinical Development of Other Immunotoxins for Solid Tumors***

To target the urokinase receptor (also called uPAR or CD87), present on many types of hematologic<sup>255,256</sup> and solid<sup>257,258</sup> tumors, the amino terminal fragment (ATF) of urokinase was fused to PE38 and PE38KDEL.<sup>259</sup> The recombinant toxins ATF-PE38 and ATF-PE38KDEL were very cytotoxic toward leukemia cells and extremely cytotoxic toward glioblastoma multiforme cells. The recombinant toxin DT388-ATF (DTAT) was also produced and found to be cytotoxic.<sup>260-263</sup> The bispecific immunotoxin DTAT13 was produced and found to be toxic to both CD87 and IL13R+ cells.<sup>264</sup> Gelonin-containing immunotoxins have been developed to target gp240 on melanoma.<sup>265,266</sup> Immunotoxins targeting the high-molecular-weight melanoma antigen have been developed for both melanoma and glioblastoma multiforme.<sup>267,268</sup> To combat prostate cancer, toxins were targeted by antibodies to prostate-specific membrane antigen.<sup>269,270</sup> To target childhood sarcomas and neuroblastoma, recombinant immunotoxins were directed to a glycoprotein using MAb 8H9 and observed to cause antitumor activity in

SCID mice at doses that were tolerated in monkeys.<sup>271</sup> Activity in neuroblastoma was also reported, targeting GD<sub>2</sub> using the recombinant immunotoxin DT5F11.<sup>272</sup> Antitumor activity was observed with the recombinant anti-Ep-CAM immunotoxin 4D5MOCB-ETA', which is undergoing phase I testing in patients with squamous cell carcinoma of the head and neck.<sup>273</sup> Finally, in an elaborate use of immunotoxins, the gene for vascular endothelial growth factor fused to truncated DT or PE was introduced into T15 T cells that were raised to recognize leukemia cells.<sup>274</sup>

## PROBLEMS AND OPPORTUNITIES IN IMMUNOTOXIN DEVELOPMENT

There are challenges associated with the development of many immunotoxins for cancer therapy. Several of these problems, including immunogenicity, unwanted toxicity, difficulty in production, limited half-life, and resistance, will be considered below, along with potential opportunities for improved development of immunotoxins.

### *Immunogenicity*

Based on a wide range of clinical trials, the incidence of immunogenicity after a single cycle of immunotoxin ranges from 50% to 100% for solid tumors, and from 0% to 40% for hematologic tumors. Patients have been reported to respond to some fusion toxins after immunogenicity is detected,<sup>128</sup> but in these cases antitoxin antibodies are detected by enzyme-linked immunosorbent assay and are probably not neutralizing. Antibodies that are neutralizing can be detected by determining whether serum containing them can block the cytotoxicity of the immunotoxin toward cultured cells. The presence of neutralizing antibodies lowers the levels of biologically active immunotoxin and compromises efficacy. Several approaches can be used to prevent immunogenicity. The method most useful for other biologic agents, such as interferon<sup>275</sup> and L-asparaginase,<sup>276</sup> is PEGylation, which not only blocks immunogenicity but also prolongs half-life. Limited success has been achieved in preclinical studies of a PEGylated form of LMB-2.<sup>277,278</sup> PEGylating a toxin appears much more challenging than PEGylating simpler molecules, since disturbing sites on a toxin reduces toxin activity. Immunologic studies have found a large number of B-cell and T-cell epitopes on *Pseudomonas* exotoxin,<sup>279-281</sup> suggesting that "humanization" of the molecule would be extremely difficult. Agents to non-specifically suppress the immune response, such as deoxyspergualin<sup>282</sup> and CTLA4Ig,<sup>283,284</sup> have shown efficacy in preclinical models but have not been tested clinically. Rituximab has proven ineffective in preventing immunogenicity in patients receiving LMB-1.<sup>285</sup> Nevertheless, it is noteworthy that no patients with CLL have ever produced neutralizing antibodies to LMB-2 or BL22. This suggests that

artificial replication of the humoral immune deficiency in CLL, from either treatment or disease, might prevent the immunogenicity of immunotoxins.

### *Unwanted Toxicity*

A variety of toxicities have been observed with immunotoxins that have limited the dose and hence the efficacy. The most common toxicity is VLS, which is not surprising, given that a cytotoxic protein must traverse endothelial cells to exit the blood vessels. Studies have shown that RTA binds directly to endothelial cells, while truncated PE requires a ligand that cross-reacts with the endothelium.<sup>225</sup> Other studies have suggested that specific residues on RTA and also truncated PE and IL-2 can bind to endothelial cells and can elicit VLS by a mechanism independent of the normal toxin-induced cell death.<sup>286,287</sup> Such studies led to a mutant form of RTA that shows less VLS in an animal model.<sup>168</sup> Hepatotoxicity, a typical side effect of recombinant immunotoxins, is attributed to the binding of basic residues on the Fv to negatively charged hepatic cells.<sup>152,288</sup> Hepatotoxicity appears to be related to cytokine production, possibly by the Kupffer cells of the liver.<sup>153</sup> Although recombinant immunotoxins that specifically bind to antigens expressed on the liver are not well tolerated systemically,<sup>109</sup> recombinant immunotoxins like LMB-2 and BL22 that cause transaminase elevations are not associated with decreased hepatic function.<sup>98,100</sup> Renal toxicity due to immunotoxins is less well defined and could be nonspecific at least in part because the kidneys are the dominant route of excretion of recombinant immunotoxin.<sup>142</sup>

### *Difficulty in Production*

Originally, chemical conjugates were made for clinical trials since manufacturers of recombinant toxins faced problems of endotoxin contamination and low yield. Advances in the production of other recombinant proteins for clinical use have solved many of these problems and have allowed large-scale production of recombinant toxins with high purity and reasonable cost. It is anticipated that corporate development will further improve yield and cut costs.

### *Potential for Future Development*

For many types of disease, immunotoxins are unlikely to work by themselves. Their half-lives may be too limited for diffusion to occur into solid tumor masses. Clinical trials of immunotoxins administered by continuous infusion have thus far not found significant improvements in efficacy over the bolus infusion route.<sup>81,289,290</sup> It is possible that combination with other therapeutic agents having nonoverlapping toxicities will result in better responses. Similarly, treatment of microscopic disease may be useful after cytoreduction by surgery, chemotherapy, or radiotherapy. Finally, the antigen

and disease targeted remain major determinants of immunotoxin efficacy and resistance. As combinations of diseases and antigen targets are chosen, we can anticipate exciting successes in the future development of immunotoxins.

## CONCLUSIONS

In the past 3 to 4 decades, a wide variety of immunotoxins have been tested against a wide variety of malignancies in cell culture, in animal models, and in patients. The most useful of these agents appear to be the relatively small recombinant fusion toxins that contain either growth factor or Fv fragments as ligands. The most sensitive diseases appear to be hematologic malignancies. Future development will need to address combinations of immunotoxins with other anticancer therapies in order to overcome problems of tumor penetration, toxicity, and immunogenicity.

## ACKNOWLEDGMENTS

This work was supported by the intramural program of the National Cancer Institute.

## REFERENCES

1. Moolten FL, Cooperband SR. Selective destruction of target cells by diphtheria toxin conjugated to antibody directed against antigens on the cells. *Science*. 1970;169:68-70.
2. Krolick KA, Villemez C, Isakson P, Uhr JW, Vitetta ES. Selective killing of normal or neoplastic B cells by antibodies coupled to the A chain of ricin. *Proc Natl Acad Sci USA*. 1980;77:5419-5423.
3. Cawley DB, Herschman HR, Gilliland DG, Collier RJ. Epidermal growth factor-toxin A chain conjugates: EGF-ricin A is a potent toxin while EGF-diphtheria fragment A is nontoxic. *Cell*. 1980;22:563-570.
4. Akhtar S, Maghfoor I. Rituximab plus CHOP for diffuse large-B-cell lymphoma. *N Engl J Med*. 2002;346:1830-1831.
5. Keating MJ, Flinn I, Jain V, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. *Blood*. 2002;99:3554-3561.
6. Cheson B. Bexxar (Corixa/GlaxoSmithKline). *Curr Opin Investig Drugs*. 2002;3:165-170.
7. Nabhan C, Tallman MS. Early phase I/II trials with gemtuzumab ozogamicin (Mylotarg(R)) in acute myeloid leukemia. *Clin Lymphoma*. 2002;2:S19-S23.
8. Francisco JA, Cerveny CG, Meyer DL, et al. cAC10-vcMMAE, an anti-CD30-monomethyl auristatin E conjugate with potent and selective antitumor activity. *Blood*. 2003;102:1458-1465.
9. Naito K, Takeshita A, Shigeno K, et al. Calicheamicin-conjugated humanized anti-CD33 monoclonal antibody (Gemtuzumab zogamicin, CMA-676) shows cytotoxic effect on CD33-positive leukemia cell lines, but is inactive on P-glycoprotein-expressing sublines. *Leukemia*. 2000;14:1436-1443.

10. Hursey M, Newton DL, Hansen HJ, Ruby D, Goldenberg DM, Rybak SM. Specifically targeting the CD22 receptor of human B-cell lymphomas with RNA damaging agents: a new generation of therapeutics. *Leuk Lymphoma*. 2002;43:953-959.
11. Yamaizumi M, Mekada E, Uchida T, Okada Y. One molecule of diphtheria toxin fragment A introduced into a cell can kill the cell. *Cell*. 1978;15:245-250.
12. Eiklid K, Olsnes S, Pihl A. Entry of lethal doses of abrin, ricin and modeccin into the cytosol of HeLa cells. *Exp Cell Res*. 1980;126:321-326.
13. Carroll SF, Collier RJ. Active site of *Pseudomonas aeruginosa* exotoxin A. Glutamic acid 553 is photolabeled by NAD and shows functional homology with glutamic acid 148 of diphtheria toxin. *J Biol Chem*. 1987;262:8707-8711.
14. Bolognesi A, Polito L, Tazzari PL, et al. In vitro anti-tumour activity of anti-CD80 and anti-CD86 immunotoxins containing type 1 ribosome-inactivating proteins. *Br J Haematol*. 2000;110:351-361.
15. Endo Y, Mitsui K, Motizuki M, Tsurugi K. The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes. *J Biol Chem*. 1987;262:5908-5912.
16. Bolognesi A, Tazzari PL, Olivieri F, Polito L, Falini B, Stirpe F. Induction of apoptosis by ribosome-inactivating proteins and related immunotoxins. *Int J Cancer*. 1996;68:349-355.
17. Hughes JN, Lindsay CD, Griffiths GD. Morphology of ricin and abrin exposed endothelial cells is consistent with apoptotic cell death. *Hum Exp Toxicol*. 1996;15:443-451.
18. Bergamaschi G, Perfetti V, Tonon L, et al. Saporin, a ribosome-inactivating protein used to prepare immunotoxins, induces cell death via apoptosis. *Br J Haematol*. 1996;93:789-794.
19. Wesche J, Rapak A, Olsnes S. Dependence of ricin toxicity on translocation of the toxin A-chain from the endoplasmic reticulum to the cytosol. *J Biol Chem*. 1999;274:34443-34449.
20. Tagge E, Harris B, Burbage C, et al. Synthesis of green fluorescent protein-ricin and monitoring of its intracellular trafficking. *Bioconjug Chem*. 1997;8:743-750.
21. Fulton RJ, Uhr JW, Vitetta ES. In vivo therapy of the BCL1 tumor: effect of immunotoxin valency and deglycosylation of the ricin A chain. *Cancer Res*. 1988;48:2626-2631.
22. Bourrie BJ, Casellas P, Blythman HE, Jansen FK. Study of the plasma clearance of antibody-ricin-A-chain immunotoxins. Evidence for specific recognition sites on the A chain that mediate rapid clearance of the immunotoxin. *Eur J Biochem*. 1986;155:1-10.
23. Blakey DC, Watson GJ, Knowles PP, Thorpe PE. Effect of chemical deglycosylation of ricin A chain on the in vivo fate and cytotoxic activity of an immunotoxin composed of ricin A chain and anti-Thy 1.1 antibody. *Cancer Res*. 1987;47:947-952.
24. Thorpe PE, Wallace PM, Knowles PP, et al. Improved antitumor effects of immunotoxins prepared with deglycosylated ricin A-chain and hindered disulfide linkages. *Cancer Res*. 1988;48:6396-6403.
25. Ramakrishnan S, Bjorn MJ, Houston LL. Recombinant ricin A chain conjugated to monoclonal antibodies: improved tumor cell inhibition in the presence of lysosomotropic compounds. *Cancer Res*. 1989;49:613-617.
26. Lambert JM, McIntyre G, Gauthier MN, et al. The galactose-binding sites of the cytotoxic lectin ricin can be chemically blocked in high yield with reactive ligands prepared by chemical modification of glycopeptides containing triantennary N-linked oligosaccharides. *Biochemistry*. 1991;30:3234-3247.

27. Mohanraj D, Ramakrishnan S. Cytotoxic effects of ricin without an interchain disulfide bond: genetic modification and chemical crosslinking studies. *Biochim Biophys Acta*. 1995;1243:399-406.
28. Kreitman RJ, Chaudhary VK, Siegall CB, FitzGerald DJ, Pastan I. Rational design of a chimeric toxin: an intramolecular location for the insertion of transforming growth factor  $\alpha$  within *Pseudomonas* exotoxin A as a targeting ligand. *Bioconjug Chem*. 1992;3:58-62.
29. Cook JP, Savage PM, Lord JM, Roberts LM. Biologically active interleukin-2-ricin A chain fusion proteins may require intracellular proteolytic cleavage to exhibit a cytotoxic effect. *Bioconjug Chem*. 1993;4:440-447.
30. Dore JM, Gras E, Wijdenes J. Expression and activity of a recombinant chimeric protein composed of pokeweed antiviral protein and of human interleukin-2. *FEBS Lett*. 1997;402:50-52.
31. Francisco JA, Gawlak SL, Siegall CB. Construction, expression, and characterization of BD1-G28-5 sFv, a single-chain anti-CD40 immunotoxin containing the ribosome-inactivating protein bryodin 1. *J Biol Chem*. 1997;272:24165-24169.
32. Fabbrini MS, Carpani D, Bello-Rivero I, Soria MR. The amino-terminal fragment of human urokinase directs a recombinant chimeric toxin to target cells: internalization is toxin mediated. *FASEB J*. 1997;11:1169-1176.
33. Tetzke TA, Caton MC, Maher PA, Parandoosh Z. Effect of fibroblast growth factor saporin mitotoxins on human bladder cell lines. *Clin Exp Metastasis*. 1997;15:620-629.
34. Kreitman RJ. Getting plant toxins to fuse. *Leuk Res*. 1997;21:997-999.
35. Phan LD, Perentesis JP, Bodley JW. Saccharomyces cerevisiae elongation factor 2. Mutagenesis of the histidine precursor of diphthamide yields a functional protein that is resistant to diphtheria toxin. *J Biol Chem*. 1993;268:8665-8668.
36. Hwang J, FitzGerald DJ, Adhya S, Pastan I. Functional domains of *Pseudomonas* exotoxin identified by deletion analysis of the gene expressed in *E. coli*. *Cell*. 1987;48:129-136.
37. Allured VS, Collier RJ, Carroll SF, McKay DB. Structure of exotoxin A of *Pseudomonas aeruginosa* at 3.0 Angstrom resolution. *Proc Natl Acad Sci USA*. 1986;83:1320-1324.
38. Ogata M, Fryling CM, Pastan I, FitzGerald DJ. Cell-mediated cleavage of *Pseudomonas* exotoxin between Arg<sup>279</sup> and Gly<sup>280</sup> generates the enzymatically active fragment which translocates to the cytosol. *J Biol Chem*. 1992;267:25396-25401.
39. Uchida T, Jr, Pappenheimer AM, Jr, Harper AA. Reconstitution of diphtheria toxin from two nontoxic cross-reacting mutant proteins. *Science*. 1972;175:901-903.
40. Rolf JM, Gaudin HM, Eidels L. Localization of the diphtheria toxin receptor-binding domain to the carboxyl-terminal M<sub>r</sub> ~ 6000 region of the toxin. *J Biol Chem*. 1990;265:7331-7337.
41. Kaul P, Silverman J, Shen WH, et al. Roles of Glu 349 and Asp 352 in membrane insertion and translocation by diphtheria toxin. *Protein Sci*. 1996;5:687-692.
42. Li M, Dyda F, Benhar I, Pastan I, Davies DR. Crystal structure of the catalytic domain of *Pseudomonas* exotoxin A complexed with a nicotinamide adenine dinucleotide analog: implications for the activation process and for ADP ribosylation. *Proc Natl Acad Sci USA*. 1996;93:6902-6906.
43. Hessler JL, Kreitman RJ. An early step in *Pseudomonas* exotoxin action is removal of the terminal lysine residue, which allows binding to the KDEL receptor. *Biochemistry*. 1997;36:14577-14582.
44. Kounnas MZ, Morris RE, Thompson MR, FitzGerald DJ, Strickland DK, Saelinger CB. The  $\alpha$ 2-macroglobulin receptor/low density lipoprotein receptor-related protein binds and internalizes *Pseudomonas* exotoxin A. *J Biol Chem*. 1992;267:12420-12423.
45. Chiron MF, Fryling CM, FitzGerald DJ. Cleavage of *Pseudomonas* exotoxin and diphtheria toxin by a furin-like enzyme prepared from beef liver. *J Biol Chem*. 1994;269:18167-18176.
46. McKee ML, FitzGerald DJ. Reduction of furin-nicked *Pseudomonas* exotoxin A: an unfolding story. *Biochemistry*. 1999;38:16507-16513.
47. Chaudhary VK, Jinno Y, FitzGerald D, Pastan I. *Pseudomonas* exotoxin contains a specific sequence at the carboxyl terminus that is required for cytotoxicity. *Proc Natl Acad Sci USA*. 1990;87:308-312.
48. Kreitman RJ, Pastan I. Importance of the glutamate residue of KDEL in increasing the cytotoxicity of *Pseudomonas* exotoxin derivatives and for increased binding to the KDEL receptor. *Biochem J*. 1995;307:29-37.
49. Theuer C, Kasturi S, Pastan I. Domain II of *Pseudomonas* exotoxin A arrests the transfer of translocating nascent chains into mammalian microsomes. *Biochemistry*. 1994;33:5894-5900.
50. Theuer CP, Buchner J, FitzGerald D, Pastan I. The N-terminal region of the 37-kDa translocated fragment of *Pseudomonas* exotoxin A aborts translocation by promoting its own export after microsomal membrane insertion. *Proc Natl Acad Sci USA*. 1993;90:7774-7778.
51. Keppler-Hafkemeyer A, Kreitman RJ, Pastan I. Apoptosis induced by immunotoxins used in the treatment of hematologic malignancies. *Int J Cancer*. 2000;87:86-94.
52. Brinkmann U, Brinkmann E, Gallo M, Pastan I. Cloning and characterization of a cellular apoptosis susceptibility gene, the human homologue to the yeast chromosome segregation gene CSE1. *Proc Natl Acad Sci USA*. 1995;92:10427-10431.
53. Choe S, Bennett MJ, Fujii G, et al. The crystal structure of diphtheria toxin. *Nature*. 1992;357:216-222.
54. Bell CE, Eisenberg D. Crystal structure of diphtheria toxin bound to nicotinamide adenine dinucleotide. *Biochemistry*. 1996;35:1137-1149.
55. Williams DP, Wen Z, Watson RS, Boyd J, Strom TB, Murphy JR. Cellular processing of the interleukin-2 fusion toxin DAB<sub>486</sub>-IL-2 and efficient delivery of diphtheria fragment A to the cytosol of target cells requires Arg<sup>194</sup>. *J Biol Chem*. 1990;265:20673-20677.
56. D'Silva PR, Lala AK. Unfolding of diphtheria toxin: identification of hydrophobic sites exposed on lowering of pH by photolabeling. *J Biol Chem*. 1998;273:16216-16222.
57. Lemichez E, Bomsel M, Devilliers G, et al. Membrane translocation of diphtheria toxin fragment A exploits early to late endosome trafficking machinery. *Mol Microbiol*. 1997;23:445-457.
58. Wilson BA, Blanke SR, Reich KA, Collier RJ. Active-site mutations of diphtheria toxin. Tryptophan 50 is a major determinant of NAD affinity. *J Biol Chem*. 1994;269:23296-23301.
59. Bennett MJ, Eisenberg D. Refined structure of monomeric diphtheria toxin at 2.3 Å resolution. *Protein Sci*. 1994;3:1464-1475.
60. Greenfield L, Johnson VG, Youle RJ. Mutations in diphtheria toxin separate binding from entry and amplify immunotoxin selectivity. *Science*. 1987;238:536-539.
61. Kondo T, FitzGerald D, Chaudhary VK, Adhya S, Pastan I. Activity of immunotoxins constructed with modified

- Pseudomonas* exotoxin A lacking the cell recognition domain. *J Biol Chem*. 1988;263:9470-9475.
62. Williams DP, Parker K, Bacha P, et al. Diphtheria toxin receptor binding domain substitution with interleukin-2: genetic construction and properties of a diphtheria toxin-related interleukin-2 fusion protein. *Protein Eng*. 1987;1:493-498.
63. Siegall CB, Chaudhary VK, FitzGerald DJ, Pastan I. Functional analysis of domains II, Ib, and III of *Pseudomonas* exotoxin. *J Biol Chem*. 1989;264:14256-14261.
64. Kreitman RJ, Batra JK, Seetharam S, Chaudhary VK, FitzGerald DJ, Pastan I. Single-chain immunotoxin fusions between anti-Tac and *Pseudomonas* exotoxin: relative importance of the two toxin disulfide bonds. *Bioconjug Chem*. 1993;4:112-120.
65. Williams DP, Snider CE, Strom TB, Murphy JR. Structure/function analysis of interleukin-2-toxin (DAB<sub>486</sub>-IL-2). Fragment B sequences required for the delivery of fragment A to the cytosol of target cells. *J Biol Chem*. 1990;265:11885-11889.
66. Chaudhary VK, FitzGerald DJ, Pastan I. A proper amino terminus of diphtheria toxin is important for cytotoxicity. *Biochem Biophys Res Commun*. 1991;180:545-551.
67. Seetharam S, Chaudhary VK, FitzGerald D, Pastan I. Increased cytotoxic activity of *Pseudomonas* exotoxin and two chimeric toxins ending in KDEL. *J Biol Chem*. 1991;266:17376-17381.
68. Theuer CP, Kreitman RJ, FitzGerald DJ, Pastan I. Immunotoxins made with a recombinant form of *Pseudomonas* exotoxin A that do not require proteolysis for activity. *Cancer Res*. 1993;53:340-347.
69. van Oosterhout YV, van Emst JL, Bakker HH, et al. Production of anti-CD3 and anti-CD7 ricin A-immunotoxins for a clinical pilot study. *Int J Pharm*. 2001;221:175-186.
70. Pai LH, Wittes R, Setser A, Willingham MC, Pastan I. Treatment of advanced solid tumors with immunotoxin LMB-1: an antibody linked to *Pseudomonas* exotoxin. *Nat Med*. 1996;2:350-353.
71. Kreitman RJ, Pastan I. Purification and characterization of IL6-PE<sup>4E</sup>, a recombinant fusion of interleukin 6 with *Pseudomonas* exotoxin. *Bioconjug Chem*. 1993;4:581-585.
72. Kreitman RJ, Pastan I. Making fusion toxins to target leukemia and lymphoma. In: Francis GE, Delgado C, eds. *Drug Targeting: Strategies, Principles, and Applications*. Totowa, NJ: Humana Press Inc; 2000:215-227. *Methods in Molecular Medicine*; vol 25.
73. Buchner J, Pastan I, Brinkmann U. A method for increasing the yield of properly folded recombinant fusion proteins: single-chain immunotoxins from renaturation of bacterial inclusion bodies. *Anal Biochem*. 1992;205:263-270.
74. Shao Y, Warman BE, Perentesis JP. Recombinant fusion toxins directed against the human granulocyte-macrophage colony stimulating factor (GM-CSF) receptor. *Methods Mol Biol*. 2001;166:31-53.
75. Choo AB, Dunn RD, Broady KW, Raison RL. Soluble expression of a functional recombinant cytolytic immunotoxin in insect cells. *Protein Expr Purif*. 2002;24:338-347.
76. Woo JH, Liu YY, Stavrou S, Neville DM. Increasing secretion of a bivalent anti-T-cell immunotoxin by *Pichia pastoris*. *Appl Environ Microbiol*. 2004;70:3370-3376.
77. Van Horssen PJ, Preijers FW, Van Oosterhout YV, De Witte TD. Highly potent CD22-recombinant ricin A results in complete cure of disseminated malignant B-cell xenografts in SCID mice but fails to cure solid xenografts in nude mice. *Int J Cancer*. 1996;68:378-383.
78. Engert A, Diehl V, Schnell R, et al. A phase-I study of an anti-CD25 ricin A-chain immunotoxin (RFT5-SMPT-dgA) in patients with refractory Hodgkin's lymphoma. *Blood*. 1997;89:403-410.
79. Schnell R, Vitetta E, Schindler J, et al. Treatment of refractory Hodgkin's lymphoma patients with an anti-CD25 ricin A-chain immunotoxin. *Leukemia*. 2000;14:129-135.
80. Amlot PL, Stone MJ, Cunningham D, et al. A phase I study of an anti-CD22-dglycosylated ricin A chain immunotoxin in the treatment of B-cell lymphomas resistant to conventional therapy. *Blood*. 1993;82:2624-2633.
81. Sausville EA, Headlee D, Stetler-Stevenson M, et al. Continuous infusion of the anti-CD22 immunotoxin IgG-RFB4-SMPT-dgA in patients with B-cell lymphoma: a phase I study. *Blood*. 1995;85:3457-3465.
82. Vitetta ES, Stone M, Amlot P, et al. Phase I immunotoxin trial in patients with B-cell lymphoma. *Cancer Res*. 1991;51:4052-4058.
83. Stone MJ, Sausville EA, Fay JW, et al. A phase I study of bolus versus continuous infusion of the anti-CD19 immunotoxin, IgG-HD37-dgA, in patients with B-cell lymphoma. *Blood*. 1996;88:1188-1197.
84. Frankel AE, Laver JH, Willingham MC, Burns LJ, Kersey JH, Vallera DA. Therapy of patients with T-cell lymphomas and leukemias using an anti-CD7 monoclonal antibody-ricin A chain immunotoxin. *Leuk Lymphoma*. 1997;26:287-298.
85. Schnell R, Staak O, Borchmann P, et al. 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*. 2002;8:1779-1786.
86. Laske DW, Youle RJ, Oldfield EH. Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors. *Nat Med*. 1997;3:1362-1368.
87. Uckun F. Immunotoxins for the treatment of leukaemia. *Br J Haematol*. 1993;85:435-438.
88. Multani PS, O'Day S, Nadler LM, Grossbard ML. 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*. 1998;4:2599-2604.
89. Grossbard ML, Niedzwiecki D, Nadler LM, et al. Anti-B4-blocked ricin (Anti-B4bR) adjuvant therapy post-autologous bone marrow transplant (ABMT) (CALGB 9254): a phase III intergroup study. *Proc Am Soc Clin Oncol*. 1998;17:3a.
90. O'Toole JE, Esseltine D, Lynch TJ, Lambert JM, Grossbard ML. Clinical trials with blocked ricin immunotoxins. *Curr Top Microbiol Immunol*. 1998;234:35-56.
91. Winkler U, Barth S, Schnell R, Diehl V, Engert A. The emerging role of immunotoxins in leukemia and lymphoma. *Ann Oncol*. 1997;8:139-146.
92. Laske DW, Muraszko KM, Oldfield EH, et al. Intraventricular immunotoxin therapy for leptomeningeal neoplasia. *Neurosurgery*. 1997;41:1039-1049.
93. Epstein C, Lynch T, Shefner J, et al. Use of the immunotoxin N901-blocked ricin in patients with small-cell lung cancer. *Int J Cancer*. 1994;8:57-59.
94. Lynch TJ, Lambert JM, Coral F, et al. Immunotoxin therapy of small-cell lung cancer: a phase I study of N901-blocked ricin. *J Clin Oncol*. 1997;15:723-734.
95. Foss FM, Bacha P, Osann KE, Demierre MF, Bell T, Kuzel T. Biological correlates of acute hypersensitivity events with DAB(389)IL-2 (denileukin diftitox, ONTAK) in cutaneous T-cell lymphoma: decreased frequency and severity with steroid premedication. *Clin Lymphoma*. 2001;1:298-302.



96. Frankel AE, Fleming DR, Hall PD, et al. A phase II study of DT fusion protein denileukin diftitox in patients with fludarabine-refractory chronic lymphocytic leukemia. *Clin Cancer Res*. 2003;9:3555-3561.
97. Dang NH, Hagemester FB, Pro B, et al. Phase II study of denileukin diftitox for relapsed/refractory B-cell non-Hodgkin's lymphoma. *J Clin Oncol*. 2004;22:4095-4102.
98. Kreitman RJ, Wilson WH, Bergeron K, et al. Efficacy of the anti-CD22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. *N Engl J Med*. 2001;345:241-247.
99. Kreitman RJ, Wilson WH, Robbins D, et al. Responses in refractory hairy cell leukemia to a recombinant immunotoxin. *Blood*. 1999;94:3340-3348.
100. Kreitman RJ, Wilson WH, White JD, et al. Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. *J Clin Oncol*. 2000;18:1622-1636.
101. Frankel AE, Powell BL, Hall PD, Case LD, Kreitman RJ. 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*. 2002;8:1004-1013.
102. Pai-Scherf LH, Kreitman RJ, Pastan I. Monoclonal antibodies: basic principles. Immunotoxins and recombinant immunotoxins. In: Rosenberg SA, ed. *Principles and Practice of the Biologic Therapy of Cancer*. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2000:382-395.
103. Benhar I, Reiter Y, Pai LH, Pastan I. Administration of disulfide-stabilized Fv-immunotoxins B1(dsFv)-PE38 and B3(dsFv)-PE38 by continuous infusion increases their efficacy in curing large tumor xenografts in nude mice. *Int J Cancer*. 1995;62:351-355.
104. Goldberg MR, Heimbrook DC, Russo P, et al. Phase I clinical study of recombinant oncotoxin TP40 in superficial bladder cancer. *Clin Cancer Res*. 1995;1:57-61.
105. Sampson JH, Akabani G, Archer GE, et al. Progress report of a Phase I study of the intracerebral microinfusion of a recombinant chimeric protein composed of transforming growth factor (TGF)-alpha and a mutated form of the Pseudomonas exotoxin termed PE-38 (TP-38) for the treatment of malignant brain tumors. *J Neurooncol*. 2003;65:27-35.
106. Posey JA, Khazaeli MB, Bookman MA, et al. Phase I trial of the single-chain immunotoxin SGN-10 (BR96 sFv-PE40) in patients with advanced solid tumors. *Clin Cancer Res*. 2002;8:3092-3099.
107. Haggerty HG, Warner WA, Comereski CR, et al. BR96 sFv-PE40 immunotoxin: nonclinical safety assessment. *Toxicol Pathol*. 1999;27:87-94.
108. Damle B, Tay L, Comereski C, Warner W, Kaul S. Influence of immunogenicity on the pharmacokinetics of BMS-191352, a Pseudomonas exotoxin immunoconjugate, in rats and dogs. *J Pharm Pharmacol*. 2000;52:671-678.
109. Pai-Scherf LH, Villa J, Pearson D, et al. Hepatotoxicity in cancer patients receiving erb-38, a recombinant immunotoxin that targets the erbB2 receptor. *Clin Cancer Res*. 1999;5:2311-2315.
110. Rand RW, Kreitman RJ, Patronas N, Varricchio F, Pastan I, Puri RK. Intratumoral administration of a recombinant circularly permuted interleukin-4-Pseudomonas exotoxin in patients with high grade glioma. *Clin Cancer Res*. 2000;6:2157-2165.
111. Weber FW, Floeth F, Asher A. Local convection enhanced delivery of IL4-Pseudomonas exotoxin (NBI-3001) for treatment of patients with recurrent malignant glioma. In: Westphal M, Tonn JC, Ram Z, eds. *Local Therapies for Glioma: Present Status and Future Developments*. Vienna, Austria: Springer-Verlag Wien; 2003:93-103.
112. Husain SR, Puri RK. Interleukin-13 receptor as a specific molecular target for cytotoxin therapy of human renal cell carcinoma in a xenograft model. *Clin Cancer Res*. 1999;5:3766s.
113. Puri RK, Leland P, Obiri NI, et al. Targeting of interleukin-13 receptor on human renal cell carcinoma cells by a recombinant chimeric protein composed of interleukin-13 and a truncated form of Pseudomonas exotoxin A (PE38QQR). *Blood*. 1996;87:4333-4339.
114. Kunwar S. Convection enhanced delivery of IL13-PE38QQR for treatment of recurrent malignant glioma: presentation of interim findings from ongoing phase I studies. In: Westphal M, Tonn JC, Ram Z, eds. *Local Therapies for Glioma: Present Status and Future Developments*. Vienna, Austria: Springer-Verlag Wien; 2003:105-111.
115. Chowdhury PS, Viner JL, Beers R, Pastan I. Isolation of a high-affinity stable single-chain Fv specific for mesothelin from DNA-immunized mice by phage display and construction of a recombinant immunotoxin with anti-tumor activity. *Proc Natl Acad Sci USA*. 1998;95:669-674.
116. Foss FM, Saleh MN, Krueger JG, Nichols JC, Murphy JR. Diphtheria toxin fusion proteins. In: Frankel AE, ed. *Clinical Applications of Immunotoxins*. Berlin, Germany: Springer-Verlag; 1998:63-81.
117. Taniguchi T, Minami Y. The IL2/IL-2 receptor system: a current overview. *Cell*. 1993;73:5-8.
118. Kodaka T, Uchiyama T, Ishikawa T, et al. Interleukin-2 receptor beta-chain (p70-75) expressed on leukemic cells from adult T cell leukemia patients. *Jpn J Cancer Res*. 1990;81:902-908.
119. Yagura H, Tamaki T, Furitsu T, et al. Demonstration of high-affinity interleukin-2 receptors on B-chronic lymphocytic leukemia cells: functional and structural characterization. *Blut*. 1990;60:181-186.
120. Kreitman RJ, Pastan I. Recombinant single-chain immunotoxins against T and B cell leukemias. *Leuk Lymphoma*. 1994;13:1-10.
121. Strauchen JA, Breakstone BA. IL-2 receptor expression in human lymphoid lesions. *Am J Pathol*. 1987;126:506-512.
122. Robb RJ, Greene WC, Rusk CM. Low and high affinity cellular receptors for interleukin 2. *J Exp Med*. 1984;160:1126-1146.
123. Bacha P, Williams DP, Waters C, Williams JM, Murphy JR, Strom TB. Interleukin 2 receptor-targeted cytotoxicity: interleukin 2 receptor-mediated action of a diphtheria toxin-related interleukin 2 fusion protein. *J Exp Med*. 1988;167:612-622.
124. LeMaistre CF, Rosenblum MG, Reuben JM, et al. Therapeutic effects of genetically engineered toxin (DAB<sub>486</sub>IL-2) in patient with chronic lymphocytic leukaemia. *Lancet*. 1991;337:1124-1125.
125. LeMaistre CF, Meneghetti C, Rosenblum M, et al. Phase I trial of an interleukin-2 (IL-2) fusion toxin (DAB<sub>486</sub>IL-2) in hematologic malignancies expressing the IL-2 receptor. *Blood*. 1992;79:2547-2554.
126. LeMaistre CF, Craig FE, Meneghetti C, et al. Phase I trial of a 90-minute infusion of the fusion toxin DAB<sub>486</sub>IL-2 in hematological cancers. *Cancer Res*. 1993;53:3930-3934.
127. LeMaistre CF, Saleh MN, Kuzel TM, et al. Phase I trial of a ligand fusion-protein (DAB<sub>389</sub>IL-2) in lymphomas expressing the receptor for interleukin-2. *Blood*. 1998;91:399-405.
128. Olsen E, Duvic M, Frankel A, et al. Pivotal phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma. *J Clin Oncol*. 2001;19:376-388.
129. Duvic M, Kuzel TM, Olsen E, Martin AG. Quality-of-life improvements in cutaneous T-cell lymphoma patients treated with Denileukin Diftitox (ONTAK). *Clin Lymphoma*. 2002;2:222-228.

130. Railan D, Fivenson DP, Wittenberg G. Capillary leak syndrome in a patient treated with interleukin 2 fusion toxin for cutaneous T-cell lymphoma. *J Am Acad Dermatol.* 2000;43:323-324.
131. Duvic M. Bexarotene and DAB{389}IL-2 (Denileukin diftitox, ONTAK) in treatment of cutaneous t-cell lymphomas: algorithms. *Clin Lymphoma.* 2000;1:S51-S55.
132. Talpur R, Apisarnthanarax N, Ward S, Duvic M. Treatment of refractory peripheral T-cell lymphoma with denileukin diftitox (ONTAK). *Leuk Lymphoma.* 2002;43:121-126.
133. McGinnis KS, Shapiro M, Junkins-Hopkins JM, et al. Denileukin diftitox for the treatment of panniculitic lymphoma. *Arch Dermatol.* 2002;138:740-742.
134. Martin A, Gutierrez E, Muglia J, et al. A multicenter dose-escalation trial with denileukin diftitox (ONTAK, DAB(389)IL-2) in patients with severe psoriasis. *J Am Acad Dermatol.* 2001;45:871-881.
135. Shao RH, Tian X, Gorgun G, Urbano AG, Foss FM. Arginine butyrate increases the cytotoxicity of DAB(389)IL-2 in leukemia and lymphoma cells by upregulation of IL-2Rbeta gene. *Leuk Res.* 2002;26:1077-1083.
136. Gorgun G, Foss F. Immunomodulatory effects of RXR rexinoids: modulation of high-affinity IL-2R expression enhances susceptibility to denileukin diftitox. *Blood.* 2002;100:1399-1403.
137. Uchiyama T, Broder S, Waldmann TA. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells, I: production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J Immunol.* 1981;126:1393-1397.
138. Weissman AM, Harford JB, Svetlik PB, et al. Only high-affinity receptors for interleukin 2 mediate internalization of ligand. *Proc Natl Acad Sci USA.* 1986;83:1463-1466.
139. Chaudhary VK, Gallo MG, FitzGerald DJ, Pastan I. A recombinant single-chain immunotoxin composed of anti-Tac variable regions and a truncated diphtheria toxin. *Proc Natl Acad Sci USA.* 1990;87:9491-9494.
140. Kreitman RJ, Pastan I. Accumulation of a recombinant immunotoxin in a tumor in vivo: fewer than 1000 molecules per cell are sufficient for complete responses. *Cancer Res.* 1998;58:968-975.
141. Bird RE, Hardman KD, Jacobson JW, et al. Single-chain antigen-binding proteins. *Science.* 1988;242:423-426.
142. Huston JS, Levinson D, Mudgett-Hunter M, et al. Protein engineering of antibody binding sites: recovery of specific activity in an antidigoxin single-chain Fv analogue produced in *Escherichia coli*. *Proc Natl Acad Sci USA.* 1988;85:5879-5883.
143. Chaudhary VK, Queen C, Junghans RP, Waldmann TA, FitzGerald DJ, Pastan I. A recombinant immunotoxin consisting of two antibody variable domains fused to *Pseudomonas* exotoxin. *Nature.* 1989;339:394-397.
144. Kobayashi H, Kao CK, Kreitman RJ, et al. Pharmacokinetics of In-111- and I-125-labeled antiTac single-chain Fv recombinant immunotoxin. *J Nucl Med.* 2000;41:755-762.
145. Kreitman RJ, Bailon P, Chaudhary VK, FitzGerald DJ, Pastan I. 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.* 1994;83:426-434.
146. Kreitman RJ, Pastan I. Targeting *Pseudomonas* exotoxin to hematologic malignancies. *Semin Cancer Biol.* 1995;6:297-306.
147. Robbins DH, Margulies I, Stetler-Stevenson M, Kreitman RJ. 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.* 2000;6:693-700.
148. Decker T, Hipp S, Kreitman RJ, Pastan I, Peschel C, Licht T. Sensitization of B-CLL cells to recombinant immunotoxin by immunostimulatory phosphorothioate oligonucleotides. *Blood.* 2002;99:1320-1326.
149. Ohno N, Kreitman RJ, Saito T, et al. Augmentation of the activity of an immunotoxin, anti-Tac(Fv)-PE40KDEL, in T cell lines infected with human T cell leukemia virus type-I. *Leuk Lymphoma.* 2002;43:885-888.
150. Onda M, Kreitman RJ, Vasmatzis G, Lee B, Pastan I. 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.* 1999;163:6072-6077.
151. Onda M, Willingham M, Wang Q, et al. Inhibition of TNF alpha produced by Kupffer cells protects against the non-specific liver toxicity of immunotoxin anti-Tac(Fv)-PE38, LMB-2. *J Immunol.* 2000;165:7150-7156.
152. Schnell R, Vitetta E, Schindler J, et al. Clinical trials with an anti-CD25 ricin A-chain experimental and immunotoxin (RFT5-SMPT-dgA) in Hodgkin's lymphoma. *Leuk Lymphoma.* 1998;30:525-537.
153. Schnell R, Borchmann P, Staak JO, et al. Clinical evaluation of ricin A-chain immunotoxins in patients with Hodgkin's lymphoma. *Ann Oncol.* 2003;14:729-736.
154. Montagna D, Yvon E, Calcatera V, et al. Depletion of alloreactive T cells by a specific anti-interleukin-2 receptor p55 chain immunotoxin does not impair in vitro antileukemia and antiviral activity. *Blood.* 1999;93:3550-3557.
155. Martin PJ, Pei J, Gooley T, et al. Evaluation of a CD25-specific immunotoxin for prevention of graft-versus-host disease after unrelated marrow transplantation. *Biol Blood Marrow Transplant.* 2004;10:552-560.
156. Reiter Y, Brinkmann U, Kreitman RJ, Jung S-H, Lee B, Pastan I. Stabilization of the Fv fragments in recombinant immunotoxins by disulfide bonds engineered into conserved framework regions. *Biochemistry.* 1994;33:5451-5459.
157. Reiter Y, Kreitman RJ, Brinkmann U, Pastan I. Cytotoxic and antitumor activity of a recombinant immunotoxin composed of disulfide-stabilized anti-Tac Fv fragment and truncated *Pseudomonas* exotoxin. *Int J Cancer.* 1994;58:142-149.
158. Kreitman RJ, Schneider WP, Queen C, et al. Mik-β1(Fv)-PE40, a recombinant immunotoxin cytotoxic toward cells bearing the β-chain of the IL-2 receptor. *J Immunol.* 1992;149:2810-2815.
159. Barth S, Huhn M, Wels W, Diehl V, Engert A. Construction and *in vitro* evaluation of RFT5(scFv)-ETA', a new recombinant single-chain immunotoxin with specific cytotoxicity toward CD25+ Hodgkin-derived cell lines. *Int J Mol Med.* 1998;1:249-256.
160. Barth S, Huhn M, Matthey B, et al. Recombinant anti-CD25 immunotoxin RFT5(ScFv)-ETA' demonstrates successful elimination of disseminated human Hodgkin lymphoma in SCID mice. *Int J Cancer.* 2000;86:718-724.
161. Matthey B, Engert A, Barth S. Recombinant immunotoxins for the treatment of Hodgkin's disease (Review). *Int J Mol Med.* 2000;6:509-514.
162. Ghetie M-A, May RD, Till M, et al. 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.* 1988;48:2610-2617.

163. Ghetie M-A, Richardson J, Tucker T, Jones D, Uhr JW, Vitetta ES. 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.* 1991;51:5876-5880.
164. Bregni M, Siena S, Formosa A, et al. B-cell restricted saporin immunotoxins: activity against B-cell lines and chronic lymphocytic leukemia cells. *Blood.* 1989;73:753-762.
165. Senderowicz AM, Vitetta E, Headlee D, et al. Complete sustained response of a refractory, post-transplantation, large B-cell lymphoma to an anti-CD22 immunotoxin. *Ann Intern Med.* 1997;126:882-885.
166. Smallshaw JE, Ghetie V, Rizo J, et al. Genetic engineering of an immunotoxin to eliminate pulmonary vascular leak in mice. *Nat Biotechnol.* 2003;21:387-391.
167. Kreitman RJ. Taming ricin toxin. *Nat Biotechnol.* 2003;21:372-374.
168. Kreitman RJ, Hansen HJ, Jones AL, FitzGerald DJ, Goldenberg DM, Pastan I. *Pseudomonas* exotoxin-based immunotoxins containing the antibody LL2 or LL2-Fab' induce regression of subcutaneous human B-cell lymphoma in mice. *Cancer Res.* 1993;53:819-825.
169. Mansfield E, Chiron MF, Amlot P, Pastan I, FitzGerald DJ. Recombinant RFB4 single-chain immunotoxin that is cytotoxic towards CD22-positive cells. *Biochem Soc Trans.* 1997;25:709-714.
170. Mansfield E, Amlot P, Pastan I, FitzGerald DJ. Recombinant RFB4 immunotoxins exhibit potent cytotoxic activity for CD22-bearing cells and tumors. *Blood.* 1997;90:2020-2026.
171. Kreitman RJ, Wang QC, FitzGerald DJ, Pastan I. 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.* 1999;81:148-155.
172. Kreitman RJ, Margulies I, Stetler-Stevenson M, Wang QC, FitzGerald DJ, Pastan I. Cytotoxic activity of disulfide-stabilized recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) towards fresh malignant cells from patients with B-cell leukemias. *Clin Cancer Res.* 2000;6:1476-1487.
173. Salvatore G, Beers R, Margulies I, Kreitman RJ, Pastan I. Improved cytotoxic activity towards cell lines and fresh leukemia cells of a mutant anti-CD22 immunotoxin obtained by antibody phage display. *Clin Cancer Res.* 2002;8:995-1002.
174. Kreitman RJ, Squires DR, Stetler-Stevenson M, et al. Phase I trial of recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) in patients with B-cell malignancies. *J Clin Oncol.* 2005;23:6719-6729.
175. Matutes E, Wotherspoon A, Brito-Babapulle V, Catovsky D. The natural history and clinico-pathological features of the variant form of hairy cell leukemia. *Leukemia.* 2001;15:184-186.
176. Tallman MS, Hakimian D, Kopecky KJ, et al. Minimal residual disease in patients with hairy cell leukemia in complete remission treated with 2-chlorodeoxyadenosine or 2-deoxycoformycin and prediction of early relapse. *Clin Cancer Res.* 1999;5:1665-1670.
177. Kreitman RJ, Pastan I. Recombinant toxins containing human granulocyte-macrophage colony-stimulating factor and either *Pseudomonas* exotoxin or diphtheria toxin kill gastrointestinal cancer and leukemia cells. *Blood.* 1997;90:252-259.
178. Hall PD, Virella G, Willoughby T, Atchley DH, Kreitman RJ, Frankel AE. Antibody response to DT-GM, a novel fusion toxin consisting of a truncated diphtheria toxin (DT) linked to human granulocyte-macrophage colony stimulating factor (GM), during a phase I trial of patients with relapsed or refractory acute myeloid leukemia. *Clin Immunol.* 2001;100:191-197.
179. Szatrowski TP, Dodge RK, Reynolds C, et al. 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.* 2003;97:1471-1480.
180. Grossbard ML, Gribben JG, Freedman AS, et al. Adjuvant immunotoxin therapy with anti-B4-blocked ricin after autologous bone marrow transplantation for patients with B-cell non-Hodgkin's lymphoma. *Blood.* 1993;81:2263-2271.
181. Scadden DT, Schenkein DP, Bernstein Z, et al. Immunotoxin combined with chemotherapy for patients with AIDS-related non-Hodgkin's lymphoma. *Cancer.* 1998;83:2580-2587.
182. Grossbard ML, Multani PS, Freedman AS, et al. 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.* 1999;5:2392-2398.
183. Longo DL, Duffey PL, Gribben JG, et al. Combination chemotherapy followed by an immunotoxin (anti-B4-blocked ricin) in patients with indolent lymphoma: results of a phase II study. *Cancer J.* 2000;6:146-150.
184. Herrera L, Yarbrough S, Ghetie V, Aquino DB, Vitetta ES. Treatment of SCID/human B cell precursor ALL with anti-CD19 and anti-CD22 immunotoxins. *Leukemia.* 2003;17:334-338.
185. Messmann RA, Vitetta ES, Headlee D, et al. 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.* 2000;6:1302-1313.
186. Schnell R, Staak O, Borchmann P, et al. 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.* 2002;8:1779-1786.
187. Hansen HP, Matthey B, Barth S, et al. Inhibition of metalloproteinases enhances the internalization of anti-CD30 antibody Ki-3 and the cytotoxic activity of Ki-3 immunotoxin. *Int J Cancer.* 2002;98:210-215.
188. Klimka A, Barth S, Matthey B, et al. 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.* 1999;80:1214-1222.
189. Barth S, Huhn M, Matthey B, et al. Ki-4(scFv)-ETA', a new recombinant anti-CD30 immunotoxin with highly specific cytotoxic activity against disseminated Hodgkin tumors in SCID mice. *Blood.* 2000;95:3909-3914.
190. Rozemuller H, Chowdhury PS, Pastan I, Kreitman RJ. 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.* 2001;92:861-870.
191. Nagata S, Onda M, Numata Y, et al. Novel anti-CD30 recombinant immunotoxins containing disulfide-stabilized Fv fragments. *Clin Cancer Res.* 2002;8:2345-2355.
192. Alexander RL, Kucera GL, Klein B, Frankel AE. In vitro interleukin-3 binding to leukemia cells predicts cytotoxicity of a diphtheria toxin/IL-3 fusion protein. *Bioconjug Chem.* 2000;11:564-568.
193. Frankel A, McCubrey J, Miller MS, et al. Diphtheria toxin fused to human interleukin-3 is toxic to blasts from patients with acute phase chronic myeloid leukemia. *Leukemia.* 2000;14:576-585.
194. Alexander RL, Ramage J, Kucera GL, Caligiuri MA, Frankel AE. High affinity interleukin-3 receptor expression on blasts from patients

with acute myelogenous leukemia correlates with cytotoxicity of a diphtheria toxin/IL-3 fusion protein. *Leuk Res.* 2001; 25:875-881.

195. Feuring-Buske M, Frankel AE, Alexander RL, Gerhard B, Hogge DE. A diphtheria toxin-interleukin 3 fusion protein is cytotoxic to primitive acute myeloid leukemia progenitors but spares normal progenitors. *Cancer Res.* 2002;62:1730-1736.

196. Black JH, McCubrey JA, Willingham MC, Ramage J, Hogge DE, Frankel AE. Diphtheria toxin-interleukin-3 fusion protein (DT(388)IL3) prolongs disease-free survival of leukemic immunocompromised mice. *Leukemia.* 2003;17:155-159.

197. Urieto JO, Liu T, Black JH, et al. Expression and purification of the recombinant diphtheria fusion toxin DT388IL3 for phase I clinical trials. *Protein Expr Purif.* 2004;33:123-133.

198. Flavell DJ, Boehm DA, Noss A, Warnes SL, Flavell SU. Therapy of human T-cell acute lymphoblastic leukaemia with a combination of anti-CD7 and anti-CD38-SAPORIN immunotoxins is significantly better than therapy with each individual immunotoxin. *Br J Cancer.* 2001;84:571-578.

199. Mehta K, Ocanas L, Malavasi F, Marks JW, Rosenblum MG. Retinoic acid-induced CD38 antigen as a target for immunotoxin-mediated killing of leukemia cells. *Mol Cancer Ther.* 2004;3: 345-352.

200. Peipp M, Kupers H, Saul D, et al. A recombinant CD7-specific single-chain immunotoxin is a potent inducer of apoptosis in acute leukemic T cells. *Cancer Res.* 2002;62:2848-2855.

201. Polito L, Bolognesi A, Tazzari PL, et al. The conjugate Rituximab/saporin-S6 completely inhibits clonogenic growth of CD20-expressing cells and produces a synergistic toxic effect with Fludarabine. *Leukemia.* 2004;18:1215-1222.

202. Zhong RK, van De Winkel JG, Thepen T, Schultz LD, Ball ED. Cytotoxicity of anti-cd64-ricin a chain immunotoxin against human acute myeloid leukemia cells in vitro and in SCID mice. *J Hematother Stem Cell Res.* 2001;10:95-105.

203. Tur MK, Huhn M, Thepen T, et al. Recombinant CD64-specific single chain immunotoxin exhibits specific cytotoxicity against acute myeloid leukemia cells. *Cancer Res.* 2003;63:8414-8419.

204. Otten HG, deGast GC, Vooijs WC. Preclinical evaluation of anti-CD86 immunotoxin in rhesus monkeys: analysis of systemic toxicity, pharmacokinetics, and effect on primary t-cell responses. *Cancer Immunol Immunother.* 2003;52:569-575.

205. Shin YK, Choi YL, Choi EY, et al. Targeted cytotoxic effect of anti-JL1 immunotoxin against a human leukemic cell line and its clinical implications. *Cancer Immunol Immunother.* 2003;52:506-512.

206. Pistillo MP, Tazzari PL, Palmisano GL, et al. CTLA-4 is not restricted to the lymphoid cell lineage and can function as a target molecule for apoptosis induction of leukemic cells. *Blood.* 2003;101:202-209.

207. FitzGerald DJ, Padmanabhan R, Pastan I, Willingham MC. Adenovirus-induced release of epidermal growth factor and pseudomonas toxin into the cytosol of KB cells during receptor-mediated endocytosis. *Cell.* 1983;32:607-617.

208. Chaudhary VK, FitzGerald DJ, Adhya S, Pastan I. Activity of a recombinant fusion protein between transforming growth factor type  $\alpha$  and *Pseudomonas* toxin. *Proc Natl Acad Sci USA.* 1987;84:4538-4542.

209. Siegall CB, Xu Y-h, Chaudhary VK, Adhya S, FitzGerald D, Pastan I. Cytotoxic activities of a fusion protein comprised of TGF $\alpha$  and *Pseudomonas* exotoxin. *FASEB J.* 1989;3:2647-2652.

210. Kreitman RJ, Siegall CB, Chaudhary VK, FitzGerald DJ, Pastan I. Properties of chimeric toxins with two recognition domains: interleukin 6 and transforming growth factor  $\alpha$  at different locations in *Pseudomonas* exotoxin. *Bioconjug Chem.* 1992;3:63-68.

211. Pai LH, Gallo MG, FitzGerald DJ, Pastan I. Antitumor activity of a transforming growth factor  $\alpha$ -*Pseudomonas* exotoxin fusion protein (TGF- $\alpha$ -PE40). *Cancer Res.* 1991;51:2808-2812.

212. Shaw JP, Degen D, Nichols JC, Bacha P, Von Hoff DD. Cytotoxicity of an epidermal growth factor receptor targeted fusion toxin for primary and cultured human tumor cells. *Proc Am Assoc Cancer Res.* 1993;34:2043.

213. Liu TF, Willingham MC, Tatter SB, et al. Diphtheria toxin-epidermal growth factor fusion protein and *Pseudomonas* exotoxin-interleukin 13 fusion protein exert synergistic toxicity against human glioblastoma multiforme cells. *Bioconjug Chem.* 2003;14:1107-1114.

214. Mishra G, Liu TF, Frankel AE. Recombinant toxin DAB389EGF is cytotoxic to human pancreatic cancer cells. *Expert Opin Biol Ther.* 2003;3:1173-1180.

215. Bruell D, Stocker M, Huhn M, et al. The recombinant anti-EGF receptor immunotoxin 425(scFv)-ETA' suppresses growth of a highly metastatic pancreatic carcinoma cell line. *Int J Oncol.* 2003;23:1179-1186.

216. Lorimer IAJ, Wikstrand CJ, Batra SK, Bigner DD, Pastan I. Immunotoxins that target an oncogenic mutant epidermal growth factor receptor expressed in human tumors. *Clin Cancer Res.* 1995;1:859-864.

217. Lorimer IA, Keppler-Hafkemeyer A, Beers RA, Pegram CN, Bigner DD, Pastan I. Recombinant immunotoxins specific for a mutant epidermal growth factor receptor: targeting with a single chain antibody variable domain isolated by phage display. *Proc Natl Acad Sci USA.* 1996;93:14815-14820.

218. Fu YM, Mesri EA, Yu ZX, Kreitman RJ, Pastan I, Epstein SE. Cytotoxic effects of vascular smooth muscle cells of the chimeric toxin, heparin binding TGF  $\alpha$ -*Pseudomonas* exotoxin. *Cardiovasc Res.* 1993;27:1691-1697.

219. Mesri EA, Kreitman RJ, Fu YM, Epstein SE, Pastan I. Heparin-binding transforming growth factor  $\alpha$ -*Pseudomonas* exotoxin A. *J Biol Chem.* 1993;268:4853-4862.

220. Mesri EA, Ono M, Kreitman RJ, Klagsbrun M, Pastan I. The heparin-binding domain of heparin-binding EGF-like growth factor can target *Pseudomonas* exotoxin to kill cells exclusively through heparan sulfate proteoglycans. *J Cell Sci.* 1994;107:2599-2608.

221. Pastan I, Lovelace ET, Gallo MG, Rutherford AV, Magnani JL, Willingham MC. Characterization of monoclonal antibodies B1 and B3 that react with mucinous adenocarcinomas. *Cancer Res.* 1991;51:3781-3787.

222. Pai LH, Kreitman RJ, Pastan I. Immunotoxin therapy. In: Devita VT Jr, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology.* 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2000:382-395.

223. Pai LH, Batra JK, FitzGerald DJ, Willingham MC, Pastan I. Antitumor effects of B3-PE and B3-LysPE40 in a nude mouse model of human breast cancer and the evaluation of B3-PE toxicity in monkeys. *Cancer Res.* 1992;52:3189-3193.

224. Pai LH, Batra JK, FitzGerald DJ, Willingham MC, Pastan I. Anti-tumor activities of immunotoxins made of monoclonal antibody B3 and various forms of *Pseudomonas* exotoxin. *Proc Natl Acad Sci USA.* 1991;88:3358-3362.

225. Kuan C, Pai LH, Pastan I. Immunotoxins containing *Pseudomonas* exotoxin targeting Le<sup>Y</sup> damage human endothelial

- cells in an antibody-specific mode: relevance to vascular leak syndrome. *Clin Cancer Res.* 1995;1:1589-1594.
226. Brinkmann U, Pai LH, FitzGerald DJ, Willingham M, Pastan I. B3(Fv)-PE38KDEL, a single-chain immunotoxin that causes complete regression of a human carcinoma in mice. *Proc Natl Acad Sci USA.* 1991;88:8616-8620.
227. Harari D, Yarden Y. Molecular mechanisms underlying ErbB2/HER2 action in breast cancer. *Oncogene.* 2000;19:6102-6114.
228. Wels W, Beerli R, Hellmann P, et al. EGF receptor and p185 (erbB-2)-specific single-chain antibody toxins differ in their cell-killing activity on tumor cells expressing both receptor proteins. *Int J Cancer.* 1995;60:137-144.
229. Schmidt M, Hynes NE, Groner B, Wels W. A bivalent single-chain antibody-toxin specific for ErbB-2 and the EGF receptor. *Int J Cancer.* 1996;65:538-546.
230. Batra JK, Kasprzyk PG, Bird RE, Pastan I, King CR. Recombinant anti-erbB2 immunotoxins containing *Pseudomonas* exotoxin. *Proc Natl Acad Sci USA.* 1992;89:5867-5871.
231. Shinohara H, Morita S, Kawai M, et al. Expression of HER2 in human gastric cancer cells directly correlates with antitumor activity of a recombinant disulfide-stabilized anti-HER2 immunotoxin. *J Surg Res.* 2002;102:169-177.
232. Azemar M, Schmidt M, Arlt F, et al. Recombinant antibody toxins specific for ErbB2 and EGF receptor inhibit the in vitro growth of human head and neck cancer cells and cause rapid tumor regression in vivo. *Int J Cancer.* 2000;86:269-275.
233. Azemar M, Djahansouzi S, Jager E, et al. Regression of cutaneous tumor lesions in patients intratumorally injected with a recombinant single-chain antibody-toxin targeted to ErbB2/HER2. *Breast Cancer Res Treat.* 2003;82:155-164.
234. Husain SR, Gill P, Kreitman RJ, Pastan I, Puri RK. Interleukin-4 receptor expression on AIDS-associated Kaposi's sarcoma cells and their targeting by a chimeric protein comprised of circularly permuted IL-4 and *Pseudomonas* exotoxin. *Mol Med.* 1997;3:327-338.
235. Debinski W, Puri RK, Kreitman RJ, Pastan I. A wide range of human cancers express interleukin 4 (IL4) receptors that can be targeted with chimeric toxin composed of IL4 and *Pseudomonas* exotoxin. *J Biol Chem.* 1993;268:14065-14070.
236. Kreitman RJ, Puri RK, Leland P, Lee B, Pastan I. Site-specific conjugation to interleukin 4 containing mutated cysteine residues produces interleukin 4-toxin conjugates with improved binding and activity. *Biochemistry.* 1994;33:11637-11644.
237. Kreitman RJ. Circularly permuted interleukin 4 retains proliferative and binding activity. *Cytokine.* 1995;7:311-318.
238. Kreitman RJ, Puri RK, Pastan I. Increased antitumor activity of a circularly permuted interleukin 4-toxin in mice with interleukin 4 receptor-bearing human carcinoma. *Cancer Res.* 1995;55:3357-3363.
239. Kreitman RJ, Puri RK, Pastan I. A circularly permuted recombinant interleukin 4 toxin with increased activity. *Proc Natl Acad Sci USA.* 1994;91:6889-6893.
240. Husain SR, Behari N, Kreitman RJ, Pastan I, Puri RK. Complete regression of established human glioblastoma tumor xenografts by interleukin-4 toxin therapy. *Cancer Res.* 1998;58:3649-3653.
241. Puri RK, Hoon DS, Leland P, et al. Preclinical development of a recombinant toxin containing circularly permuted interleukin 4 and truncated *Pseudomonas* exotoxin for therapy of malignant astrocytoma. *Cancer Res.* 1996;56:5631-5637.
242. Obiri NI, Debinski W, Leonard WJ, Puri RK. Receptor for interleukin 13. Interaction with interleukin 4 by a mechanism that does not involve the common gamma chain shared by receptors for interleukins 2, 4, 7, 9, and 15. *J Biol Chem.* 1995;270:8797-8804.
243. Obiri NI, Leland P, Murata T, Debinski W, Puri RK. The IL-13 receptor structure differs on various cell types and may share more than one component with IL-4 receptor. *J Immunol.* 1997;158:756-764.
244. Weaver M, Laske DW. Transferrin receptor ligand-targeted toxin conjugate (Tf-CRM107) for therapy of malignant gliomas. *J Neurooncol.* 2003;65:3-13.
245. Hagihara N, Walbridge S, Olson AW, Oldfield EH, Youle RJ. Vascular protection by chloroquine during brain tumor therapy with Tf-CRM107. *Cancer Res.* 2000;60:230-234.
246. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. *Proc Natl Acad Sci USA.* 1996;93:136-140.
247. Chowdhury PS, Chang K, Pastan I. Isolation of anti-mesothelin antibodies from a phage display library. *Mol Immunol.* 1997;34:9-20.
248. Hassan R, Benbrook DM, Lightfoot SA, et al. SS1(dsFv)-PE38, a recombinant anti-mesothelin immunotoxin targets human gynecologic tumors overexpressing mesothelin. *Proc Am Soc Clin Oncol.* 2000;19:383a.
249. Hassan R, Lerner MR, Benbrook D, et al. Antitumor activity of SS(dsFv)PE38 and SS1(dsFv)PE38, recombinant antimesothelin immunotoxins against human gynecologic cancers grown in organotypic culture in vitro. *Clin Cancer Res.* 2002;8:3520-3526.
250. Li Q, Verschraegen CF, Mendoza J, Hassan R. Cytotoxic activity of the recombinant anti-mesothelin immunotoxin, SS1(dsFv)PE38, towards tumor cell lines established from ascites of patients with peritoneal mesotheliomas. *Anticancer Res.* 2004;24:1327-1335.
251. Fan D, Yano S, Shinohara H, et al. Targeted therapy against human lung cancer in nude mice by high-affinity recombinant antimesothelin single-chain Fv immunotoxin. *Mol Cancer Ther.* 2002;1:595-600.
252. Bera TK, Williams-Gould J, Beers R, Chowdhury P, Pastan I. Bivalent disulfide-stabilized fragment variable immunotoxin directed against mesotheliomas and ovarian cancer. *Mol Cancer Ther.* 2001;1:79-84.
253. Lynch TJ. Immunotoxin therapy of small-cell lung cancer. N901-blocked ricin for relapsed small-cell lung cancer. *Chest.* 1993;103:436s-439s.
254. Fidiias P, Jr, Grossbard M, Jr, Lynch TJ, Jr. A phase II study of the immunotoxin N901-blocked ricin in small-cell lung cancer. *Clin Lung Cancer.* 2002;3:219-222.
255. Lanza F, Castoldi GL, Castagnari B, et al. Expression and functional role of urokinase-type plasminogen activator receptor in normal and acute leukaemic cells. *Br J Haematol.* 1998;103:110-123.
256. Plesner T, Ralfkiaer E, Wittrup M, et al. Expression of the receptor for urokinase-type plasminogen activator in normal and neoplastic blood cells and hematopoietic tissue. *Am J Clin Pathol.* 1994;102:835-841.
257. Taniguchi T, Kakkar AK, Tuddenham EGD, Williamson RCN, Lemoine NR. Enhanced expression of urokinase receptor induced through the tissue factor-factor VIIa pathway in human pancreatic cancer. *Cancer Res.* 1998;58:4461-4467.
258. Sier CFM, Stephens R, Bizik J, et al. The level of urokinase-type plasminogen activator receptor is increased in serum of ovarian cancer patients. *Cancer Res.* 1998;58:1843-1849.

259. Rajagopal V, Kreitman RJ. Recombinant toxins which bind to the urokinase receptor are cytotoxic without requiring binding to the  $\alpha 2$  macroglobulin receptor. *J Biol Chem*. 2000;275:7566-7573.
260. Ramage JG, Vallera DA, Black JH, Aplan PD, Kees UR, Frankel AE. The diphtheria toxin/urokinase fusion protein (DTAT) is selectively toxic to CD87 expressing leukemic cells. *Leuk Res*. 2003;27:79-84.
261. Frankel AE, Beran M, Hogge DE, et al. Malignant progenitors from patients with CD87+ acute myelogenous leukemia are sensitive to a diphtheria toxin-urokinase fusion protein. *Exp Hematol*. 2002;30:1316-1323.
262. Vallera DA, Li C, Jin N, Panoskaltis-Mortari A, Hall WA. Targeting urokinase-type plasminogen activator receptor on human glioblastoma tumors with diphtheria toxin fusion protein DTAT. *J Natl Cancer Inst*. 2002;94:597-606.
263. Rustamzadeh E, Li C, Doumbia S, Hall WA, Vallera DA. Targeting the over-expressed urokinase-type plasminogen activator receptor on glioblastoma multiforme. *J Neurooncol*. 2003;65:63-75.
264. Todhunter DA, Hall WA, Rustamzadeh E, Shu Y, Doumbia SO, Vallera DA. A bispecific immunotoxin (DTAT13) targeting human IL-13 receptor (IL-13R) and urokinase-type plasminogen activator receptor (uPAR) in a mouse xenograft model. *Protein Eng Des Sel*. 2004;17:157-164.
265. Rosenblum MG, Murray JL, Cheung L, Rifkin R, Salmon S, Bartholomew R. A specific and potent immunotoxin composed of antibody ZME-018 and the plant toxin gelonin. *Mol Biother*. 1991;3:6-13.
266. Rosenblum MG, 3rd, Cheung LH, 3rd, Liu Y, 3rd, Marks JW, 3rd. Design, expression, purification, and characterization, in vitro and in vivo, of an antimelanoma single-chain Fv antibody fused to the toxin gelonin. *Cancer Res*. 2003;63:3995-4002.
267. Chan MC, Murphy RM. Kinetics of cellular trafficking and cytotoxicity of 9.2.27-gelonin immunotoxins targeted against the high-molecular-weight melanoma-associated antigen. *Cancer Immunol Immunother*. 1999;47:321-329.
268. Hjortland GO, Garman-Vik SS, Juell S, et al. Immunotoxin treatment targeted to the high-molecular-weight melanoma-associated antigen prolonging the survival of immunodeficient rats with invasive intracranial human glioblastoma multiforme. *J Neurosurg*. 2004;100:320-327.
269. Fracasso G, Bellisola G, Cingarlini S, et al. Anti-tumor effects of toxins targeted to the prostate specific membrane antigen. *Prostate*. 2002;53:9-23.
270. Huang X, Bennett M, Thorpe PE. Anti-tumor effects and lack of side effects in mice of an immunotoxin directed against human and mouse prostate-specific membrane antigen. *Prostate*. 2004;61:1-11.
271. Onda M, Wang QC, Guo HF, Cheung NK, Pastan I. In vitro and in vivo cytotoxic activities of recombinant immunotoxin 8H9(Fv)-PE38 against breast cancer, osteosarcoma, and neuroblastoma. *Cancer Res*. 2004;64:1419-1424.
272. Thomas PB, Delatte SJ, Sutphin A, Frankel AE, Tagge EP. Effective targeted cytotoxicity of neuroblastoma cells. *J Pediatr Surg*. 2002;37:539-544.
273. Di Paolo C, Willuda J, Kubetzko S, et al. A recombinant immunotoxin derived from a humanized epithelial cell adhesion molecule-specific single-chain antibody fragment has potent and selective antitumor activity. *Clin Cancer Res*. 2003;9:2837-2848.
274. Jin N, Chen W, Blazar BR, Ramakrishnan S, Vallera DA. Gene therapy of murine solid tumors with T cells transduced with a retroviral vascular endothelial growth factor—immunotoxin target gene. *Hum Gene Ther*. 2002;13:497-508.
275. Reddy KR. Development and pharmacokinetics and pharmacodynamics of pegylated interferon alfa-2a (40 kD). *Semin Liver Dis*. 2004;24:33-38.
276. Graham ML. Pegaspargase: a review of clinical studies. *Adv Drug Deliv Rev*. 2003;55:1293-1302.
277. Onda M, Vincent JJ, Lee B, Pastan I. Mutants of immunotoxin anti-Tac(dsFv)-PE38 with variable number of lysine residues as candidates for site-specific chemical modification, I: properties of mutant molecules. *Bioconjug Chem*. 2003;14:480-487.
278. Tsutsumi Y, Onda M, Nagata S, Lee B, Kreitman RJ, Pastan I. Site-specific chemical modification with polyethylene glycol of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) improves antitumor activity and reduces animal toxicity and immunogenicity. *Proc Natl Acad Sci USA*. 2000;97:8548-8553.
279. Roscoe DM, Pai LH, Pastan I. Identification of epitopes on a mutant form of Pseudomonas exotoxin using serum from humans treated with Pseudomonas exotoxin containing immunotoxins. *Eur J Immunol*. 1997;27:1459-1468.
280. Roscoe DM, Jung SH, Benhar I, Pai L, Lee BK, Pastan I. Primate antibody response to immunotoxin: serological and computer-aided analysis of epitopes on a truncated form of Pseudomonas exotoxin. *Infect Immun*. 1994;62:5055-5065.
281. Nagata S, Numata Y, Onda M, et al. Rapid grouping of monoclonal antibodies based on their topographical epitopes by a label-free competitive immunoassay. *J Immunol Methods*. 2004;292:141-155.
282. Pai LH, FitzGerald DJ, Tepper M, Schacter B, Spitalny G, Pastan I. Inhibition of antibody response to Pseudomonas exotoxin and an immunotoxin containing Pseudomonas exotoxin by 15-deoxyspergualin in mice. *Cancer Res*. 1990;50:7750-7753.
283. Siegall CB, Haggerty HG, Warner GL, et al. Prevention of immunotoxin-induced immunogenicity by coadministration with CTLA4Ig enhances antitumor efficacy. *J Immunol*. 1997;159:5168-5173.
284. Gelber EE, Vitetta ES. Effect of immunosuppressive agents on the immunogenicity and efficacy of an immunotoxin in mice. *Clin Cancer Res*. 1998;4:1297-1304.
285. Hassan R, Williams-Gould J, Watson T, Pai-Scherf L, Pastan I. Pretreatment with rituximab does not inhibit the human immune response against the immunogenic protein LMB-1. *Clin Cancer Res*. 2004;10:16-18.
286. Baluna R, Rizo J, Gordon BE, Ghetie V, Vitetta ES. Evidence for a structural motif in toxins and interleukin-2 that may be responsible for binding to endothelial cells and initiating vascular leak syndrome. *Proc Natl Acad Sci USA*. 1999;96:3957-3962.
287. Baluna R, Coleman E, Jones C, Ghetie V, Vitetta ES. The effect of a monoclonal antibody coupled to ricin A chain-derived peptides on endothelial cells in vitro: insights into toxin-mediated vascular damage. *Exp Cell Res*. 2000;258:417-424.
288. Onda M, Nagata S, Tsutsumi Y, et al. Lowering the isoelectric point of the Fv portion of recombinant immunotoxins leads to decreased nonspecific animal toxicity without affecting antitumor activity. *Cancer Res*. 2001;61:5070-5077.
289. Grossbard ML, Lambert JM, Goldmacher VS, et al. Anti-B4-blocked ricin: a phase I trial of 7-day continuous infusion in patients with B-cell neoplasms. *J Clin Oncol*. 1993;11:726-737.
290. Grossbard ML, Fidias P, Kinsella J, et al. Anti-B4-blocked ricin: a phase II trial of 7 day continuous infusion in patients with multiple myeloma. *Br J Haematol*. 1998;102:509-515.