

## EDITORIAL

### Immunotoxins: Will Their Clinical Promise Be Fulfilled?

By Daniel A. Vallera

#### A NEW ERA OF SELECTIVE TARGETING OF THE LYMPHOHEMATOPOIETIC SYSTEM WITH IMMUNOTOXINS

**I**MMUNOTOXINS (ITs) are pharmacologic agents consisting of catalytic toxins linked to an appropriate targeting ligand. Monoclonal antibodies (MoAbs) are the ligands used most often and are directed against determinants expressed on disease-causing target cells. The sole purpose of these synthetic hybrid molecules is to more selectively deliver therapy than the more general chemotherapeutic or radiologic approaches currently used. It appears that a renewed interest has occurred primarily fueled by the following facts. (1) Some degree of clinical success has been reported in phase I and phase II clinical trials in the areas of B-cell lymphoma, Hodgkin's disease, T-cell lymphoma, B-cell leukemia, and steroid-resistant graft-versus-host disease (GVHD).<sup>1</sup> These responses have occurred mostly in targeting the lymphohematopoietic system because these cells might be more easily accessed by ITs than solid tumors that have poor blood supply and large interstitial pressures. Thus, this editorial will deal mostly with targeting lymphohematopoietic cells. (2) Despite these partial successes, the majority of patients diagnosed with hematologic neoplasms ultimately succumb to their disease. Thus, there still exists an urgent clinical need for more effective therapies for targeting residual disease in adult and childhood hematopoietic malignancies, for more effective GVHD therapies, and for a more efficient elimination of host T cells that contribute to organ graft or bone marrow rejection. (3) We are armed with sophisticated molecular techniques that have permitted modification of the older genre of ITs. Polymerase chain reaction (PCR) technology has expedited the molecular engineering of new fusion proteins with cytokine genes or truncated Ig genes as ligands and toxin subunits as a new generation of IT, which will be discussed. PCR has revolutionized molecular genetics by enabling us to produce enormous numbers of specified gene sequences without the complexity of cloning. (4) We have an expanded repertoire of biochemical techniques and scale-up technology that has permitted productive modifications to toxin, MoAbs, and chemical cross-linkers, which will be discussed. (5) The single most important *in vivo* requirement for taking an IT to clinical trial is efficacy and new animal modeling strategies have been devised for its determination.

#### IS ONE TOXIN BETTER THAN ANOTHER?

When building an IT, one immediately realizes that there is an extensive choice of catalytic toxins, all of which kill target cells by protein synthesis inhibition. All toxins in clinical trials are either bacterial or plant. All are irreversibly catalytic and amazingly active in nanogram quantities. And yet, at this early stage of clinical evaluation, there is not any obvious advantage of one over another. The toxins tested in clinical trials include the plant toxins ricin toxin A chain (RTA), saporin, and pokeweed antiviral protein; recombinant bacterial toxins include genetically modified *Pseudomonas* exotoxin (PE) and diphtheria toxin (DT).

Toxins such as PE and DT contain separate toxin domains that are depicted in Fig 1. Three separate domains are responsible for target cell binding, enhancement of translocation through the membrane into the cell interior, and the destruction of cellular protein synthesis by an effect on elongation factor 2 (EF-2).<sup>2,3</sup> As shown in Fig 1, B chain containing the native site for binding human cells can be either removed, as in RTA<sup>4</sup>; chemically blocked, as in blocked ricin<sup>5</sup>; or genetically deleted, as in DT or PE<sup>6,7</sup> to prevent non-specific interaction of IT with nontarget cells. Another approach is to use ribosome inactivating proteins (RIPs) such as saporin or pokeweed antiviral protein that do not have a B chain but have the same enzymatic properties of RTA.<sup>8</sup> Although B chain appears unnecessary for the production of a potent IT, the use of certain portions of B chain containing the translocation enhancing region has been important in engineering ITs that have much higher potency than ITs made without these regions.<sup>6</sup>

Do toxicities among different toxins differ? Most clinical studies have involved RTA-based ITs. These have shown different phase I dose-limiting toxicities, including vascular leak syndrome (VLS), which involves extravasation of fluids and proteins from the vasculature into the periphery.<sup>1</sup> Also occurring is hypoalbuminemia and myalgia. In contrast, ITs

---

*From the Section on Experimental Cancer Immunology, Department of Therapeutic Radiology, University of Minnesota, Minneapolis.*

*Address reprint requests to Daniel A. Vallera, MD, Section on Experimental Cancer Immunology, Department of Therapeutic Radiology, University of Minnesota, Minneapolis, MN 55455.*

© 1994 by The American Society of Hematology.

0006-4971/94/8302-0043\$3.00/0

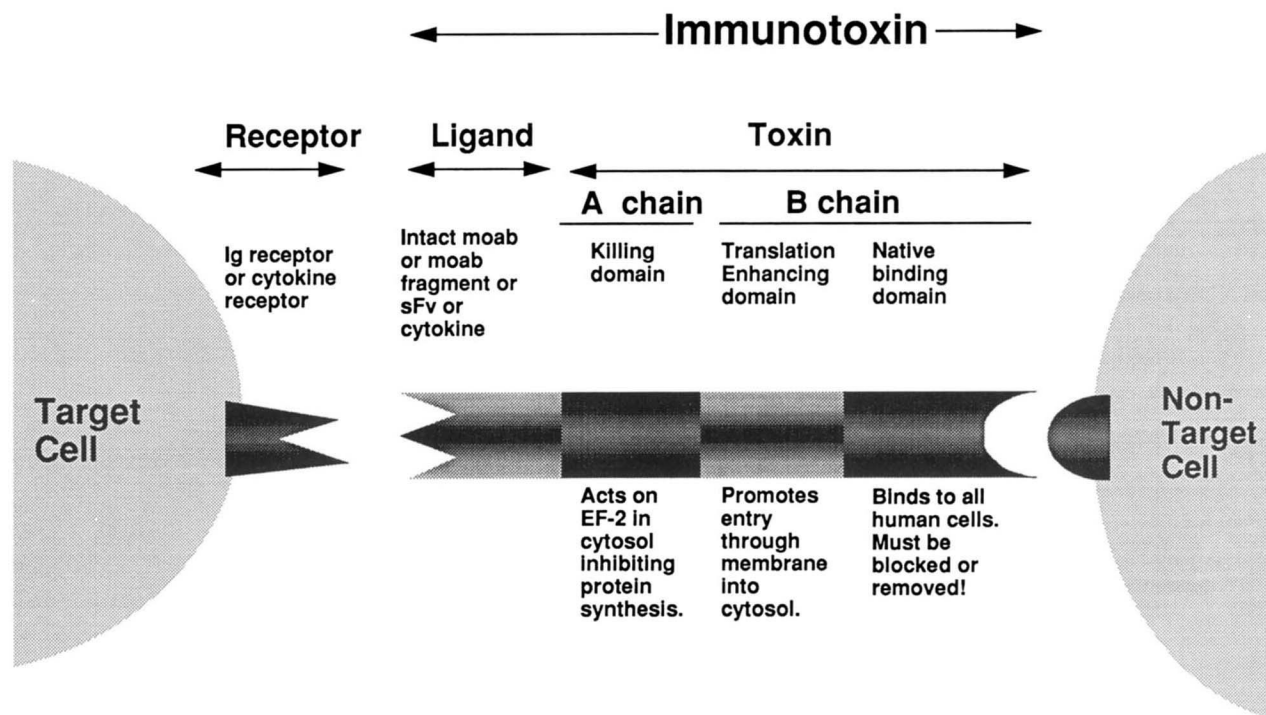


Fig 1. The general principle of an IT. Regardless of whether biochemically linked or genetically engineered, the IT principle is the same. The ligand portion of the molecule, MoAb or cytokine, binds to target-cell-specific surface determinants. The catalytic toxin gains entry to the cytosol through its association with the ligand, thus inhibiting protein synthesis by some effect on EF-2. For bacterial toxins, such as DT and PE, there are three separate toxin domains associated with cell killing, translocation, and binding human cells. The domain that normally binds human cells must be deleted or blocked.

made with blocked ricin B chain<sup>9</sup> or PE induce hepatotoxicity.<sup>10</sup> Because different ITs display different side effects and we have clinically tested a limited array of toxins, it will be important to identify a toxin with limited side effects. If no such toxin can be identified, the field must await new approaches to control toxicity such as the use of pentoxifylline to treat VLS.<sup>11</sup>

Is one toxin more immunogenic than another? At this stage, it does not appear so. All ITs used clinically induce antibody responses in patients to both toxin and MoAb regions of ITs. However, only anti-IT antibodies that neutralize the enzymatic portion of the molecule or interfere with selective binding should be problematic. Thus, despite their immunogenicity, only a proportion of anti-IT antibodies may actually interfere with efficacy. This may be why ITs made with DT have shown clinical responses, even though the general population is immunized to DT.

#### BEATING PLOWSHARES INTO SWORDS

Over the years, many problems have become apparent in efforts to develop suitable ITs for in vivo use. These problems and some of the modifications that have attempted to address these are listed in Table 1. (1) One problem is that carbohydrate associated with some toxin molecules increases nonspecific IT binding in vivo. For example, ricin-associated mannose nonspecifically binds to macrophages enhancing IT interaction with the reticuloendothelial system.<sup>12</sup> To prevent this, RTA can be chemically deglycosylated,<sup>13</sup> which increases serum half life. Clinical studies for

B-cell lymphoma<sup>1,14</sup> and T-cell acute lymphocytic leukemia (ALL) (unpublished data, University of Minnesota and Medical University of South Carolina) are currently underway using deglycosylated RTA. In lieu of chemical treatment, a fraction of RTA called RTA<sub>30</sub> that is naturally low in oligosaccharide content can be purified<sup>15</sup> or toxins that are naturally nonglycosylated can be chosen. Also, glycosylation is not a problem in genetically engineered ITs, because prokaryotic expression systems produce RTA with no oligosaccharide chains.<sup>16</sup> (2) Generally, modulation of surface determinants is regarded as a benefit with ITs rather than a problem, as in the case of radiolabeled antibodies. Better internalization should result in better access to the cell's interior where ITs exert their damage. However, a successful voyage from the membrane to the cytosol may require the internalization of thousands of IT molecules. An IT that is unstable in vivo could provide a source of free MoAb that would modulate the relevant determinants. More stable cross-linkers have been developed to deal with this problem.<sup>17</sup>

(3) Many investigators have sought to increase IT potency, which can be accomplished by biochemically obstructing the binding region of ricin B chain,<sup>5</sup> and still take advantage of the B chain enhancing properties of intact ricin.<sup>18</sup> Portions of the B chain associated with translocation enhancement can be incorporated into engineered ITs.<sup>6</sup> Potency can also be increased by screening for higher affinity antibodies and the use of potentiators such as ammonium chloride, chloroquine, or monensin,<sup>19</sup> which might in them-

Table 1. IT Problems and Possible Solutions

IT Problems	Possible Solutions
IT-associated carbohydrate shortens half life	Chemical deglycosylation, low oligosaccharide forms, prokaryotic expression systems, nonglycosylated toxins
Modulation of target antigen	Stable MoAb to toxin linkage
IT not potent enough	Use higher MoAb affinity, use B chain when possible, use potentiator if possible
IT immunogenic	PEG, humanize MoAb, naturally occurring toxins such as ribonuclease, use different toxins
Heterogeneous target antigen expression	IT mixtures, continuous infusion
Not targeting tumor progenitors	Use clonogenic assays to identify progenitors, use adjunctive therapy such as chemotherapy or cytokines
IT-resistant mutants	Adjunctive therapy
IT cannot penetrate tumor, IT too large	Single-chain Fv IT, antibody fragments attack vascular endothelium with IT, compartmentalized therapy
Nontarget toxicity	Deglycosylation, remove Fc, stable cross-linkers, check for activation
Target antigen shedding	Target antigens that are not shed

selves increase nontarget toxicity. (4) Another problem is that injected patients produce both anti-antibody and anti-toxin antibodies in response to IT administration. This may interfere with a course of IT treatment, which often goes for 1 week or more and often requires several courses. To reduce antibody immunogenicity, rodent variable Ig regions are "humanized" with human Ig gene sequences that present fewer xenogenic peptide sequences and are thus less immunogenic.<sup>20</sup> Another approach is attachment of certain chemical agents such as polyethylene glycol (PEG), which render IT less immunogenic.<sup>21</sup> Naturally occurring human ribonuclease has been used in place of toxin to construct ITs that are nonimmunogenic.<sup>22</sup> However, it is still a concern that the combination of these natural toxins with ligand may generate new immune responses to this new hapten/carrier complex. (5) Another problem is that tumors may be heterogeneous in expressing certain antigens. Targeting tumor does not guarantee that tumor progenitor cells are being targeted. Also, IT-resistant mutants have been described.<sup>23,24</sup> These will be discussed later.

(6) Tumor penetration of ITs may be improved by synthesizing smaller ITs. This can be done using MoAb fragments. Comparative clinical studies with anti-CD22IT made with intact MoAb and monomeric Fab' fragments were undertaken because intact MoAb ITs had the advantage of high potency, longevity, and stability<sup>25</sup> and the Fab' ITs may be more effective in localizing to tumor tissue.<sup>26</sup> Thus far, the results of the trials are similar.<sup>1,2</sup> Another exciting development is the generation of single-chain antibodies (Fv) by genetically fusing the coding region of MoAb light ( $V_L$ ) and heavy ( $V_H$ ) variable Ig genes, as illustrated by Kreitman et al<sup>27</sup> in this issue. These molecules are smaller than Fab' and still bind target antigen with high affinity. In-

stead of penetrating larger tumors, it may be possible to use ITs to attack the vascular bed that nourishes them.<sup>28</sup> Thus, tumors could be nutritionally and oxygen deprived. (7) Toxicity to nontarget tissue has been another major problem with ITs. Mistaken targets have been easy to identify in some cases such as when ITs were cross-reactive with nervous tissue.<sup>29</sup> In other studies, targets have not been so easy to identify. Toxicity may be decreased by deglycosylation, removal of the Fc region, or use of more stable cross-linkers. However, none of these are present in recombinant ITs such as DAB<sub>486</sub>IL-2 and dose-limiting toxicity still occurred in clinical trials.<sup>30</sup> In some ITs, activation of target cells by the MoAb portion has been problematic, such as in the case of anti-CD3, but activation toxicity was reduced by Fc region removal.<sup>31</sup> The solution will not always prove this simple.

#### THE MOUSE—MAN'S BEST FRIEND

Animal models weigh heavily in determining the therapeutic potential of ITs because several years and high costs are involved in new drug development. There are numerous examples of preclinical mouse models driving clinical studies. For example, a mouse BCL1 tumor of B-cell origin was used to study the comparative advantages of intact MoAb IT and Fab' IT before clinical studies with anti-CD22dg RTA and anti-CD22Fab' RTA.<sup>32</sup> For GVHD studies, mouse bone marrow transplants were performed into fully mismatched recipients and showed that IT could finely differentiate GVHD-causing T cells from bone marrow stem cells in vitro preventing lethal GVHD.<sup>33</sup> These studies proceeded the clinical use of ITs for in vitro T-cell depletion<sup>34</sup> and later for the in vivo administration for steroid refractory GVHD.<sup>35</sup> An important advantage of mouse modeling is the availability of numerous congenic mouse strains. For example, congenics have been bred differing from the wild type in the expression of single allelic form of a particular cell surface marker such as Thy1 on lymphocytes. ITs against the Thy1.2 allele of Thy1 will target Thy1.2-expressing leukemia cells and not Thy1.1 leukemia cells.<sup>36</sup> Thus, by using congenics, we can test IT efficacy in targeting tumor-specific markers.

The latest technologies in molecular biology are being combined with recent discoveries in mouse models to devise new animal modeling strategies to more comprehensively evaluate new reagents. Two examples are in this issue of the journal. Kreitman et al<sup>27</sup> have recognized the fact that establishing the in vivo efficacy of ITs containing anti-TAC has been difficult. The antibody binds to the p55 subunit of the IL-2 receptor expressed on cells from humans or other primates, but most IL-2 receptor-expressing lines will not grow in vivo. Also, primate studies are slow, expensive, and require special facilities available to few institutions. The investigators chose to transfect human cells with the human p55 IL-2 receptor inserted into a retroviral vector. The resulting new cell line formed tumors in nude mice and permitted the evaluation of a promising new recombinant IT, anti-TAC(Fv)-PE40. Their single-chain Fv IT composed of the variable domains of the MoAb anti-TAC and a modified form of the PE showed significant antitumor activity at 1% of the LD<sub>50</sub>.

Also in this issue, Winkler et al<sup>37</sup> illustrate another important animal model widely used to test reagent efficacy against disseminated human tumor implanted in mice with severe combine immunodeficiency disease (SCID). Although first described in the early 1980s, SCID mice today are preferentially used over nude mice for studying certain human tumors that are not disseminated in nudes. The investigators evaluated the effects of deglycosylated ricin toxin A chain ITs made with the MoAb RFT5 (CD25) and an anti-70-kD MoAb. Their report shows these have potential for therapy of Hodgkin's lymphoma. Because the human tumor disseminated in this model, the investigators were able to extract human DNA from different organs and probe for identity with human DNA to quantify the extent of organ infiltration, a clear advantage of using a murine model.

Those who have studied ITs for treating T-cell malignancies have experienced difficulty in getting human T-ALL cell lines to metastasize in nude mice. Tumor dissemination in nudes was possible only by coinjecting an accessory cell line.<sup>38</sup> A model more closely depicting metastatic human T-cell cancer has been devised in SCID mice and we have adapted several frozen T-ALL samples to the SCID model<sup>39</sup> (unpublished results). These models and others should prove useful to further optimize IT treatment strategies, including scheduling and dosing. Although it can be argued that rodent systems may fall short in providing appropriate toxicity assessments for clinical trials, they have become the choice for rapidly evaluating ITs. This is mainly because of FDA requirements for the demonstration of efficacy in animal systems, limited cost, speed in providing preclinical data, and the availability of numerous congenic strains.

#### TREATING THE BONE MARROW, NOT THE PATIENT

In the early 1980s, the use of fiercely catalytic toxins such as DT and ricin for selectively killing antigen expressing targets in animals was an exciting new approach, but injecting them into patients was viewed as high risk. The use of ITs in the field of bone marrow transplantation offered an opportunity to test the clinical selectivity of ITs without directly injecting them into patients and risking catastrophic side effects. Bone marrow was treated with a mixture of intact ricin ITs to remove GVHD-causing T cells that contaminated the donor graft in HLA-matched sibling transplants.<sup>40</sup> These studies showed that T cells in bone marrow could be killed without killing critical pluripotent stem cells ultimately responsible for lymphohematopoietic recovery and showed a clinically significant impact on GVHD.<sup>41</sup> ITs were also used to purge patient bone marrow of leukemia cells before aggressive conditioning in autologous bone marrow transplants.<sup>42</sup> These studies showed that ITs can effectively and selectively eliminate clonogenic leukemia cells without impairing lymphohematopoietic engraftment. However, the failure of conventional conditioning regimens for eliminating residual disease have limited this strategy.

#### THE FIRST IN VIVO CLINICAL SKIRMISHES IN A PROTRACTED WAR ON B-CELL MALIGNANCY

B-cell lymphoma has been the first clinical testing grounds for ITs for several reasons. (1) B cells express mark-

ers such as CD19 and CD22, which are lineage restricted.<sup>43</sup> (2) Historically, B-cell diseases are responsive to anticancer therapy with drugs or x-ray. (3) The tumors are more accessible to circulating IT. (4) Immunogenicity is less of a problem when B cells are targeted because the destruction of normal B cells can prevent the formation of human antitoxin or anti-Ig. However, anti-IT responses still occur in a significant proportion of treated patients.

Several IT strategies are currently being used and the results are provocative. From these studies, we have learned (1) with ITs, there is a higher partial/complete response rate in phase I studies (12% to 75%) than observed for the drugs that are currently marketed today for the treatment of cancer.<sup>1</sup> (2) Maximal tolerated doses range from 0.25 mg/kg to 1.8 mg/kg depending on the IT. The highest levels of hepatotoxicity and the lowest maximum tolerated dose (MTD) appear to be associated with those ITs synthesized with blocked B chain,<sup>5</sup> but a therapeutic advantage of the blocked ricin ITs may be the presence of B chain. This may result in a more potent IT because B chain contains translocation enhancing properties. ITs made with deglycosylated RTA have also proven efficacious, but treatment of any disease is associated with the onset of vascular leak problems. (3) There is no difference in clinical findings comparing Fab' anti-CD22 ITs to intact anti-CD22 ITs for treatment of non-Hodgkin's lymphoma.<sup>1</sup> (4) Multiple courses have been well tolerated, indicating that toxicity is not cumulative. (5) Toxicities that have occurred do not seem related to MoAb-variable region cross-reactivity with markers expressed on other vital organ systems. Thus, CD19, which is expressed on leukemic progenitor cells,<sup>44</sup> and CD22 appear to be good choices for targeting B-cell malignancies. Such has not always been the case. For example, when ITs were used to treat breast cancer, ITs unexpectedly recognized neural markers resulting in severe neurotoxicity.<sup>29</sup>

#### KILLING T CELLS—A DOUBLE EDGED SWORD

T cells can create clinical problems in the form of T-cell malignancies, autoimmune disease, acquired immunodeficiency syndrome (AIDS), diabetes, organ rejection, and GVHD. So why have we found it difficult to kill T cells without creating new problems? Animal models predicted that the simple elimination of T cells from bone marrow grafts to prevent GVHD lead to problems in sustained donor bone marrow engraftment possibly related to interference of ITs with graft-promoting interactions between T cells and stem cells.<sup>45</sup> In trials designed to explore the anti-GVHD potential of donor T-cell elimination with ITs, *in vitro* treatment was found to reduce the severity of, but not completely prevent clinical GVHD.<sup>41</sup> Clinical phase II studies designed to test ITs in ongoing steroid-resistant GVHD showed a number of partial and complete responses in a phase 2 trial administering anti-CD5-RTA and engraftment was not a problem in this setting.<sup>35</sup>

These and other studies<sup>46</sup> taught us that, although T cells can be targeted, IT/T-cell interactions are complicated by (1) low MTDs and anti-IT antibodies and (2) differing routes of marker internalization. For example, in the case of T cells, CD5 ITs are more toxic than CD2 ITs, which is

caused by preferential routing of anti-CD2 ITs to lysosomes where degradation of the A chain takes place.<sup>47</sup> (3) The exact nature of the GVHD target also complicates interactions because GVHD is a dynamic and complicated syndrome involving various lymphocyte types and antigen-presenting cells.<sup>48</sup> Several T-cell types may be involved and different cells may be involved in the induction versus the effector phase of the disease. (4) In eliminating abnormal T cells, we also eliminate normal T cells that can result in further complications to an already complicated immune system.

New knowledge of T-cell biology has had a major impact on the targeting field. Five years ago, an anti-CD3 MoAb directed against a component of the T-cell receptor (TCR) was administered to patients to prevent organ graft rejection. Toxicity occurred that was later associated with newly discovered activation properties of T cells caused by TCR recognition in association with Fc binding.<sup>49</sup> This caused cytokine release and side effects resulting in toxic anti-CD3 IT, which could be prevented by Fc removal.<sup>31</sup> With a new understanding of T-cell biology, new T-cell elimination strategies have developed. T-cell regulatory molecules such as the IL-2 receptor (IL-2R) have been targeted. Cytokine targeting takes advantage of the high affinity of cytokine ligands for their naturally occurring receptors and offers the advantage of nonimmunogenicity. IL-2 has been spliced to DT<sup>50</sup> or PE.<sup>51</sup> Truncated toxin genes lacking the binding domain but retaining the catalytic domain are used to prepare ITs with minimal nonspecific toxicity.

Kreitman et al<sup>27</sup> in this issue describe another clever approach involving a new molecular construct encompassing anti-IL-2R Fv and truncated toxin for *in vivo* therapy. Despite the advantages of reduced size (about 65 kD), no chemical bond, and targeting with a nonimmunogenic naturally occurring cytokine, there may be disadvantages because circulating levels of IL-2 or soluble IL-2R secreted by tumor or as part of the rejection process may interfere with IT binding and subsequent IT activity. Winkler et al<sup>36</sup> in this issue measure circulating IL-2 receptor (CD25) in their animal model and suggest that shedding of soluble CD25 in SCID mice is dependent on tumor mass. They point out that it may be impossible to differentiate between CD25 shed from tumor and soluble CD25 shed from activated lymphocytes, but that this may not be a problem in ITs used to treat residual disease, rather than more advanced bulky disease. This may be correct because their conventional anti-IL-2R IT was less effective against more advanced animal tumors. Another problem may be modulation of cytokine receptor because evaluation of mice with progressive lymphoma after IT treatment showed a lack of CD25 antigen that was initially present.

Another new approach is bispecific IT. Duke-Cohan et al<sup>52</sup> in the October 1, 1993 issue of *Blood* described a bispecific antibody with 2 different variable regions recognizing CD4 and CD26 antigens simultaneously on an activated memory T-cell subset that provides help for Ig synthesis and responses against recall antigens. When linked to blocked ricin, the bispecific antibody did not inhibit resting T cells. These agents may be useful for inhibiting graft rejection or GVHD. Also, future approaches may take advantage of T-

cell-associated recognition structures such as CD28 in a manner similar to the targeting of CD3.

Many diseases that are the focus of today's healthcare issues involve T cells, so investigation of novel and more effective means of targeting them will undoubtedly continue. In AIDS, ribosome-inactivating proteins have antiviral properties and CD4<sup>+</sup> T cells are a target of viral attack.<sup>53</sup> Autoimmune disease such as type 1 autoimmune diabetes involve T cells and anti-T cell IT show efficacy in animal models.<sup>54</sup> Rheumatoid arthritis has been treated with provocative clinical results.<sup>55</sup> T-cell malignancies continue to remain a problem, with few alternatives existing for patients undergoing drug refractory relapse. Although IT therapy can eliminate resting and dividing target cells in contrast to some drugs that preferentially inhibit dividing cells, potency still remains a major issue. In GVHD, which still requires better treatment approaches, preliminary studies have been encouraging. In graft rejection, the elimination of host T cells is still a paramount issue. Thousands remain on dialysis awaiting kidneys. A specific method of eliminating graft-rejecting recipient T cells would undoubtedly result in more transplants with less concern for rigorous matching. Also the elimination of T cells that prevent bone marrow rejection could reduce the need for aggressive, damaging recipient conditioning that is currently administered in the form of lethal total body irradiation (TBI). Preclinical studies show that ITs can be used with nonlethal irradiation to promote donor bone marrow engraftment across the major histocompatibility barrier.<sup>56</sup>

#### TARGETING OTHER ASPECTS OF THE LYMPHOHEMATOPOIETIC SYSTEM

ITs can be used to treat other hematopoietic malignancies. Winkler et al<sup>37</sup> describe 2 new ITs that can be used to treat the most frequently diagnosed lymphoma, Hodgkin's disease. Clinical trials are underway using ITs constructed by linking the RIP saporin to anti-CD30<sup>57</sup> or using a fusion protein consisting of the IL-2 gene spliced to truncated diphtheria toxin.<sup>58</sup> Hodgkin/Reed Sternberg cells causing Hodgkin's disease express large numbers of CD25 and CD30 determinants that are present only on a minority of normal cells. Although ITs have not been used clinically to treat acute myeloid leukemia (AML) previous *in vitro* IT studies show that myeloid cells can be selectively targeted.<sup>59</sup> Phase I trials with antimyeloid antibodies labeled with the radioisotope <sup>131</sup>I already show that AML is responsive to treatment.<sup>60</sup> Perhaps antimyeloid ITs that kill cells by a mechanism entirely unrelated to irradiation would kill radiation-resistant target cells or perhaps would enhance the killing of irradiated targets by protein synthesis inhibition of enzymes that repair DNA.

ITs may also be useful for specifically targeting the lymphohematopoietic system while avoiding other organ systems. Anti-CD45, which broadly targets lymphohematopoietic cells, could be used for treating various lymphohematopoietic malignancies.<sup>61,62</sup> Perhaps anti-CD45 IT could also be used to condition recipients by depleting lymphohematopoietic recipient cells before bone marrow transplant. Currently, aggressive conditioning of recipients

with TBI must be used to clear an abnormal lymphohematopoietic system to replace it with a healthy donor graft. ITs could be used to specifically deplete lymphohematopoietic cells without targeting other organ systems that are often injured by high-dose TBI. Such approaches may not replace our need for irradiation, but may reduce TBI levels currently necessary to achieve engraftment using matched, but unrelated donor marrow. If such an approach also requires depletion of pluripotent stem cells, ITs could be used to target them as well.

#### SMART MISSILES OR DUDS?

One of the most important questions is yet to be answered. Are ITs potent enough to eliminate all the tumor in stage 3 or stage 4 disease ( $10^{11}$  to  $10^{12}$  tumor cells)? The best kill delivered *in vitro* is about 5 to 7 logs. *In vivo*, ITs are limited by their ability to penetrate large tumors. This is not as great a problem in the case of lymphohematopoietic tumors, which are more accessible to systemic ITs. Also, there are antigen-deficient or transport-defective IT mutants that may be selected by prolonged IT courses.<sup>23,24</sup> Potency may be increased in these cases using mixtures of ITs reactive against different cell surface antigens. Mixtures may give enhanced killing compared with individual ITs.<sup>40</sup> This is not always the case. Sometimes the potency of the mixture is overshadowed by the activity of the most potent immunotoxin in the mixture, which is reported in this issue by Winkler et al.<sup>37</sup> Differences in IT potency may be explained by differences in antigen expression, intracellular compartmentalization of IT, and IT affinity for target, all which might be improved with the right IT or IT mixture. Even if we are unable to obtain levels of potencies needed to cure large tumors or relapsed leukemia, it is clear that ITs that disrupt protein synthesis kill by a different mechanism than conventional therapies such as DNA-targeting drugs or radiation. Studies indicate that, when ITs are combined with DNA-disrupting therapies such as cyclophosphamide, potency is greatly enhanced against tumor progenitor cells *in vitro*<sup>63</sup> and against progressing tumors *in vivo*.<sup>64</sup> The mechanisms of IT-enhanced drug killing are unknown, but it is likely that toxins inhibit the synthesis of cellular enzymes that facilitate drug breakdown such as aldehyde dehydrogenase. Thus, this approach may be important in eradication of residual disease after treatment with drugs or irradiation. Perhaps the best way to improve IT potency is through genetic engineering.

#### "BRINGING GE (GENETIC ENGINEERING) TO LIGHT"

Can we improve nature's design? The ability to scramble and unscramble toxin and Ig genes has generated excitement and certain clinical anticipation. But can PCR technology deliver better therapeutic agents than nature has naturally selected? One important philosophy has emerged at this stage: the simpler, the better. The likelihood of producing a functional protein decreases as the number of domains in a fusion toxin increase, because even if only one domain folds incorrectly it may nullify the usefulness of the entire molecule. The anti-TAC(Fv) made by Kreitman et al<sup>27</sup> in

this issue is an excellent example of improving IT design. The cell binding domain was removed from PE while retaining the translocation-enhancing domain. Nonessential amino acids 365-380 were deleted to make the molecule smaller. KDEL, a classic endoplasmic reticulum retention sequence, was included to increase potency. Only the variable domains of anti-Tac antibody were used to form a single chain Fv. Together, these modifications resulted in smaller, simpler ITs capable of inhibiting tumor cells *in vitro* at a doses less than one-tenth of a billionth of a gram. Other modifications have also proven useful. (1) New hybrid toxins have been engineered consisting of the RTA and DT genes retaining both N-glycosidase and ADP-ribosylating activity, respectively.<sup>65</sup> Conjugates made with this hybrid toxin are 100- and 1,000-fold more effective than conjugates made with RTA or DT alone. (2) We are gaining a better understanding of the interchain domains. For example, certain mutations in the peptides connecting single chain Fv to PE decreases IT aggregation and increases the yield of active IT molecules.<sup>66</sup> (3) Human antibodies of a desired specificity can now be selected from large display phage libraries ( $>10^7$  members) of human  $V_H$  and  $V_L$  Ig genes. Purified antigen is used to select the phage with the appropriate specificity, thus bypassing both hybridoma technology and immunization.<sup>67</sup> The Fv can then be spliced to an appropriate toxin gene. (4) Bispecific antibodies can now be engineered using leucine zippers.<sup>68</sup> Leucine zippers are specific amino acid sequences about 30 residues long, with leucine occurring every seventh residue. Such sequences form amphipathic  $\alpha$  helices. Because peptides corresponding to the leucine zippers of Fos and Jun preferentially form heterodimers, the attachment of these to different Fab' by gene fusion results in bispecific antibody formation. These could be biochemically or genetically linked to toxin. (5) Lysine residues have been introduced for modification of IT with PEG to prolong circulation time and reduce immunogenicity.<sup>69</sup> Despite the dazzling display of genetic manipulation currently being published, it is much too early to determine the superiority of fusion proteins over conventional ITs whose value can only be determined in the clinic.

#### TIL DEATH DO US PART

Only clinical success will insure a future for ITs. Partial successes will likely continue in studies designed to target lymphohematopoietic tumors because this approach partly side-steps the immense problem of IT penetration of tumor that will hamper treatment of advanced solid tumors. For the same reasons, strategies using ITs for compartmentalized therapy as in intrathecal IT administration for inoperable nervous system tumors should meet with some degree of success.<sup>70</sup> Many investigators studying systemic treatment with ITs in clinical trials have already recognized that their ITs are partially effective, but are not curative, and perhaps may never be curative by themselves because they are limited by their toxic side effects. These doses are mostly determined in very sick patients with bulky, end-stage disease and may be much higher than doses required to treat less-advanced cancers in healthier patients. This could lead

to abandonment of valuable new therapies unless approaches are discovered for increasing MTDs. Perhaps it should be recognized that ITs may require different strategies than the current system designed largely for drug testing. The reason is that dose escalation in terminal patients in phase I studies will establish an MTD, but the poor condition of these patients may not provide accurate information regarding clinical benefit of the treatment against less bulky or minimal disease. Perhaps a more reasonable approach would be to establish an efficacious dose based on the ability to obtain convincing responses in an animal model, such as in the same tumor grown in SCID mice. For example, anti-CD7-dRTA IT<sup>39</sup> currently in clinical trial was efficacious at 400  $\mu\text{g}/\text{kg}$  against human T-cell leukemia in SCID mice. This dose is eightfold less than the MTD reported for RTA IT in T-cell lymphoma phase I studies.<sup>71</sup> If clinical responses are observed with this lower dose, it might be more logical to use this dose as an adjunctive therapy in combination with established anticancer drugs that have proven partially effective in treating healthier patients with less disease, thus maximizing antitumor effects. While treatment with this current generation of IT alone will unlikely be effective for advanced disease, the evidence is clear and encouraging that ITs should have a role at least in combined therapies. The use of ITs as single-agent therapy will likely arise only from continued efforts to better understand the complex issues of targeting. At this time, it would appear that the clinical promise of ITs has not been fulfilled, but continues to grow in the face of our expanding technologic and biologic advances in the assembly ligands and toxins.

## REFERENCES

- Vitetta E, Thorpe PE, Uhr JW: Immunotoxins: Magic bullets or misguided missiles? *Immunol Today* 14:252, 1993
- Endo Y, Mitsui K, Motizuki M, Tsurugui K: The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes. *J Biol Chem* 262:5908, 1987
- Pappenheimer AM Jr: Diphtheria toxin. *Annu Rev Biochem* 46:69, 1977
- Nicolson G, Blaustein J: The interaction of *Ricinus communis* agglutinin with normal and tumor cell surfaces. *Biochim Biophys Acta* 266:543, 1972
- Lambert JM, McIntyre G, Gauthier MN, Zullo D, Rao V, Steeves RM, Goldmacher VS, Blattler WA: 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* 30:3234, 1991
- Colombatti M, Greenfield L, Youle RJ: Cloned fragment of diphtheria toxin linked to T cell-specific antibody identifies regions of B chain active in cell entry. *J Biol Chem* 261:3030, 1986
- Murphy JR, Bishai W, Borowski M, Miyanojara A, Boyd J, Nagle S: Genetic construction, expression, and melanoma-selective cytotoxicity of a diphtheria toxin-related  $\alpha$ -melanocyte stimulating hormone fusion protein. *Proc Natl Acad Sci USA* 83:8258, 1986
- Stirpe F, Barbieri L: Ribosome-inactivation proteins up to date. *FEBS Lett* 195:1, 1985
- Grossbard ML, Freedman AS, Ritz J, Coral F, Goldmacher VS, Eliseo L, Spector N, Dear K, Lambert JM, Blattler WA, Taylor JA, Nadler LM: Serotherapy of B-cell neoplasms with anti-B-blocked ricin: A phase I trial of daily bolus infusion. *Blood* 79:576, 1992
- Bookman MA, Godfrey S, Padavic K: Anti-transferrin receptor immunotoxin (IT) therapy: A phase-I intraperitoneal (i.p.) trial. *Proc Am Soc Clin Oncol* 9:187, 1990
- Edwards MJ, Heniford BT, Klar EA, Doak KW, Miller FN: Pentoxifylline inhibits interleukin-2-induced toxicity in C57BL/6 mice but preserves antitumor efficacy. *J Clin Invest* 90:637, 1992
- Simmons BM, Stahl PD, Russell JH: Mannose receptor-mediated uptake of ricin toxin and ricin A chain by macrophages. *J Biol Chem* 261:7912, 1986
- Thorpe PE, Wallace PM, Knowles PP, Relf MG, Brown ANF, Watson GJ, Blakey DC, Newell DR: Improved antitumor effects of immunotoxins prepared with deglycosylated ricin A-chain and hindered disulfide linkages. *Cancer Res* 48:6396, 1988
- Amlot PL, Stone MJ, Cunningham D, Fay J, Newman R, Collins R, May R, McCarthy M, Richardson J, Ghetie V, Ramilo O, Thorpe PE, Uhr JW, Vitetta ES: Phase I study of an anti-CD22-deglycosylated ricin A chain immunotoxin in the treatment of B-cell lymphomas resistant to conventional therapy. *Blood* 82:2624, 1993
- Fulton RJ, Blakey DC, Knowles PP, Uhr JW, Thorpe PE, Vitetta ES: Purification of ricin A<sub>1</sub>, A<sub>2</sub> and B chains and characterization of their toxicity. *J Biol Chem* 261:5314, 1986
- Piatek M, Lane JA, Laird W, Bjorn MJ, Wang A, Williams M: Expression of soluble and fully functional ricin A chain in *Escherichia coli* is temperature-sensitive. *J Biol Chem* 263:4837, 1988
- Thorpe PE, Wallace PM, Knowles PP, Relf MG, Brown ANF, Watson GJ, Knybe RE, Wawrzynczak EJ, Blakey DC: New coupling agents for the synthesis of immunotoxins containing a hindered disulfide bond with improved stability *in vivo*. *Cancer Res* 47:5924, 1987
- Youle RJ, Neville DM Jr: Kinetics of protein synthesis inactivation by ricin-anti-Thy 1.1 monoclonal antibody hybrids. *J Biol Chem* 257:1598, 1982
- Jansen FK, Blythman HE, Carriere D, Casellas P, Gros O, Gros P, Laurent JC, Paolucci F, Pau B, Poncelet P, Richer G, Vidal H, Voisin GA: Immunotoxins: Hybrid molecules combining high specificity and potent cytotoxicity. *Immunol Rev* 62:185, 1982
- Hale G, Dyer MJS, Clark MR, Phillips JM, Marcus R, Riechmann L, Winter G, Waldmann H: Remission induction in non-Hodgkin lymphoma with reshaped human monoclonal antibody CAMPATH-1H. *Lancet* 2:1394, 1988
- Sehon AH: Suppression of IgE antibody responses with tolerogenic conjugates of allergens and haptens. *Prog Allergy* 32:161, 1982
- Youle RJ, Newton D, Wu YN, Gadina M, Rybak SM: Cytotoxic ribonucleases and chimeras in cancer therapy. *Crit Rev Ther Drug Carrier Syst* 10:1, 1993
- Engert A, Martin G, Pfreundschuh M, Amlot P, Hsu S-M, Diehl V, Thorpe P: Antitumor effects of ricin A chain immunotoxins prepared from intact antibodies and Fab' fragments on solid human Hodgkin's disease tumors in mice. *Cancer Res* 50:2929, 1990
- Ghetie MA, Tucker K, Richardson J, Uhr JW, Vitetta ES: The antitumor activity of an anti-CD22 immunotoxin in SCID mice with disseminated Daudi lymphoma is enhanced by either an anti-CD19 antibody or an anti-CD19 immunotoxin. *Blood* 80:2315, 1992
- Reinherz EL, Haynes BF, Nadler LM, Bernstein ID (eds): *Leukocyte Typing II*, vol 2. New York, NY, Springer-Verlag, 1986, p 3
- Spitler LE, del Rio M, Khentigan A, Wedel NI, Brophy NA, Miller LL, Harkonen WS, Rosendorf LL, Lee HM, Mischak RP, Kawahata RT, Stoudemire JB, Fradkin LB, Bautista EE, Scannon

PJ: Therapy of patients with malignant melanoma using a monoclonal antimelanoma antibody-ricin A chain immunotoxin. *Cancer Res* 47:1717, 1987

27. Kreitman RJ, Bailon P, Chaudhary VK, FitzGerald DJP, 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* 83:426, 1994

28. Burrows FJ, Watanabe Y, Thorpe PE: A murine model for antibody-directed targeting of vascular endothelial cells in solid tumors. *Cancer Res* 52:5954, 1992

29. Gould BJ, Borowitz MJ, Groves ES, Carter PW, Anthony D, Weiner LM, Frankel A: Phase I study of an anti-breast cancer immunotoxin by continuous infusion: Report of a targeted toxic effect not predicted by animal studies. *J Natl Cancer Inst* 81:775, 1989

30. LeMaistre F, Rosenblum M, Reuben J, Parkinson D, Meneghetti C, Parker K, Shaw J, Deisseroth A, Woodworth T: Phase I study of genetically engineered DAB<sub>486</sub>IL-2 receptor expressing malignancies. *Blood* 76:360a, 1990 (abstr, suppl 1)

31. Hirsch R, Bluestone A, DeNenno L, Gress RE: Anti-CD3F(ab)<sub>2</sub> fragments are immunosuppressive in vivo without evoking either the strong humoral response or morbidity associated with the whole monoclonal antibody. *Transplantation* 49:1117, 1990

32. Krolick KA, Uhr JW, Slavin S, Vitetta ES: *In vivo* therapy of a murine B cell tumor (BCL<sub>1</sub>) using antibody ricin A chain immunotoxins. *J Exp Med* 155:1797, 1982

33. Valleria DA, Youle RJ, Neville DM Jr, Kersey JH: Bone marrow transplantation across major histocompatibility barriers. V. Protection of mice from lethal GVHD by pretreatment of donor cells with monoclonal anti-Thy-1.2 coupled to the toxin ricin. *J Exp Med* 155:949, 1982

34. Filipovich AH, Valleria DA, Youle RJ, Quinones RR, Neville DM Jr, Kersey JH: *Ex vivo* treatment of donor bone marrow with anti-T cell immunotoxins for the prevention of graft-versus-host disease. *Lancet* 8375:469, 1984

35. Byers VS, Henslee PJ, Kernan NA, Blazar BR, Gingrich R, Phillips GL, LeMaistre CF, Gilliland G, Antin JH, Martin P, Tutscha PJ, Trown P, Ackerman SK, O'Reilly RJ, Scannon PJ: Use of an anti-pan T-lymphocyte ricin A chain immunotoxin in steroid-resistant acute graft-versus-host disease. *Blood* 75:1426, 1990

36. Youle RJ, Neville DM Jr: Anti-Thy 1.2 monoclonal antibody linked to ricin is a potent cell-type-specific toxin. *Proc Natl Acad Sci USA* 77:5483, 1980

37. Winkler U, Gottstein C, Schön G, Kapp U, Wolf J, Hansmann M-L, Bohlen H, Thorpe P, Diehl V, Engert A: Successful treatment of disseminated human Hodgkin's disease in SCID mice with deglycosylated ricin A-chain immunotoxins. *Blood* 83:466, 1994

38. Ziegler HW, Frizzera G, Bach FH: Successful transplantation of a human leukemia cell line into nude mice: Conditions optimizing graft acceptance. *J Natl Cancer Inst* 68:15, 1992

39. Jansen B, Valleria DA, Jaszcz WB, Nguyen D, Kersey JH: Successful treatment of human acute T-cell leukemia in SCID mice using anti-CD7-deglycosylated ricin A-chain immunotoxin DA7. *Cancer Res* 52:1314, 1992

40. Valleria DA, Ash RC, Zanjani ID, Kersey JH, LeBien TW, Beverly PCL, Neville DM Jr, Youle RJ: Anti-T cell reagents for human bone marrow transplantation: Ricin linked to three monoclonal antibodies. *Science* 222:512, 1983

41. Filipovich AH, Valleria DA, Youle RJ, Haake R, Blazar B, Arthur D, Neville DM Jr, Ramsay NKC, McGlave P, Kersey JH: Graft-versus-host disease prevention in allogeneic bone marrow

transplantation from histocompatible siblings: A pilot study using immunotoxins for T cell depletion of donor bone marrow. *Transplantation* 44:62, 1987

42. Uckun FM, Kersey JH, Valleria DA, Ledbetter JA, Weisdorf D, Myers DE, Haake R, Ramsay NKC: Autologous bone marrow transplantation in high risk remission T-lineage ALL using immunotoxins plus 4-HC for marrow purging: I. Application of multiparameter flow cytometry and colony assays for a quantitative analysis of autografts for the presence of residual T-lineage leukemic lymphoid progenitor cells. *Blood* 79:1723, 1990

43. Grossbard ML, Press OW, Appelbaum FR, Bernstein ID, Nadler LM: Monoclonal antibody-based therapies of leukemia and lymphoma. *Blood* 80:863, 1992

44. Uckun FM, Gajl-Peczalska KJ, Kersey JH, Houston LL, Valleria DA: Use of a novel colony assay to evaluate the cytotoxicity of an immunotoxin containing pokeweed antiviral protein against blast progenitor cells freshly obtained from patients with common B-lineage acute lymphoblastic leukemia. *J Exp Med* 163:347, 1986

45. Valleria DA, Filipovich A, Soderling CCB, Kersey JH: Bone marrow transplantation across major histocompatibility barriers in mice. IV. Graft-versus-host disease in TLI-conditioned mice. *Clin Immunol Immunopathol* 23:437, 1982

46. Valleria D: The use of immunotoxins in bone marrow transplantation: Eradication of T cells and leukemic cells, in Vogel C-W (ed): *Immunoconjugates*. New York, NY, Oxford, 1987, p 217

47. Press OW, Vitetta ES, Farr AG, Hansen JA, Martin PJ: Evaluation of ricin A-chain immunotoxin directed against human T cells. *Cell Immunol* 102:10, 1986

48. Grebe SC, Streilein WJ: Graft-versus-host reactions: A review. *Adv Immunol* 22:119, 1976

49. Hirsch R, Gress RE, Plutnick DH, Eckhaus M, Bluestone JA: Effects of *in vivo* administration of anti-CD3 monoclonal antibody on T cell function in mice. II. *In vivo* activation of T cells. *J Immunol* 142:737, 1989

50. Williams DP, Parker K, Bacha P, Bishai W, Borowski M, Genbauffe F, Strom TB, Murphy JR: Diphtheria toxin receptor binding domain substitution with interleukin-2: Genetic construction and properties of a diphtheria toxin-related interleukin-2 fusion protein. *Protein Eng* 1:492, 1987

51. Bailon P, Weber DV, Gately M, Smart JE, Lorberboum-Galski H, Fitzgerald D, Pastan I: Purification and partial characterization of an interleukin 2-*Pseudomonas* exotoxin fusion protein. *Biotechnology* 6:1326, 1988

52. Duke-Cohan JS, Morimoto C, Schlossman ST: Targetting of an activated T-cell subset using a bispecific antibody-toxin conjugate directed against CD4 and CD26. *Blood* 82:2224, 1993

53. Kim YW, Fung MSC, Sun NC, Sun CRY, Chang NT, Chang TW: Immunoconjugates that neutralize HIV virions kill T-cells infected with diverse strains of HIV-1<sup>1</sup>. *J Immunol* 144:1257, 1990

54. Valleria DA, Carroll SF, Brief S, Blazar BR: Anti-CD3 immunotoxin prevents low-dose STZ/interferon-induced autoimmune diabetes in mouse. *Diabetes* 41:457, 1992

55. Byers VS, Caperton E, Ackerman S, Shepard J, Scannon P: Modification of the immune system in patients with rheumatoid arthritis treated with anti-CD5 ricin A chain immunotoxin. *FASEB J* 3:5205, 1989

56. Blazar BR, Hirsch R, Gress RE, Carroll SF, Valleria DA: *In vivo* administration of anti-CD3 monoclonal antibodies or immunotoxins in murine recipients of allogeneic T-cell depleted marrow for the promotion of engraftment. *J Immunol* 147:1492, 1991

57. Falini B, Bolognesi A, Flenghi L, Tazzare PL, Broe MK, Stein H, Durkop H, Aversa F, Corneli P, Pizzolo G, Barbabietola G, Sabatini E, Pileri S, Martelli MF, Stirpe F: Response of refractory Hodgkin's disease to monoclonal anti-CD30 immunotoxin. *Lancet* 339:1195, 1992

58. Woodworth TG, LeMaistre CF, McCaffrey R, Schnipper L, Rosen S, Ratain M: Phase I/II clinical studies of DAB<sub>486</sub>IL-2 fusion toxin in patients with refractory IL-2 receptor expressing malignancies. *Blood* 80:158a, 1992 (abstr, suppl 1)
59. Myers DE, Uckun FM, Ball ED, Valleria DA: Immunotoxins for ex vivo marrow purging in autologous bone marrow transplantation for acute nonlymphocytic leukemia. *Transplantation* 46:240, 1988
60. Schwartz MA, Lovett DR, Redner A, Gulati S, Divgi CR, Graham MC, Finn R, Gee TS, Andreef MD, Old LJ, Larson SM, Scheinberg DA: Therapeutic trial of radiolabelled monoclonal antibody M195 in relapsed or refractory myeloid leukemias. *Blood* 78:54a, 1991 (abstr, suppl 1)
61. Matthews DC, Applebaum FR, Eary JF, Hui TE, Fisher DR, Martin PJ, Durack LD, Nelp WB, Press OW, Badger CC, Bernstein ID: Radiolabeled anti-CD45 monoclonal antibodies target lymphohematopoietic tissue in the macaque. *Blood* 78:1864, 1991
62. Omary MN, Trowbridge IS, Battifor HA: Human homologue of murine T-200 glycoprotein. *J Exp Med* 152:842, 1980
63. Uckun FM, Stong RC, Youle RJ, Valleria DA: Combined ex vivo treatment with immunotoxins and mafosfamid: A novel immunochemotherapeutic approach for elimination of neoplastic T-cells from autologous marrow grafts. *J Immunol* 134:3504, 1985
64. Weil-Hillman G, Uckun FM, Manske JM, Valleria DA: Combined immunochemotherapy of human solid tumors in nude mice. *Cancer Res* 47:579, 1987
65. Li B-Y, Ramakrishnan S: Recombinant hybrid toxin with dual enzymatic activities. Potential use in preparing highly effective immunotoxins. *J Biol Chem* 269:1, 1994
66. Brinkmann U, Buchner J, Pastan I: Independent domain folding of *Pseudomonas* exotoxin and single-chain immunotoxins: Influence of interdomain connections. *Proc Natl Acad Sci USA* 89:3075, 1992
67. Marks MD, Hoogenboom HR, Bonnert TP, McCafferty J, Griffiths AD, Winter G: By-passing immunization. Human antibodies from V-gene libraries displayed on phage. *J Mol Biol* 222:581, 1991
68. Kostelny SA, Cole MS, Tso JY: Formation of a bispecific antibody by the use of leucine zippers. *J Immunol* 148:1547, 1992
69. Wang Q, Pai LH, Debinski W, FitzGerald DJ, Pastan I: Polyethylene glycol-modified chimeric toxin composed of transforming growth factor alpha and pseudomonas exotoxin. *Cancer Res* 53:4588, 1993
70. Zovickian J, Youle RJ: Efficacy of intrathecal immunotoxin therapy in an animal model of leptomeningeal neoplasia. *J Neurosurg* 68:767, 1988
71. LeMaistre CF, Rosen S, Frankel A, Kornfeld S, Saria E, Meneghetti C, Drajesk J, Fishwild D, Scannon P, Byers V: Phase I trial of H65-RTA immunoconjugate in patients with cutaneous T-cell lymphoma. *Blood* 78:1173, 1991