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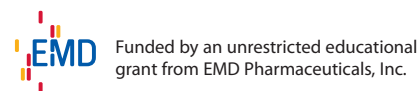
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Immunotoxins: The Next Generation

Reviewed by Philip D. Bonomi, MD

Immunotoxins* are proteins used to treat cancers; they are composed of an antibody fragment or a growth factor linked to a toxin, either *Pseudomonas aeruginosa* exotoxin A or diphtheria toxin. The immunotoxin binds to a surface antigen on a cancer cell; it is internalized by the target cells and the enzymatic fragment of the toxin translocates to the cytosol. Once in the cytosol, one molecule is capable of killing the cell, making immunotoxins potentially the most potent killing agents. Immunotoxins derive their potency from the toxin and their specificity from the antibody or antibody fragment to which they are attached.

First-generation immunotoxins that were developed by chemically conjugating whole toxins to antibodies were typically ineffective in animal models because the toxin also killed normal cells. In the second-generation products, the removal of the cell-binding domain from the toxin and attachment of this modified toxin to various antibodies produced better tolerated immunotoxins with some antitumor activity. More recently, the third-generation immunotoxins have been developed using recombinant DNA techniques and protein engineering to overcome the problems associated with the earlier products.

Immunotoxins are now designed to contain only the elements needed to recognize and kill tumor cells. This occurs by replacing the cell-binding domain of the toxin with the Fv portion of an antibody and retaining and translocating and cell-killing domains; these recombinant chimeric proteins can be produced economically in large quantities in *Escherichia coli*.

Another means of targeting toxins to cells is to replace the cell-binding domain with a growth factor or cytokine. Among the agents that have been utilized are the interleukins (2, 4, and 13), transforming growth factor- α , and granulocyte-macrophage colony stimulating factor.

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Toxicity Associated With Immunotoxins

Two types of toxicity are associated with immunotoxins: (1) non-specific and (2) targeted. A common type of nonspecific toxicity is vascular leak syndrome, in which fluid leaks from the capillaries, serum albumin falls, and fluid retention, edema, and weight gain occur. This type of adverse event usually can be managed by adequate hydration. Targeted toxicity is caused by targeting the toxin to normal tissues that contain the same target antigen as the cancer cell. This is not a problem if immunotoxins are targeted to antigens on B- or T-cell malignancies, because normal B and T cells can be regenerated from antigen-negative stem cells, but it may be a serious problem if solid tumors are targeted, since the antigen may be present on vital organs and the immunotoxin will kill normal cells in such tissues.

Clinical Trials of Immunotoxins

Individual immunotoxins are designed to treat specific cancers. To date, most of the clinical success with immunotoxins has been achieved in the treatment of hematologic tumors. Obstacles to successful treatment of solid tumors include poor penetration into tumor masses and the immune response to the toxin component of the immunotoxin, which limits the number of cycles that can be given. Strategies to overcome these limitations are under consideration.

Immunotoxins Targeting the IL-2 Receptor

Denileukin diftitox (known as Ontak), produced using recombinant DNA technology to target diphtheria toxin to cells that express the high-affinity IL-2 receptor, became available several years ago and has been shown to be a useful and important agent in the treatment of patients with persistent or recurrent cutaneous T-cell lymphoma (CTCL).¹ In clinical trials in patients with cutaneous T-cell lymphoma, denileukin diftitox produced response rates of 30%, which included 10% complete remissions; response rates of 33% and 25% have been noted in chronic lymphocytic leukemia² and non-Hodgkin's lymphoma.³ Denileukin diftitox is being studied to eliminate regulatory T cells that express CD25 to increase the antitumor activity of cancer vaccines.⁴

Anti-CD25 Immunotoxins

LMB-2, which targets the α -subunit of IL-2R, contains the Fv of the anti-CD25 monoclonal antibody anti-Tac fused to the N terminus of PE38, the 38 kDa fragment of *P aeruginosa* exotoxin A. LMB-2 is currently undergoing testing at the National Institutes of Health (NIH) in Bethesda, Maryland, in patients with chronic lymphocytic leukemia (CLL), CTCL, hairy cell leukemia (HCL), and Hodgkin's disease.

In a phase I trial in which LMB-2 was administered to 35 patients with CD25+ hematologic malignancies for whom standard and salvage therapies failed. LMB-2 was given intravenously at dose levels ranging from 2 to 63 $\mu\text{g}/\text{kg}$, over 30 minutes on alternate days for 3 days.⁵ Toxicity was transient and most commonly included transaminase elevations and fever. Less than 20% of patients developed neutralizing antibodies after the first cycle of administration. Phase II trials are currently in progress in patients with CLL or CTCL, with results anticipated in about a year. Activity has been observed in all diagnoses being tested.

BL22 Immunotoxin

BL22 is a recombinant immunotoxin containing a truncated form of the *P aeruginosa* exotoxin A attached to an Fv fragment of an anti-CD22 monoclonal antibody. Clinical trials with this agent began in 1999.

In phase I studies of patients with various B-cell malignancies, BL22 was found to be very active in chemoresistant HCL, with 19 (61%) of 31 patients achieving complete remission (CR).⁶ The cytopenias characteristic of HCL improved in all complete and partial responders. The dose-limiting toxicity in HCL was a reversible hemolytic uremic syndrome, observed only during cycles 2 or 3. The maximum tolerated dose was established as 40 $\mu\text{g}/\text{kg}$ intravenously every 2 days x3 for cycle 1.

Now under way at the NIH are a phase II trial of BL22 in patients with HCL and phase I trials in CLL, non-Hodgkin's lymphoma, and pediatric acute lymphocytic leukemia (ALL).

Immunotoxins Targeting the GM-CSF Receptor

A recombinant toxin that targeted the GM-CSF receptor present

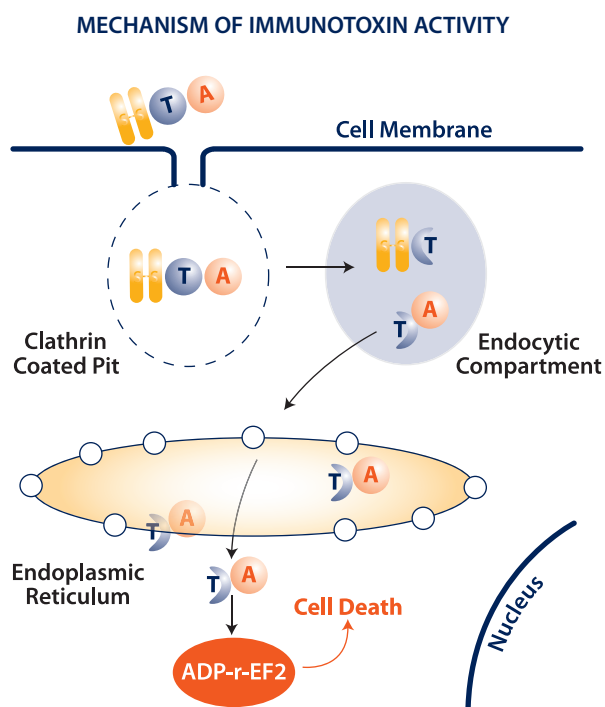


Figure. Tumor cell killing by a recombinant immunotoxin.

The figure shows a disulfide stabilized recombinant immunotoxin connected to a truncated PE toxin lacking the cell binding domain but retaining the translocation (T) and ADP ribosylation domain (A). After attaching to the tumor antigen, the immunotoxin enters the cells via clathrin-coated pits, goes to the endocytic compartment, and undergoes processing. The C-terminal fragment of the immunotoxin, which retains the ADP ribosylation domain, is transported to the endoplasmic reticulum. It is then translocated to the cytosol, where it ADP ribosylates elongation factor 2, leading to inhibition of protein synthesis and cell death.

on acute myeloid leukemia (AML) cells was developed but was associated with cytokine release and liver toxicity. An alternate approach currently under investigation in patients with AML is to target the IL-3 receptor using the recombinant toxin DT388-IL3.⁷

Immunotoxins Targeting Mesotheliomas and Ovarian and Pancreatic Cancers

Mesothelin is a differentiation antigen that is highly expressed in several types of cancer, such as mesothelioma and ovarian and pancreatic cancers.⁸ An immunotoxin, SS1P, with a high affinity for mesothelin has been produced and is under study at the National Cancer Institute (NCI).

“Mesothelin is highly expressed on a number of tumors, and it appears to play a role in cancer spread,” said Raffit Hassan, MD, Chief, Solid Tumor Immunotherapy Section in the Laboratory of Molecular Biology at the NCI Center for Cancer Research who is studying this immunotoxin. “These characteristics make it a very important molecule for targeted therapies.”

In a recently completed trial, SS1P was administered by continuous infusion over 10 days; in another, SS1P was given by 30-minute infusion every 2 days for 3 to 6 doses. In both trials, the dose-limiting toxicity was pleuritis. Minor but significant antitumor responses were seen with both methods of administration. A phase II trial is currently being planned in which patients with mesothelioma will receive a combination of SS1P and chemotherapy

SS1P is the first targeted therapy for mesothelioma, an aggressive cancer and one for which there is no effective treatment currently available, according to Dr. Hassan.

Immunotoxins Targeting IL-4, IL-13, and EGFRs

Truncated *P aeruginosa* exotoxin A has been fused to several cytokines and growth factors including IL-4, IL-13, and epidermal growth factor receptors (EGFRs). Such agents appear to be suited for local therapy, and 3 of these agents that target the EGF, IL-4, and IL-13 receptors have been evaluated for the therapy of glioblastoma. Agents known as TGF α -PE38 and IL-13-PE38QQR have produced complete responses in some patients during phase I and II trials and are actively under development.^{9,10}

Refining the Immunotoxin Strategy

The many investigators working on immunotoxin development have reported that clinical responses have been observed primarily in hematologic malignancies and not solid tumors. This appears to be the case because cancer cells found in the blood, bone marrow, and spleen are more readily accessible to immunotoxins than those found in solid tumors. Additionally, in patients with hematologic malignancies, the immune system may be damaged by prior chemotherapy, so that there is little production of anti-immunotoxin antibodies. Thus, more than 1 cycle of immunotoxin treatment can be administered. Rituximab and high-dose steroids have been investigated as potential means of preventing neutralizing antibody formation in patients with solid tumors, but did not prove effective. Chemical modification of the immunotoxin with high-molecular-weight polyethylene glycol appears to decrease immunogenicity.¹¹

What's Ahead in Immunotoxin Therapy?

There is now a body of evidence suggesting that immunotoxins may play an important therapeutic role in the treatment of hematologic malignancies. Unfortunately, the utility of these agents in solid tumors remains to be established, because there appears to be poor uptake of the therapeutic proteins. A potential means of overcoming this barrier might be combining immunotoxin therapy with cytotoxic agents that damage blood vessels and reduce the high interstitial pressure within tumors that impedes the entry of the immunotoxins. One promising target is the mesothelin protein that is expressed on ovarian cancers and mesotheliomas.

Another challenge that remains is to render the toxin portion of the immunotoxin less immunogenic, to prevent the development of neutralizing antibodies and ensure that more cycles of treatment can be given. Among the approaches under consideration are identifying and removing T- or B-cell epitopes, modifying the protein with high-molecular-weight polyethylene glycol, or treating patients with immunosuppressive agents.

“Immunotoxin therapy may have several potential advantages over other forms of surface-targeted therapy,” said NIH researcher Robert J. Kreitman, MD, Chief of the Clinical Immunotherapy Section, Laboratory of Molecular Biology, at the NCI, NIH. “They do not rely on the patient’s immune system for killing, they can kill tumor cells that do not undergo apoptosis, they are non-toxic to neighboring normal cells that do not internalize them, and they kill chemoresistant tumor cells. Most importantly, they, unlike less potent molecules, can kill cells expressing only a limited number of antigen sites per cell.” ■

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