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**Review Article**

# Immunotoxins - a new class of anticancer drugs

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**Abstract**

Immunotoxins deliver a toxin specifically to tumor cells by targeting tumor cell-surface antigens, without harming the normal cell. Once inside the cell, the toxin kills the tumor cell by either inhibiting protein synthesis or altering signal transduction pathways. Immunotoxins improve the therapeutic index, with the potential to enhance efficacy and decrease systemic toxicity. Several immunotoxins have shown impressive anticancer activity in various stages of clinical trials. We present a brief overview of recent progress in the clinical development of immunotoxins.

## Introduction

Immunotoxins consist of two elements, a tumor-targeting ligand and a modified toxin. The tumor-targeting ligand is primarily an antibody or antibody fragment that recognizes the tumor cell-surface antigen. It may also be a cytokine, growth factor or peptide hormone that binds to a specific receptor distributed on the surface of tumor cells. The tumor-targeting ligand binds a malignant cell-surface antigen, triggers internalization and delivers toxin to the cytosol, where the toxin can either hinder cellular protein synthesis or modify signal transduction pathways, leading to cell apoptosis (1-4).

Immunotoxins have obvious advantages over traditional chemotherapy, since they spare normal cells and thereby reduce systemic toxicity. In addition, immunotoxins are also a therapeutic option for chemotherapy-refractory malignancies because of the different killing mechanism compared to traditional chemotherapy. Furthermore, immunotoxins may offer synergistic effects in the treatment of tumors in combination with traditional chemotherapy.

Recent advances in genetic engineering have resulted in several monoclonal antibodies, including Herceptin® (trastuzumab), Rituxan® (rituximab), Avastin® (bevacizumab) and Erbitux® (cetuximab), playing remarkable roles in oncology (Table I). The tumor-killing mechanism covers from targeting amplified oncogene products to inhibiting angiogenesis. Our growing knowledge in antibody tumor targeting will expedite the development of antibody derivatives, including radiolabeled antibodies, drug-antibody conjugates and immunotoxins. Several immunotoxins (Table II) have shown impressive anticancer activity, although many problems also emerged in various stages of clinical trials. We will provide an overview of the recent progress in the development and clinical evaluation of immunotoxins.

## Clinical trials

Hematological malignancies are excellent candidates for immunotoxin therapy since the tumor cell-surface markers are usually highly expressed and easily accessible. One of the earliest immunotoxins, anti-B4-blocked ricin, was evaluated in two phase II trials in patients with acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL). Anti-B4-blocked ricin is a chemically conjugated immunotoxin composed of an antibody (the anti-CD19 murine monoclonal antibody B4) and a modified ricin toxin. Ricin toxin is a heterodimer consisting of a functional subunit and a binding subunit. The functional subunit acts as an rRNA *N*-glycosidase enzyme that blocks cellular protein synthesis, while the binding subunit (lectin) binds to normal mammalian cell-surface oligosaccharides. In order to reduce the nonspecific binding of ricin toxin, the binding subunit was blocked before conjugating ricin toxin to the antibody.

In the first study, Szatrowski *et al.* (5) compared anti-B4-blocked ricin in CD19-positive ALL patients with high-dose cytarabine in CD19-negative ALL patients. Although 66 of 78 patients (85%) achieved complete remission, the patients showed no additional clinical benefit from

*Table I: Monoclonal antibodies and their derivatives for cancer therapy.*

| Generic name | Trade name | Target | Indication |
|---|---|---|---|
| Rituzimab | Rituxan | CD20 | Non-Hodgkin's lymphoma |
| Trastuzumab | Herceptin | HER-2/neu | Breast cancer |
| Gemtuzumab | Mylotarg | CD33 | Acute myeloid leukemia |
| Alemtuzumab | Campath | CD52 | Chronic lymphocytic leukemia |
| Y-90 Ibritumomab tiuxetan | Zevalin | CD20 | Non-Hodgkin's lymphoma |
| I-131 Tositumomab | Bexxar | CD20 | Non-Hodgkin's lymphoma |
| Cetuximab | Erbitux | EGFR | Refractory metastatic colorectal cancer |
| Bevacizumab | Avastin | VEGF | Metastatic colorectal cancer |

*Table II: Immunotoxins in clinical development.*

| Name | Component | Indication | Phase |
|---|---|---|---|
| Anti-B4-blocked ricin | Anti-CD19/ricin | Non-Hodgkin's lymphoma | III |
| TGFα-PE38 (TP-38) | Anti-EGFR/PE | High-grade glioma | II |
| RFB4(dsFv)-PE38 (BL22) | Anti-CD22/PE | Hairy cell leukemia<br>CD22+ chronic lymphocytic leukemia | II |
| Anti-Tac(Fv)-PE38 (LMB2) | Anti-CD25/PE | CD25+ CLL or cutaneous T-cell lymphoma | II |
| Ki-4.dgA | Anti-CD30/dgA | Hodgkin's lymphoma | I/II |
| IL-13PE38 | Interleukin/PE | Malignant glioma | I/II |
| RFT5.dgA | Anti-CD22/dgA | Hodgkin's lymphoma | I/II |
| DT388GMCSF | GMCSF/DT | Acute myeloid leukemia | I |
| BU12-saporin | Anti-CD19/saporin | CD19+ lymphoma | I |
| SS1-PE38 | Anti-mesothelin/PE | Mesothelin+ solid tumor | I |
| IgG-RFB4-SMPT-dgA | Anti-CD22/dgA | CD22+ B-cell lymphoma | I |
| Transferrin-CRM107 | Transferrin/DT | Glioblastoma multiforme | I |
| Hum-195/rGel | Anti-CD33/gelonin | Advanced myeloid malignancy | I |

DT = diphtheria toxin; dgA = deglycosylated ricin A; PE = *Pseudomonas* exotoxin; GMCSF = granulocyte-macrophage colony-stimulating factor; EGFR = epidermal growth factor receptor; CLL = chronic lymphocytic leukemia.

anti-B4-blocked ricin. The most common toxicities were asymptomatic transient elevation of liver function tests, lymphopenia and immune response to anti-B4-blocked ricin. No objective responses were observed in another phase II study of anti-B4-blocked ricin in CLL patients (6).

In addition to ricin with a blocked galactose binding site, deglycosylated ricin A subunit (dgA) was also used to construct immunotoxins in order to reduce the frequently associated toxicities, such as hepatotoxicity, vascular leak syndrome and anti-ricin immune response. Schnell *et al.* (7) reported results from phase I/II clinical studies with RFT5.dgA (anti-CD25/dgA) and Ki-4.dgA (anti-CD30/dgA) in patients with refractory Hodgkin's lymphoma. Twenty-seven patients with refractory Hodgkin's lymphoma participated in the RFT5.dgA study and 17 patients in the Ki-4.dgA study. In both trials, the use of deglycosylated ricin A was associated with significantly reduced hepatotoxicity. However, vascular leak syndrome and anti-ricin immune response remained a problem. At the maximum tolerated dose of RFT5.dgA, 2 of 17 patients achieved partial remissions, 1 of 17 a minor response and 5 of 17 stable disease. At the maximum tolerated dose of Ki-4.dgA, 1 of 15 achieved a partial remission, 1 of 15 a minor response and 2 of 15 stable disease.

*Pseudomonas aeruginosa* exotoxin is a polypeptide bacterial toxin with a COOH-terminal ADP-ribosylation domain. With modern genetic engineering, *Pseudomonas* exotoxin has been fused to various antibody fragments to produce recombinant immunotoxins. The recombinant immunotoxin has a consistent final product, smaller molecular size, reduced immunogenicity and controllable pharmacokinetics in comparison to chemical conjugates.

Today, more and more recombinant immunotoxins are being produced and tested in clinical trials. One example is RFB4(dsFv)-PE38 (BL22) containing an anti-CD22 variable domain (Fv) fused to truncated *Pseudomonas* exotoxin, which was successfully tested against refractory hairy cell leukemia (8). In a clinical study of BL22, 11 of 16 patients who were resistant to cladribine had a complete remission and 2 of 16 had a partial remission. Of the 11 patients who had complete remission, 2 had minimal residual disease in the bone marrow or blood. During a median follow-up of 16 months, 3 of the 11 patients who had a complete response relapsed and were retreated, and all of these patients had a second complete remission. Frequent toxic effects included transient hypoalbuminemia and elevated aminotransferase levels.

So far, the most exciting immunotoxin is $DAB_{389}$IL-2 (Ontak®, denileukin diftitox), a fusion protein containing diphtheria toxin and interleukin-2. The FDA approved

$DAB_{389}$IL-2 in 2002 for the treatment of chemotherapy-resistant cutaneous T-cell lymphoma. Some other immunotoxins which contain a diphtheria toxin have shown promising results, such as DT388GMCSF (9). DT388GMCSF is a recombinant immunotoxin consisting of granulocyte-macrophage colony-stimulating factor (GM-CSF) and diphtheria toxin for the treatment of refractory or relapsed acute myeloid leukemia. DT388GMCSF contains the catalytic and translocation domains of DT388 fused to human GM-CSF. The dose-limiting toxicity is liver injury. Of 31 patients resistant to chemotherapy, 1 had a complete remission and 2 had partial remissions.

The relatively successful story of immunotoxins for hematological malignancies can not be translated, however, to solid tumors. Solid tumors have many different biological features and a different microenvironment than hematological malignancies, which creates difficulties for treatment with immunotoxins. Compared to hematological malignancies, solid tumors tend to be more heterogeneous, and the surface antigens are not evenly distributed and not easily accessible. However, local intratumoral administration instead of systemic administration of immunotoxins may overcome some of these problems.

One example is intratumoral infusion of NBI-3001 for the treatment of brain tumors (10, 11). NBI-3001 is a recombinant immunotoxin consisting of the receptor-binding domain of human IL-4 and *P. aeruginosa* exotoxin. A single infusion of NBI-3001 led to 3 years' survival with a durable tumor response. In a subsequent open-label, dose-escalation trial in patients with recurrent malignant glioma, NBI-3001 appeared to have an acceptable safety and toxicity profile.

The intratumoral delivery of immunotoxin can be further improved by convection-enhanced delivery systems, which use positive pressure to generate a pressure gradient that optimizes distribution of immunotoxin within the brain. By this method, local intratumoral administration of IL13PE38QQR provided prolonged survival in malignant glioma patients (12). IL13PE38QQR is a recombinant immunotoxin composed of *Pseudomonas* exotoxin A and human IL-13. In clinical studies, local infusion with or without resection was fairly well tolerated.

## Discussion

Most of the early immunotoxins were chemical conjugates of antitumor monoclonal antibodies and toxins such as ricin and *Pseudomonas* exotoxin. These toxins are either chemically or genetically modified to reduce severe side effects or nonspecific binding. These immunotoxins have generated limited antitumor responses in patients with hematological malignancies. One of them, anti-B4-blocked ricin, is currently being evaluated in a phase III clinical trial for the treatment of non-Hodgkin's lymphoma. The dose-limiting toxicities observed in clinical studies are vascular leak syndrome, thrombocytopenia and hepatic damage.

Modern genetic engineering has produced recombinant fusion immunotoxins. The recombinant immunotoxins were created by fusing the functional domain of toxins (diphtheria toxin or *Pseudomonas* exotoxin) to an antibody fragment or other targeting ligand, such as a cytokine or growth factor receptor. In this group, $DAB_{389}$IL-2, containing human IL-2 and diphtheria toxin, became the first FDA-approved immunotoxin and is effective in chemotherapy-resistant cutaneous T-cell lymphoma. Several other candidates also showed promising clinical activity, including anti-Tac(Fv)-PE38 (LMB2), anti-CD22 (dsFv)-PE38 (BL22) and transferrin-CRM107. The recombinant immunotoxins are usually less toxic and less immunogenic than conventional chemical conjugates and they can therefore be administered repeatedly at high doses to induce optimal clinical responses.

The progress in the application of immunotoxins for the treatment of solid tumors is very slow. Much needs to be done in order to generate clinically usable immunotoxins. First, the selection of tumor target is very important. In early 2004, the FDA approved new classes of antibodies, including Avastin® and Erbitux® , for the treatment of metastatic and refractory colorectal carcinomas. This has paved the way for the future design of immunotoxins for solid tumor therapy. For example, over 50% of colon cancers express the epidermal growth factor receptor (EGFR) and the expression of growth factors and growth factor receptors contributes to the proliferation, invasion and metastasis of the tumor. Therefore, immunotoxins targeting EGFR may block EGFR-mediated signaling and eventually interrupt tumor cell proliferation. Second, reduction of immunogenicity will permit repeated dosing of immunotoxins for optimal clinical outcomes. Besides genetic modification, polyethylene glycol (PEG)-modified immunotoxins or the use of RNases can be alternative choices (13, 14). Third, the search for novel targeted toxins continues. Many patients have pre-existing antibodies against the currently clinically used toxins and some toxins are inactive against tumor cells because of rapid degradation in lysosomes. Anthrax (*Bacillus anthracis*) toxin is an excellent choice for building immunotoxins since most people lack pre-existing antibodies and it is more potent than most of the other peptide toxins (15).

Currently, immunotoxins are still new to medicine. With the advances in genetic engineering and knowledge gained in genomics and proteomics, the future should see an expanded list of immunotoxins for the treatment of primary malignancies or minimal residual disease, either as single agents or part of combination protocols.

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