

# Advances in the Development of Anti-CD22 Immunotoxins Containing *Pseudomonas* Exotoxin for Treatment of Hematologic Malignancies

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## Abstract

Immunotoxins composed of monoclonal antibodies linked to bacterial or plant protein toxins have been studied during the past 30 years as a potential targeted therapy for cancer. A series of refinements in the design of immunotoxins containing the bacterial toxin *Pseudomonas* exotoxin A (PE) led to the development of the current smaller, more selective, and more stable recombinant immunotoxins that are composed of a truncated form of PE fused to the variable domain of a monoclonal antibody. Immunotoxins targeting CD22, an antigen commonly expressed on B-cell malignancies, including BL22 (also known as CAT-3888) and its improved, higher affinity derivative, moxetumomab pasudotox (also known as HA22 or CAT-8015) are being evaluated in patients with hematologic malignancies. BL22 induced complete remissions in the majority of patients with chemoresistant hairy cell leukemia (HCL) during phase 1 and 2 studies, and showed promising antitumor activity in patients with chronic lymphocytic leukemia (CLL). Moxetumomab pasudotox is currently undergoing phase 1 testing in refractory HCL, as well as in CLL, non-Hodgkin lymphoma, and pediatric acute lymphoblastic leukemia (ALL). Thus far, clinical activity has been observed in approximately 80% of adult patients with HCL and complete remissions have been reported in 25% of children with ALL. No dose-limiting toxicities have been reported in the adult study in patients with HCL or in patients in the pediatric study who also received dexamethasone.

**Keywords:** CAT-8015; CD22; Immunotoxin; Hematologic malignancies; Moxetumomab pasudotox

## Introduction

Bacterial protein toxins are among the most potent cell-killing molecules known. They bind to cell surface antigens, enter cells by endocytosis, reach the cytoplasm, and disrupt protein synthesis via interference with mRNA translation, leading to cell death usually via apoptosis.

In the early 1980s, a new concept in cancer therapy started to evolve: enhance the selectivity of cytotoxic or cell-killing molecules by directly delivering them to cancer cells via the targeting of antigens that are unique to or highly expressed on the surface of cancer cells. Among these emerging therapeutic agents were the immunotoxins—hybrid molecules composed of protein toxins derived from bacteria or plants linked to specific antibodies directed to cancer cells to achieve selective death of these cells.

With colleagues at the National Cancer Institute (NCI), we have studied for many years how peptide hormones bind to and enter living cells via the endocytic pathway. In the mid 1980s, David FitzGerald came to NCI as a postgraduate fellow to study the pathway by which the bacterial toxin *Pseudomonas* exotoxin A (PE) entered cells. These studies made Pastan acutely aware that extremely small amounts of the toxin could kill cells. Based on these findings, we were encouraged to make immunotoxins by attaching PE to various antibodies. Since then, the Laboratory of Molecular Biology at the Center for Cancer Research, NCI, has focused its research efforts on the design and production of the optimum PE-based immunotoxin for cancer therapy, as summarized below.

## Historical stages in developing immunotoxins

**Chemical conjugates of full-length and truncated PE:** The first PE-based immunotoxins were composed of full-length PE protein attached to whole monoclonal antibodies (MAbs). Unfortunately, these immunotoxins retained some ability to bind to normal noncancerous

cells, resulting in high toxicity in animals. Evolving data about the structure and function of PE during the 1980s enabled us to identify and delete regions of the toxin that were not needed for cell killing. The most important of these regions was the cell binding domain of PE. Eventually, we produced PE38, a smaller, truncated toxin, which by itself did not bind to or kill cells, but could be directed to cells by attaching it to an antibody. These early immunotoxins containing either full-length or truncated forms of PE were attached to MAbs via conventional chemical coupling methods. Their chemical heterogeneity, large molecular size that limited their entry into bulky tumors, and high production costs were among the tribulations associated with these antibody-toxin conjugates.

**Recombinant immunotoxins:** In the 1990s, advances in recombinant DNA techniques enabled the development of single-chain recombinant immunotoxins. DNA sequences encoding only the antigen-binding site of the antibody (the Fv portion) were fused to DNA sequences encoding PE38, the truncated form of PE. These single-chain recombinant proteins were homogeneous in composition and had a smaller molecular size than their chemical conjugate predecessors, and they could be produced in *Escherichia coli*, resulting in lower production costs.

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The first recombinant immunotoxin that was developed at NCI and that showed evidence of clinical activity in hematologic malignancies was LMB2, an immunotoxin targeting the CD25 antigen.

**PE-based anti-CD22 immunotoxins for B-cell malignancies:** CD22, an antigen expressed on the surface of normal B cells and in the vast majority of B-cell leukemias and lymphomas, subsequently became a new target for immunotoxin development. In 1997, using a murine anti-CD22 antibody developed by Peter Amlot (Royal Free Hospital, London, United Kingdom), we (FitzGerald and Pastan) synthesized a single-chain immunotoxin, RFB4(scFv)-PE38, that was cytotoxic to CD22-expressing cells in vitro [1].

**BL22 [RFB4(dsFV)PE38, CAT-3888]:** Further improvements in anti-CD22-PE38 immunotoxins occurred with the introduction of a disulfide bond as the link between the heavy and light chain domains of the Fv antibody fragment to replace the peptide linker. This led to RFB4(dsFv)-PE38, an immunotoxin with significant improvement in stability compared with its predecessor, RFB4(scFv)-PE38 [2]. This new immunotoxin was named BL22.

BL22 exhibited high activity both in vitro and in vivo against B-cell malignancies, supporting clinical evaluation. BL22 was then clinically evaluated in patients with refractory/resistant hairy cell leukemia (HCL), non-Hodgkin lymphoma (NHL), and chronic lymphocytic leukemia (CLL) in a phase 1 study and in HCL patients in a phase 2 study. In the phase 1 study (ClinicalTrials.gov identifier: NCT00021983), the highest activity was obtained among HCL patients—81% overall response rate (ORR), 61% complete response (CR), and 19% partial response (PR) [3]. Dose-limiting toxicities were reversible hemolytic uremic syndrome (HUS) and vascular leak syndrome. Reversible HUS was observed in 5 of 46 patients (11%) enrolled in the study. In the phase 2 study (ClinicalTrials.gov identifier: NCT00074048), the ORR was 72% (CR, 47%; PR, 25%). Best responses to BL22 after cladribine failure were achieved before the patients developed massive splenomegaly or underwent splenectomy. Grade 3 reversible HUS developed in 2 patients (6%) and grade 1 HUS in 1 patient (3%) [4]. Other common adverse events observed in patients treated with BL22 were grade 1 or 2 hypoalbuminemia, elevated alanine and aspartate aminotransferases, edema, myalgia, proteinuria, fatigue, nausea, and fever. The mechanism underlying HUS is not completely understood; patients develop thrombocytopenia, hyperbilirubinemia, hemolysis, creatinine elevations, and proteinuria; and in most instances, HUS is completely reversible.

BL22 has also been studied in pediatric acute lymphoblastic leukemia (ALL) and NHL. BL22 exhibited modest activity during the phase 1 study (ClinicalTrials.gov identifier: NCT00077493) in 23 children and adolescents with ALL and NHL. No ORRs were obtained and a modest hematologic response was seen among ALL patients [5].

BL22 was patented and initially produced at the NCI. In 2005, Cambridge Antibody Technology (CAT) licensed BL22 and named it CAT-3888.

**Moxetumomab pasudotox (HA22, CAT-8015):** To further optimize the anti-CD22-PE38 immunotoxin, three point mutations were introduced to the heavy chain domain of BL22 using phage display. This resulted in an immunotoxin with a higher binding affinity to CD22 and a 10-fold higher cytotoxicity against malignant B cells in vitro. This new immunotoxin was named HA22.

In 2005, HA22 was licensed to CAT, which named the drug CAT-

8015. In 2007, AstraZeneca acquired CAT, and currently MedImmune, a subsidiary of AstraZeneca, is developing the immunotoxin in collaboration with the NCI. Its generic name, moxetumomab pasudotox, was recently approved.

**Clinical development of moxetumomab pasudotox:** MedImmune undertook the clinical development of moxetumomab pasudotox upon acquisition of CAT in late 2007. Moxetumomab pasudotox is currently being evaluated in various B-cell malignancies. Several phase 1 clinical studies that accrued patients with various B-cell malignancies were initially supported by CAT, and two of these have been continued under MedImmune guidance. The most advanced of these studies is a dose-escalation phase 1 study of moxetumomab pasudotox in adult patients with relapsed/refractory HCL requiring treatment (ClinicalTrials.gov identifier: NCT00462189). So far, an ORR of 81% has been observed across all dose cohorts (CR, 37.5%; PR, 43.8%). No dose-limiting toxicities have been reported at the highest dose tested, and the safety profile suggests a lower incidence of HUS in patients treated with moxetumomab pasudotox compared with BL22: reversible grade 2 HUS in 2 patients (6%) [6]. The clinical manifestation of HUS appears to be similar to that observed in patients treated with BL22.

The second clinical study initially sponsored by CAT and continued by MedImmune is a phase 1 study in pediatric patients with refractory or relapsed ALL or NHL (ClinicalTrials.gov identifier: NCT00659425). Thus far, the immunotoxin has exhibited some activity in this patient population: 3 of 12 evaluable patients (25%) achieved CR; 5 (42%) showed hematologic activity (blood count improvement, blast reduction), 3 (25%) had stable disease, and 1 (8%) had progressive disease [7].

In order to more efficiently evaluate the use of moxetumomab pasudotox in other B-cell malignancies, the remaining CAT studies were closed and a new phase 1 clinical study was initiated to evaluate the immunotoxin in patients with NHL and CLL (ClinicalTrials.gov identifier: NCT01030536). This clinical study is ongoing at multiple sites within the United States and Europe, but data are not yet available.

## Conclusions

Advances in the design and production of immunotoxins containing the bacterial protein toxin PE have made possible the clinical testing of promising new targeted agents for treatment of hematologic malignancies. PE-based immunotoxins targeting the CD22 antigen, namely, BL22 and its improved, higher affinity derivative moxetumomab pasudotox, have demonstrated promising clinical activity and a safety profile in early clinical studies that support further development of moxetumomab pasudotox in HCL and other B-cell malignancies expressing CD22.

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## Disclosures

Dr. Pastan is a co-inventor on patents assigned to the United States of America, as represented by the Department of Health and Human Services, for the investigational products. Dr. Lechleider is an employee of MedImmune, LLC.

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