Removing immuno-toxin immunogenicity

By Kai-Jye Lou, Staff Writer

Researchers at the **National Cancer Institute** have developed an approach to decrease the immunogenicity of therapeutic proteins that involves identifying and removing immunogenic B cell epitopes.¹ As proof of concept, the group applied its method to **MedImmune LLC**'s moxetumomab pasudotox and engineered an analog that was nonimmunogenic in mice.

There are multiple approaches to avoid immunogenicity associated with therapeutic peptides and proteins from nonhuman sources. One option is modifying the biologics with polyethylene glycol (PEG), which can mask immunogenic regions of a molecule but also can attenuate its activity.² A second option is coadministering immunosuppressive drugs, but this can expose patients to greater risk for serious infections.

The NCI researchers think they have a better alternative.

"We are interested in the big picture, which is, how can we deimmunize proteins without also reducing their biological activity," said Ira Pastan, an **NIH** distinguished investigator and co-chief of the laboratory of molecular biology at NCI. "Our strategy is to identify the immunogenic B cell epitopes on a protein and mutate these epitopes so they no longer trigger an immune response."

AstraZeneca plc's MedImmune unit is developing moxetumomab pasudotox (formerly CAT-8015, GCR-8015 and HA22), which is an anti-CD22 antibody variable fragment fused to a 38 kDa fragment of *Pseudomonas* exotoxin A called PE38. The compound is in Phase I testing for hairy cell leukemia, pediatric acute lymphoblastic leukemia (ALL), chronic lymphocytic lymphoma (CLL) and non-Hodgkin's lymphoma (NHL).

"Patients with B cell malignancies typically have very suppressed immune systems, and many of the treatments used to treat these diseases also have an immunosuppressive mechanism," Pastan told *SciBX*. "Thus, antibody formation against an immunotoxin-based therapy like moxetumomab pasudotox would not be a major concern in these patients. We are more concerned about antibody formation in patients with solid tumors, where the immune system is generally still intact."

Pastan's lab discovered moxetumomab pasudotox, and NCI licensed it to Genencor (now part of **Danisco A/S**) in 2004. AstraZeneca gained the molecule after buying Cambridge Antibody Technology Group plc, which had acquired it from Genencor.^{3,4}

Robert Lechleider, director of clinical development oncology at MedImmune, noted that "in the clinical data we've reported thus far, we have not seen a correlation between the immunogenicity of moxetumomab pasudotox and reduced safety or efficacy."

Whereas MedImmune has been advancing moxetumomab pasudotox in the clinic, Pastan's group has been developing strategies to create analogs with decreased immunogenicity.

Previously, the NCI researchers removed one of the two domains from the toxin portion of moxetumomab pasudotox after identifying a set of immunogenic B cell epitopes.⁵ The domain removal did not significantly reduce the molecule's cytotoxic activity against cancer cells. Pastan thinks his group got lucky in this case, as removing entire domains from a protein usually results in decreased functionality.

The modified molecule, designated HA22-LR, was less immunogenic than moxetumomab pasudotox but also had a shorter half-life due to its smaller size.⁶

Now, the researchers have mapped out the remaining immunogenic B cell epitopes in HA22-LR's toxin fragment and identified point mutations in these regions that decrease the binding of anti-*Pseudomonas* exotoxin A mAbs by more than 90% (see **Figure 1, "Identifying key mutations that decrease immunogenicity"**).

The binding of antibodies to B cell epitopes can trigger an immune response that neutralizes the molecule's biological effect.

The NCI group used this information to generate HA22-LR-8M, which is HA22-LR with

eight such point mutations.

In a mouse model of human Burkitt's lymphoma, a total of 3 injections of HA22-LR-8M given in 5 mg/kg doses every other day caused complete tumor regression in all mice and no signs of illness. In contrast, 0.4 mg/kg doses of moxetumomab pasudotox were the maximum that could be safely given to mice. At this dose, also given a total of three times, moxetumomab pasudotox inhibited tumor growth but did not cause tumor regression.

HA22-LR-8M did not trigger an antibody response in healthy mice. Nor did it trigger an antibody response in mice previously immunized with moxetumomab pasudotox.

Results were published in the *Proceedings of the National Academy of Sciences*.

New candidates; new analogs

According to Pastan, the group's approach could encourage the development of therapeutic candidates based on proteins previously considered too immunogenic for human use. The approach also could be used to design nonimmunogenic analogs of existing immunotoxin-based therapeutics that could be used in a broader range of patients and indications.

"In principle, our method could be applied to any immunogenic protein," said Pastan, who is corresponding author on the paper. "If we have the structure of the protein, we can map out the B cell epitopes to

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National Cancer Institute

Figure 1. Identifying key mutations that decrease immunogenicity.

Immunogenicity needs to be considered when designing therapeutic proteins and peptides. Immunogenic molecules can stimulate the production of antibodies that block their therapeutic effect or even trigger a harmful immune response.

Researchers from the **National Cancer Institute** report in the *Proceedings of the National Academy of Sciences* a systematic approach for identifying the B cell epitopes in domain III of the *Pseudomonas* exotoxin A and point mutations in these regions that decrease the binding of *Pseudomonas* exotoxin A-specific mAbs.

First, researchers generate a panel of mAbs and select for those that bind to domain III using immune complex capture ELISA [a]. Next, researchers generate a set of domain III mutants, in which large, surface-exposed amino acid residues are substituted with smaller ones [b].

In a second ELISA step, researchers load individual wells of a microtiter plate with a domain III mutant and mAb such that every mutant is tested for reactivity with every mAb [c]. A secondary antibody is added to generate a signal that measures the degree of protein-antibody reactivity [d]. This step is used to identify key mutations that reduce the binding of one or more mAbs by over 90% compared to a domain III mutant with a control mutation.

Researchers then use the information obtained from the previous step to deduce the sequence of a domain III mutant containing a set of mutations that should decrease reactivity to most, if not all, the tested mAbs.

find the immunogenic regions and then remove them. We know that the large, hydrophilic amino acid residues of these epitopes are usually involved in antibody binding, so we replace them with smaller residues like alanine and glycine.”

Although the approach is labor intensive and would be difficult to carry out in an academic lab, Pastan said companies should have the capability to apply the method on an industrial scale.

“I think the described approach is the appropriate direction to go,” said Arthur Frankel, a professor of medicine at the **Texas A&M Health Science Center College of Medicine**. “Immune responses against foreign proteins are ubiquitous, and one could only treat patients for a short period of time with compounds derived from such proteins” before the patient’s body mounts an immune response that renders the treatment ineffective.

“Targeted protein therapies, if not humanized or made nonimmunogenic, are not going to be practical for treating chronic diseases,” he added. “There are many diseases including cancers that we want be able to treat in the chronic setting.”

Humanization is used to decrease the immunogenicity of therapeutic antibodies but is generally not applicable to therapeutics derived from proteins not found in humans.

Going forward, Pastan said his group is working to identify additional B cell epitopes in HA22-LR-8M. “There are several epitopes found in humans that are not found in mice, so our task now is to identify those and remove them,” he told *SciBX*.

Pastan added that his team also plans to apply its approach to another immunotoxin that AstraZeneca received via its acquisition of Cambridge Antibody Technology: CAT-5001 (SS1P). The murine anti-mesothelin disulfide-stabilized Fv antibody fragment linked to PE38 had previously completed Phase I testing in mesothelioma and ovarian and pancreatic cancers. AstraZeneca disclosed that CAT-5001 was discontinued in its 2007 annual report.

“We plan to use the same approach to deimmunize this immunotoxin since patients with these cancers have a normal immune system and the antibodies that develop after one or two treatment cycles can limit efficacy,” he said.

Pastan’s work with moxetumomab pasudotox is supported by a Cooperative Research and Development Agreement (CRADA) between MedImmune, NIH and NCI.

NIH has multiple issued and pending patents that describe mutations to make immunotoxins more active and less immunogenic. MedImmune has exclusive rights from NCI for using moxetumomab pasudotox to treat hematological malignancies. The work involving immunotoxins that use *Pseudomonas* exotoxin A variants is available for licensing from NIH in select fields of use.

Lechleider noted that because moxetumomab pasudotox is a targeted therapy for B cell malignancies that selectively express CD22, the company would not develop the compound for solid tumors.

Lou, K.-J. *SciBX* 4(15); doi:10.1038/scibx.2011.418
Published online April 14, 2011

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COMPANIES AND INSTITUTIONS MENTIONED

AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Danisco A/S (CSE:DCO), Copenhagen, Denmark
MedImmune LLC, Gaithersburg, Md.
National Cancer Institute, Bethesda, Md.
National Institutes of Health, Bethesda, Md.
Texas A&M Health Science Center College of Medicine, Bryan, Texas