Designer lipocalins

By Lauren Martz, Staff Writer

Researchers at the **Technical University Munich** and **Pieris AG** have developed next-generation anticalins, a family of high-affinity, highly specific target binders, based on a detailed SAR analysis of their binding mechanism.¹ The new anticalins have antibody-like affinities and specificities that, together with their small size and ability to bind difficult targets, may provide an advantage over traditional antibodies.

Pieris, which was founded in 2001 based on first-generation technology from the university, is incorporating the new findings into the design of its preclinical anticalin that binds cytotoxic T lymphocyte-associated protein 4 (CTLA4; CD152). CTLA4 is currently targeted by antibodies in the clinic for cancer.

Anticalins are binding molecules based on lipocalins, natural binding proteins that transport, store and sequester compounds that have a low molecular weight.² In humans, there are about 10 types

of lipocalins, all of which share a conserved eight-stranded β -barrel structure with an attached α -helix. Four variable loops are connected to one end of the β -barrel and determine the binding specificity of small molecules and peptides such as vitamins and hormones³ (see **Figure 1**, “Antibodies vs. anticalins”).

In their latest work, researchers from the Technical University and Pieris used *in vitro* mutagenesis to reshape these loops in order to engineer anticalins that bind proteins. In the *Proceedings of the National Academy of Sciences*, Arne Skerra and colleagues report on the structural plasticity of the anticalins and the improvement of the binding affinity and specificity of engineered lipocalin 2 (LCN2; NGAL) for CTLA4 based on a detailed structural analysis of the binding mechanism.

Skerra is a founder of Pieris and chair of the Department of Biological Chemistry at the Technical University.

Using Pieris’ preclinical CTLA4-binding anticalin, PRS-010, version #003, an analysis of the crystal structures of the anticalin alone and in complex with the protein target showed that the anticalin’s binding site changes its conformation upon antigen binding.

In vitro studies led to the identification of modified versions of the anticalin—#004 and #005—that had binding affinities and biological effects comparable to CTLA4-specific antibodies. In a mouse model of *Leishmania donovani*, one of the anticalins significantly decreased

Figure 1. Antibodies vs. anticalins. In the *Proceedings of the National Academy of Sciences*, Schonfeld *et al.* describe the design and engineering of anticalins that bind cytotoxic T lymphocyte-associated protein 4 (CTLA4; CD152). Anticalins have several advantages over classical antibodies including size, tissue permeability, versatility and adjustable half-life.

[a] Classical antibodies are comprised of four polypeptide chains: a pair of identical heavy chains (denoted with a subscript H in the figure) and a pair of identical light chains (subscript L). Each polypeptide contains constant regions (C_H and C_L) and variable regions (V_H and V_L), the latter of which form ligand-specific binding sites.

[b] In contrast, the substantially smaller anticalins are comprised of a single polypeptide chain with a conserved, antiparallel β -barrel structure. One end of the barrel structure is closed with small loops and side chains, whereas the other has four loops with a variable amino acid sequence that makes up the ligand-specific binding site.

parasite burden compared with a nonspecific control lipocalin and showed efficacy similar to an anti-CTLA4 antibody.

“Our successful selection of a CTLA4-specific anticalin nicely illustrates the potential of the lipocalin scaffold to form binding sites with different shapes with its set of four structurally variable loops,” Skerra told *SciBX*.

He added: “Engineered lipocalins provide a combining site that is capable of tightly and specifically binding protein antigens using structural principles that are extremely similar to antigen-specific antibodies. We are not aware that for any other engineered protein scaffold—outside the Ig superfamily—it has ever been demonstrated that a set of loops can form a binding site with such a large structural plasticity in combination with an induced fit.”

Skerra said his group has generated several anticalins with picomolar binding concentrations for their targets, including the CTLA4 anticalin described in the paper.

“With this method, in principle, you could design an anticalin against anything. The sky’s the limit on the indications for which these compounds could be used, and you could potentially interact with any pathway in the body,” said Roland Strong, member of the Division of Basic Sciences at the **Fred Hutchinson Cancer Research Center**.

An antibody alternative

Given the comparable binding affinity and efficacy of anticalins and antibodies, the Technical University researchers think the selling point for their molecules will be their small size, which, compared with antibodies, allows for better tissue penetration. Anticalins are about 20 kDa, whereas antibodies are 150 kDa.

“Anticalins also can bind small, organic compounds such as peptides and lipids that can be difficult to target antibodies against,” noted Kristian Jensen, COO of Pieris.

However, Skerra told *SciBX* that the plasma half-life of the new compounds still needs to be adjusted to achieve the optimal amount of time in circulation. “Half-life extension methods are necessary for the anticalins, but this is common among small proteins. We are addressing the problem with methods such as pegylation, which have been shown to improve half-life,” he said.

Jensen argued that the ability to control the half-life of anticalins is actually an advantage. “There are antibodies against CTLA4 in late-stage clinical trials with the same mechanism as our anticalin. But antibodies can cause continuous stimulation of the immune system, so autoimmunity can result. Our anticalins have an adjustable half-life, so you can stimulate them for however long you like,” he told *SciBX*.

Ipilimumab, a mAb against CTLA4 from **Medarex Inc.** and **Bristol-**

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Myers Squibb Co., is in Phase III testing to treat melanoma. In Phase II trials, the compound produced immune-related adverse events including rash, diarrhea and hepatitis. Neither company returned requests for comment.

Pieris’ lead compound is Angiocal, an anticalin against VEGF that is expected to enter Phase I testing this year to treat cancer.

Looking forward

The structural information reported in the *PNAS* paper can be used to engineer other lipocalin scaffolds to produce anticalins specific for different targets and capable of potentially treating a variety of conditions, noted Skerra. “We already have proof-of-principle data for another lipocalin, lipocalin 1, and we are working internally with yet other scaffolds,” he said.

Skerra told *SciBX* that the team is using lipocalin 1- and lipocalin 2-based anticalin libraries to select medically relevant protein targets. Because both lipocalins are human and lack effector functions, there is at the moment no reason to prefer one over the other for therapeutic anticalin development.

For the anticalin that targets CTLA4, Pieris is still considering an indication. In addition to fine-tuning its half-life, Skerra said, an appropriate treatment schedule must be determined in animal disease models before advancing the molecule into clinical trials.

“The signaling cascade of the molecule is more complicated than one would think. Our next step is to test the anticalin in various animal models to determine the most promising indication,” he said. Possible therapeutic settings could include cancer and infectious diseases.

Jensen expects that program to enter the clinic in about two years.

Skerra said the patent portfolio for anticalins, including the molecule that binds CTLA4, is owned by Pieris. The company is seeking strategic partners for the anticalin technology.

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COMPANIES AND INSTITUTIONS MENTIONED

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