Fixing vascular leak in IL-2 immunotherapy

By Kai-Jye Lou, Staff Writer

Neither of the two marketed IL-2 immunotherapies for cancer is widely used due to potentially severe side effects such as vascular leak syndrome. Now, Swiss researchers have shown that lung endothelial cells magnify IL-2's toxicity. They have gone on to develop cytokine-antibody complexes that uncouple the efficacy of IL-2 therapeutics from their side effects by reducing IL-2 receptor activation on these cells.[1]

Nascent Biologics Inc. has licensed the work and is humanizing the mAb component of the compounds.

Marketed IL-2 drugs include **Novartis AG**'s Proleukin aldesleukin IL-2 to treat metastatic melanoma and renal cell carcinoma (RCC) and Ontak denileukin diftitox, an IL-2 plus diphtheria toxin fusion protein from **Eisai Co. Ltd.** that is approved to treat cutaneous T cell lymphoma (CTCL).

The immunotherapies activate the IL-2 receptor on immune cells that are important for the antitumor immune response, including NK cells and $CD8^+$ T cells, and trigger their expansion.

But both drugs can cause vascular leak syndrome, a dose-limiting side effect that increases vascular permeability and can lead to edema and organ failure. Side effects associated with IL-2 immunotherapy are managed by withholding or delaying scheduled doses or by discontinuing therapy altogether.

"It was believed that the toxicity of IL-2 immunotherapy goes hand in hand with the antitumor immune response it induces, so it was thought that you cannot reduce the adverse effects of IL-2 without also taking away from its antitumor effect," noted Onur Boyman, a cofounder of Nascent Biologics and a professor in the Department of Dermatology at the **University Hospital Zurich**.

A pair of 2003 papers provided hints that IL-2's efficacy and side effects could be separated.[2,3] That year, researchers led by Alan Epstein identified the peptide fragment of IL-2 responsible for vascular leak syndrome and showed that a single amino acid substitution in that region of the cytokine eliminated the adverse effect with only a marginal reduction in the immunostimulatory effect. Epstein is cofounder of **Pivotal Biosciences Inc.** and a professor in the Department of Pathology at the **University of Southern California**'s Keck School of Medicine.

Although the studies identified the parts of IL-2 responsible for vascular leak syndrome, the cell populations that the cytokine acted on weren't known. A team co-led by Boyman and Carsten Krieg, a senior research associate at the University Hospital Zurich, now has found that lung endothelial cells are the likely culprits.

IL-2 receptors are formed as trimers consisting of the IL-2 receptor α-chain (IL2RA; CD25), IL-2 receptor β-chain (IL2RB; CD122) and IL-2 receptor γ-chain (IL2RG; CD132) or as dimers between the latter two. IL-2 receptors with all three chains have a much higher affinity for IL-2 than those containing only CD122 and CD132.

The group found that mouse lung endothelial cells express the $CD25^+$, high-affinity IL-2 receptor variant. The researchers showed that direct binding of IL-2 to these cells induced pulmonary edema.

The Swiss group also showed that lung endothelial cell populations have relatively higher CD25 expression and lower CD122 expression than antitumor immune cells. Thus, the team generated cytokine-mAb complexes consisting of IL-2 and an anti-IL-2 mAb that directs the cytokine to cells expressing high levels of CD122. Their goal was to use these complexes to preferentially activate IL-2 receptors on immune cells and avoid receptor activation on lung endothelial cells.

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> *—Onur Boyman, University of Zurich*

In a mouse model of melanoma, these CD122-selective IL-2-mAb complexes stimulated an antitumor immune response, whereas a CD25-selective IL-2-mAb complex did not.

Moreover, the CD122-selective complexes did not cause pulmonary edema in immune-depleted mice, whereas the CD25-selective complexes elicited severe pulmonary edema that was comparable to that caused by IL-2 itself.

The researchers used immune-depleted mice and an anti-CD25 mAb to confirm that IL-2 binding to $CD25^+$ lung endothelial cells—and not $CD25^+$ immune cells—is the major contributor to IL-2-induced pulmonary edema.

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

"What we show in our study is that lung endothelial cells are the major contributors to the toxicity seen with IL-2 immunotherapy," said Boyman, who is corresponding author on the paper. "We also proposed a solution to the observed IL-2-associated toxicity by developing these CD122-selective IL-2-mAb complexes that avoid IL-2 receptor stimulation on $CD25^+$ lung endothelial cells."

The Swiss group conducted the research at its previous post at the **University Hospital of Lausanne**. Krieg is the paper's lead author.

"The most interesting finding from the current study is that functional IL-2 receptors, which are known to be expressed on lymphoid cells, were also found on nonlymphoid cells, specifically lung endothelial cells," said Jonathan Sprent, a cofounder of Nascent Biologics and a professor of immunology at the **Garvan Institute**.

"We have known that vascular leak syndrome is caused by nitric oxide release, but what we did not know was the origin of release," said Epstein.

"It could be conceivable that the nitric oxide that's causing vascular leak is being released from lung epithelial cells."

Pivotal Biosciences' PB1, an analog of IL-2, is in preclinical development for cancer. Epstein said PB1 contains an amino acid substitution that avoids the vascular permeability seen with recombinant human IL-2.

"The approach described in this paper uses off-the-shelf IL-2 and an antibody to form a complex that's specifically targeted, whereas our approach uses an IL-2 that has an arginine to tryptophan substitution at the 38th amino acid," he told *SciBX*. "I think both molecules will be effective in reducing vascular permeability."

"It would be very important in this case to know the half-life for this antibody complex, as it may not be desirable to continuously stimulate the intermediate affinity IL-2 receptor."

—Elizabeth Grimm, The University of Texas M.D. Anderson Cancer Center

The complex debate

In addition to the reduced potential for toxicity, Sprent noted that the complex described in the *PNAS* article "is more potent than soluble IL-2 at stimulating the expansion of tumor-reactive $CD8^+$ and NK cells. This means that lower doses of the compound will be needed to achieve the same antitumor response."

"You could either use a low dose of the IL-2-mAb complexes that shows comparable efficacy to high doses of IL-2 but without significant toxicity or you could provide high doses of the IL-2-mAb complexes and thus push the antitumor effect of IL-2 immunotherapy to beyond what is achievable with a safe dose of IL-2," added Boyman. "What we have developed is a more efficient way to activate IL-2 receptors that generates fewer side effects."

In the *PNAS* study, the researchers showed that the highest tolerated dose of the complex achieved greater expansion of immune cell populations in mice compared with the highest tolerated dose of soluble IL-2.

However, Stephen Gillies, cofounder, president and CEO of **Provenance Biopharmaceuticals Corp.**, thinks that immune cell overexpansion alone "may not be the ideal approach for designing a more effective immunotherapy." He said T cell receptor stimulation is also an important element in generating an effective and persistent antitumor immune response.

"The complex they used in the study only stimulates cells through the IL-2 receptor, so the expanded immune cell populations may not be very effective at targeting tumor cells" due to their lack of specificity for tumor antigens, Gillies told *SciBX*. "You also won't have the antigen specificity needed for the long-term immunological memory that is important for preventing a tumor from coming back."

Provenance's DI-Leu16-IL2 is a recombinant fusion protein composed of the humanized anti-CD20 mAb Leu16 fused to human IL-2. The **Beckman Research Institute at City of Hope** is running a Phase I trial evaluating DI-Leu16-IL2 in patients with B cell non-Hodgkin's lymphoma (NHL).

"DI-Leu16-IL2 binds to tumor cells and, by doing so, presents IL-2 to T cells in the tumor microenvironment at the same time they engage antigen with their T cell receptors," Gillies told *SciBX*. "Earlier antibody-IL-2 fusion proteins have been shown in preclinical models to activate and expand antigen-specific T cells that kill the tumor and generate long-term cytotoxic T cell memory that protects the animal from later tumor challenge."

The IL-2-mAb complex approach described in *PNAS* also could encounter manufacturing and regulatory challenges.

"The complex is formed using two protein molecules, so there could be batch-to-batch variation when it's being made," said Epstein. "Running validation studies is also going to be more difficult than with a single molecule."

Elizabeth Grimm, a professor in the Department of Experimental Therapeutics and the Department of Melanoma Medical Oncology at **The University of Texas M.D. Anderson Cancer Center**, noted that the pharmacokinetic profile of the complex will need to be determined.

"It would be very important in this case to know the half-life for this antibody complex, as it may not be desirable to continuously stimulate the intermediate affinity IL-2 receptor," she told *SciBX*. "I would also want to know what happens to the complex *in vivo*. Does the complex eventually come apart, and how does it get degraded? These points would need to be addressed before the compound could be tested in the clinic."

Grimm, who also is a member of Pivotal's scientific advisory board, added that it would be important to determine whether CD25 is expressed in human pulmonary endothelial cells and how serum cytokine levels are affected by the complex. "Serum cytokines are currently considered to be the major stimulus of vascular leak in the human," she said.

Boyman said the group at the University Hospital Zurich and collaborators including Sprent will be testing the IL-2-mAb complexes in additional models of cancer. Nascent is humanizing the anti-IL-2 mAb component in preparation for clinical testing.

Nascent has a pending patent covering the technology used to create the cytokine-mAb complexes. The complexes are available for licensing from the company.

Epstein said Pivotal Biosciences will need to obtain funding or an industry partner to run clinical trials of PB1.

Gillies said Provenance plans to fund a Phase I/IIa trial of DI-Leu16-IL2 and, based on the results, begin licensing discussions.

Lou, K.-J. *SciBX* 3(27); doi:10.1038/scibx.2010.815
Published online July 15, 2010

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   Contact: Onur Boyman, University Hospital of Lausanne, Lausanne, Switzerland
   e-mail: onur.boyman@chuv.ch
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COMPANIES AND INSTITUTIONS MENTIONED

Beckman Research Institute at City of Hope, Duarte, Calif.
Eisai Co. Ltd. (Tokyo:4523; Osaka:4523), Tokyo, Japan
Garvan Institute, Sydney, New South Wales, Australia
Nascent Biologics Inc., San Diego, Calif.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Pivotal Biosciences Inc., Los Angeles, Calif.
Provenance Biopharmaceuticals Corp., Waltham, Mass.
University Hospital of Lausanne, Lausanne, Switzerland
University Hospital Zurich, Zurich, Switzerland
University of Southern California, Los Angeles, Calif.
The University of Texas M.D. Anderson Cancer Center, Houston, Texas