A TEM to kill

By Chris Cain, Staff Writer

Novartis AG and the **National Cancer Institute** have developed mAbs against anthrax toxin receptor 1 that inhibit tumor angiogenesis in multiple mouse models of cancer with fewer side effects than anti-VEGF therapies.¹ A key next step is to determine how widely the target is expressed in human tumor samples.

Drugs that block VEGF signaling do not differentiate between tumors and healthy blood vessels, and thus they can cause side effects including delayed wound healing, pulmonary hemorrhage and gastrointestinal perforation.

To find a more specific way to target tumor angiogenesis, the NCI's Brad St. Croix had previously identified genes that were specifically upregulated in the tumor vasculature compared with healthy blood vessels.² In that 2000 study, his group selected nine genes to validate as tumor endothelial markers. Of those, tumor endothelial marker 8 showed the most significant tumor-specific expression.

St. Croix is head of the tumor angiogenesis section at the NCI and the investigator who led the collaboration with Novartis.

In 2001, researchers at the **University of Wisconsin–Madison** and **Harvard Medical School** independently identified tumor endothelial marker 8 as a receptor for anthrax toxin and renamed it anthrax toxin receptor 1 (ANTXR1; TEM8).³

Although some researchers have since been working on understanding how ANTXR1 mediates anthrax toxicity, other groups sought to develop derivatives of anthrax toxin to specifically kill tumor cells, because of ANTXR1's tumor-specific expression. Even though the latter efforts effectively inhibited tumor growth, residual systemic toxicity limited the translation of this approach.⁴

Six year later, a team made the key discovery that a noncytotoxic anthrax toxin subunit could inhibit tumor angiogenesis—an effect that was dependent on receptor binding.⁵ This suggested ANTXR1 not only was a tumor cell-specific marker but also might play a direct role in regulating tumor angiogenesis.

The next logical step was to develop antibodies against the target.

To create potent mAbs, the NCI researchers turned to Novartis, which helped develop ANTXR1-specific mAbs using phage display libraries screened against ANTXR1 purified from mammalian cell culture. St. Croix told *SciBX* that talks with Novartis began in 2007, and a cooperative research and development agreement (CRADA) was signed in 2008.

With antibodies in hand, the team started to probe the effect of targeting ANTXR1 *in vivo*. In xenograft mouse models of colon cancer and a syngeneic mouse model of melanoma, an anti-ANTXR1 antibody significantly decreased tumor growth and tumor-associated

vasculature compared with vehicle control. The molecule had no effect on normal wound healing.

The antibody also was effective in combination with other cancer drugs. In the colon cancer model, the anti-ANTXR1 antibody plus the generic chemotherapeutics irinotecan or 5-fluorouracil (5-FU) lowered tumor growth compared with either treatment alone. In the melanoma model, the antibody plus an inhibitor of VEGF receptor 2 (KDR/Flk-1; VEGFR-2) decreased tumor growth compared with either treatment alone. The antibody also bound the tumor endothelium of six colorectal cancer patient samples but did not bind to adjacent normal tissue.

Results were published in *Cancer Cell*.

"This is a proof-of-concept study that clearly demonstrates these anti-ANTXR1 antibodies or antibodies with similar activity are likely to show good activity in cancer," said Michael Rogers, assistant professor of surgery at Harvard Medical School and research associate in vascular biology at **Children's Hospital**

Boston. "Without this study, the impetus to aggressively investigate ANTXR1 has not been there. My guess is there will be a rush of new people interested in the target after this work."

Rogers led the team that showed the anthrax toxin protective antigen inhibits tumor angiogenesis, and the group is continuing to study how ANTXR1 and ANTXR2 (CMG2) regulate angiogenesis.⁵

Lots to learn

Additional studies are needed to get a clearer understanding of how ANTXR1 functions and how widely it is expressed in people with cancer.

"The biology surrounding ANTXR1 needs to be resolved, but we know from cell culture experiments that the protein is needed for endothelial cells to form tubes during vasculogenesis," said Bradley Fletcher, associate professor of pharmacology and medicine at the **University of Florida**. "It is likely ANTXR serves as a bridge between extracellular matrix proteins such as type IV collagen and internal skeletal proteins like actin. Therefore, ANTXR1 may give external clues to endothelial cells about where to be and where to form vessels."

ANTXR1 has a conserved integrin-like domain which mediates interactions with protein ligands. Fletcher said it is likely the antibodies are disrupting ANTXR1 function by binding to its integrin-like domain and thus blocking ligand binding. In his own lab, he is developing fusion proteins that deliver toxic peptides to ANTXR1-expressing cells.

Rogers wanted to see a larger study looking at how widely ANTXR1 is expressed in patient tissue samples from diverse tumor types.

Arthur Frankel, director of the Division of Hematology and Oncology and executive director of the **Scott & White Cancer**

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Harvard Medical School

Research Institute, agreed, adding that “the real challenge right now is to get an antibody that binds tumor blood vessels.”

Frankel is developing his own set of human mAbs independently from St. Croix and has not yet published his work. “We are now developing a panel of ANTXR1-specific antibodies that we hope to screen against patient samples.”

St. Croix’s lab is now focusing on the development of new ANTXR1 antibodies for immunohistochemistry and exploring which ANTXR1-expressing cell types contribute to tumor growth. He told *SciBX* the CRADA with Novartis is no longer in effect.

The NCI has filed for a patent related to this work, and its licensing status is undisclosed. Novartis declined to comment on this work.

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COMPANIES AND INSTITUTIONS MENTIONED

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