CD73: double-breasted suit

By Michael J. Haas, Senior Writer

Australian researchers have shown that an anti-CD73 antibody can reduce both primary tumor growth and metastasis of breast cancer.¹ Because CD73 may be expressed on breast tumors that do not respond to current therapies, the antibody could be particularly useful in patients with advanced disease for whom there are few options. However, additional studies need to explore how a systemic anti-CD73 therapy would affect other immune cells that also express the target.

CD73 is expressed on T_{regs} and other immune cells and plays a role in regulating normal immune responses to inflammation by converting extracellular adenosine monophosphate (AMP) to adenosine. Adenosine attenuates the recruitment of immune cells to the site of inflammation, thus dampening immune responses that could otherwise lead to excessive inflammation and tissue damage.

CD73 is also expressed on many types of tumor cells, where its role in producing extracellular adenosine is thought to help the tumor evade host immunity. *In vitro* studies have suggested that CD73 also plays a part in breast cancer cell migration and metastasis,² but the mechanism was never fully understood.

The Australian researchers set out to elucidate CD73's role in immune evasion and metastasis of breast cancer and to determine whether CD73-targeted therapy could help treat primary and metastatic breast cancer.

First, the team showed that murine breast cancer cell lines prone to metastasize expressed higher levels of Cd73 and produced more extracellular adenosine than nonmetastatic cancer cells. A small molecule CD73 inhibitor decreased adenosine production of the prometastatic cells.

Next, they injected immunocompetent and immunocompromised mice with the prometastatic breast cancer cells and treated the animals with an anti-CD73 mAb. The antibody reduced primary tumor growth only in the immunocompetent mice. The team reasoned that decreased adenosine production allowed immune cells to attack tumors.

In contrast to its effect on primary tumors, the antibody reduced the number of lung metastases in both immunocompetent and immunocompromised mice compared with no treatment. *In vitro* assays showed that the antibody blocked the migration and metastasis of murine breast cancer cells by inhibiting chemotactic signaling between Cd73 and adenosine A_{2B} receptor (Adora2b).

The team was led by Mark Smyth, professor of cancer immunology at the **Peter MacCallum Cancer Centre**, and included researchers from **St. Vincent's Hospital** and **The University of Melbourne**. Data were reported in the *Proceedings of the National Academy of Sciences*.

Getting expressive

"The authors argue that the tumor is promoting adenosine production" that enables immune evasion, noted Andries Zijlstra, assistant professor of pathology at **Vanderbilt University**. Thus the concept of targeting CD73 "could be a powerful anticancer tool and one which might be resilient to resistance" because the *PNAS* authors are reinstating the host's normal physiological responses to cancer instead of killing the tumor directly.

Tim Hoey, SVP of cancer biology at **OncoMed Pharmaceuticals Inc.**, agreed. "I think the approach of immune stimulation in cancer research is very interesting and underutilized in the field," he said.

"These first data of treating a tumor with an anti-CD73 antibody in immunocompetent mice are very impressive," said Jürgen Hess, manager of scientific affairs at cancer company **Trion Pharma GmbH**. He added that the team's results suggest anti-CD73 therapies might be most beneficial in patients with advanced metastatic cancer.

Emma Shtivelman, director of cancer research at **Bionovo Inc.**, noted that CD73 expression has been correlated to breast cancer cell lines that do not express estrogen receptor,³ which is one of the three missing receptors in

triple-negative breast cancers.

"Existing treatment options for metastatic breast cancer are mostly ineffective against estrogen receptor-negative cancers, and very few existing and experimental therapies are effective for triple-negative breast cancers," she said. "Therefore any promising treatment targeting this group of tumors would be very welcome indeed."

Bionovo's Bezielle (BZL101), an aqueous extract from the herb *Scutellaria barbata* that inhibits glycolysis, is in Phase II testing to treat advanced breast cancer and in Phase I/II testing to treat pancreatic cancer.

Shtivelman did say she wanted to see studies of CD73 expression in primary breast tumors. "There is no extensive evidence documenting CD73 expression in primary breast cancer or other cancer types," she said.

As an example, Shtivelman cited a 1991 study that found CD73 expression in only 9 of 102 primary breast carcinomas.⁴ "The majority of tumors showed CD73 expression in the surrounding tumor stroma," in which adenosine production could help the tumor evade host immunity—though additional studies would be needed to confirm that possibility, she said.

Shtivelman also noted that it was unclear whether stromal CD73 expression could aid tumor cell migration and metastasis.

John Stagg, a postdoctoral research fellow at Peter MacCallum and first author on the *PNAS* paper, agreed that CD73 needs to be validated

"In my view this is a great start, but most of the data presented in this paper are based on only one model of mouse breast cancer."

— Tim Hoey,
OncoMed Pharmaceuticals Inc.

as a therapeutic target in human breast cancer cells. However, he said there is evidence for CD73 expression in primary human tumors and that other researchers have shown a correlation between CD73 expression and lack of estrogen receptor in primary breast tumors.³

Zijlstra said additional studies might be needed to confirm the antibody's mechanism of action in reducing primary tumor growth. He said it wasn't clear whether the anti-CD73 antibody was working by shutting off adenosine production or through antibody-dependent cellular cytotoxicity (ADCC).

Stagg suggested the latter mechanism was not a factor.

"Our observation that the anti-CD73 mAb was ineffective in the immunocompromised mice, together with our results with tumor cells in which CD73 expression is decreased, suggest that blocking CD73 can induce adaptive antitumor immune responses independently of ADCC," he said.

Both Shtivelman and Hess also wanted to see preclinical studies of the anti-CD73 mAb in combination with other therapies that have a direct, cell-killing effect on tumors.

OncoMed's Hoey agreed that additional studies would be needed to validate CD73 as a cancer target. "The *PNAS* paper provides good *in vivo* evidence that targeting CD73 reduces breast cancer metastasis," he said. "In my view this is a great start, but most of the data presented in this paper are based on only one model of mouse breast cancer."

He also called for additional evidence of efficacy in other models and "experimental evidence that targeting human CD73 would have an effect on metastatic growth."

OncoMed and partner **GlaxoSmithKline plc** are developing OMP-21M18, a human mAb targeting delta-like ligand 4 (DLL4). The antibody is in Phase I testing to treat advanced solid tumors.

Antibody tailoring

Researchers also wanted to know how systemic CD73 inhibition would affect normal cells and tissues.

"There are many potential toxicities associated with inhibiting CD73," Hoey said. "Adenosine has many diverse functions across a range of cell types, and CD73 expressed on T_{regs} serves to limit immune and inflammatory responses. Thus, targeting CD73 may result in deregulated immune responses and autoimmunity."

"As B and T lymphocytes also express CD73, the question is still open about what will happen to these cell types under anti-CD73 antibody therapy," said Hess.

Indeed, Shtivelman noted that studies in knockout mice have shown that Cd73 was required for proper vascular tone and platelet aggregation and to protect the heart, kidney and lungs from ischemia or acute injury. Those results, she said, "raise the possibility of a spectrum of unpleasant side effects that could be associated with the systemic anti-CD73 immunotherapy."

Stagg added that CD73 also plays roles in the migration of lymphocytes into lymph nodes and in the maturation of B cells aided by follicular

dendritic cells (FDC).

Nevertheless, Zijlstra argued that developing an anti-CD73 antibody as a cancer therapy should not present insurmountable challenges.

"A great benefit of working with antibodies is that the development process for them is very slick and very efficient," he said. "If you can demonstrate a function for an antibody—diminishing CD73 activity, in this case—then there are ways to modify it for use in the clinic."

Smyth did acknowledge that CD73 inhibition could adversely affect renal functions and responses to inflammation. But he told *SciBX* that the Melbourne team saw no significant signs of toxicity in mice treated with the anti-CD73 mAb.

Stagg said the team is testing the anti-CD73 antibody in animal models of spontaneous breast and prostate cancer, and in combination with other cancer therapies such as chemotherapies, Herceptin trastuzumab and agonists of tumor necrosis factor-related apoptosis-inducing ligand receptors (TRAILRs).

Roche and its **Genentech Inc.** unit market Herceptin, a humanized mAb against HER2 (ERBB2; neu), to treat breast cancer. The antibody is also in registration to treat gastric cancer.

Smyth said the team is interested in developing the anti-CD73 mAb to treat patients with very established disease for whom there are few therapeutic options, including those with triple-negative breast cancer. For such patients, he said CD73 may be "an ideal target because metastatic cells may express the highest levels of it and these are the lesions that will kill cancer patients."

He said the findings reported in *PNAS* are not patented and are available for licensing or partnering.

Haas, M.J. *SciBX* 3(4); doi:10.1038/scibx.2010.107

Published online Jan. 28, 2010

REFERENCES

1. Stagg, J. *et al. Proc. Natl. Acad. Sci. USA*; published online Jan. 4, 2010; doi:10.1073/pnas.0908801107
Contact: Mark J. Smyth, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia
e-mail: Mark.Smyth@petermac.org
Contact: John Stagg, same affiliation as above
e-mail: john.stagg@petermac.org
2. Wang, L. *et al. J. Cancer Res. Clin. Oncol.* **134**, 365–372 (2008)
3. Spychala, J. *et al. Clin. Cancer Res.* **10**, 708–717 (2004)
4. Krüger, K. *et al. Br. J. Cancer* **63**, 114–118 (1991)

COMPANIES AND INSTITUTIONS MENTIONED

Bionovo Inc. (NASDAQ:BNVI), Emeryville, Calif.
Genentech Inc., South San Francisco, Calif.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
OncoMed Pharmaceuticals Inc., Redwood City, Calif.
Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
St. Vincent's Hospital, Melbourne, Victoria, Australia
Trion Pharma GmbH, Munich, Germany
The University of Melbourne, Melbourne, Victoria, Australia
Vanderbilt University, Nashville, Tenn.