BIOCENTURY
Innovations
FROM IDEA TO IND

REPRINT FROM NOVEMBER 12, 2015

PRODUCT R&D

PROBODIES, ANTICANCER

By Lauren Martz, Senior Writer

How successful checkpoint inhibitors and chimeric antigen receptor (CAR) T cells will be at revolutionizing oncology hangs largely on whether it will be possible to curb their on-target but off-tissue toxicities. CytomX Therapeutics Inc. is using its Probody platform to restrict activity to tumors by capitalizing on enzymes unique to the tumor microenvironment. The company believes that by improving safety, its technology can expand the range of targets, indications and therapeutic combinations possible in cancer immunotherapy.

Over the past eight weeks, CytomX has presented the first data on its Probody platform for three different types of cancer immunotherapies — checkpoint inhibitors, antibody-drug conjugates (ADCs) and T cell-engaging bispecific antibodies (TCBs) — and announced a new partnership on a fourth, CAR-NK cells.

"Probodies are designed to bind tumors to protect healthy tissue. The rationale is that antibody therapeutics, particularly the recent wave of highly potent oncology modalities, come with significant side effects that can limit utility," said CytomX CEO Sean McCarthy.

At the September meeting of the American Association for Cancer Research (AACR), Cancer Research Institute (CRI), Association for Cancer Immunotherapy (CIMT) and European Academy of Tumor Immunology (EATI), CytomX presented preclinical data showing its lead candidate CX-072, an anti-PD-L1 probody, decreased tumor growth as effectively as standard antibodies, but without the autoimmune toxicities.

Earlier this week, CytomX presented additional preclinical data at the AACR-National Cancer Institute (NCI)-European Organization for Research and Treatment of Cancer (EORTC) meeting in Boston, showing its Probody platform can improve the safety of two other types of cancer immunotherapies: ADCs and TCBs.

And on November 5th, the company announced a research collaboration with the University of Texas MD Anderson Cancer Center to apply the Probody technology to CAR-NK cells. Like CAR T cells, CAR-NK cells are engineered *ex vivo* to express antigen-binding receptors that direct the cells to tumor antigens.

The Probody platform involves modifying therapeutic antibodies with an N-terminal, cleavable chemical mask that blocks activity. The molecules contain an antibody and a chemical mask at the antibody-binding site, linked by a substrate specific for proteases in the tumor microenvironment. The design prevents the agents from binding antigens in circulation, but allows them to be activated upon exposure to the proteases, which cleave the linker and release the chemical mask. (See "CytomX's Probodies")

Although CytomX originally developed the technology to produce antibodies against a range of tumor antigens, about three years ago it switched gears to looking at safety problems of antibody-based therapies with a specific focus on immuno-oncology.

"Around 2011 and 2012, the field of cancer immunotherapy was just coming into focus, and the seriousness of toxicities of checkpoints began to emerge," said McCarthy.

As the field developed, he said, on-target, off-tissue toxicity became a problem for different classes of immunotherapies. For checkpoint inhibitors, toxicity limits the therapeutic combinations that can be safely used; for ADCs, it limits the range of potential targets; and for bispecifics and CAR T cells, it makes the therapies difficult to use outside of blood cancers.

Because CytomX's technology has the potential to minimize systemic exposure while maintaining activity in tumors, the team thought that by substituting probodies for antibodies, it could solve many of those problems.

PD-L1 PROBODIES

For checkpoint inhibition, CytomX plans to develop Probodies as single agents or combination therapies. It started with anti-CTLA-4 Probodies partnered with Bristol-Myers Squibb Co., and now has its first wholly owned lead candidate in CX-072.

At the September meeting, the company showed that in *in vitro* assays, a probody with cross-reactivity against human, rodent and monkey PD-L1 bound and blocked the human protein in the presence of tumor-specific proteases with efficacy efficacy to the parent mAb, but had virtually no activity when the enzymes were absent. The probody studied was CX-072 modified with a mask and cleavable linker optimized for use in mice.

In a syngeneic mouse model of colon cancer, the probody decreased tumor volume compared with a control mAb, and was as effective as the parent mAb and a standard anti-PD-L1 reference mAb at shrinking tumors.

McCarthy told BioCentury the company hasn't compared CX-072 with anti-PD-L1 compounds in the clinic because those are specific for the human target and can't be evaluated in mice.

CytomX also presented data on the toxicity advantage of the Probody platform.

Because autoimmune toxicity occurs when T cells are activated in the periphery and attack healthy tissues, the team tested whether the probody caused autoimmunity in mice predisposed to developing Type I diabetes.

In the non-obese diabetic mouse model, autoimmune diabetes occurs when autoreactive T cells controlled by the PD-L1 pathway attack the pancreas. While 62% of mice treated with the parent antibody developed diabetes, none of the animals that received the probody developed the disease.

In the colon cancer model, the probody bound about half as many T cells as the mAb did in peripheral blood, indicating that systemic T cell activation was decreased by the Probody design.

CytomX plans to pursue CX-072 in the clinic as a monotherapy, but hopes to quickly begin conducting trials in combination with other checkpoint inhibitors or molecules that synergize with checkpoint inhibitors, including Yervoy ipilimumab, an anti-CTLA-4 antibody from BMS.

McCarthy told BioCentury that in Phase I, the company will evaluate CX-072 in a broad variety of cancer types, with a specific focus on melanoma and lung cancers, to determine the best indications. He added that the substrate linkers in the molecules can be cleaved by several different tumor-specific proteases, allowing the same probody to be used in different solid tumor types.

In addition, McCarthy said, preclinical combination studies are underway but will be limited because of the differences between human and animal immune systems. "This is a rare class of drugs that actually work much better in patients than in mice," he told BioCentury.

CytomX CMO Rachel Humphrey said that while the PD-1 family has a low dropout rate of about 8% due to toxicity in trials for the agents as stand-alone therapies, when used in combination with other therapies "the synergy they create in the periphery is a big challenge."

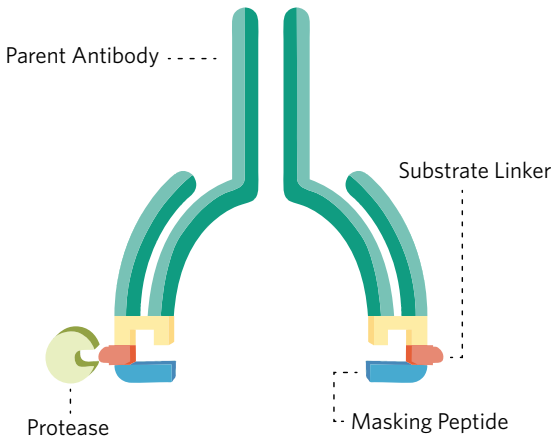
"If this product acts as we expect it to, it will be the first in generation and truly differentiated from all others out there," she said. "It has the potential to expand the market to otherwise intolerable combinations."

PROBODY-DRUG CONJUGATES

CYTOMX'S PROBODIES

CytomX Therapeutics Inc. is developing antibody prodrugs, dubbed Probodies, that restrict antibody binding and activity to the tumor microenvironment. Probodies are composed of standard antibodies **(dark green)**, modified with peptide masks **(blue)** that prevent antibody binding in normal circulation.

The masks are attached to the parent antibody's antigen-binding sites **(yellow)** by substrate linkers **(red)**. The substrates are specific for individual proteases found in the tumor microenvironment. In the presence of the corresponding protease **(green C)**, the linkers are cleaved to release the masks and expose the antigen-binding sites for normal antibody activity. This design prevents the probody from binding to its target antigens in normal tissues while allowing binding inside solid tumors. *Source: Reproduced from a company presentation, with permission from CytomX Therapeutics Inc.*



For improving upon ADCs, CytomX is using the platform on targets inaccessible to conventional conjugates, and wants to "go after first-in-class targets that the ADC community can't," said CSO Michael Kavanaugh.

For typical ADCs, said Kavanaugh, the target has to be highly expressed in tumors "because the higher the expression, the higher payload delivery and more tumor killing. At the same time, it needs to be absent or present at very low levels on non-tumor tissues."

According to Kavanaugh, the number of targets that meet those criteria is very small, and those that do meet it are not ideal.

CytomX's first target for the program, CD166, is highly expressed on a broad range of tumor types, and is involved in T cell recruitment to the tumors through interaction with CD6 on

> "It has the potential to expand the market to otherwise intolerable combinations."
>
> Rachel Humphrey, CytomX

T cell surfaces. The problem is that CD166 is also expressed on healthy tissues, including the liver.

At this week's meeting, CytomX presented its first data from the program, and showed a probody conjugated to a maytansinoid — a class of cytotoxic agents commonly used in ADCs — had activity comparable to, but better safety than, ADCs.

In mice with triple-negative breast cancer (TNBC), non-small cell lung cancer (NSCLC) or ovarian cancer xenografts, a CD166 probody-drug conjugate (CD166 PDC), designed for both the human and monkey target, decreased tumor volume with efficacy comparable to an CD166 ADC.

The CD166 PDCs remained in circulation in monkeys longer than CD166-targeted ADCs, indicating less binding to normal healthy tissues. At therapeutically relevant doses in monkeys, the probody conjugates produced no adverse effects on the liver or blood, where the target is most highly expressed.

CytomX is currently conducting lead optimization studies and is moving toward nomination of a clinical candidate. The company plans to begin clinical testing of an CD166 PDC in 1H17.

BEYOND BLOOD CANCERS

CytomX's third angle, expanding cell-based immunotherapies to solid tumors, aims to avoid a problem that is most acute for TCBs and CAR-based immunotherapies: the compounds are highly potent against all cells expressing the T cell targets and therefore are generally only acceptable for blood cancer treatments.

Kavanaugh said that cell therapies can be tolerated for blood cancers because the targets are often expressed at low levels on normal B cells which then often get depleted, but the "patients don't really need them." For solid tumors, targets are often expressed on cells in other organs such as the liver, lung or colon that patients can't do without, making the therapies "unusable" in solid tumors.

"If there were such a thing as a tumor target with absolute specificity, this wouldn't be a problem, but these two modalities at present are very unforgiving for even a small amount of normal tissue expression," he told BioCentury.

TCBs are antibodies designed to target two antigens: a tumor-specific antigen and a T cell-specific antigen, which work together to direct the T cells to the tumors and elicit an antitumor immune response. CytomX has modified bispecific antibodies using its Probody platform to produce molecules that contain two masks, one blocking each antigen-binding domain. Like the simple probodies, the T cell-engaging bispecific probodies (Pb-TCBs) are activated only in the presence of specific proteases in the solid tumor microenvironment.

This week, CytomX presented data on the Pb-TCB segment of the platform, showing a bispecific that contained binding sites for the T cell receptor CD3 and the solid tumor cell surface antigen EGFR had antitumor efficacy comparable to antibody bispecifics (Ab-TCBs) against the same targets, with a 30-fold higher maximum tolerated dose.

In the absence of proteases to cleave both masks, binding of the Pb-TCB to both targets and cytotoxicity against human blood and colon cancer cells was more than 1,000-fold lower than Ab-TCBs alone or Pb-TCBs in the presence of PLAU, a protease that cleaves the Probody masks. In mice with established colon cancer tumor xenografts, Ab-TCBs and Pb-TCBs comparably decreased tumor volume. In contrast, a Pb-TCB variant without a cleavable substrate did not decrease tumor volume.

As a measure of safety, the company found that Pb-TCBs were well tolerated at 30-fold higher doses than Ab-TCBs without causing significant weight loss or affecting markers of liver or kidney toxicity.

The company has not yet disclosed timelines for clinical development of Pb-TCBs.

CytomX has also signed a new research collaboration with MD Anderson to explore whether it can use the same bispecific principle in CAR-NK cell therapies.

McCarthy told BioCentury that CAR T cells are engineered to express single-chain variable segments that recognize the

antigen outside of the cell to bind the cancer target. "We can engineer those antibody-like molecules to have our Probody masks, driving CAR T or CAR-NK cells to activate at solid tumors and not healthy tissues," he said.

The partners are developing CAR-NKs against several undisclosed targets, and CytomX has an option to license resulting products for clinical and commercial development.

"The CAR T field has really exploded onto the scene with dramatic clinical results, but the focus is now on how to increase the therapeutic window for CAR T therapies to drive antitumor activity," said McCarthy. He added that this avenue may turn out to be the "ultimate application" for CytomX's Probody platform based on the high therapeutic potential for the modality and the pressing need for ways to improve safety.

COMPANIES AND INSTITUTIONS MENTIONED

American Association for Cancer Research (AACR), Philadelphia, Pa.
Association for Cancer Immunotherapy (CIMT), Mainz, Germany
Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.
Cancer Research Institute, New York, N.Y.
CytomX Therapeutics Inc. (NASDAQ:CTMX), South San Francisco, Calif.
European Academy of Tumor Immunology (EATI), Paris, France
European Organization for Research and Treatment of Cancer (EORTC), Brussels, Belgium
National Cancer Institute (NCI), Bethesda, Md.
University of Texas MD Anderson Cancer Center, Houston, Texas

TARGETS AND COMPOUNDS

CD166 (ALCAM) - Activated leukocyte cell adhesion molecule
CTLA-4 (CD152)
EGFR - Epidermal growth factor receptor
PD-L1 (B7-H1; CD274) - Programmed cell death 1 ligand 1
PLAU (uPA) - Urokinase-type plasminogen activator

BIOCENTURY PUBLICATIONS, INC.

NEWSROOM
pressreleases@biocentury.com

SAN CARLOS, CA
+1 650-595-5333; Fax: +1 650-595-5589

CHICAGO
+1 312-755-0798; Fax: +1 650-595-5589

WASHINGTON, DC
+1 202-462-9582; Fax: +1 202-667-2922

UNITED KINGDOM
+44 (0)1865-512184; Fax: +1 650-595-5589