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mAbs that live long and prosper

By Lev Osherovich, Senior Writer

Independent teams at **The University of Texas at Austin** and **Xencor Inc.** have come up with strategies to improve the effectiveness and durability of cancer mAbs. Both approaches involve modifying the invariant region common to all mAbs to increase their binding to receptors on innate immune cells and endothelial cells.

The biotech is using its strategy to develop an extended half-life mAb and has licensed the technology to several pharma companies.

Antibodies generally feature two antigen-specific variable regions called the Fab (fragment antigen binding) regions, which are held together by one invariant portion called the Fc (fragment crystallizable) region. The Fab regions selectively bind to the mAb's target and are thus the focus of antibody engineering efforts.

The Fc region binds to complement proteins and to a family of cell-surface receptors on immune cells and vascular endothelial cells. It is these Fc interactions that determine the effects of injectable mAb therapeutics. Depending on which type of Fc receptor it binds to, a mAb can be internalized and degraded by macrophages or endothelial cells or it can stimulate an immune response that boosts the mAb's therapeutic efficacy.

The University of Texas (UT) paper, published in the *Proceedings of the National Academy of Sciences*, showed that engineered mutations in the Fc domain can improve innate immune cell recognition by mAbs manufactured in bacteria.[1] Previously, bacterially derived mAbs were unable to recruit innate immune cells and were thus ineffective at raising an attack against tumor cells.

Meanwhile, a report by Xencor in *Nature Biotechnology* showed that other mutations in the Fc region can enhance the *in vivo* stability of tumor-targeting mAbs manufactured in mammalian cells.[2]

"It's been known for years that the Fc region has important interactions with the immune system, but people were previously focused on engineering the variable region" of antibodies to optimize antigen binding, said Xencor president and CEO Bassil Dahiyat. "We are now starting to see hints of data validating the therapeutic aspects" of improving the Fc end of the antibody.

No sugar? No problem

The UT study stems from efforts to make therapeutically useful mAbs in bacteria. A key roadblock is that bacterially manufactured antibodies lack Fc region glycosylation.

"Antibodies that are not glycosylated cannot be recognized by immune cells," said George Georgiou, lead author of the study in *PNAS* and professor of chemical engineering, biomedical engineering, molecular genetics and microbiology at UT. "Activation of the innate immune response is very important for the action of cancer therapies like Herceptin."

Herceptin trastuzumab, an antibody against HER2 (ERBB2; neu), is marketed by **Roche**'s **Genentech Inc.** unit to treat breast cancer.

To correct the lack of receptor binding by bacterial mAbs, Georgiou's team set up an *in vitro* screen for mutated versions of bacterially produced Fc fragments that bound to Fc γ-receptor I (CD64; FCGR1), an activating receptor found on the surface of dendritic cells (DCs). The team found two mutant Fc fragments that had higher FCGR1 binding than wild-type Fc fragments.

The UT team then fused these mutant Fc domains to the antigen-recognizing region of Herceptin and expressed the hybrid protein in *Escherichia coli*. Although the bacterially derived Herceptin variants were not glycosylated, they nonetheless were recognized by DCs.

Moreover, the *E. coli*–produced Herceptin stimulated tumor cell killing by DCs in cell culture more effectively than normally glycosylated, mammalian cell–derived Herceptin.

Georgiou thinks the extra potency of the mutant mAb results from both higher affinity for the activating FCGR1 receptor and lower affinity for Fc γ-receptor IIb (CD32B; FCGR2b). The latter receptor antagonizes DC activity, so antibodies that don't bind to it send a purely stimulatory signal to the DCs.

Because the Fc region is shared among all mAbs, Georgiou believes his team's process should work for other cancer mAbs that enlist innate immune cells to kill tumor cells.

"All antibodies targeted toward a cell-surface antigen could be improved by this method," said Georgiou. As examples, he cited Erbitux cetuximab and Campath alemtuzumab.

Erbitux is marketed by **Bristol-Myers Squibb Co.**, **Eli Lilly and Co.**'s **ImClone Systems Inc.** unit and **Merck KGaA** for non–small cell lung cancer (NSCLC), metastatic colorectal cancer (mCRC) and squamous cell carcinoma of the head and neck (SCCHN). Campath is marketed by **Genzyme Corp.** to treat B cell chronic lymphocytic leukemia (CLL).

Georgiou's discoveries also should pave the way to cheaper manufacturing of cancer mAbs, according to Hans de Haard, CSO of antibody discovery company **arGEN-X B.V.**

arGEN-X uses camelid antibodies to generate leads for making full-length and humanized mAbs.

"The possibility of expressing aglycosylated Fc versions of therapeutic antibodies that are still able to recruit effector functions is a big step forward," said de Haard. Making mAbs in bacteria "will reduce the cost of goods and might enable treatment of a broader range of diseases" for which effective mammalian mAbs cannot be made.

"One of the challenges of making mAbs is the time and expense of manufacturing them in mammalian cells," said Debbie Law, CSO of **Ablynx N.V.** "*E. coli* is a very attractive expression system, but because the antibodies come out in an aglycosylated form, there are issues with folding and multimerization," which are processes thought to go awry when the sugars decorating the antibody's surface are missing.

Ablynx develops nanobodies, which are minimized Fab fragments derived from the single-protein antibodies of camelids and are smaller than antibodies. Because nanobodies have only a single protein chain and lack a glycosylated Fc region, they can be made in bacteria and fungi; however, they do not readily interact with the innate immune system.

Recycle and reuse

The Xencor study, from a team led by VP of research John Desjarlais, showed that the Fc region also can be modified to increase mAb survival in the blood stream.

Dahiyat said the relatively short half-life of injected mAbs is a result of internalization and degradation of circulating antibodies by vascular endothelial cells. This process occurs naturally as part of the body's effort to dispose of unnecessary serum proteins.

"Anything circulating in the blood will be taken up nonspecifically inside endothelial cells," said Dahiyat. "Most injected biologics have a pretty short half-life because of this mechanism."

Drawing on evidence that this internalization process was antagonized by an Fc-binding receptor called Fc fragment of IgG receptor transporter-α (FCGRT; FCRN), the Xencor team designed a mutant Fc domain that had higher affinity for FCRN.

The team then put these mutations into Erbitux and Avastin bevacizumab. Avastin is an anti-VEGF antibody marketed by Genentech and Roche to treat colorectal, lung, breast and renal cancers.

In monkeys, the modified versions of Erbitux and Avastin persisted longer in the serum than the parent antibodies. In mouse xenograft cancer models, the longer persistence correlated with greater reductions in tumor volume.

Dahiyat thinks Xencor's Fc mutations cause greater *in vivo* persistence by increasing the recycling of mAbs trapped by the endothelial cell surface.

He noted that FCRN ordinarily releases its cargo once it has entered the low-pH environment of the endosome, which degrades captured serum proteins. Once free of its load, FCRN returns to the surface and repeats the cycle.

Xencor's mutations appear to make FCRN hold on to antibodies more tightly in the endosome but do not affect binding in the neutral pH of the blood.

Because antibodies held tightly by FCRN in the endosome eventually return to the cell surface and are released back into the blood, the mutant mAbs are essentially recycled, said Dahiyat.

Mix and match

The two reports pave a path for further optimization of mAbs beyond antigen binding, which has been the traditional area of focus. After selection of the best Fab region, "the Fc part itself can now be engineered for improving serum half-life or effector functions," said arGEN-X's de Haard.

Conceivably, the UT and Xencor techniques could be combined to generate antibodies that are cheaper to produce, kill cancer cells more effectively and remain active for longer than cancer mAbs currently on the market and in the clinic. However, additional engineering likely would be needed to maximize the combined benefits.

For example, Georgiou noted that the same mutations that render bacterially derived glycosylation-free mAbs more potent appear not to work in mammalian cell–derived glycosylated mAbs.

Likewise, Dahiyat noted that Georgiou's mutations improved antibody stimulation of DCs but appeared to reduce their effect on natural killer cells and macrophages compared with the effects of unmodified control mAbs.

Georgiou maintained that DCs are likely to be the main players in tumor cell killing by the innate immune system but acknowledged that additional engineering could further improve mAb interactions with the other innate immune cells, which use a different set of Fc receptors.

Georgiou's patents on the discoveries are available for licensing from **Clayton Biotechnologies Inc.**, an IP-holding company that is a subsidiary of the **Clayton Foundation for Research**. The foundation partially funded Georgiou's work.

Dahiyat said Xencor has patented and nonexclusively licensed the Fc modifications described in the *Nature Biotechnology* paper to **Pfizer Inc.**, **Merck & Co. Inc.** and **Johnson & Johnson**'s **Centocor Inc.** unit. Xencor also has an internal mAb in preclinical development for an undisclosed indication that uses the half-life–enhancing mutations.

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   Contact: George Georgiou, The University of Texas at Austin, Austin, Texas
   e-mail: gg@che.utexas.edu
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   Contact: John R. Desjarlais, Xencor Inc., Monrovia, Calif.
   e-mail: jrd@xencor.com

COMPANIES AND INSTITUTIONS MENTIONED

Ablynx N.V. (Euronext:ABLX), Ghent, Belgium
arGEN-X B.V., Rotterdam, the Netherlands
Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.
Centocor Inc. (NASDAQ:CNTO), Malvern, Pa.
Clayton Biotechnologies Inc., Houston, Texas
Clayton Foundation for Research, Houston, Texas
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The University of Texas at Austin, Austin, Texas
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