Genentech plays BACE

By Lev Osherovich, Senior Writer

Researchers at Roche's Genentech Inc. unit have engineered antibodies that slip past the blood brain barrier and hit BACE1, an Alzheimer's disease target. This approach departs from the conventional wisdom that BACE1 inhibitors should be small molecules and shows that custom-tailored mAbs can also do the job. Genentech is pursuing preclinical development of the antibody.

Beyond hitting β -site APP-cleaving enzyme 1 (BACE1), the new antibody engineering techniques used to get the mAbs into the brain open up a platform for hitting other CNS targets with peripherally injected mAbs. The findings were reported in two back-to-back papers in *Science Translational Medicine*.^{1,2}

BACE1 is involved in the conversion of amyloid precursor protein (APP) into the aggregation-prone β -amyloid (A β), which is believed to promote neurodegeneration.

Blocking BACE1 would presumably decrease A β levels, but BACE1 is primarily an intracellular target that resides behind the blood brain barrier (BBB). Thus, hitting it has proven to be a major challenge. Only one compound—CTS-21166 from CoMentis Inc. and Astellas Pharma Inc.—has made it into Phase I testing.

Ryan Watts, associate director of neuroscience at Genentech, said the company had initially set out to identify BACE1 antibodies as research tools and only later discovered their therapeutic potential.

"We raised antibodies that are exclusively specific for BACE1" and not related proteases, said Watts. "When we did cell culture experiments, we saw that the antibodies were very potent, and we realized that we could potentially turn this into a therapeutic."

Watts' first version of the antibody was derived from a phage display library and blocked the activity of BACE1 *in vitro*.

The surprise came when the team treated neurons with the BACE1 antibody. Watts said although the majority of BACE1 protein resides in the endosomal membranes and is thought to be inaccessible to antibodies, the team's antibody bound to the small fraction of BACE1 present at the cell surface, was gradually internalized and decreased BACE1 activity.

Indeed, mice and monkeys treated with the antibody had lower

serum and brain levels of A β than animals treated with nonspecific antibody controls.¹

Back to BACEics

Watts' initial antibody didn't readily cross the BBB, so the team needed to dose mice at a very high level to see a reduction in brain A β .

To improve on the brain delivery of the BACE1 mAb, Watts collaborated with Mark Dennis, senior scientist of antibody engineering at Genentech, to develop a BACE1 antibody that slips into the brain.

Dennis and Watts drew on prior research suggesting transferrin receptor protein 1 (TFRC; TFR; CD71), a protein in the brain's vasculature, could act as a shuttle from one side of the BBB to the other.

TFRC's normal function is to transport transferrin, an iron-binding protein, across endothelial cell layers such as the one that forms the BBB. Other researchers have previously delivered therapeutic agents into the brain by conjugating them to TFRC-binding mAbs.³ But these high-affinity conjugates had limited success at crossing the BBB.

"Antibodies against the transferrin receptor do get transported into the brain to a certain extent, but mostly get trapped in the endothelium," said Dennis. To achieve higher yields in the brain, "we realized that we wanted to reduce the affinity of the antibody for the receptor."

Dennis said that in the blood, in which concentrations of the mAb are high after dosing, a small fraction of the weakened mAbs can still bind to TFRC and undergo transport. But on the other side of the barrier, the weak affinity allows the transported mAbs to fall away from TFRC and drift off into the brain.

Thanks to the law of mass action, the net result is the gradual accumulation of the mAb in the brain.

Indeed, low-affinity TFRC antibodies injected into mice accumulated in the brain at a greater concentration than high-affinity antibodies.

The team next put Dennis' trick to the test by engineering a bifunctional mAb with two distinct epitope-bindings sites—one for TFRC and the other for BACE1.

In treated mice, the engineered mAb accumulated in the brains and decreased A β levels at a lower dose than the conventional BACE1 mAb.²

Watts also said adding the weak TFRC-binding arm to the BACE1 mAb led to equivalent BACE1 inhibition at about a fivefold lower dose than the original BACE1 mAb.

But William Pardridge, professor of medicine at the University of California, Los Angeles, thinks the team would have gotten better BACE1 inhibition with a conventional mAb engineered to cross the BBB by high-affinity binding to TFRC or other transporter proteins.

"The problem is how to engineer a bispecific antibody," said Pardridge. "The low affinity of their mAb leads predictably to low brain uptake in the mouse, which leads predictably to the requirement

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for massive doses, around 25–50 mg/kg, to get a therapeutic effect.”

Watts countered that mice have more rapid pharmacokinetic clearance of mAbs and thus require higher doses than humans, so he expects that a lower dose of the bifunctional mAb will be effective in the clinic.

Pardridge is also CSO of **ArmaGen Technologies**, which has fusion proteins that deliver a therapeutic payload across the BBB via insulin receptor (INSR). ArmaGen's preclinical Alzheimer's disease (AD) pipeline consists of ATG-110, a tumor necrosis factor- α receptor fragment fused to high-affinity INSR antibodies, and ATG-160, an INSR mAb fused to an A β mAb.

BACE against time

Watts and Dennis said the engineered BACE1 antibody is already in preclinical development as an AD therapeutic, although they didn't disclose a timeline for starting human trials.

Genentech already has a footprint in the AD space: the company's MABT5102A, a mAb against A β , started Phase II testing in May. MABT5102A was co-developed with **AC Immune S.A.**

Although a plethora of A β -lowering strategies have been tried in the clinic, Watts said no therapeutic candidate has yet shown a substantial decrease of A β in the brains of patients, primarily because of the BBB.

Thus, if the team's new mAb works in humans the way it does in animal models, Watts thinks the molecule could be the first informative test of the hypothesis that A β causes AD.

Samuel Gandy, professor of neurology and psychiatry at **Mount Sinai School of Medicine**, said Genentech's transporter technology was “a nice technical advance,” but it's not clear whether BACE1-targeting mAbs are better than antibodies that directly target A β .

Gandy said that *in vivo* imaging and postmortem studies have already hinted that anti-A β immunotherapy can lower A β levels in the brain.⁴ But these studies were small in scale, showed only modest effects on brain A β levels and did not significantly reduce AD progression.

Gandy noted that brain penetration might not be required for mAbs that directly target A β , citing evidence that peripherally restricted mAbs can lower brain A β by perturbing the equilibrium of A β in the blood and brain, the so-called peripheral sink effect.

However, Genentech's mAb potentially opens an opportunity to block A β production at an earlier step and with a greater effect than previous immunotherapies.

“We know that we can lower brain plaque burden with the antibodies that we already have,” said Gandy, “Conceivably removing more [A β] faster could be superior to what has been achieved so far.”

Watts noted that no anti-A β immunotherapy has yet shown a meaningful clinical effect, so the jury is still out on whether targeting A β or BACE1 will be better.

Genentech has filed for patents on the BACE1 mAb and the TFR- based delivery strategy. The IP is not available for licensing.

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e-mail: rwatts@gene.com
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Contact: Ryan J. Watts, Genentech Inc., South San Francisco, Calif.
e-mail: rwatts@gene.com
Contact: Mark S. Dennis, same affiliation as above
e-mail: msd@gene.com
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COMPANIES AND INSTITUTIONS MENTIONED

AC Immune S.A., Lausanne, Switzerland
ArmaGen Technologies, Santa Monica, Calif.
Astellas Pharma Inc. (Tokyo:4503), Tokyo, Japan
CoMentis Inc., South San Francisco, Calif.
Genentech Inc., South San Francisco, Calif.
Mount Sinai School of Medicine, New York, N.Y.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
University of California, Los Angeles, Calif.