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Opening the brain

By Lev Osherovich, Senior Writer

A **Cornell University** team has proposed a new strategy for delivering large-molecule therapeutics into the CNS: agonizing adenosine receptors in the brain's vasculature to loosen up the blood brain barrier.[1] Private equity company **KensaGroup LLC** has formed **Adenios Inc.** to commercialize the approach.

The blood brain barrier (BBB) is maintained by a set of pumps, barriers and cytoskeletal structures that work in the endothelial cells of the brain's blood vessels to keep out foreign substances. The barrier prevents many small molecules and most therapeutic proteins from entering the brain.

Current strategies for slipping large molecules past the BBB include targeting surface receptors on endothelial cells with bifunctional mAbs that transport a therapeutic cargo from one side of the cell layer to the other.[2] This Trojan horse technology from **ArmaGen Technologies** is in preclinical development. **Roche**'s **Genentech Inc.** unit has a mAb-based approach in preclinical development for Alzheimer's disease (AD).

Instead of antibody engineering, Margaret Bynoe has opted to tackle the BBB by targeting adenosine receptors. "Adenosine is an endogenous molecule that has receptors expressed on the endothelial cells that we can modulate to open or close the blood brain barrier," said Bynoe, who is an associate professor of immunology at Cornell.

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>
> ***—Margaret Bynoe, Cornell University***

In rodents, Bynoe's team showed that agonists of two adenosine receptors—adenosine A_1 receptor ($ADORA_1$) and $ADORA_{2A}$—increased absorption into the brain of high molecular weight dextrans and a therapeutic mAb targeting β-amyloid (Aβ).

Knock knock

Bynoe's team had previously shown that adenosine receptors regulate the ability of lymphocytes to migrate into the brain in a mouse model of multiple sclerosis (MS).[3] Her group then hypothesized that the receptors could affect the brain uptake of large molecules.

To test the idea, the team injected mice with fluorescently labeled dextran polymers and NECA, a D-ribofuranuronamide agonist of multiple adenosine receptors. Brain lysates from mice given dextran and NECA had detectable fluorescent signals, whereas lysates from mice receiving dextran plus an inert vehicle control did not.

The researchers then narrowed the mediators of NECA's effect to two specific adenosine receptors: $ADORA_1$ and $ADORA_{2A}$. Both receptors are highly expressed on brain endothelial cells, and knockout mice for either receptor had lower levels of dextran absorption after NECA treatment than wild-type controls.

Selective $ADORA_1$ and $ADORA_{2A}$ agonists each partially mimicked the effect of NECA on dextran absorption and in combination produced an effect comparable to that of NECA. Among the $ADORA_{2A}$ agonists used by the team was Lexiscan regadenoson, which is marketed by **Gilead Sciences Inc.** and **Astellas Pharma Inc.** as a cardiovascular imaging agent.

The team also found that NECA helped facilitate the entry of a fluorescently labeled anti-Aβ mAb across the BBB. In transgenic mice expressing the AD-associated protein, animals receiving NECA and the antibody had higher fluorescent labeling of amyloid plaques than mice given vehicle and the antibody.

Results were reported in *The Journal of Neuroscience*.

Bynoe has filed for patents on her findings, and the IP is licensed to Adenios.

Inside or outside?

The mechanism by which adenosine receptor agonists exert effects on the BBB remains uncertain. Bynoe's team reported cell culture data suggesting that adenosine receptor agonists lead to alterations in the cytoskeletal structure of endothelial cells. This, in turn, could lead to opening of the tight junctions that hold the cells together (*see* **Figure 1, "Opening the blood brain barrier"**).

It is also unclear whether the large molecules the team tested penetrated into the brain in sufficient quantities to produce a therapeutic effect.

One issue is that Bynoe's team "used immunofluorescence measurements that are semiquantitative," said Edward Neuwelt, professor of neurology and neurosurgery at **Oregon Health & Science University** and the **Portland VA Medical Center**.

Roland Bainton, associate professor of anesthesia and preoperative care at the **University of California, San Francisco**, added that measuring fluorescently labeled molecules may overestimate the extent of brain penetration.

The team measured total fluorescence in brain homogenates, but Bainton said some of this signal could be caused by molecules getting stuck in the vascular endothelium.

"They assume that their methods are showing that the barrier is open, but they don't really show the dextrans getting in," said Bainton. He suggested the team could address this concern by preparing brain slices showing staining of neurons with fluorescent dextrans or mAbs.

To accurately measure what fraction of the dextrans and mAbs actually enters the brain, Neuwelt said the team would need to use more rigorous methods such as radiolabeling or PET tracer imaging.

Neuwelt has developed an osmotic method for opening the BBB that involves an interarterial injection of mannitol. The method "opens

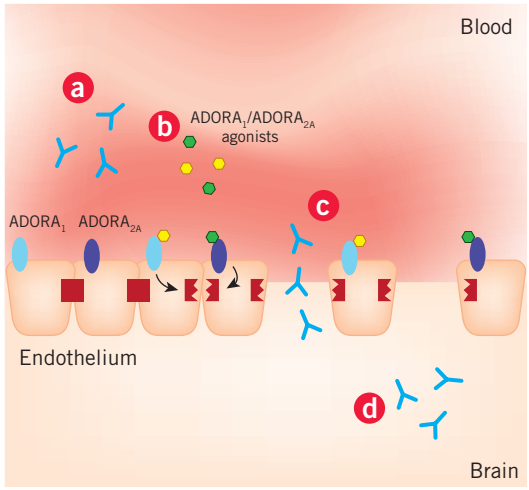

Figure 1. Opening the blood brain barrier. Carman *et al.* propose a strategy for opening the blood brain barrier using adenosine receptor agonists.

Ordinarily, tight junctions (red squares) between endothelial cells block the passage of large molecules such as therapeutic mAbs from the blood to the brain [**a**]. Carman *et al.* show that adenosine A_1 receptor ($ADORA_1$) and $ADORA_{2A}$ agonists [**b**] trigger alterations in the tight junctions that loosen the gaps between endothelial cells [**c**]. In mice, coadministration of $ADORA_1$ and $ADORA_{2A}$ agonists increased brain penetration of a model therapeutic mAb [**d**].

Inotek Pharmaceuticals Corp. has the $ADORA_1$ agonist INO-8875 in Phase II testing for glaucoma. **Gilead Sciences Inc.** and **Astellas Pharma Inc.** market the short-acting $ADORA_{2A}$ agonist Lexiscan regadenoson as a coronary vasodilator for myocardial perfusion imaging.

Pfizer Inc. has the $ADORA_{2A}$ agonist CorVue binodenoson in registration for cardiovascular imaging. **Forest Laboratories Inc.** has the $ADORA_{2A}$ agonist Stedivaze apadenoson in Phase III trials for cardiovascular imaging.

Forest Laboratories also has the $ADORA_{2A}$ agonist ATL313 in preclinical development for cancer, pain, rheumatoid arthritis (RA) and glaucoma, in partnership with various companies. **AnaMar AB** has the $ADORA_{2A}$ agonist AM 230-DAR in preclinical development for glaucoma.

the blood brain barrier for about 15 minutes" but requires general anesthesia, he noted.

If Bynoe's method indeed works, it "would be a lot easier to give an intravenous drug like an adenosine receptor agonist" than to use the mannitol procedure, said Neuwelt.

Adenios amigos

The biggest test of the method will be whether adenosine receptor agonists increase the efficacy of mAbs or other large molecules in mouse models of neurological diseases such as AD or MS. Bynoe said these experiments are ongoing.

Meanwhile, Adenios is optimizing the compounds in Bynoe's study and is developing them as adjunct therapies for AD, MS and brain tumors. The company was cofounded by Bynoe in 2009.

KensaGroup managing director Eric Eisenhut said the company is seeking to develop a tailored adenosine receptor agonist with optimal pharmacodynamics for BBB opening while decreasing other undesired effects of adenosine receptor agonists, such as myocardial permeabilization.

"Adenosine receptors are not limited to just the blood brain barrier," so the best candidate should be optimized to minimize effects on other tissues, said Eisenhut, who sits on Adenios' board of directors.

Indeed, $ADORA_1$ and $ADORA_{2A}$ are involved in a range of neurological, cardiovascular and inflammatory indications.

Pfizer Inc.'s CorVue binodenoson $ADORA_{2A}$ agonist is in registration for cardiovascular imaging. **AnaMar AB**, **Forest Laboratories Inc.** and **Inotek Pharmaceuticals Corp.** have $ADORA_1$ and $ADORA_{2A}$ agonists in various stages of development for a range of indications.

Eisenhut said Adenios "is in the process of selecting a lead compound that targets one or both receptors" and the next step is preclinical testing. Adenios also is considering the possibility of repurposing an approved $ADORA_{2A}$ agonist like Lexiscan, he said.

Bynoe thinks the ideal candidate would have a relatively short biological half-life. This would allow entry of protein therapeutics without compromising the BBB's ability to keep the brain free of potentially harmful peripheral molecules.

"The most important thing for modulation of the blood brain barrier is to both open and close it," added Bynoe. "The mechanism must be reversible."

KensaGroup has launched 10 companies in the past 12 years, including polymer synthesis company **Novomer Inc.** and cancer player **Zuma Biosciences LLC**.

Osherovich, L. *SciBX* 4(39); doi:10.1038/scibx.2011.1080
Published online Oct. 6, 2011

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COMPANIES AND INSTITUTIONS MENTIONED

Adenios Inc., Ithaca, N.Y.
AnaMar AB, Goeteborg, Sweden
ArmaGen Technologies, Santa Monica, Calif.
Astellas Pharma Inc. (Tokyo:4503), Tokyo, Japan
Cornell University, Ithaca, N.Y.
Forest Laboratories Inc. (NYSE:FRX), New York, N.Y.
Genentech Inc., South San Francisco, Calif.
Gilead Sciences Inc. (NASDAQ:GILD), Foster City, Calif.
Inotek Pharmaceuticals Corp., Lexington, Mass.
KensaGroup LLC, Ithaca, N.Y.
Novomer Inc., Waltham, Mass.
Oregon Health & Science University, Portland, Ore.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Portland VA Medical Center, Portland, Ore.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
University of California, San Francisco, Calif.
Zuma Biosciences LLC, Ithaca, N.Y.