APOE in AD

By Tim Fulmer, Senior Writer

U.S. researchers have shown that **Eisai Co. Ltd.**'s cancer drug Targretin bexarotene raises apolipoprotein E levels in the brain and partially reverses cognitive and behavioral deficits in a mouse model of Alzheimer's disease.¹ Based on the findings, the team founded **ReXceptor Inc.**, which plans to begin a proof-of-concept AD trial in about two months.

Apolipoprotein E (APOE) occurs in three isoforms—APOE2, APOE3 and APOE4—that serve as lipid-carrying proteins in the serum and brain.

Prior work by ReXceptor founder Gary Landreth, a professor of neuroscience at the **Case Western Reserve University School of Medicine**, and others showed that raising APOE levels led to downstream clearance of β -amyloid (A β) in mouse models of AD.^{2,3} The challenge was figuring out the best way to increase those levels.

To do that, Landreth and colleagues decided to focus on activating the three nuclear receptors that upregulate APOE expression: peroxisome proliferation-activated receptor- γ (PPARG; PPAR γ), liver X receptor (LXR) and retinoid X receptor (RXR).

In earlier work, Landreth's team had shown that an LXR agonist enhanced APOE levels in the brain, which in turn increased both A β degradation and cognition in mice.⁴ The study provided proof of concept that agonizing the nuclear receptors could lead to improvements in AD, but the researchers did not pursue the LXR angle because agonists of that target have a poor side-effect profile.

Landreth was further dissuaded from targeting PPAR γ , at least partly, because of data from a Phase III trial in AD disclosed by **GlaxoSmithKline plc** in 2009, in which GSK's PPAR γ agonist Avandia rosiglitazone failed to increase cognition and memory compared with placebo. GSK markets the drug to treat type 2 diabetes.

Thus, Landreth focused on activating RXR, which is the target of at least one approved drug: Targretin to treat cutaneous T cell lymphoma (CTCL). Targretin was developed by **Ligand Pharmaceuticals Inc.**, which sold the drug, along with the rest of its cancer portfolio, to Eisai in 2006.

Eisai did not return calls or e-mails requesting comment.

In cultured microglia and astrocytes, Targretin stimulated expression of APOE, promoted degradation of soluble A β and increased secretion of high-density lipoprotein (HDL) particles compared with no treatment.

In a mouse model of AD, Targretin led to greater APOE expression,

increased HDL, a 30% decrease in soluble A β and a 75% decrease in A β plaques in the hippocampus and cortex compared with vehicle. The lower A β levels were associated with behavioral and cognitive improvements in the mice.

Analysis of brain tissue showed that Targretin-treated mice also had more A β -laden microglia, suggesting that Targretin lowered A β levels by a dual mechanism: increasing both APOE levels and the phagocytic activity of microglia.

"Thus, activation of RXR by Targretin stimulates two distinct cellular processes—increased APOE/HDL levels as well as enhanced microglial activation—both of which are disease modifying," said Landreth.

Prior work by other labs has shown that activated microglia can migrate to A β depositions and clear them from the brain via phagocytosis.⁵⁻⁷

The new findings were published in *Science*.

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—Gary Landreth,
Case Western Reserve University
School of Medicine

Finding the right model

Robert Mahley, president emeritus of **The J. David Gladstone Institutes** and founder of **ApoBiopharma Inc.**, told *SciBX* the mouse model used in the paper is poorly predictive of success in the clinic. "Mouse ApoE does not behave structurally or functionally like human APOE3 and APOE4. Moreover, this is an exaggerated mouse model of excess production

of the amyloid precursor protein and A β ," he said. "We cannot draw conclusions about a response in humans from the design of this study."

A more predictive mouse model would express the human APOE isoforms. Indeed, David Holtzman wanted to see "if the same *Science* findings are replicated in the presence of human APOE isoforms" in APOE knock-in mice.

In 2011, Holtzman and colleagues published in *The Journal of Neuroscience* that such mice have varying levels of A β plaque burden depending on the number of copies of APOE3 and APOE4 genes.⁸

Holtzman is professor of neurology and developmental biology and associate director of the Alzheimer's Disease Research Center at **Washington University in St. Louis**.

Landreth said he and Holtzman are discussing a collaboration to study the mechanism of RXR activation in the APOE knock-in mice.

Divided we stand

Despite sharing the common goal of targeting APOE to lower downstream levels of toxic A β , the approaches of ReXceptor and ApoBiopharma are separated by "a wide philosophical divide," said Landreth (see Figure 1, "Two theories on APOE in AD").

ReXceptor is trying to increase extracellular levels of all APOE isoforms, whereas ApoBiopharma wants to decrease intracellular levels of a single isoform—APOE4. That isoform is converted into cytotoxic fragments by intracellular proteases. The differing strategies reflect uncertainty about the exact mechanism of action of APOE in AD.

"I believe, based on our work, that any benefit of removing amyloid by increasing APOE levels will be significantly offset by increased

Figure 1. Two theories on APOE in AD.

Two biotechs are targeting apolipoprotein E (APOE) isoforms in the brain to lower levels of toxic β -amyloid ($A\beta$) and treat Alzheimer's disease (AD). Their approaches differ and are guided by two divergent hypotheses of how APOE contributes to disease.

APOE occurs in three different isoforms—APOE2, APOE3 and APOE4—which carry lipids between various cells in the brain. Genetic studies have linked APOE4 to increased risk of late-onset AD compared with APOE2 or APOE3. However, the precise mechanistic roles the three isoforms play in AD pathophysiology is unclear.

In a paper in *Science*, **ReXceptor Inc.** founder Gary Landreth and colleagues at the **Case Western Reserve University School of Medicine** showed that the retinoid X receptor (RXR) agonist Targretin bexarotene upregulated all APOE isoforms, [a(1)] which blocked $A\beta$ aggregates [b] and decreased AD-associated behavioral and cognitive deficits in mice [c].

Targretin is marketed by **Eisai Co. Ltd.** to treat cutaneous T cell lymphoma (CTCL). ReXceptor is developing its own formulations of RXR agonists based on Targretin.

An alternative theory proposed by **ApoBiopharma Inc.** founder Robert Mahley of **The J. David Gladstone Institutes** postulates that the APOE4 isoform is intrinsically toxic to neurons compared with the other isoforms. The toxicity derives from the cleavage products of APOE4 when it is degraded by cellular proteases [a(2)]. Therefore, the company's goal is to treat disease by selectively lowering APOE4 levels and decreasing downstream toxicity that leads to sick and dying neurons [b and c].

In 2011, Mahley and colleagues published in *The Journal of Biological Chemistry* that small molecule 'structure correctors', which convert the toxic structure of APOE4 into less toxic forms, showed lower toxicity in cultured wild-type mouse neurons than no treatment.

production of the APOE4 isoform, which is a neurotoxic protein,” said Mahley. “Our approach is to attack the detrimental effects of APOE4.”

ApoBiopharma is taking a two-pronged approach to lowering APOE4 levels.

One strategy is to identify small molecules that bind APOE4 and convert it into a nontoxic form that resembles APOE3. In 2011, Mahley and colleagues published the identification of small molecule ‘structure correctors’ of APOE4 that decreased the protein’s neurotoxic effects in cultured neurons.⁹

Mahley and the **University of California, San Francisco** have licensed the structure correctors to ApoBiopharma.

The second approach is to identify small molecule inhibitors of the proteases that convert APOE4 into toxic fragments.

“We are currently performing *in vivo* mouse studies to demonstrate that the structure correctors can correct learning and memory problems that exist in mice expressing APOE4. This will provide evidence that our approach could be useful in humans,” said Mahley.

Landreth said ReXceptor plans to start a small pilot trial of Targretin in a couple of months. “This will be a proof-of-concept trial in 12 healthy volunteers—6 receiving Targretin, 6 placebo—in which we monitor CSF [cerebrospinal fluid] levels of $A\beta$ and APOE over a period of 4 months,” he said. “Those data should give us an initial idea of the activity of

Targretin in patients and whether there are any potential safety issues.”

Based on the results, ReXceptor hopes to determine a formulation and dosing regimen of Targretin to treat AD, said Landreth. He added that IP based around that formulation should help ReXceptor compete with generic forms of the drug, which goes off patent in April.

The *Science* findings are covered by patents that are exclusively licensed to ReXceptor from Case Western.

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COMPANIES AND INSTITUTIONS MENTIONED

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