AZ goes APOE

By Lev Osherovich, Senior Writer

AstraZeneca plc has assembled an academia-industry consortium to unravel the role of apolipoprotein E in Alzheimer's disease. The team, dubbed the A5 alliance, hopes to uncover basic mechanisms about how apolipoprotein E drives the disease process and to discover therapeutics tailored for carriers of the Alzheimer's disease-associated *apolipoprotein E4* allele.

Even though apolipoprotein E (APOE)'s contribution to AD risk has been known since the early 1990s, the underlying mechanism remains a mystery. APOE pathophysiology has proven hard to study because the protein plays multiple roles in cholesterol transport, cardiovascular function and inflammation, all of which are thought to affect AD.¹

AstraZeneca's Neuroscience Innovative Medicines Unit (iMed) will provide drug screening expertise and \$5 million over 4 years to fund work by academic teams at **Weill Cornell Medical College, Washington University in St. Louis, The Feinstein Institute for Medical Research and The University of British Columbia.**

AstraZeneca's ultimate aim is to seed development of a new AD therapeutic class that modulates the activity of APOE itself. Such an approach is fundamentally different from existing symptomatic therapies or drug candidates that hit another key AD target, β -amyloid (A β).

APOE is a component of the lipoprotein particles that transport cholesterol in the bloodstream. In the brain, APOE is thought to affect the activity of microglia and astrocytes, which are glial cells that help maintain the environment around neurons.

In AD, that environment goes awry, leading to a cascade of malfunctioning brain circuitry, protein aggregation and neuronal death. In *APOE4* carriers, this disease process occurs more quickly than it does in individuals with the disease-neutral *APOE3* allele.

The A5 alliance is "focused on the biology of the genetic predisposition for AD by *APOE4*," said Michael Wood, project leader of AstraZeneca's iMed. "We don't understand the underlying contribution of APOE to pathology."

The A5 alliance hopes to crack this hard nut using new cell culture, genetic and imaging assays developed in academic laboratories as well as pharmacological tools from the AstraZeneca side.

The problem

Human genetic evidence suggests *APOE4* is a major player in AD disease

risk, "dwarfing the effect of other risk genes," said Andrew Saykin, professor of medical and molecular genetics and radiology and director of the Center for Neuroimaging at the **Indiana University School of Medicine**. Saykin is not a member of the A5 alliance.

Saykin said that the presence of *APOE4* likely contributes to the majority of cases of late-onset AD, the most prevalent form of the disease.

"In the general population, maybe 25% of Caucasians carry one or more copies of *APOE4*," said Saykin. "But if you take a clinical sample of patients with AD, often as many as 40%–65% of patients have *APOE4*."

"Inheriting one copy of *APOE4* increases your risk of AD by a third, but inheriting two copies increases your risk by an astonishing 15-fold in contrast to the so-called wild-type *APOE3* allele," said A5 alliance leader Steven Paul.

Paul is professor of neuroscience and psychiatry and director of the

Helen and Robert Appel Alzheimer's Disease Research Institute at Weill Cornell and a venture partner at **Third Rock Ventures**. Paul was previously EVP of science and technology and president of the Lilly Research Laboratories unit of **Eli Lilly and Co.**

Most patients with AD carry *APOE4*, and the outlook for these patients with therapies currently in late-stage development is poor.

Because of the aggressive course of disease caused by *APOE4*, current AD trials typically segregate carriers and noncarriers into separate arms. Such stratified trials have so far suggested that therapeutics aimed at reducing levels of

aggregated A β are less effective in *APOE4* carriers than in noncarriers.

As an example, **Pfizer Inc.** and **Johnson & Johnson** reported on July 26 that bapineuzumab (AAB-001), a mAb targeting A β , did not meet endpoints in a Phase III trial in patients with mild to moderate AD who carry *APOE4*. Data from ongoing trials of bapineuzumab in noncarriers are expected next year.

Less or more?

The A5 alliance plans to tackle the mechanism of APOE using cell culture, mouse models and imaging techniques. The central premise of the consortium is that APOE affects production or activity of A β and that *APOE4* performs this function poorly compared with *APOE3*. If so, raising levels of *APOE4* would presumably ameliorate AD.

Along these lines, a team led by Gary Landreth, professor of neuroscience at **Case Western Reserve University School of Medicine**, reported earlier this year that a retinoid X receptor (RXR) agonist that upregulates expression of APOE had a beneficial effect in a mouse model of AD.²

That technology has been licensed to **ReXceptor Inc.**, which plans to start a Phase I AD trial this year.

On the other hand, other researchers believe that *APOE4* has a distinct toxic effect in neurons. If that were the case, it would be best to reduce levels of *APOE4*.

Indeed, **Merck & Co. Inc.** and researchers at the **Gladstone Institute of Neurological Disease** looked into the potential toxic effects of *APOE4*

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on mitochondrial function and screened for compounds to prevent this toxicity.

That project was terminated by Merck in 2011, and preclinical compounds arising from the collaboration were out-licensed to **ApoBiopharma Inc.**³

Altogether, the existing data on APOE's role in AD paint a complex and seemingly contradictory picture, making it hard for pharma to decide how to tackle the target.

"The bulk of the evidence generated in preclinical models points toward APOE playing a role in clearing A β , thereby probably reducing amyloid burden," said Wood. "But there has been a long-standing hypothesis that there's a toxic gain of function in *APOE4*. That's something we want to sort out."

Paul and Wood believe that academic members of the A5 alliance have lined up the tools and techniques to clear the air about how to hit APOE.

Prior mouse studies by Paul and fellow A5 alliance member David Holtzman suggest that the *APOE* genotype affects clearance of A β in the brain.⁴

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

Holtzman's team has created AD mice in which the murine version of *ApoE* has been replaced with human *APOE* variants, including *APOE4*, *APOE3* and *APOE2*, a rare variant that actually reduces AD risk.

"I'm a strong believer in our work with Holtzman that suggests that APOE influences how much amyloid develops in the brain," said Paul. In mice, "*APOE4* carriers have more amyloid than *APOE3*, and in turn *APOE3* carriers have more than *APOE2*. This has been substantiated in human imaging studies."

What is still unknown is which specific step in A β production is affected by *APOE* and how the three human variants of *APOE* differ with respect to that activity. Answering these questions will require using cell culture assays.

Paul said the biggest unanswered question about APOE in AD is whether it is better to have more or less of it. The consortium will address this with mouse genetics and *in vivo* imaging studies in mice.

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"We want to establish a model that we think is representative of Alzheimer's disease and then alter the expression of different APOE proteins," said Wood. "This will help to guide subsequent drug discovery and development."

Paul mentioned the possibility that APOE3 and APOE4 might have different effects in disease and would thus require different types of intervention.

"There's still the question of whether treating disease in E4 is the same as treating it in E3 carriers," he noted.

Paul said that once the question of whether to agonize or antagonize APOE is resolved, the consortium will launch drug screening efforts with the help of AstraZeneca's compound libraries and medicinal chemistry

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expertise. AstraZeneca will retain rights to the company's compounds. Terms of ownership of new patents emerging from the collaboration are undisclosed.

Shrunk heads

The A5 alliance is an example of how AstraZeneca hopes to replenish its early discovery pipeline with compounds from external collaborators. Last year, the pharma shrank its internal neuroscience discovery unit down to 40–50 people focused on external partnering.⁵

AstraZeneca has only three compounds in clinical development for AD, all of which aim to relieve AD symptoms but do not halt the disease process.

Those compounds are AZD3480 (TC-1734) and AZD1446, neuronal nicotinic acetylcholine receptor $\alpha_4\beta_2$ agonists partnered with **Targacept Inc.** that are respectively in Phase II and Phase I testing for AD; and AZD5213, a histamine H3 receptor (HRH3) antagonist in Phase II trials.

"Even though we have closed our neuroscience-focused laboratories, we still have a lot of internal drug discovery capabilities," said Wood. "We're looking toward this collaboration as a way to guide discovery efforts to identify a treatment for AD."

Despite the A5 alliance's focus on APOE, Wood said AstraZeneca is open to other targets for next-generation AD therapeutics. He noted that the ultimate yardstick for a disease-modifying agent in AD will be its effect on A β .

"We still believe that amyloid is a key component of AD. You have to have amyloid plaques to be considered an AD patient," said Wood.

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REFERENCES

1. Hauser, P.S. *et al. Prog. Lipid Res.* **50**, 62–74 (2011)
2. Cramer, P.E. *et al. Science* **335**, 1503–1506 (2012)
3. Fulmer, T. *SciBX* 5(9); doi:10.1038/scibx.2012.221
4. Castellano, J.M. *et al. Sci. Transl. Med.* **3**, 89ra57 (2011)
5. Hansen, S. *BioCentury* **20**(6) A1; Feb. 6, 2012

COMPANIES AND INSTITUTIONS MENTIONED

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Case Western Reserve University School of Medicine, Cleveland, Ohio
Eli Lilly and Co. (NYSE:LLY), Indianapolis, Ind.
The Feinstein Institute for Medical Research, Manhasset, N.Y.
Gladstone Institute of Neurological Disease, San Francisco, Calif.
Indiana University School of Medicine, Indianapolis, Ind.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
ReXceptor Inc., Cleveland, Ohio
Targacept Inc. (NASDAQ:TRGT), Winston-Salem, N.C.
Third Rock Ventures, Boston, Mass.
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Weill Cornell Medical College, New York, N.Y.