



## Philadelphia: Targets in a Barely Tapped Market Keep Big Pharma Focused on AD

29 November 2007. At the [17th Annual Marian S. Ware Neurodegenerative Diseases Research Retreat](#), held on 9 November 2007 at the University of Pennsylvania in Philadelphia, hosts Virginia Lee and John Trojanowski pulled together a formidable roster of speakers bearing news of big pharma's efforts in the Alzheimer arena. In a rare summit of sorts, AD leaders from Merck, Pfizer, Wyeth, and Eli Lilly met to talk about the challenges and promises of developing new medicines to treat AD. About 200 researchers and clinicians from academia and industry showed up for updates on drug discovery for Alzheimer disease.



Barry Greenberg

There was ample chance for discussion and commiserating about the difficulties of running AD trials, and the need for biomarkers, after **Barry Greenberg** of Neurochem presented data from the tramiprosate trial (see [ARF related news story](#)). Poignantly, Trojanowski asked how many failed trials before pharma loses its appetite for AD therapies? In answer, the presenters talked about strategies and targets too numerous to leave the observer with doubt about their commitment to the disease, where the need is so great and the market so large. If the day was any indication, they are all planning to keep on trying.



Peter Reinhart

What are they trying? **Peter Reinhart**, Senior Director of Neurodegeneration Research at Wyeth Research in Princeton, New Jersey, described his company's strategy of moving forward on multiple targets simultaneously. The Wyeth pipeline includes both passive and active immunization approaches (see [ARF related news story](#)), inhibitors of A $\beta$  production by both  $\beta$ - and  $\gamma$ -secretase, a plasminogen activator inhibitor (PAI)-targeted compound to boost A $\beta$  degradation, and symptomatic treatments using neurotransmitter analogs. Reinhart said that Wyeth has a dozen candidate AD programs in the clinic, plus twice as many in preclinical stages.

Reinhart highlighted two of these preclinical programs, a  $\beta$ -secretase inhibitor and a plasmin activator. In the  $\beta$ -secretase program, Wyeth identified selective, small, and potent inhibitors that knock down A $\beta$  production in Tg2576 mice. Besides reducing plasma A $\beta$  levels and brain plaques, the inhibitors produce a dose-dependent reversal of hippocampal-dependent memory deficits as measured by a contextual fear-conditioning trial. At the optimal dose of one such inhibitor, the AD animals remember just as well as non-transgenic animals, Reinhart said. The company is now trying to put their preclinical data together to move the inhibitor forward into human experiments. A recurring theme at the meeting was the uneasy dependence on mouse models for evaluating targets, with the outstanding question of how well those models predict important aspects of human disease. "Our initial clinical trials will only be as good as the models they're based on," was how Reinhart put it.

On the other side of the A $\beta$  equation, Wyeth is also working on enhancing A $\beta$  degradation by the protease plasmin, which chews up both monomeric and aggregated A $\beta$  (see [ARF related news story](#)). Previous work has shown that A $\beta$  aggregates induce tissue plasminogen activator (tPA), which cleaves plasminogen to yield active plasmin. There is evidence that the plasmin cascade is less active in AD. An inhibitor of tPA, plasmin activator inhibitor-1 (PAI-1), is upregulated in mouse models of AD and in human AD brain. Wyeth has identified small molecule inhibitors of PAI-1 that activate cleavage of A $\beta$  in vitro assays. In Tg2576 mice, the inhibitor causes a dose-dependent decrease in plasma A $\beta$  and reduces brain A $\beta$  by about one-third after a single dose. Like the  $\beta$ -secretase inhibitor, the PAI-1 inhibitor reverses the memory deficits in the foot shock contextual fear-conditioning test. A naturally occurring mutation among some Amish families creates essentially a human knockout of PAI-1. These people have no obvious phenotype, suggesting that blocking PAI-1 may be a safe way to reduce A $\beta$ , Reinhart said.



Seabrook, Lee, Hutton, Trojanowski

**Guy Seabrook**, Senior Director and Head of AD Research at Merck's site in West Point, Pennsylvania, talked about that company's immunotherapy programs. This program includes an active vaccine based on a multiple antigenic peptide (MAP) vaccine

construct. For passive immunotherapy, the company has recently focused on anti-oligomer antibodies, which they produced by immunizing mice with stable preparations of oligomers, or A $\beta$ -derived diffusible ligands (ADDLs, licensed from [Acumen](#)). After affinity maturation and humanization, the scientists end up with highly selective anti-oligomer antibodies, Seabrook said. The antibodies preferentially block binding of ADDLs to cultured neurons, compared to A $\beta$  monomers. When infused into rhesus monkeys, the antibodies elevated plasma A $\beta$ . The speculation is that this A $\beta$  is coming from the brain, which is consistent with data that the antibodies lower brain A $\beta$  in several transgenic mouse lines, Seabrook said.

Merck is also actively seeking disease state biomarkers. On that goal, Seabrook showed results using CSF from subjects in the Oxford Project to Investigate Memory and Ageing (OPTIMA), in collaboration with A. David Smith of the University of Oxford in England. By combining information on the levels of A $\beta$ 42, tau, phospho-tau, and  $\beta$ -secretase, he said, his company achieved

99 percent accuracy in assigning AD in a group of elderly people with AD diagnoses confirmed postmortem.

In his keynote talk, **Steven Paul**, President of Lilly Research Labs in Indianapolis, focused on apolipoprotein E (ApoE) in AD. Despite being a well-established risk factor for AD, little is understood about how ApoE functions in the disease. AlzGene meta-analysis calculated that two E4 alleles elevate risk 15-fold, though a recent study with pathologically verified cases put that number as high as 25-fold (see [ARF related news story](#)). How does the E4 protein, which differs by just two amino acids from the protective E2 isoform, increase risk so dramatically? Paul thinks if researchers can sort out the role of ApoE4, the pathogenesis of AD will become much clearer. Earlier work by Paul, Kelly Bales, and Ron DeMattos at Lilly and their collaborator David Holtzman at Washington University in St. Louis, Missouri, has shown that human ApoE effectively reduces A $\beta$  deposition/amyloid plaque formation in aging PDAPP mice. The ApoE2 and ApoE3 isoforms are more effective than the ApoE4 isoform. Consequently, Paul and colleagues have postulated that drugs that increase ApoE expression or secretion in the CNS may prevent or slow the progression of AD (see [ARF related news story](#)).

What is clear is that cholesterol transport pathways are intimately involved with the production and clearance of A $\beta$  in the brain. ApoE is the major lipoprotein in the brain, where it is made mostly by astrocytes and microglia. With recent work showing that the lipid transporter ABCA1, which is required to transfer cholesterol to ApoE, also functions in A $\beta$  clearance, both ABCA1 and ApoE have become important drug targets. Agonists of the liver X receptor (LXR), a steroid receptor that regulates the expression of both genes ([Liang et al., 2004](#); [Lefterov et al., 2007](#); [ARF related news story](#)), have been proposed as therapeutics. However, Paul cautioned, the approach may require selective compounds that would stimulate expression in brain more than in peripheral organs to avoid unwanted side effects such as a fatty liver.

ApoE may also play a role in the inflammatory response to A $\beta$ , Paul showed. When the researchers exposed PDAPP brain slices to astrocytes in a culture dish, they found that astrocytes from adult mice avidly degraded A $\beta$ . The adult cells picked up A $\beta$  via receptor-mediated endocytosis in a process that requires ApoE; astrocytes from ApoE knockout mice did not do it, Paul reported.

In the wake of reports that peripheral monocytes can enter the brain and phagocytose A $\beta$  (see [ARF related news story](#), but see also [ARF news story](#)), Paul and his coworkers tested the effect of peripheral macrophages on the brain slices. They found that the peritoneal macrophages gobbled up A $\beta$  without harming the brain tissue. These cells took up A $\beta$  from diffuse plaques, but also ate amyloid, whereas neither microglia nor astrocytes could destroy amyloid. Similar to what the scientists saw with astrocytes, macrophages from ApoE knockout mice were far less effective in clearing A $\beta$  or amyloid than cells from wild-type mice. And when macrophages expressing the human ApoE isoforms were tested, E2-expressing cells proved much better at clearing amyloid than E4-expressing cells. The activity of the A $\beta$ -degrading enzyme matrix metalloprotease 9 was higher in ApoE2-expressing macrophages than in E4 expressers. "ApoE2-expressing macrophages are plaque-eating machines," Paul said. Paul's results jibe with recent reports from several groups about clearance of plaque by brain-infiltrating macrophages (see [ARF related news story](#) and [ARF related conference story](#)). Now, Paul's group is trying to replicate the results in vivo by transplanting bone marrow from E2-expressing mice into PDAPP mice, to see if those cells can clean up plaque.

**Michael Hutton** of Merck presented an alternative to the A $\beta$ -centric view in his talk on tau-targeted therapies. Hutton recently left academia to take a position as Senior Director of Neuroscience Drug Discovery at Merck Research Labs in Boston, where he is in charge of non-A $\beta$ -directed therapies for AD.

Hutton said that arguments in favor of targeting tau in AD only start with the fact that tau pathology exists side-by-side with amyloid as a hallmark of AD. Tau itself can cause neurodegeneration, as evidenced by the 20 primary tauopathies caused by familial mutations in tau. In addition, tauopathy correlates with neuronal loss and memory decline, and its proximity to neurodegeneration may mean that tau therapies offer the best chance for disease modification. Mouse models with robust neurofibrillary tangle pathology show widespread neurodegeneration, cell loss, and functional decline, which might increase the likelihood that they will be good reporters for therapeutic effects compared to amyloid-only models. In addition, multiple rare tauopathies (considered orphan diseases) present the opportunity for proof-of-concept studies, and Hutton said he feels a responsibility for including these patients in clinical studies. Finally, elevation of phospho-tau, a validated biomarker in AD CSF, could provide a simple way to monitor therapies.

The downsides of targeting tau include its key role in microtubule function and its propensity to form intracellular lesions. In AD, no tau mutations are known, and only a weak association of risk is seen with the tau H1c haplotype variant, suggesting that tauopathy may play but a peripheral role in the disease. So far, findings in tauopathies do not show a clear pathogenic mechanism, and cell models for tauopathies are poor, Hutton said. Despite the availability of transgenic mouse models, no unambiguous or consistent therapeutic prevention or disease modification studies have been reported. Hutton said that may just be because the field is still young.

Even with these drawbacks, potential tau-directed therapies abound: kinase inhibitors, aggregation inhibitors, reducers of tau expression, and microtubule stabilizers are all possibilities that are under study in various labs. Enhanced clearance of tau by HSP90 inhibitors is another angle (see [ARF related news story](#)). Hutton noted his surprise that even tau immunotherapy shows some promise (see [ARF related news story](#)).



Christopher  
Austin

Tau aggregation inhibitors have been identified in several high throughput screens. Hutton highlighted one from the Mandelkow lab in Hamburg ([Pickhardt et al., 2007](#)), and **Christopher Austin**, director of the [NIH Chemical Genomics Center](#), talked about two more at his center. As part of the Molecular Libraries Screening Initiative, Austin's lab screens a growing library of more than 200,000 compounds, and has developed "quantitative HTS." This method allows the government scientists to

use multiple concentrations already in a first screen of each compound, rather than one high concentration, as is done in many screens. By going right to dose-response curves, Austin says, his group saves time, identifies many more positive hits, and can come out of a first screen with structure-activity relationships. "This method has revolutionized our ability to draw conclusions relatively rapidly after screening," Austin said. Last year, working with Jeffrey Kuret of Ohio State University in Columbus, they identified compounds that either inhibited or enhanced tau aggregation ([Honson et al., 2007](#)). Now, Austin says they are working with Virginia Lee on another large-scale screen of tau.



Holly Soares

Nearly every speaker expressed a wish for better biomarkers, both to nail an early diagnosis and to quickly assess the effects of new treatments. As Trojanowski put it, "We are not going to get very far very fast without ways to monitor disease with biomarkers." With that, he introduced **Holly Soares**, a Director of Translational Medicine at Pfizer Research in Groton, Connecticut, who talked about her group's work on serum biomarkers.

Interest is high in blood-derived markers, with their promise of easily accessible, non-invasive diagnosis or tracking of disease progression. For example, a panel of 18 proteins was recently described by Tony Wyss-Coray and coauthors (see [ARF related news story](#)) that could distinguish elderly people with AD from those without. Those markers were discovered by measuring a larger group of 120 proteins and then comparing disease and control samples to find the set that discriminated the two populations.

Soares described a similar approach, comparing blood from 20 patients with mild to moderate AD and 20 matched controls. Blood was tested at 3, 6, and 9 months, using HumanMAP from RBM in Austin, Texas, a bead-based multiplex assay for 89 different proteins that included cardiovascular risk factors, inflammatory, and cancer markers. Of the proteins assayed in the recent Wyss-Coray paper, 44 percent were also included in the Pfizer experiment. Their test found no discriminatory power for AD versus control in these overlapping analytes. Soares said they had trouble with variability in the inflammatory markers, but did see changes in lipoproteins and tumor necrosis factor  $\alpha$  between cases and controls. She said the beads can differentiate AD versus control plasma, but not with the same set of proteins that Wyss-Coray and coworkers reported. Soares did not see changes in the profiles over time, at least up to 1 year, but wants to do longer studies.

In another study, Soares showed data on biomarker measurements in serum samples from Pfizer's Alzheimer Disease Cholesterol-lowering Treatment trial. That trial tested the cholesterol-lowering drug atorvastatin, and in a small study the scientists looked at the effect of cholesterol-lowering on several potential biomarkers. They saw little or no changes in plasma A $\beta$  levels or ratios. A brain-specific cholesterol marker, cerebrosterol, was decreased in patients on the drug. When Soares applied the multiplex assay, she found significant decreases in serum amyloid P, a potential seed for amyloid fibrils, in the patients on drug. That was unexpected, and may reflect different mechanisms of action of the statins, beyond their effects on cholesterol synthesis via HMG-CoA reductase. The multiplex assays are very powerful, Soares said, because they can give a fuller picture of the effects of drugs in the context of a disease. She concluded with this optimistic assessment: "Finding biomarkers in plasma is not easy, but they are there."

At the end of the day, John Trojanowski observed that no one had addressed the issue of trying to slow aging. What did pharma think about setting aside specific AD targets and going after aging pathways, such as the resveratrol approach? This topic seemed to be on the speakers' radars, but still just as a blip, caught at the early stage where people are thinking about how to design animal models to test anti-aging pathways.—Pat McCaffrey.