ALZHEIMER RESEARCH FORUM — NETWORKING FOR A CURE

8th International Winter Conference on Alzheimer's Disease
(http://www.alzforum.org/new/detail.asp?id=3363)

By Gabrielle Strobel

Zuers—Meeting Mixes Translational News and Debate

In a snowy mountain town in Austria, 75 scientists met from 7-10 December 2012 for the 8th International Winter Conference on Alzheimer's Disease. They traded data and discussion of treatment approaches in the kind of cooped-up, homey atmosphere that brings down barriers when a diverse group of scientists stay together from breakfast until dinner to present, question, and debate science. (Yes, skiing in Zuers is great but, unlike in Keystone, the scientific program called for a daily 8 a.m. to 7 p.m. marathon, and the conference is so small that folks would notice if you played hooky on the slopes.) Started 10 years ago, this meeting is organized by **Manfred Windisch** of the contract research organization QPS, formerly JSW, in Graz, Austria, with help from **Abraham Fisher** and **Ezio Giacobini**. "The intention is to complement the big meetings with a small, interactive one where you can network and maybe start new projects," said Windisch.

The sun illuminates the mountaintops as it rises over the town of Zuers. *Image courtesy of Gabrielle Strobel*

The meeting started off with **Niels Andreasen**, who heads the Department of Clinical Drug Research at Karolinska University Hospital in Huddinge, Sweden. His center runs some 20 intervention studies at any given time, half sponsored by industry and half by the

European Union or other sources. On the approved cholinesterase inhibitor drugs, Andreasen cited a three-year trial, a three-year open-label comparison of treated patients versus historical controls, and his personal experience treating patients to say that these medications are better than their reputation (Minthon et al., 2009). "The drugs do have a real effect," Andreasen said. Combination therapy of cholinesterase inhibitors and memantine is not approved in Sweden, but Andreasen noted that clinical practice in his country has shifted toward using this combination, in part because of a U.S. study reporting that combination therapy delayed nursing home placement (Lopez et al., 2009). "Nursing homes are much more expensive than these treatments. It is both better for the patient and more cost effective for society if patients can stay in their homes," said Andreasen.

Besides anti-amyloids, drugs tested at Andreasen's site that have been largely negative include NSAIDs, estrogen, antioxidants, rosiglitazone, lithium, gingko biloba, and various nutraceuticals. Gingko has been definitively shown not to work (see ARF related news story), but omega 3 fatty acids generated a signal in early-stage patients and are now being retested as part of the Lipididiet®/Souvenaid® program (see ARF related news story).

Discussing recent trial setbacks, Andreasen shared these observations: The scyllo-inositol trial saw deaths in the highest-dose groups, and the Rember® trial was not truly double-blinded because the study drug colors urine. Insufficient blinding may have had a hand in the apparent success of Dimebon in Phase 2. In Phase 3 at his Karolinska site, Andreasen said, "I could see who was on drug, because Dimebon, being an antihistamine, makes people tired." From his vantage point, the preponderance of negative trials over the past decade reflects that neither drug selection nor dosing and treatment duration have been optimized in the AD field.

Andreasen urged the field to test combination therapy, such as an antibody and a BACE inhibitor, and move toward tailored, individualized therapy. Alzheimer's dementia is the endstage of a group of diseases that are still often misdiagnosed even in dementia clinics (Beach et al., 2012). This may be due in part to the mixed pathologies and extensive vascular damage apparent on autopsy. Neuropathologists have said this for many years. In Zuers, **Kurt Jellinger** of the Institute of Clinical Neurobiology in Vienna, Austria, updated the audience by saying that newer molecular pathology techniques have reconfirmed this old finding. As the Viennese scientists see it, significant mixed pathology is present in up to 70 percent of cases diagnosed with dementia during life. Therefore, a range of drugs must be developed for different types of patients, Jellinger believes. One common theme echoing through the conference was that biomarkers should be used more widely to enrich trial populations for the particular aspect of Alzheimer's disease the drug is thought to target, and to generate much more data on the theragnostic potential of a given marker-drug pairing.

That the disease starts some 20 years before symptoms clearly implies that the presymptomatic patient has AD, Andreasen said, but old diagnostic criteria did not allow for that to be recognized. "This is like watching a drowning man stick up his arms for a

rescue buoy and saying: 'No no, I need to see a blue face before I know you are drowning,'" Andreasen said. "It was, therefore, with great enthusiasm that I read the Dubois criteria. They took us from exclusion to inclusion" (see ARF related news story on Dubois et al., 2007). Since then, longitudinal CSF biomarker studies have shown that it is possible to identify people who will go on to develop AD dementia eight years before they would have met the old diagnostic criteria, Andreasen said.

On biomarkers, Andreasen urged the field to work to overcome the reluctance, particularly of general practitioners, to use lumbar punctures (LP). An LP costs €100 compared to an amyloid scan's thousands, and in the hands of a trained neurologist is no more dangerous than the radioisotope injection, he argued. "Widespread fears about lumbar puncture in some countries are misplaced," Andreasen said, citing four published studies about headache, the only proven side effect of the procedure that put its incidence at 0.9 to 4 percent. That rate improves when the physician uses a needle that requires no drawing but allows the CSF sample to drip out on its own, Andreasen said (see ARF Webinar). There are no publications about paralysis or infections produced by lumbar punctures. That said, the unrelated recent U.S. meningitis scandal caused by fungus-infested steroid injections into the spines of people with chronic pain may reinforce those fears in the minds of patients and of physicians, many of whom have little training in lumbar puncture.

With regard to infections, Andreasen said, a lumbar puncture can actually provide helpful information at trial screening. He cited the examples of a 70-year-old man and of a female hairdresser who both met inclusion criteria for an AD immunotherapy trial. Their LP showed that they had Lyme neuroborreliosis, a neuroinflammatory condition that needs to be treated. Their Lyme disease could have confounded these patients' response to the immunotherapy, and side effects would likely be attributed to the immunotherapy (Andreasen et al., 2010).

At the scientific level, CSF samples taken in the course of clinical trials yield insight into new pharmacodynamic biomarkers for drugs, said **Erik Portelius** of the University of Gothenburg, Sweden. In Zuers, Portelius summarized ongoing work to define theragnostic biomarkers for AD drug development by combining targeted proteomics with SRM mass spectrometry. For example, analyses of mouse, dog, and human CSF in response to various drugs targeting APP processing indicate that Aâ37, 40, and 42 are useful pharmacodynamic markers for ã-secretase modulation. Aâ15, 16, and 34 are markers of ã-secretase inhibition, and Aâ5-40 may be a new marker of BACE-1 inhibition. What role, if any, these new forms of Aâ may play in Alzheimer's disease the scientists do not yet know. Even so, monitoring these isoforms in early-stage clinical studies could help researchers decide which drugs to take forward into larger, more expensive trials, Portelius added.

In Zuers, researchers reviewed preclinical data of three immunotherapies that are incorporating biomarkers in ongoing Phase 2 and 3 trials (see prior ARF stories on gantenerumab, Q&A With Luca Santarelli, on BIIB037, and BAN2401). **Hansruedi Loetscher** of Roche in Basel, Switzerland, noted that the biomarker packages for

bapineuzumab and solanezumab were too limited for these programs to have fully tested the amyloid hypothesis. Most trials have CSF and amyloid PET substudies on too few participants for the resulting data to settle open questions. For Roche's gantenerumab, a previous Phase 1 dosing study indicated an 11 percent difference in the amount of amyloid change between placebo and the higher dose at six months, but that group had only 16 participants. In gantenerumab's ongoing Phase 2/3 trial of 770 patients, amyloid PET scans are to be done on a substudy of 90 participants. So far, baseline PET scans indicate, at least, that the trial is not enrolling many amyloid-negative participants, said Loetscher said, who expects outcome data in 2015.

BIIB037 is currently in Phase 1b testing with Biogen Idec of Cambridge, Massachusetts. It was developed at Neurimmune in Schlieren, also in Switzerland. In Zuers, **Jan Grimm** of Neurimmune recounted this antibody's generation from a healthy cohort of very old donors with intact cognition. Grimm noted that the researchers also found antibodies against tau, á-synuclein, and TDP-43 in these volunteers. "It is amazing what the human immune system is producing in people resistant to these diseases," Grimm said. BIIB037 is an IgG4 that binds to aggregated forms of Aâ but not monomer. In the brain, BIIB037 preferentially binds parenchymal over vascular amyloid, and after chronic dosing in older mice, plaques of all sizes shrank, but vascular amyloid stayed unchanged. Dosing and brain exposure studies in mice suggest that microhemorrhages do not happen up until a dose more than 100 times higher than the minimally effective dose, Grimm added.

As is true for gantenerumab, microglia become recruited and clear amyloid with BIIB037 treatment via an Fc receptor-mediated mechanism. In the current Phase 3 gantenerumab trial, people with prodromal AD get a monthly injection under the skin. BIIB037 is delivered by IV, and while Grimm anticipates no more than monthly delivery for this antibody, too, defining a suitable regimen is a goal of the current trial in prodromal AD.

Whereas Grimm's company developed a particular natural antibody from donors into a recombinant biologic, plasma manufacturers are pushing into the Alzheimer's therapy market with intravenous immunoglobulin (IVIG), a pooled product. IVIG reflects the cumulative antigen exposure of the thousands of donors whose plasma gets pooled in the manufacturing process. Baxter's IVIG, called Gammagard, is arguably the most advanced potential Alzheimer's therapy at present. Of its two Phase 3 trials (NCT00818662 and NCT01524887), one is due to read out in early 2013. But it is not alone. Sensing an opportunity, other plasma manufacturers are moving in. Octapharma, Grifols Biologicals, Inc., and Sutter Health are already testing their own plasma products in Phase 2 or 3 trials in Alzheimer's and mild cognitive impairment, and Behring AG in Bern, Switzerland, is entering the fray.

Much of the published research on immunoglobulin in Alzheimer's has focused on its possible effects on Aâ. In Zuers, **Susann Cattepoel** of CSL Behring AG said she is taking a closer look at what her company's IVIG product might do in AD. She is finding that it, too, contains a small but active anti-Aâ component. Cattepoel said that affinity purification of CSL Behring's Privigen® showed it indeed contains 0.1 percent of polyclonal Aâ antibodies. They bind to synthetic Aâ oligomers and are active in various

established Aâ assays of fibril formation, toxicity, and LTP inhibition. These anti-Aâ antibodies, as well as—less potently—total IVIG, activated microglial phagocytosis, Cattepoel said.

Beyond that, however, it is likely that IVIG exerts whatever effect it may have on Alzheimer's disease, at least in part, via its broad anti-inflammatory mechanisms of action. IVIG inhibits complement-mediated damage, modulates the production of cytokines away from the inflammatory ones such as Il-1b and IFN-ã, and increases expression of genes linked to reduced production of radical oxygen species.

Discussion about microglial activation pervaded the Zuers conference. How strong does it need to be to phagocytose pathology without causing collateral damage? How can therapies turn on phagocytic activation while avoiding cytokine-spewing activation? What role might the pathways of the microglial receptor TREM2, and other innate immunity genes implicated in AD, play in a person's response to drug treatment? At this conference, on Alzforum (see ARF Webinar on neuroinflammation), and in research groups around the world (see ARF related news story), these questions are moving to the top of the agenda.

Alpha, Beta, Sigma: Which Will Yield New AD Drug?

This conference was titled "AD Drug Therapy—Hope and Reality. New Targets in Sight?" True to form, then, new targets and compounds with which to hit them took up much space on the program. Here are some examples. Researchers are drilling down on targets found in the area of receptor neuropharmacology, such as the ó-1 receptor, as well as among the defining pathogenic proteins of neurodegenerative diseases, such as the synucleins.

First, ó-1 receptors. They are strangers to published AD drug development, though pharmaceutical industry research on compounds active in the CNS comes across them frequently. First identified in the 1970s, these receptors were studied pharmacologically until Austrian researchers cloned the gene (Hanner et al., 1996). In the years after, scientists learned that many endogenous peptides, for example, pregnanolone and neuropeptide Y, interact with these receptors, and subsequent knockout and other biological studies showed they act as chaperones associated with the endoplasmic reticulum (Hayashi and Su, 2007). Unusually for chaperones, a slew of endogenous ligands that act as agonists or antagonists operate the ó-1 receptor, **Tangui Maurice** at INSERM in Montpellier, France, told the audience in Zuers.

The ó-1 receptor's role in neurodegenerative diseases is poorly understood. One mutation appears to cause juvenile ALS (Al-Saif et al., 2011), but beyond that, data on its genetic contribution remain sparse (see AlzGene listing).

The receptor is ubiquitous. Besides neurons, astrocytes, oligodendrocytes, and Schwann cells, the liver, spleen, heart, kidney, intestine, and other organs express it. It is a transmembrane protein that lives in the ER, particularly at touch points with mitochondria. There, it modulates calcium and influences the composition of lipid rafts.

The receptor becomes active in response to ligand binding and in conditions of ER stress. It protects mitochondria by influencing production of radical oxygen species and expression of the anti-apoptotic gene BCL2. It also sits in the plasma membrane, where it interacts with receptors ranging from TrkB, the muscarinic acetylcholine receptor, to sodium and potassium channels. “The ó-1 receptor is an important activity-dependent signaling modulator of multiple intracellular pathways in the cell,” Maurice told the audience in Zuers.

How can the ó-1 receptor influence myriad functions in cells? It does so by way of cooperating with other receptors. At least in some cases, it forms heteromers with them, said **Abraham Fisher**, Israel Institute for Biological Research, Ness-Ziona (e.g., see Navarro et al., 2010). This constellation is how the ó-1 receptor may work as a target for certain drugs. For example, haloperidol treats schizophrenia by acting on the dopamine D2 and ó-1 receptors; fluoxetine treats depression through a combined effect on the serotonin and ó-1 receptors, and donepezil treats AD through an effect on a cholinesterase and the ó-1 receptor.

In Zuers, both Fisher and Maurice presented their respective efforts at finding small molecules that tickle this receptor in a way that might treat AD better than current drugs do. Fisher introduced AF710B, a bicyclic heterocyclic spiro-compound that he said selectively activates both the muscarinic M1 receptor and the ó-1 receptor. In detailing its effects on a list of phenotypic parameters—it increases sAPPá secretion, decreases tau hyperphosphorylation and GSK-3â activity, decreases Bax, and increases BCL2 expression in mitochondria—Fisher emphasized that the former two effects come through the M1 receptor and the latter two through the ó-1 receptor. Unlike previous compounds Fisher developed, which were mainly M1-selective orthosteric agonists, AF710B is an allosteric M1 receptor agonist. Its heteromer-specific effects differentiate it from other M1 agonists and modulators. Fisher claimed that the new compound is exquisitely potent, acting as a cognitive enhancer in rats at 1 to 30 micrograms (not milligrams) per kilogram body weight. According to Fisher, the compound is orally available with a safety margin of more than 50,000 times the minimally active dose. “Those are orders of magnitude more potent than donepezil and other agonists acting either on the M1 or the ó-1 receptors, respectively,” Fisher said.

Fisher proposed that the compound has a unique mechanism of action, whereby it sensitizes the M1 receptor through heterodimerization with the ó-1 receptor in the membrane of the ER, adding, however, that heteromerization of these receptors has not been formally proven. “We are looking to license it for drug development,” Fisher said.

For his part, Maurice presented data on two joint M1/ó-1 agonists called Anavex1-41 and Anavex2-73, developed by the biotech company Amylgen. Maurice tested these compounds by injecting them into the brain of a mouse model that develops an Alzheimer's-like phenotype after brain injection of synthetic Aâ oligomers (e.g., Villard et al., 2009). In this model, the compounds both treated and prevented the model’s learning and memory deficits, hippocampal cell death, astrogliosis, and tau hyperphosphorylation via GSK-3â, Maurice told the audience. Chronic oral treatment of

Tg2576 mice at 3 milligrams per kilogram per day is ongoing, but preliminary data suggest that compound 2-73 reduces both Aâ load and memory deficits. “Compounds that act on both the M1 and ó-1 receptors could have therapeutic potential,” Maurice said.

Several scientists quizzed about this approach praised the science, but they also cautioned that the ó-1 receptor is generally considered a challenging drug target because it is so ubiquitously expressed not just in the body, but also in several organelles within cells. Its modulation by a plethora of ligands is tied to a range of diseases on both sides of the agonist-antagonist divide. Therefore, these scientists said, drugs against it might be prone to side effects, and safety for chronic treatment in the elderly would have to be demonstrated with special care. Emphasizing the large therapeutic safety window his compound had shown thus far, Fisher countered that future toxicology studies would test the safety of targeting the modulatory action of these two receptors.

Another alternative treatment approach featured in this session focused on synucleins. á-synuclein is such a fixture in dementing and movement disorders that a drug against it might pay off broadly. “If we target á-synuclein, it might be useful not just for Parkinson’s, but also for dementia with Lewy bodies, Parkinson’s disease dementia, multiple systems atrophy, perhaps even some cases of AD,” said **Eliezer Masliah** of the University of San California, San Diego. Its cousin â-synuclein is a poorly understood homologue expressed in the brain that is thought to counteract á-synuclein and even Aâ42 fibril formation, much in the way Aâ40 appears to lessen Aâ42 aggregation in some circumstances.

Manfred Windisch of the CRO QPS, formerly JSW, in Graz, Austria, cued up a pair of talks on peptidomimetic drug discovery for synucleins. First, he reviewed a published study testing three active â-synuclein peptides in transgenic mice expressing the Swedish and London APP mutations, and in mice transgenic for mutant á-synuclein (Windisch et al., 2004). Both intraperitoneal and intranasal delivery of these peptides reduced pathology while improving spatial learning and memory; however, the results were so variable that they would have required large studies going forward. Moreover, the peptides were degraded within an hour. “This was not good enough,” Windisch said.

Next, the Austrian researchers created D-enantiomeric peptides, which stay intact in the body much longer than L-enantiomers, and tested these compounds in preventive and treatment studies in transgenic mice. The new peptides seemed to work like the original ones. They reduced Aâ load measured in different fractions and improved performance on memory tests. Apparently, the D-stereoisomer works both via aggregation and an anti-apoptotic pathway, Windisch said. He hopes its structure and activity can be recapitulated in a small-molecule compound; otherwise, the peptide itself might become a candidate for drug development. “I do believe there is something there, because I have seen very many amyloid-reducing drugs that do not have the pronounced behavioral improvements we see with this peptidomimetic,” Windisch said. Until recently, Windisch headed JSW, an Austrian contract research organization that measures anti-amyloid and behavioral effects of hundreds of putative therapeutics on behalf of academic, biotech, and pharma

labs around the globe. In August 2012, he sold JSW to QPS, a larger CRO headquartered in Newark, Delaware.

A separate effort to move from peptides to small molecules is underway for á-synuclein. Masliah presented work done by a biotech partnership set up between the University of California, San Diego, and EVER Neuro Pharma, the Austrian purveyor of cerebrolysin, nutritional supplements for "mental agility," and apomorphin, a dopamine agonist injection for advanced Parkinson's. Called Neuropore Therapies, Inc., the new company tries to turn insight gleaned from peptides that block á-synuclein aggregation into small molecules that can prevent dimerization and subsequent formation of oligomers. The idea is to stabilize monomeric á-synuclein into a functional molecule that does not enter a toxic aggregation pathway, Masliah said. In Zuers, he described how scientists in his research group had found a motif in the C-terminus of á-synuclein that seemed suitable for peptide docking. They tested some peptides for aggregation blocking and then turned them into a peptidomimetic to create analogues. Many assays later, the scientists came out with an isoindole pyrimido pyrazine compound called NPT100-18A.

This molecule blocks the formation of dimers and higher species in cell-free blood, and performed to expectations on calcium flux and neurite formation readouts in neuroblastoma and primary cell culture, Masliah said. In á-synuclein transgenic mice, the compound improved motor and memory performance and other readouts in young, but not old, mice. Masliah believes the compound works by blocking the dimerization of á-synuclein somewhere near glutamine 38 of the peptide. Mutating that site blocks the ability of the compound to keep á-synuclein from aggregating. Because the pharmacokinetics of this compound are not right just yet, the scientists are currently working on finding a series of derivatives that would more readily get into the brain and reach an effective concentration there when given by mouth.

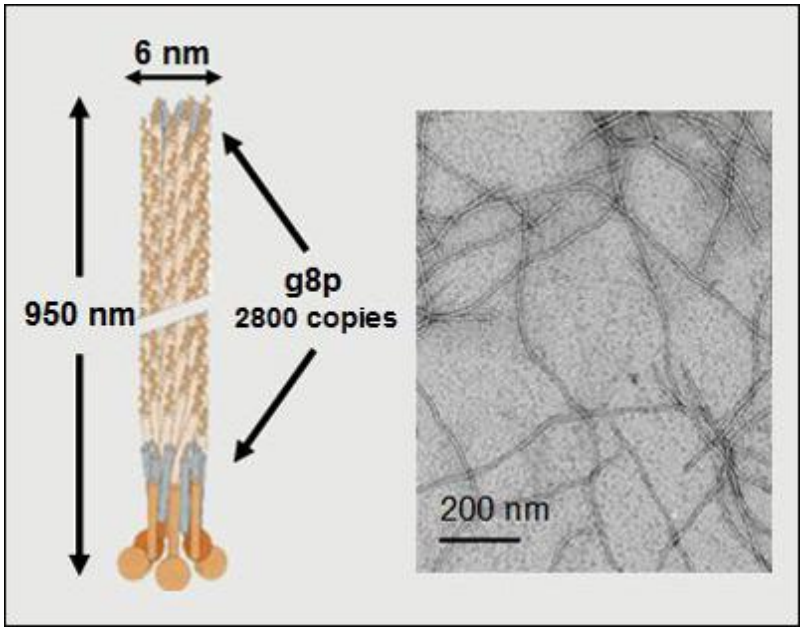
No Pill or Drip: Scientists Inject Phage Drug Into CSF

The meeting featured alternative treatment approaches that are trying to reach the starting line for clinical trials as some of the more advanced therapies have underwhelmed in Phase 2 and 3. Take NeuroPhage in Cambridge, Massachusetts. Funded in 2007, this biotech company is trying to turn a filamentous phage into something of a pan-therapy for neurodegenerative diseases marked by amyloid deposits of misfolded proteins. In Zuers, NeuroPhage's **Kimberley Gannon** rattled off a rapid-fire list of experimental results. She tried to prove to the research community that her company's work turning a bacteriophage into a therapy has come in from the lonesome fringes of research. Gannon said the therapy is holding its own when subjected to the scrutiny of established research assays applied internally and in a variety of external collaborators' laboratories.

Last year, Alzforum reported from a conference in Sweden on data showing the phage's ability to dissolve Aâ plaques and remediate behavior in some mouse models of Alzheimer's amyloidosis (see prior ARF related news story). In Zuers, Gannon expanded on these findings, claiming that the phage works for tau, á-synuclein, and other fibrils. "We hope to treat Alzheimer's, Parkinson's, dementia with Lewy bodies, and multiple orphan diseases such as tauopathies," Gannon said. She also said the company has

pinpointed its mechanism of action and is building it into a new biological therapy that isn't saddled with the perceived "ick factor" of what is, after all, a virus.

The bacteriophage M13 is a harmless wisp of DNA and protein about one micrometer long and 6 nanometers wide. M13 surrounds human life. Found in tap water, it infects *E. coli* in the gut of mammals and is sometimes even deployed in sewage treatment plants. Bacteriophages were used to fight bacterial infections in the former Soviet Union and Eastern Europe in the decades before the modern era of antibiotics, and later drew renewed interest as a potential niche therapy in the face of multidrug-resistant bacteria (see NYT Sunday Magazine, 2000; Stone, 2002; Matsuzaki et al., 2005).



NeuroPhage's lead treatment is a natural variant of bacteriophage M13, depicted graphically and by electron microscopy. *Image courtesy of NeuroPhage Pharmaceuticals*

Unlike some other types of phage, M13 does not carry genes for toxins. It has a long record of being innocuous to humans, and in NeuroPhage's hands, as well, experiments in some 300 aged transgenic mice and a dozen monkeys thus far have flagged no safety concerns. "We have done extensive safety studies and have no adverse findings. We also always look for microhemorrhage in APP-overexpressing mice, for example, and have found none," Gannon said. These were transgenic mouse safety, tolerability, and monkey toxicology studies; separate toxicology studies done according to good laboratory practice (GLP) standards will start in 2013, said Gannon.

Will regulators bless a bacteriophage as an AD therapeutic? They responded positively in three meetings held in 2011 and 2012 with the FDA and requisite agencies in France and Sweden, Gannon claimed, saying that an IND will follow the outstanding toxicology data in early 2014.

In Zuers, Gannon said the phage, trademarked as NPT002®, performed well in more than a dozen different assays. It binds Aâ fibrils in vitro, and colocalizes and dose-dependently disrupts them in vivo. It also destabilizes fibers made of tau, á-synuclein, and yeast prion in a filter retention assay, and remodels such fibers in detergent solubility assays. Adenylate kinase toxicity assays showed protection from cytotoxicity against Aâ oligomers prepared as per Stine et al., 2011, and tau fibers as per Gamblin et al., 2000. Binding, assembly inhibition, and fiber destabilization have been shown for Aâ, tau, and á-synuclein using several different assays for each parameter, Gannon said. That work is ongoing for prion protein and SOD1 fibers.

The phage reduces Aâ load within days of injection into the brain in the PDAPP, 3xTg, APPSweLon, and Tg2576 mouse models, Gannon said. The effect of a single dose lasts for six weeks. More recently, much the same findings on tau came out of studies of the Tg4510 and 3xTg mice. The same was found on á-synuclein in two mouse strains in studies performed in the laboratory of **Eliezer Masliah** at the University of California, San Diego. In a large dose-response study funded by the Michael J. Fox Foundation, the treatment reduced á-synuclein, increased tyrosine hydroxylase levels, and improved motor function, Gannon said.

How might the phage do this? In the past year, researchers at NeuroPhage cloned and expressed a 25 kDa active motif on M13. They studied its structure-activity relationship and, with NMR, defined the key peptide residues as a basis for making a second-generation compound. Gannon did not show what those residues are, but said that an IgGFc fusion molecule containing them—basically an antibody engineered to display recombinant phage bits as its antigen binding site—seems to be as potent as the phage itself at reducing plaque in hippocampus and cortex of transgenic mice. A head-to-head comparison has not been done yet.

This IgGFc fusion protein, researchers said at the conference, puts NeuroPhage more in line with other immunotherapies, and might overcome lingering resistance on the part of physicians (and clinical trial funders) to using phage. The fusion protein is behind the phage in terms of its preclinical characterization, and would compete with other antibodies that are wending their way through the clinical pipeline. Even so, countered Gannon, the IgGFc fusion molecule, if it behaves as the phage, would dissolve all major aggregates implicated in age-related neurodegeneration, and in this way might address the mixed pathology frequently seen in AD, DLB, and PD.

A big hurdle NeuroPhage faces at present is that researchers will have to inject the phage directly into the CSF to get a therapeutic dose of it into the brain. In the U.S., where physicians treating AD patients, and even some specialized research physicians, are famously loath to perform lumbar punctures—and receive little training for the procedure—any invasive therapy for age-related neurodegenerative disease faces an uphill battle. "Nice data, but the delivery is a problem," was the general tenor of opinion at the Zuers conference. One scientist said that if current immunotherapies continue to fall short in the clinic, as have bapineuzumab and less so solanezumab, then direct-to-brain delivery of phage might become more widely accepted.

Clinicians who frequently perform lumbar punctures see it more positively. “In principle, I am interested in performing a trial. I would need a great deal more information. Will it be given through an LP? Which volume? How many injections and intervals? Over how long a time frame? How to insure sterility? But the idea is good, and if these questions are answered, such a project may be possible in Sweden,” said **Niels Andreasen**, who heads the department of Clinical Drug Research at Karolinska University Hospital in Huddinge, Sweden, and serves as national coordinator of Alzheimer’s clinical trials in that country.

To study delivery methods, NeuroPhage scientists contracted with Northern Biomedical Research, an animal research company in Michigan, and with scientists at Massachusetts General Hospital, Rush University in Chicago, and the National Institutes of Health (see Ksendzovsky et al., 2012; Papisov et al., 2012). These groups compared how bolus dosing into the intrathecal space in the lower back—basically a lumbar puncture with injection of drug—compared to continuous infusion or injection directly into the brain. Using immunohistochemistry, whole-body positron emission tomography, and quantitative PCR in monkeys, they determined that a bolus injection of one milliliter of phage solution distributed broadly throughout the cortex and subcortical brain areas at exposure levels sufficient to expect a drug effect. “We will go into human trials with lumbar intrathecal bolus,” Gannon said. For more details, see Q&A below.

“There is resistance to lumbar punctures for CSF collection, especially in non-Scandinavian countries. If that slows enrollment into a trial, I predict that intrathecal bolus delivery might be even harder. But it is not out of the question,” said **Enchi Liu** with Janssen AI. For more on this issue, read the Q&A with Gannon below.

Q&A with Kimberley Gannon. Questions by Gabrielle Strobel

Q: What would this treatment look like in people? Would patients see their doctor for an intrathecal injection every month? Every quarter? Would they get a port implanted once and live with it, and get a bolus at a local clinic every so often? Help us picture it.

A: We are anticipating intermittent dosing with an inter-dose interval that will be estimated initially from the imaging data in the Phase 1 trial. Since we know that the significant effect on reducing Aâ lasts for over six weeks in an aggressive model of plaque deposition (18- to 20-month-old female Tg2576 at injection), and that human plaque deposition is much more gradual, we anticipate a fairly long inter-dose interval. This is hard to predict, but we anticipate it to be three months or longer. If the data support that, IT bolus is a feasible route of administration.

Q: Would NPT088 be administered intrathecally, too?

A: We are in the process of de-immunizing NPT088 for systemic administration in humans. We are also testing NPT088 using intraperitoneal administration in mice to assess feasibility of systemic dosing.

Q: Are all your mouse studies single dose? What about long-term data?

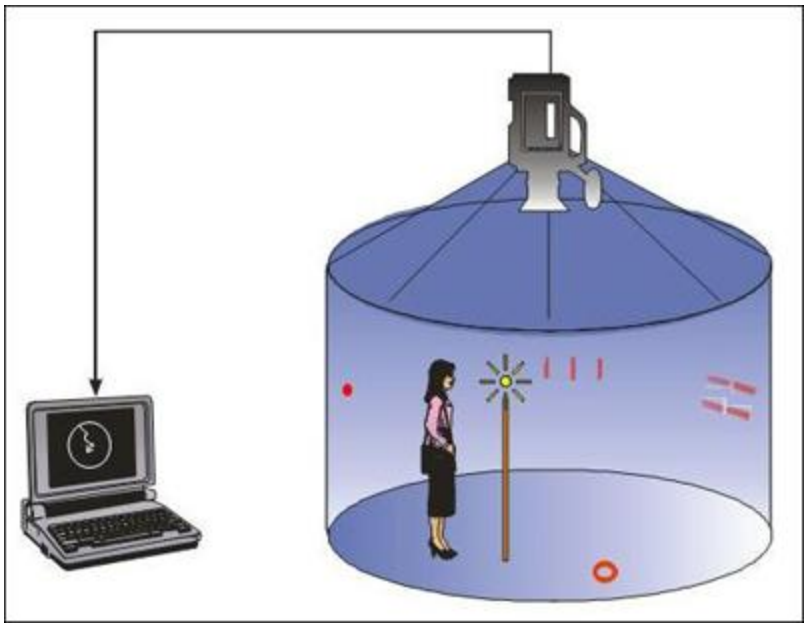
A: Most of our mouse efficacy studies thus far have involved a single intrahippocampal dose. We have one 14-day ICV continuous infusion study. Since we sacrifice the animals at predetermined time points, we have never looked at survival. The longest duration post-dosing that we have evaluated has been 12 weeks following a single intrahippocampal injection.

Q: Were the animal studies blinded? We often hear that preclinical mouse studies should be blinded just like drug studies, such that the investigators/collaborators do not know if they handle drug or placebo.

A: Yes, we always run our animal studies blinded, both behavior and analyses. The drug vials are shipped with labels indicating test article or vehicle for injections. However, all animals are coded with respect to the treatment that they received so that anyone running behavioral assessment or looking at brain tissue is blinded to treatment. The individuals doing the injections are never the same individuals performing behavioral testing or conducting the neuropathological evaluations. Therefore, behavioral testing and the neuropath analyses are conducted in a blinded fashion. Behavioral testing and neuropath analyses typically occur around five to 14 days post-injection.

Can Spatial Navigation Guide Clinical Trials?

Like many Alzheimer's disease research conferences these days, this one hosted ample discussion of how a field lost in the rubble of negative trials can find its way out. As part of that conversation, it featured a little-known proposal. What if prodromal AD patients could be tested before and after treatment in a human version of the Morris water maze? After all, this mainstay of spatial navigation testing worked for tarenflurbil, bapineuzumab, and other drugs whose promising mouse effects withered when measured by the ADAS-cog in Phase 3. No one is suggesting nudging AD patients into a pool. Instead, **Jakub Hort** of Charles University in Prague, Czech Republic, and **John Harrison** at Metis Cognition in Kilmington, U,K., are pitching a real-life, dry version of the Morris water maze in hopes that the AD field will explore whether this kind of testing might be useful in trials. If so, most prospective patients may end up "navigating" a computerized version.



Human water maze for testing spatial navigation in prodromal Alzheimer's disease. *Image courtesy of Jakub Hort*

The scientists' argument goes like this: One reason why preclinical data have failed to translate to patients may be that different phases of drug development tap different cognitive domains. At the mouse stage, it is spatial navigation; in humans, it is primarily episodic memory with verbal or visual tasks. "After all, mice don't speak and AD patients don't swim," said Hort. The Morris water maze is as entrenched in mouse treatment studies as is the ADAS-cog in late-stage AD trials, said Harrison. "It is there; you have to work with it." The disconnect can't easily be bridged by going backwards from humans, as mice won't start remembering word lists any time soon. However, Hort thought that forward continuity from preclinical through Phase 3 could be achieved by testing spatial memory in humans. It is something people use every day. "A test for finding your way across space will be more valid across cultures than the paper-and-pencil tests that were originally developed for white Anglo-Saxon college students," Harrison quipped.

The new spatial navigation measure, Hort believes, could identify people with prodromal AD and detect drug response in clinical trials in this sought-after population. His is not the first attempt to provide much-needed predictive validity to the Morris water maze. The eight-arm spatial memory task of the CANTAB was developed with the same intention. Alas, its execution did not measure up to the idea, and it never found wide application in AD clinical trials, said Harrison. "There are no studies that show it is predictive clinically of what you have seen preclinically," he said.

The Blue Velvet Arena

Hort developed a human analogue of the water maze, in which the person enters a circular tent 2.9 meters wide, enveloped by a dark-blue velvet curtain. Called Blue Velvet Arena, the installation's name is eerily similar to *Blue Velvet*, a popular 1986 motion

picture by David Lynch about a creepy, sinister world most people would not care to enter. According to the website of Polyhymnia TS, a company that classics aficionado Harrison founded with Hort and **Manfred Windisch**, the tent's new name is Urania, after the daughter of Zeus and Mnemosyne. Readers who remember their Greek mythology may have already guessed that the computerized version goes by the name of Clio, Urania's sister.

Inside, the arena features navigation signposts projected onto the curtains along its perimeter which guide volunteers in their task of locating a target. This exercise distinguishes allocentric navigation, which is independent of the seekers' starting position and uses the hippocampus and vision, from egocentric navigation, which incorporates people's starting position and taxes primarily their parietal cortex and proprioception. Of the two, allocentric navigation is the kind that declines more at the prodromal stage of typical AD.

Initial performance data on cognitively normal volunteers and people with amnestic MCI and AD were published five years ago (see ARF related news story on Hort et al., 2007). Since then, follow-up papers have expanded the finding. One characterized the measured cognitive impairment (Laczó et al., 2009), one reported that ApoE4 status worsened spatial navigation in the arena (Laczó et al., 2010), and one linked allocentric navigation in prodromal AD to right hippocampal volume (Nedelska et al., 2012). This latest paper also reports data on how well a 2-D computer version, which would be cheaper to install in clinical sites than a physical arena, corresponds to the real-space test. For a review on spatial navigation in AD, see Gazova et al., 2012).

Hort noted in Zuers that he will soon publish data on 200 patients that suggests spatial navigation is a separate cognitive entity. Data on memory, language, executive, and visuospatial functions do not fully explain the variance he measures. "We think spatial navigation is a promising cognitive domain. We would like to evaluate our test in the context of clinical trials," Hort said.

As a first step toward knowing whether the test responds to drug treatment, Hort reported on two small trials, one with donepezil in mild AD, one with scopolamine in healthy volunteers. When 18 newly diagnosed patients with mild AD started taking donepezil, their egocentric navigation stayed unchanged but their allocentric navigation improved slightly after three months, along with a small improvement on standard tests of delayed recall. The scopolamine trial—a crossover design in which each volunteer was subjected to each intervention at some point—disrupted their navigation at several hourly time points after they received a single dose of scopolamine; donepezil partially reversed this deficit.

The first paper in a major journal on the human Morris water maze came out in PNAS in 2007, but thus far, AD researchers have not adopted it. Why not? The research is sound, according to **John Growdon** at Massachusetts General Hospital in Boston, who called it an innovative approach to neuropsychology. "I have no qualms about the quality of the science; in fact, I am a proponent of this. To have a cognitive test in humans that mimics

that in animal models is clearly an advantage for drug discovery," said Growdon, who had intended to try out the virtual version at MGH until a grant application to this effect went unfunded.

A sometimes contentious bunch, neuropsychologists tend to hold strong beliefs in their own tests—or their favorite tests. It can take a while for a newbie to break in. Hort is known to the Alzheimer's research community; for example, he co-organized the 2009 AD/PD Conference in Prague. The test might gain prominence if Hort made it readily available to a dozen or so leading neuropsychologists in the U.S. and Europe, inviting them to validate it with their local patients and comparing it to other tests they use for the prodromal/MCI stage. "That would be the U.S. style of gaining acceptance within the research community. It worked very well when the transgenic mice came out, for example," said Growdon. This kind of outreach early on can persuade leaders in the field that the first published reports on a new test are true and robust. Hort and Harrison are licensing academic use of the technology to other interested research teams.

Commercialization may be a consideration in researchers' minds as well. In the United States, there is movement away from fee- or license-based tests; for example, the National Alzheimer's Coordinating Center (NACC) is substituting some proprietary tests for free alternatives, even though the former have been used in observational research (see ARF related news story). "The fees can be an irritant and make consortia move away from such tests, even if that undercuts the longitudinal aspect of some studies," said Growdon.

In Zuers, the audience urged Hort to compare the human Morris water maze directly to the ADAS-cog. "Head-to-head comparison has not been the primary objective of the ongoing research yet. This will be the next step. Our priority so far has been to translate animal spatial navigation data to clinical practice," Hort wrote to Alzforum.

So far, one Blue Velvet Arena exists at the University of Prague, and similar installations exist in Kiel, Germany, and Warsaw, Poland, Hort said. One is being built at the research offices in Graz, Austria, of JSW, the CRO founded by Polyhymnia partner Windisch. Clinical trials within the catchment area of those cities could use these installations. "The limit for wider dissemination of the real-space version lies in its complexity, cost, and need for an experienced staff," Hort wrote.

The computerized test could become more widely applied. Its correlation with the real thing is not perfect, the scientists agreed. At present it explains about 64 percent of the results in the real-space version, Hort wrote. "I suspect that ultimately we will be asked to show proof of concept using the Blue Velvet Arena, and that the computerized version will be employed in later confirmatory studies," Harrison wrote to Alzforum.