Madrid: News from the Vaccine Front—Phase 2 Postmortem, Part 1

Immunotherapy may not be the most eclectic choice of subject for those news aficionados who thirst for something completely different. Yet as the bearer of much hope and expectation throughout the field, the topic clearly warrants an update from every conference that offers news. And news there was at the 10th International Conference on Alzheimer's Disease and Related Disorders, held July 15 to 20 in Madrid. Here is a quick summary of some of the presentations this reporter managed to attend. Whip out your notes, and send in additions or corrections! (And for those who still want something completely different, check out our closing story, in part 3 of this series, of **Beka Solomon's** phage-only nose spray.)

Remains of AN1792—did it do some good, after all?

Followers of the field have heard proponents repeat the refrain that the trial of Elan/Wyeth's active immunotherapy prototype was valuable despite the aseptic meningoencephalitis that halted it prematurely. Skeptics might say: "Yes, yes, we've heard that the trial proved immunotherapy can remove Aβ from human brain. And yes, we know that the responders of the Zurich cohort appear to be doing a tad better than the non-responders even if that's but a handful of people." But what most may not know yet is that an ongoing follow-up study of the entire trial population is beginning to show inklings that the same may be true throughout.

Mike Grundman, who formerly worked at the University of California, San Diego site of the Alzheimer Disease Cooperative Study (ADCS) and now is at Elan Pharmaceuticals in South San Francisco, heads the study. UCSD's **Leon Thal**, who is chief investigator of the ADCS, encouraged Elan/Wyeth to conduct the study and presented the data available to date. At a packed symposium sponsored by Elan, Thal first reminded the audience that the trial participants, who received one or two injections of antigen, showed a small but significant improvement in three memory and three executive function tests (Gilman et al., 2005). But how well they fare beyond this assessment is unknown. Indeed, for a while scientists worried that there would not be any formal follow-up study of the trial participants. This study now under way asks these questions:

- How are the participants doing clinically in the long term?
- Do their antibody titers persist?
- Do their puzzling MRI findings persist?
- Did they develop anymore side effects after the study ended?

The study began this past January, 4.5 years after the trial began. It is being conducted blinded. Participants who are still able to visit the clinics are asked to come in, while others receive home visits or phone calls from investigators. Many of the study participants have progressed in their AD so that neuropsychological testing is no longer possible. For this reason, the investigators are applying a dependence scale and interviews with caregivers instead, though neuropsychology tests and MRI scans are still taken where possible. To date, about 40 percent of the original study—372 participants—have been contacted, and 85 of them agreed to participate. "We expect to have twice the data eventually that we have now," Thal said.

What does that initial data suggest? This long after the trial, the mean MMSE for the treated group is 13, and for those on placebo it is 10. Both groups started out at a mean of 20. Seven of the eight responders (defined as people who produced antibodies in response to the vaccine) whose blood has been tested so far have retained "respectable" titers, Thal said. Ninety-five percent of placebo recipients now require total care, whereas 65 percent of the responders do. On activities of daily living, all groups declined comparably, and the clinical dementia rating (CDR) changed for the worse in all groups, as well. The ADAS-Cog data are too premature to make a statement, Thal noted. Follow-up MRI scans are slowly trickling in; the two available to date from the placebo group and three from the responder group no longer show the differential brain shrinkage that had startled the field after an initial post-dose scan (see Fox et al., 2005). There, too, the data is too premature to make a claim, Thal cautioned.

A curious finding popped up around the basic measure of age. At baseline, both treatment and placebo groups had a mean age of just over 71. At the 2006 follow-up, the treatment group had aged, as one would expect, to a mean age of 76; however, the placebo recipients who have been contacted so far still come in at a mean age of 71. Time has not stood still for them; rather, it is possible that the older AD patients among the placebo group might have died at a higher rate than those in the treatment group.

Finally, no further cases of encephalitis beyond the 18 reported ones have cropped up since then, nor did other drug-related serious side effects, Thal noted. He emphasized that even though the data show a trend favoring patients who received AN1792 and responded to it, this data is highly preliminary and not yet fit for conclusions.

For his part, **Roger Nitsch** of University of Zurich offered further tidbits of data on the AN1792 trial Zurich cohort that his group is following separately. On the meningoencephalitis, Nitsch noted that of the three Zurich patients who developed it, two have antibodies and their Alzheimer disease remains stable to date, whereas one did not have antibodies and died three years after the immunization.

Nitsch then described a fourth autopsy case in addition to three published ones, from Southampton (Nicoll et al., 2003), Barcelona (Ferrer et al., 2004), and Arizona (Masliah et al., 2005). A 79-year-old man with a 7-year history of dementia from the Zurich group stopped speaking after a final MMSE of 12, then died four years after having received two shots of vaccine. He did not suffer the encephalitis and had low antibody titers in his blood and CSF. His Aβ levels in frontal and temporal cortex were low, as was amyloid deposition, Nitsch reported. Amyloid plaques had microglia around them, which stained with the 6E10 Aβ antibody, indicating the cells were ingesting the amyloid. This patient showed severe neuronal loss, gliosis, but no cerebral amyloid angiopathy. Alzforum has followed conference updates on this trial closely; for recent news, see ARF Eibsee report; ARF Sorrento story; and ARF St. Moritz story).

Madrid: News from the Vaccine Front—Phase 1 Hopefuls, Part 2

Whatever the fallout of the AN1792 debacle, it has not stopped commercial or academic interest in immunotherapy. Elan Pharmaceuticals and its partner Wyeth have moved a passive vaccine based on a version of the plaque-binding 3D6 antibody into a multicenter phase 2 trial. Other companies, too, are beginning to report first clinical experiences with their own vaccines. In Madrid, two such vaccines that have entered human studies were made public in some detail, one by Novartis and its partner Cytos Biotechnology in Zurich, Switzerland, and another by Eli Lilly and Co. in Indianapolis.

Matthias Staufenbiel, of Novartis Institutes for BioMedical Research in Basel, disclosed preclinical data for a new active vaccine, dubbed CAD106, that the company is currently testing in humans in Sweden. Staufenbiel described the vaccine as having grown out of efforts to avoid the T cell activation widely blamed for the inflammatory side effect that stymied Elan/Wyeth's AN1792 trial. The Swiss scientists fashioned a therapeutic vaccine out of the first six N-terminal amino acids of Aβ—a snippet able to stimulate human B cells but devoid of epitopes that arouse human T cells. The trick lies in hitching this stub of Aβ to a virus-like particle that generates the sort of T cell reaction needed to mount a full-fledged antibody response and to break the body's self-tolerance against Aβ (see also Li et al., 2004). The virus-like particle comes with the added benefit that it is sufficiently potent at marshaling the immune system's troops as to render additional adjuvants unnecessary.

All mice injected with this vaccine, young and old, mounted an antibody response with high titers, Staufenbiel said. Rabbits did, too. The researchers used two different strains of APP-transgenic mice—one depositing mostly diffuse amyloid but none around blood vessels, and one depositing plaques on blood vessel walls as well as between neurons (see part 3 for more on vessel amyloid). Both mice strains had reductions in Aβ levels and in their predicted amyloid pathology. Vaccination of young versus old mice indicated that the vaccine is more potent at reducing the accumulation of new plaques than removing existing plaques, Staufenbiel said. CAD106 removes parenchymal and vascular amyloid but, importantly, it does not cause the microhemorrhages that some other vaccines are reported to have caused in mice, Staufenbiel noted.

Tests with spleen cells isolated from immunized mice showed that CAD106 does not appear to stimulate T cells. Studies of CAD106 in rhesus monkeys confirmed the mouse data in that the primate antibodies stain plaques in APP-transgenic mouse brain and AD postmortem brain, do not cross-react with APP, and block Aβ-induced toxicity in cell-based assays. Results from the phase 1 trial are expected in 2007, according to a press release issued by Cytos Biotechnology.

Lilly's **Eric Siemers** described an initial single-dose trial of a so-called capture antibody. The rationale of this trial is based on the peripheral sink hypothesis. The approach grew out of a widely noted study showing that a single injection of the m266 antibody, which sticks with high affinity to soluble Aβ, improved cognition in mice overnight (Dodart et al., 2002). Together with a paper describing how peripheral injection of the m266 antibody bound plasma Aβ (DeMattos et al., 2001), this line of investigation raised hope that certain antibodies might be able to "draw" Aβ out of the

brain by way of shifting a series of presumably connected transport equilibria across the brain, CSF, and blood toward the side of the blood. The vision of a peripheral therapy for AD, or perhaps a diagnostic test similar to an insulin challenge shot for diabetes, took shape.

After reviewing preclinical research in PDAPP mice and rats, Siemers described the first human study in Lilly's clinical program using LY206430. This humanized version of the m266 monoclonal antibody binds to Aβ16-23, the peptide's midsection. Investigators infused one of four doses into the veins of four AD patients per dose, plus three placebo controls. Their mean age was 69, mean MMSE 20. The investigators took CSF samples and ran MRI scans at baseline and 21 days later. They tested the participants' performance in the ADAS-Cog battery at baseline, three days later to check for immediate effects as seen in the animal studies, and again at 21 days. The patients were then followed for one year.

This is what the Lilly scientists found: The antibody appeared safe in terms of standard laboratory values such as liver enzymes, and it also produced no evidence of the side effects that make AD vaccinologists jittery these days, that is, inflammation or microhemorrhage. The antibody produced a typical infusion reaction in five patients, which soon resolved on its own, Siemers said.

Plasma Aβ40 levels shot up between roughly 150- to 600-fold, depending on how much antibody was infused. CSF Aβ40 levels nudged up roughly 1.2- to 1.8-fold; Siemers estimated that 0.1 percent of this antibody enters the CSF and binds Aβ there. He added that the ADAS-Cog values signaled a hint of improvement but that this single-dose study was not designed to support a statement on efficacy.

Other scientists noted that more work lies ahead for this approach. One question they debate concerns the origin and precise nature of the Aβ that binds to the antibody in plasma. How much of it comes from the brain as compared to coming from other large organs known to secrete Aβ, such as muscle? The body-wide economy of Aβ will become clearer as this approach progresses. In that regard, a poster by **Yona Levites**, in **Todd Golde**'s group at the Mayo Clinic in Jacksonville, showed that peripheral injection of an anti-Aβ1-16 monoclonal antibody caused a steep rise in plasma Aβ but did not change brain Aβ in wild-type and young APP-transgenic mice. This raises questions about stabilization of peripheral Aβ by the antibody, and appears to complicate the notion of a diagnostic challenge test based on injected antibody. **Peter Seubert**, of Elan Pharmaceuticals in South San Francisco, reported that in his group's hands, even long-term treatment of PDAPP mice with the m266 capture antibody did not lower the mice's cerebral amyloid burden or improve neuritic dystrophy, but it did reduce soluble brain Aβ levels significantly and produced some improvement in tests of acute function and of synaptic health. Seubert's and Golde's findings would suggest that a capture antibody might prolong the peripheral clearance rate of Aβ by stabilizing Aβ in the blood.

On a more general note, the plasma half-life of all antibodies that are being developed as passive AD vaccines is roughly around a month. This raises questions about how often it would have to be infused and what price for such a therapy health care systems would be able, or willing, to sustain.

Madrid: News from the Vaccine Front—Bloody Complicated? Part 3

Scientists continue to debate the relative merit of using N-terminal versus mid-section or C-terminal antibodies, and of using antibodies against soluble versus against fibrillar Aβ. The discussion turns, in part, on the issue of microhemorrhages. Concern about small bleeds inside the brain arose when several groups began reporting them in the brains of immunized mice, especially mice who carry a heavy load of amyloid deposits in the blood vessel walls of their brains, not just in the parenchymal spaces. Called cerebral amyloid angiopathy (CAA), this pathology exists in the majority of AD patients. CAA is estimated to occur in up to 30 percent of elderly people, though that number varies. CAA can cause hemorrhagic strokes and cognitive impairment, and by itself is thought to cause numerous small bleeds that often go unnoticed.

The condition is attracting increasing attention from researchers. At the 10th ICAD meeting, held July 15 to 20 in Madrid, 23 presentations dealt with the topic of CAA and microbleeds. Presentations ranged from one showing that CAA pathology intensifies along with AD pathology, albeit in somewhat different brain regions, to another suggesting that the amyloid imaging agent PIB-PET can detect the region-specific CAA pattern in non-demented, living people. At the epidemiological level, scientists led by **Meike Vernooij** and colleagues at Erasmus University Medical School in Rotterdam used MRI to measure the frequency of microbleeds in 491 community-based participants of the Rotterdam Scan study. They found that 17 percent had such bleeds in their brains. Older people were at higher risk than younger people, and high blood pressure and use of thrombolytic agents upped the risk some more.

The worry with regard to CAA and AD immunotherapy is that clearance of blood vessel amyloid by anti-Aβ antibodies might lead to ruptures of the already dysfunctional vessel wall (Pfeifer et al., 2002; Burbach et al., 2006). A related worry is that overly fast removal of parenchymal amyloid would overwhelm the clearance capacity at nearby blood vessels and lead to renewed deposition of the amyloid there (Wilcock et al., 2004). Intriguingly, the sugar groups decorating a given antibody appear to influence this phenomenon (Wilcock et al., 2006). RN1219, a C-terminal Aβ antibody being developed by the San Francisco biotechnology company Rinat Neuroscience, a Genentech spinoff, is said to have an edge on that score.

This issue is in flux, and in Madrid, **Sally Schroeter** of Elan Pharmaceuticals addressed it with data from a 6-month study comparing the effects of several Aβ antibodies on CAA in the PDAPP mouse model. In short, Schroeter reported that, to her surprise, the 3D6 antibody, which recognizes fibrillar Aβ, not only did not worsen CAA, but instead cleared it in a dose-dependent fashion. The m266 capture antibody predictably had no effect on CAA. A multiphoton imaging study by **Claudia Prada** and colleagues at Massachusetts General Hospital in Charlestown paralleled this data by observing in live mice that a different antibody given to Tg2576 mice caused CAA to regress.

Furthermore, in Schroeter's study, microhemorrhages did occur in conjunction with the CAA clearance, she acknowledged, but could be limited by reducing the antibody dose and exposure time, essentially tuning down amyloid clearance to a manageable rate. **David Morgan**, of University of Southern Florida, who has collaborated with

Rinat Neuroscience but has no financial interest in the company, pointed out that at the age at which Schroeter and colleagues began treating the PDAPP mice—12 months—the mice's brains do not yet have a full load of parenchymal amyloid and also have less CAA than many AD patients. Repeating the study in older mice might more closely mimic the amyloid and CAA burden of AD patients and would test the findings raised by Wilcock et al. and Pfeifer et al. under more comparable conditions. For a prior comparison of 3D6 and m266 in older PDAPP mice, led by **Ron DeMattos** at Lilly, see Racke et al., 2005.

Yet another factor that young PDAPP mice represent poorly is the inflammation in blood vessels laden with CAA. In Madrid, **Manuel Buttini** and colleagues of Elan Pharmaceuticals in South San Francisco characterized inflammatory infiltrates in brain blood vessels of elderly people with AD and young normal controls, and found that more than twice the percentage of CAA vessels than CAA-free vessels had monocytes and T cells near them. "Age still is the most important risk factor in AD," Morgan summed up.

But Morgan also emphasized that it's all but clear how important the microbleeds will prove to be clinically in people. Clot busters such as the stroke treatment tissue plasminogen activator are known to cause microbleeds and, at least in acute situations, the risk is tolerated. Morgan's antibody-treated mice, for all they are worth, retained the behavioral improvement despite having more of these bleeds. "Minute hemorrhages are not the big worry. Large ones are," Morgan said. In summary, numerous scientists asked about this issue tended to agree that they expected some form of immunotherapy to slow progression of AD. But they also worry that some patients who receive these therapies for extended periods of time might develop larger hemorrhages, forcing the FDA's hand against the approach.

Little phage to the rescue?If reading all this leaves you scratching your head about how the pitfalls of AD immunotherapy can possibly be avoided, you are not alone. Some scientists are also looking for alternatives. One of them, **Beka Solomon** of Tel Aviv University, who has worked on AD immunotherapy for a decade (Solomon et al., 1997) happily announced a fortuitous observation that led her to begin exploring such an alternative. Solomon has long studied the potential of bacteriophages for ferrying either Aβ antigens or antibodies into the brain. It was during one of those studies that a control—naked phage—surprised her when it performed just as well as the study drug—phage studded with single-chain Aβ antibody—on her measures of reduction in amyloid burden. It seemed to do so more safely, too. Perhaps this humble life form could make for a treatment?

Bacteriophages come in two basic varieties. The better-known ones that lyse bacteria have been used as an alternative to antibiotics in the former Soviet Union, but Solomon uses the non-lytic phages. At almost a micrometer in length but only nine nanometers in width, they look like microscopic filaments of DNA packaged into a narrow protein sheath. Their physicochemical properties enable them to slip through membranes easily. In prior years, Solomon has explored the phages' potential to carry foreign peptides into the brain of animals (e.g., Lavie et al., 2004), to serve as immunogens (Frenkel et al., 2000), and to exert effects in the brain when sprayed into the nose (Frenkel and Solomon, 2002). (Incidentally, the scent of intranasal delivery wafted through the ICAD conference, with some presentations extolling the benefits of this route and others describing its use in the delivery of specific experimental

drugs. Examples include a talk by **Suzanne Craft** at the University of Washington, Seattle, on intranasal insulin and verbal memory in early AD, and another by **Illana Gozes** of Tel Aviv University on the neuroprotective peptide NAP, which acts to stabilize microtubules and is in early clinical trials with an intranasal formulation.)

In her studies with the phages, Solomon discovered that they alone, even without sporting Aβ or an antibody at their tips, had useful effects in AD models. Apparently, their size and structure allow them not only to penetrate the brain when given through the nose but also to intercalate into the β-sheet structure of amyloid and disrupt it. Electron microscopy images showed immuno-gold labeled Aβ fibrils alone, and amorphous Aβ aggregates in the presence of filamentous phage. The phages' threadlike shape did the trick, because when hooked into spheres, the phages no longer busted amyloid fibrils.

Solomon then showed in-vitro data on the phage's disaggregating properties, and on their ability to stain amyloid plaques. Injection of filamentous phage alone into the brains of Tg2576 mice reduced the mice's amyloid load over the course of three days. A subsequent one-year study of biweekly, then monthly, administration of phage sprayed up the noses of PDAPP mice improved the mice's memory performance in an object recognition test and a smell test. No water maze data were given. The phage also reduced the amyloid burden in the mice's brain and increased their synaptophysin levels, Solomon said. Notably, unlike the untreated transgenic mice, the phage-treated mice had no astrocytosis in their hippocampuses. Microgliosis showed no difference between the groups. Microhemorrhages were undetectable in the phage-treated mice, Solomon stressed. Peripheral organs also suffered no ill effects, in keeping with the established safety profile of these organisms. The phages leave the body within 3 weeks, Solomon said. They exit the brain with the help of microglia and are concomitantly eliminated from the body by urine and feces.

Solomon did not show data about the detailed in vitro-in vivo correlations, pharmacokinetic data, and dose-effect curves that would become necessary once drug developers became interested in this approach. Likewise, other scientists were curious to see short-term in-vivo studies that track how Aβ levels change in brain and CSF soon after phage delivery. Presumably, levels of Aβ would initially go up as it gets freed from fibrils, before eventually being cleared. Solomon said this was her initial presentation on the approach and much remains to be done. But already, it is clear that the phages are safe and ubiquitous in the environment, she said. "You and I can pick them up by swallowing a bit of water while swimming outdoors, and we won't even know it," Solomon added. They are dirt cheap, too.—Gabrielle Strobel.