From Australia, Impulse for a New Alzheimer's Research Agenda
By Gabrielle Strobel

Picture an expansive canyon wilderness with vistas of untouched land, where exotic birds soar above eucalyptus trees shrouded in a bluish haze. Can such a place free the mind to take flight toward new frontiers? A group of 25 Australian, U.S., and European research leaders on neurodegenerative diseases and aging put the idea to the test. On 22-24 August 2011, they huddled in Katoomba, a picturesque former mining town perched at the edge of the Blue Mountains National Park west of Sydney, Australia, for three days of animated discussion. Their goal was to rise above a certain funk that beset the field after the recent clinical trial setbacks, and to think broadly about new directions Alzheimer's disease research could take. Conference attendees are listed at the end of this report. Hosted by Bryce Vissel of Sydney's Garvan Institute, the conference intentionally kept time for data presentation short and instead encouraged conversation that articulated an informal set of recommendations for where research might go next.

The number of people with dementia worldwide stands at around 30 million and is forecast to rise to 115 million by 2050 (Alzheimer's Disease International, World Alzheimer Report, 2009). Research on Alzheimer's disease (AD) has made great strides in the past 25 years, but the advances have not paid off thus far in the form of disease-modifying treatments available to patients. A series of negative clinical trials in the past few years has challenged the public image of Alzheimer's research. The absence to date of robust clinical improvement from the current anti-amyloid drugs tested in Phases 2 and 3, and even cognitive worsening with a γ-secretase inhibitor in Phase 3, has intensified controversy around the amyloid hypothesis. The field is divided. Some scientists charge that the amyloid hypothesis is misguided. Most scientists maintain that it has still not been rigorously tested in the clinic, and that much is being learned from the negative trials. They counsel patience as a growing number of other investigational treatments in the clinical pipeline are wending their way through testing.

Other types of treatment, from Dimebon to rosiglitazone, from DHEA to statins and NSAIDs, from gingko biloba to valproic acid, have fared no better in clinical testing. Collectively, the trials have left in their wake a glum mood at a time when an aging Baby Boom generation is becoming increasingly aware and fearful of the disease.

For their part, the group emphasized that Alzheimer's research is underfunded in the U.S. compared to other major diseases. For example, the 2011 NIH budget request for HIV/AIDS research was $ 3.1 billion (see NIH Budget Statements); this amounts to about a tenth of the annual NIH budget and about $3,100 per case. (One million people are estimated to be living with HIV in the U.S.) Fiscal year 2012 NIH funding for Alzheimer's is expected to be $458 million (see NIH RePORT accessed 3 October 2011), or about $92 per case. (Five million people are estimated to have AD in the U.S. currently.) Also on NIH RePORT, 2012 cancer research draws 5.9 billion, cardiovascular disease 2.2 billion, nutrition 1.5 billion. In terms of NIH research dollars, AD is on par with alcoholism and below complementary/alternative medicine, for example. The National Institute on Aging, which supports aging and AD research, in 2010 received

$1.11 billion from the NIH, compared to $5.1 billion for the National Cancer Institute and $4.5 billion for the NIAID (see NIH Almanac Appropriations section).

Pointing to progress in the basic research underpinning Alzheimer's disease, particularly in biomarkers, the scientists at Katoomba articulated the need to drastically improve funding for translation into new clinical paradigms. In particular, the group emphasized a scientific imperative for pressing ahead with treatment trials in mutation carriers and biomarker-positive individuals such as those being proposed by three initiatives: the Dominantly Inherited Alzheimer Network (DIAN), the Alzheimer's Prevention Initiative (Reiman et al., 2010), and the Alzheimer's Disease Cooperative Study's Anti-Amyloid Treatment in Asymptomatic (A4) trial (for details, see ARF DIAN news series, ARF API news series, and ARF Webinar). These projects have complementary aspects that should be advanced collaboratively in order to forge an accelerated regulatory path and maximize scientific benefit for the field as a whole.

How to move ahead? In Katoomba, the scientists broke this question into seven parts, each time paying heed to both fundamental research and bridges toward translation. They focused on these areas:

- Genetics and Aging
- Protein Aggregation, Selective Vulnerability, Spreading
- Aβ Toxicity, ApoE
- Inflammation
- Validation of Candidate Therapeutics
- Lessons From a Clinical Trial—Too Little Too Late
- The Way Forward: Pre-symptomatic Alzheimer's Disease

Genetics and Aging

After a decade of little progress, the genetics of AD is once again becoming better elucidated. Most genes causing the rarest, Mendelian forms of AD are known. Less rare medium-risk variants are expected to be found over the course of the next years through sequencing of GWAS loci, exome sequencing, or whole genomic deep sequencing in families with a clustering of cases. Common variants, from ApoE4 on down to low-risk ones, have already come out of recent genomewide association studies (GWAS). These three categories cover the spectrum in terms of frequency and risk of the genetic burden of AD.

Collectively, the GWAS have redrawn the list of AD risk genes with a new set that is reproducible across 20,000 samples (see AlzGene Top Hits). The AD GWAS era will largely come to a close after one final, ongoing mega-merger of U.K., French, and American samples, plus several smaller studies in ethnic populations. Already, research is beginning to shift toward identifying the actual pathogenic variants near the risk SNPs, and understanding how, as part of their respective molecular pathways, they interact and contribute to AD. This is a lot of work. In aggregate, it appears at present that variation in the aging brain's response to underlying injury contributes to a person's risk for AD.

Within the AD field, as has been the case in schizophrenia and multiple sclerosis (MS), GWAS are facing controversy as some researchers question if they are worth their price tag. The low odds ratio of most hits discourages some molecular biologists from taking up the study of the genes responsible for those hits. The pharmaceutical industry regards GWAS data as leads to implicated pathways, not as drug targets per se. Some successful drugs hit targets that are low on their respective disease's list of risk genes as per GWAS, for example, PPARγ agonists in diabetes or interferon β, natalizumab or fingolimod in MS. In contrast, a disease's dominant genetic risk factor can sometimes prove undruggable. This is true for HLA, the dominant risk gene in MS and, despite ongoing effort, appears to be the case so far for ApoE, the dominant risk gene in AD. In both MS and AD, expected genes—that is, those related to myelin in MS, and APP and presenilin in AD—have not turned up in GWAS, but other risk factors have, and they point toward helpful directions for research and treatment.

On balance, GWAS have been more successful in AD research than prior candidate gene studies. They have yielded reliable hits, which help researchers choose pathways for mechanistic study. For example, some 100 ways of changing amyloid deposition in mice have been published; this makes it difficult to decide what is truly relevant to the human disease. The GWAS data validate certain areas as worthy of investigation. This includes known ones such as Aβ metabolism and cholesterol metabolism, as well as newer concepts such as endocytosis, the complement cascade, and innate immunity.

Other ongoing genetics research is beginning to blur the distinction between genes blamed for early-onset familial AD (APP, the presenilins) and those thought to cause LOAD (all other risk genes). Pathogenic APP and presenilin mutations have recently been found in late-onset familial as well as late-onset sporadic cases. These and other data suggest that AD genetics represent a complex spectrum, where modifier genes can reduce the penetrance of an otherwise high-risk variant in one person, delaying age of onset, or where several low-risk genes act additively, bringing on disease faster in that person. The strongest known protective allele is ApoE2; it remains grossly understudied.

Aging is the biggest risk factor not only for AD, but more broadly for protein aggregation of all types. There is a growing view that AD represents a quantitative trait with broad overlap to brain aging. One fruitful approach the group endorsed would be to understand the cell biological changes during brain aging that allow proteins to aggregate. For example, in small-animal models such as the worm *C. elegans*, gene mutations that delay age-related protein aggregation also extend lifespan. A deeper understanding of the underlying pathways is desirable. They include protein homeostasis/proteostasis, clearance, and autophagy. How are these pathways affected in AD? In animals, mutations affecting pathways involved in nutrient, stress, and energy sensation can extend lifespan and delay protein aggregation toxicity. Can these gene changes be exploited to develop therapies that replicate their effect in humans? Screens can be done already for small molecules that turn on longevity pathways.

Aging studies in worms, flies, and yeast should intersect more with human genetics. For example, geneticists can look for genes that segregate in long-lived families and search for

connections between genotype and resistance to AD. This requires proper genetic analysis of very old people who are ascertained to be cognitively normal and amyloid free. (Note that a prominent paper in this area has been retracted, see ARF related news brief). To back up human genetics, molecular biologists can define pathways in small-animal models to see which ones prevent or correct age-related protein aggregation. Natural suppressors found in these models can then be analyzed in human genetics. The current datasets of neurodegenerative disease and aging GWAS should be mined for information on enrichment of proteostasis genes. Finding these would, in turn, aid definition of the relevant pathways.

Furthermore, potential biomarkers of the aging human brain can be incorporated into studies that track aging versus AD. In particular, longitudinal brain imaging studies have identified a pattern of frontal hypometabolism as a signature of normal aging. Longitudinal studies increasingly collect blood for genotyping; hence, human analogs of age-related genes from small-animal research or primary findings from human genetics could be correlated with imaging changes. For example, FOXO, the human version of the *C. elegans* longevity gene DAF-16, has been linked to human longevity in eight different human populations. In this way, biomarkers for human brain aging could be found. Also, going from animal research to human genetics, the genes for secreted factors in blood that are known to influence brain aging (see Villeda et al., 2011) could be analyzed in GWAS data and then correlated with longitudinal imaging findings.

The GWAS signals from each of the major age-related protein aggregation diseases of the brain (Alzheimer's, Parkinson's, FTD, ALS) are different, pointing out disease-specific pathways. However, their top 2,000 SNPs should be cross-compared, meaning these respective GWAS could merge their samples to see if additional hits emerge that point to the commonalities between these diseases. While each of these diseases is different, protein aggregation is a common attribute they share.

Genetics research should integrate epigenetics, for example, methylation and histone deacetylation, across different brain areas at early stages of disease. This is challenging because it is rare that scientists obtain tissue from early-stage patients, and postmortem tissue from people who died with late-stage AD contains secondary epigenetic changes that make results difficult to interpret. In general, studies of gene-environment interactions in aging versus AD remain underdeveloped.

One way to encourage them is to ensure that extensive phenotypic information is gathered and databased properly for everyone entering a GWAS so both genotype and phenotype can be mined. Then GWAS data can be analyzed specifically for people who were exposed to head trauma or infections, or people who exercise or follow certain diets. In phenotypically characterized subgroups, signals that formerly fell short of genomewide significance might rise above that threshold.

In addition, GWAS data could be re-analyzed for variants of all known genes of a given pathway. This would reveal if their relative contributions to risk are additive, as was seen in fly genetics. High-risk but rare variants might come up when analyzed as part of a

pathway. For example, both genetics and molecular biology indicate that calcineurin may represent one pathway connecting Aβ and tau; GWAS datasets could be probed in search of additional variants in this pathway. (One note of caution: When re-analyzing publicly available data such as GWAS, scientists should acknowledge the problem of multiple testing by disclosing what database was used.)

A practical impediment to this research is that, to date, AD GWAS have not collected phenotypic information extensively. A better source of phenotypic information than current GWAS samples are longitudinal aging cohorts and brain banks. Some of them are adding GWAS, and at that point they will provide a rich dataset for focused genetic analysis of phenotypic profiles. To add another well-phenotyped source of data, the NIA-funded federal network of Alzheimer's Disease Research Centers could ensure that every patient seen in their memory clinics is encouraged to have a GWAS as part of their Uniform Data Set, which is uploaded to the National Alzheimer's Coordinating Center.

Large-scale sequencing now proceeds in different centers. Therefore, an initiative to build and host a publicly accessible database into which geneticists can upload all sequence data for the research community to mine becomes an infrastructure priority to advance the field.

Importantly for studies of aging and age-related disease, researchers need to conduct more studies in aged mice. Due to cost and logistic constraints, many studies of aging and AD use young to middle-aged mice. The Jackson Laboratory Alzheimer's Disease Mouse Model Resource represents one nonprofit mechanism by which scientists can obtain aged mice.

Overall, the neurodegenerative disease and aging research communities are too segregated. The group noted that the existence of separate sections focused on AD and on aging at the National Institute on Aging tends to fragment AD research. The NIA supports segregated networks at universities nationwide of Claude Pepper Centers for Aging Research and of Alzheimer's Disease Research Centers; it should encourage more interaction between them. Another way to accomplish interaction is for conferences such as the Keystone, Gordon, and Cold Spring Harbor series to integrate aging and neurodegeneration to foster greater communication and collaboration among these communities.

Protein Aggregation, Selective Vulnerability, Spreading

Because protein aggregation occurs in all age-related neurodegenerative diseases, understanding protein homeostasis inside neurons is a priority. Research into the cellular proteostasis network has yielded basic insight into proteome stability in some model systems. The cell maintains a relative balance of synthesis, folding, degradation, and aggregation; the factors controlling this homeostasis and how they change with age should be identified. In small-animal models, it has become clear that neurodegenerative disease proteins challenge the stability of the proteome and have adverse consequences for other proteins and the cell globally. For example, an aggregation-prone protein such as Aβ42 can amplify aggregation of different proteins, wreaking "collateral damage." Once

translated to humans, these processes could explain some of the mixed pathologies that are common to Alzheimer's, Parkinson's and dementia with Lewy bodies (DLB), or to frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS).

Small-molecule screens for compounds that activate known proteostasis regulators such as certain chaperones, the unfolded protein response, or an antioxidant response may generate therapies that could complement protein-specific therapies such as anti-Aβ antibodies or protease inhibitors.

The absence of sensors of the proteostasis network is a key bottleneck in the field. Scientists need sensors to follow in real time how the network changes in age and disease. Such sensors could monitor how changes in the environment (i.e., oxidative or ER stress) alter the relative proportion of specific disease proteins. Vice versa, sensors could track how the accumulation of a single aggregation-prone protein (e.g., Aβ, tau, or α-synuclein) affects other metastable proteins and the proteome at large. The majority of protein components in amyloid plaques, neurofibrillary tangles, and Lewy bodies are prone to aggregation in *C. elegans*. Sensing reagents and localization tools are needed for all major proteins that build up in AD, as well as for chaperones and autophagy.

In this area, specific instances of human translation are already possible, for example, stable isotope labeling of Aβ metabolism in human CSF. This method could address how production and clearance changes between young and old people. Beyond Aβ, such labeling could be developed for other proteins, particularly ApoE, α-synuclein, and tau. For example, a slight distortion of the splicing of tau mRNA into 3-repeat (3R) and 4R tau is known to upset the balance of tau protein isoforms and lead to FTD. But no one knows the relative amounts and compartmentalization of these species in human brain normally, with age, and pathologically in FTD. Reagents are needed that can define the gemischte of tau protein components that are likely to co-occur in the human brain. This knowledge could then be back-translated into model systems for mechanistic study. This kind of research calls for collaborations of labs with the requisite skill sets, as few labs have expertise in both human tissue and animal tissue/small-animal models.

More generally, intensive discovery work on tau could greatly advance drug development across a range of neurodegenerative diseases and build the needed components for combination therapies. This has yet to happen; to this day, few tau-based treatments have entered clinical trials.

Beyond tau, methods should be developed to measure protein turnover rate in a proteomic fashion, or for certain groups of proteins in a disease-enriched fashion. This fundamental biology can be applied to understand if amyloid causes the co-occurring proteopathies in AD, i.e., deposits of tau, α-synuclein, and TDP-43. What is it that makes some young people with presumably normal proteostasis develop Aβ and tau or α-synuclein aggregation as they age? Does aggregation of one protein seed the other, directly or indirectly? Does this happen in people? A directed study of the interaction of these proteins' proteostasis should be done.

Based largely on postmortem pathology, the observation of mixed disease is robust; however, debate abounds regarding the underlying toxic mechanisms early on in disease. Scientists increasingly doubt whether microscopic deposits of any of these disease proteins are to blame. Instead, smaller aggregates show toxicity in a growing number of experimental systems. And for some proteins, such as TDP-43, loss of nuclear function brought on by mislocalization to the cytoplasm is thought to be the root of the problem.

As in genetics, known environmental risk factors should be examined in the context of proteostasis. Does head trauma affect proteostasis sensors? A ketogenic diet? Sleep? Metabolic syndrome? Exercise?

An old question in AD research is, What makes some regions supremely vulnerable to the disease but relatively spares others. This question, too, might be freshly approached from the perspective of protein homeostasis. Unpublished mouse models expressing an FTD mutant of human tau predominantly in the entorhinal cortex (EC) display a regional progression of neurofibrillary pathology as seen in AD. From its expression site in the EC, the aggregated tau propagates, presumably monosynaptically, to anatomically connected projection fields in the hippocampus and later to subcortical nuclei and cortical regions. Separate cell culture experiments suggest that tau fluxes in and out of cells continuously, entering recipient cells through endocytosis and possibly "converting" tau there into an aggregated form. Cellular mechanisms of this movement, or any subsequent induction of misfolding, are unclear. Research needs to identify the species that spread, and investigate whether this spreading is the consequence of differences in protein isoform or post-translational modification in the neurons that take up the "seed" and may be selectively vulnerable to it. The human relevance of this phenomenon needs to be clarified.

In essence, the new mouse strains model the staging of AD pathology as laid out by Braak. Similarly, spread of pathology along functionally connected regions has been postulated for α-synuclein in Parkinson's disease (see ARF series). Functional MRI of the connectivity of the affected networks, as well as emerging methods for minimally invasive optical connectivity imaging in mice (White et al., 2011), now enable integrated study of functional and pathologic spread of disease; proteostasis sensors would amplify these studies further. In general, mouse neuroimaging methods should be more tightly integrated with molecular and anatomic research of the requisite models. In human neuroimaging, information from the ongoing Human Connectome Project of normal brain could be integrated with the rapidly growing connectivity data from AD patients. In particular, the selective vulnerability of the default-mode network deserves close study. Overall, research on selective vulnerability should move beyond cell-autonomous processes to examine neural circuits and networks.

Aβ Toxicity, ApoE

Despite intensive study, and with some frustration, the question of how Aβ is toxic to the cell in Alzheimer's disease remains unsolved. The group suggested several areas of focus.

First, the range of Aβ species found in human brain has broadened considerably from the two that have received the most attention in the past—Aβ42 and 40—to now include both

shorter and longer forms, as well as post-translationally modified and N-truncated forms. Mass spectrometry of CSF has identified additional species, notably Aβ16. Enzymology of the cleavage mechanism at the epsilon site of γ-secretase suggests two parallel processing lines, one generating Aβ49, Aβ46, 43, and 40, the other, Aβ48, 45, 42, and 38. Longer forms are generally considered more toxic, and all pathogenic presenilin mutations tested to date act by prematurely releasing Aβ during enzymatic cleavage, generating more of the longer species. Given this multitude of Aβ products, the scientific rationale for the widely used Aβ40/42 ratio appears arbitrary and requires reconsideration. Each of the species whose production increases as part of therapeutic γ-secretase modulation needs careful investigation of its relative toxicity to the brain, particularly Aβ38. For both toxicity research as well as compound selection, it is advisable to profile the range of Aβ species rather than assessing one of the Aβ40/42 ratios. These data would help define which of these species are most important in AD, both mechanistically and as therapeutic targets.

Second, the group recommended that the field define what exactly the term "Aβ toxicity" means in human brain. In cell or slice culture experiments, death, effects on synaptic physiology, and many other measures serve as outcomes of experimental Aβ toxicity studies. In the AD brain, however, the available outcome measures—structural MRI, functional MRI, cognitive testing, postmortem cell counts—are not proven to be due to Aβ toxicity.

Third, and perhaps most vexing to the group, was concern about the enigmatic relationship between the Aβ monomer, dimers, trimers, Aβ*56, protofibrils, other oligomers, fibrils, and plaques. Indeed, several researchers at the meeting noted their inability to measure oligomeric species in interstitial fluid, and raised the possibility that Aβ assemblies rather than a particular oligomer residing on the cell membrane may have a role in the pathogenesis of AD. Clarity on this score is crucial, in part because some of the anti-amyloid treatments might potentially release small assemblies from plaques at least temporarily. A way needs to be found to see them in brain and use them as theranostic markers.

An urgent need in the Aβ toxicity field, then, is to develop standardized protocols to detect, quantify, and categorize non-fibrillar assemblies robustly and reproducibly. Equally needed is some consensus around the source of relevant oligomer species—synthetic, cell secreted, or tissue extraction. An immediate step the group requested is for authors to describe precisely in their papers how their Aβ preparation was made. Beyond that, several groups could mount a round robin effort of exchanging and reproducing protocols, jointly generating standard protocols for synthetic and human oligomers, and endorsing some standard terminology. These protocols can then be shared openly with the research community, for example, via Alzforum's freely available protocol database. (For an upcoming expert discussion of this topic, see the October 13 Alzforum Webinar.)

Fourth, the group articulated definition of the most toxic aspect of Aβ assemblies as a research goal. Do they act intracellularly (if so, in which compartment?), extracellularly, on neurons, on glia, specifically on particular types of candidate receptors or non-

specifically on membranes? Considerable controversy has arisen about whether a single most pathogenic species exists or whether a soup of species—or the mere process of aggregation itself—is what damages cells. The answer to this question may remain elusive until the field has converged around a way to better characterize the species at hand, preferably with rigorous biophysical methods. Regarding oligomers made synthetically versus from brain extract, one stubborn finding is that in experiments to date, only the latter potently seeds amyloid deposition in aggregation-prone mice. This is true for many preparations tested at physiologic concentrations. This has been consistently the case for some years, highlighting the question of what is the difference between synthetic Aβ versus the "natural Aβ seed"? The group identified this as an exciting research priority.

The current problems with multiple preparations, terms, irreproducible effects, and general lack of consistency in the Aβ oligomer field are solvable. Doing this soon would head off similar confusion in the emerging field of tau oligomers. That field is too young for investigators to agree on standard protocols, but they are well advised to look back at the lessons learned with amyloid and start by defining with great precision what preparation they used in their study and to adopt consistent terminology early on. At this early stage, the field might benefit if many different labs explore different tau oligomers, ask other labs to replicate, and then build consensus about which ones are relevant in human tauopathy. If consensus were attempted too early on, important discoveries could be missed.

Despite receiving the lion's share of research attention, Aβ still mystifies the field on other counts, as well. The detailed molecular mechanism by which Aβ interacts with ApoE remains unknown. This has come to the fore as a research priority following robust demonstration that Aβ clearance is one of the major ways in which ApoE isoforms influence a person's risk for AD. Human brain imaging studies at numerous academic centers, as well as the Australian AIBL and ADNI in the U.S. and Japan, consistently show that carriers of the E4 allele of ApoE begin depositing amyloid plaques about a decade younger than do ApoE3 and 2 carriers. This is a huge step forward. Interstitial fluid measurements and other studies have shown that ApoE4 slows down Aβ clearance by some 30 percent. But how? A priority in this area is to understand tissue physiology around blood vessels, particularly how interstitial fluid flow in the brain leads to Aβ buildup. Early original work by Roy Weller, postulating age- and atherosclerosis-related slowdown of perivascular drainage, could be revisited with newer technologies. In this context, a concerted focus on how pericytes and other cells of the blood-brain barrier influence fluid dynamics may be fruitful.

For all its importance, Aβ clearance and aggregation is not necessarily the last word on ApoE in Alzheimer's. Genetics studies controlling for Aβ leave some fraction of the ApoE risk potentially unexplained. Separate research suggests that deceased young adult ApoE4 carriers already have a measurable deficit in cytochrome oxidase activity in their brain's mitochondria before detectable elevations in soluble or fibrillar Aβ42 (Valla et al., 2010). Differences in brain activity were present in young adult ApoE4 heterozygotes more than four decades before their estimated average age at clinical onset (Reiman et al., 2004; Filippini et al., 2009), raising the question of whether ApoE4 might exert a

bioenergetic or developmental effect on the brain. There remain opportunities to discover what that might be, and whether these effects are important to AD, or minor compared to ApoE's known effects.

The group agreed that renewed attempts to define the role of APP metabolites and function would be timely. Considerable work on this has fallen short of generating broad consensus, partly due to the frequent use of gross APP overexpression. Preparations using physiological expression levels or, at most, mild overexpression could validate the relevance of existing data. These include knock-in models with endogenous expression levels, or the study of changes in APP and its metabolites with sensors that indicate change over time. In general, moving away from snapshots and toward longitudinal observation would strengthen this field. Also, translation to human is key at this point. An integrated longitudinal proteomics study of APP metabolites would go a long way toward establishing the importance of these data.

Inflammation

The role of the immune system in Alzheimer's disease (AD) remains poorly understood, in part because the tools are not at hand to distinguish myeloid-derived cells from microglia, the brain's resident phagocytes. The field also needs better biomarkers of immune function and the inflammatory response in the brain, both fluid factors and imaging tracers. Along the same lines, peripheral cytokines might be developed to serve as biosensors for damage in the aging brain (see Villeda et al., 2011). This is of particular interest for understanding the aging brain's decline in neurogenesis; however, the role and importance of neurogenesis in AD remains to be firmly established. There is great interest in understanding whether systemic inflammation triggers protein aggregation in the brain, perhaps as a "second hit" on an already stressed, aging proteostasis system.

One research priority could easily be addressed. It is a common observation in hospital care that when some older patients come in delirious from an acute urinary tract or other common infection, treatment of the infection goes well but the patients emerge with dementia, although prior to hospitalization they had appeared cognitively intact or only mildly impaired. These patients do not improve back to baseline, and some cannot resume independent life at home. Did the infection unmask and accelerate a preclinical dementia? Similar questions surround surgery/anesthesia as a potential second hit for neurodegeneration in AD. Specifically for urinary tract infection, research could attempt to biochemically purify what it is about it that leads to rapid cognitive worsening. Is it a bacterial component, or a part of the immune system's response such as acute phase proteins? These factors could be isolated from human cases, identified, and reverse-translated for study in animal models. From such work might come relevant biomarkers associated with inflammation-triggered dementia. Toward therapeutic application, the age and immune system components of donor plasma used for transfusion in older hospitalized patients could be analyzed for differential benefits on cognitive outcomes.

Viewed broadly, the importance of the inflammatory response in age-related neurodegenerative disease remains debated. Agreement exists on the presence of an immune inflammatory event in the AD brain and on a worsening of dementia by systemic

inflammation. It is also undisputed that GWAS have identified loci associated with innate immunity. But there is no general consensus on which immune processes are activated in the run-up to AD, when in the long road toward symptomatic AD they come into play, or which particular immune pathways are beneficial and which are detrimental.

One problem leading to confusion is that research papers frequently call an observation "inflammatory" without defining whether it is phagocytic or cytotoxic. This lack of precision arises because scientists lack cell-type-specific markers. Research is needed to distinguish the lineages of myeloid cells that infiltrate from resident microglia and perivascular macrophages, all of which can respond to infection. All plastic cells that respond to injury in the brain should be characterized. This is important for translation, because treatments would address blood-borne and microglial macrophages in a different way. If monocytes prove troublesome in AD, they can be kept out of the brain by catching them systemically, whereas microglia would have to be treated with drugs that enter the brain. In this context, as well, the blood-brain barrier requires more focused attention.

Validate Candidate Therapeutics

Frequently in Alzheimer's disease research, primary academic research papers about potential new treatment avenues arouse keen interest among fellow scientists in academia and pharma, sometimes even attracting media attention and raising hope among the general public. The literature is replete with purported treatment strategies in mouse models that subsequently appear to fade away, as if they had been ignored. In many cases, multiple laboratories in pharma and academia have, indeed, attempted to replicate the original finding and failed, but never published their negative result. The group's discussion of this problem was reinforced by a simultaneously published review (Prinz et al., 2011), and the topic echoes through industry blogs. Likewise, few academic scientists who try in vain to replicate prominent findings go to the trouble of publishing a negative experience, which, if they do, often appears in a low-impact journal and possibly burdens a collegial relationship with the original authors and potential grant reviewers. This situation leads to wasted effort as other scientists, unaware of the negative findings, continue to try to validate the original finding, making it harder for any one investigator to decide what not to pursue amid a growing literature.

Importantly, the group noted, failure to replicate is not inherently a lack of skill. When a pharma laboratory attempts to repeat an original preclinical study, it generally brings to bear considerable resources, for example, often using larger groups of mice than the original paper, from well-characterized colonies. Ph.D. scientists in pharma repeat experiments multiple times, sometimes in multiple strains and different species. If they cannot repeat a published finding, or if the finding is shown to be dependent on one particular transgenic strain, then it is not considered robust enough for human AD drug discovery. Frequently, multiple academic and pharma labs have the same experience, but the field at large does not see this and therefore cannot learn from it.

The group agreed that scientists engaged in preclinical AD research would benefit from a resource for sharing replication data, positive or negative, coming from pharma as well as academic labs. One general mechanism exists in the peer-reviewed open-access Journal of

Negative Results in Biomedicine (see ARF related news story). A less formal mechanism that focuses on preclinical neurodegenerative disease, lowers the bar for participation, and incentivizes scientists might serve the AD research community. For their part, researchers would do well to demonstrate preclinical effects in multiple mouse models to give a first indication that the effect is robust.

Equally important, academic investigators may want to focus more attention on understanding the pharmacology of their compound of interest. For example, proper dose-finding research is frequently absent from papers on preclinical treatment effects. Instead, 10 mg/kg is sometimes used seemingly at random, without experimental evidence why that would be the correct dose. Other reports of treatment effects fail to gain traction because the compound at hand does not enter the mouse or human brain. Studies in the AD mouse models should aim to include evidence of target engagement. This kind of pharmacology research can be done collaboratively with experts in this area. The NIA's Translational Research Program exists to support preclinical pharmacology of candidate drugs. See a basic tutorial on drug development.

The group further noted that cognitive/behavioral endpoints in mice have been unable to predict similar benefit in humans. For drug development purposes, murine behavioral endpoints are not necessarily objective markers for translation to AD. Rigorous pharmacology—showing that the compound is safe enough, and enough of it reaches its target in the brain with suitable kinetics and with a strong rationale of why the target should play a role in disease—offers the best basis at present for taking the leap into treatment trials. Mouse behavioral assays are, however, useful for identifying proteins and pathways that play a role in cognition.

Lessons From a Clinical Trial—Too Little Too Late

This meeting could not avoid a discussion of semagacestat/LY450139, a γ-secretase inhibitor whose development ended in 2010 following cognitive worsening and other side effects in Phase 3.

The pharmacology of semagacestat included a reported IC50 in cell-free systems of 2.6 nanomolar for Aβ production and 14 nanomolar for processing of Notch, the substrate whose cleavage inhibition likely gave rise to intestinal and skin side effects. IC50 measurements vary widely across the in-vitro systems used, however, and some labs have even measured a higher, not lower, semagacestat IC50 in cell-free systems for APP than for Notch. Therefore, it is difficult to separate an efficacious dose on Aβ processing and Notch processing.

A key point is that in humans, the drug's appetite for Notch limited its tolerability so severely that the maximal concentration that could be achieved in humans was much lower than the efficacious dose. The published Phase 2 data further showed that in acute dosing, the already low maximal concentration occurred at six hours. This means a single administration achieved what is called "drug coverage" only for a short period of time. According to ClinicalTrials.gov, the drug was given once daily in Phase 3, likely not enough to keep a lid on APP cleavage.

Based on a 14-week study in Phase 2, the doses tested in Phase 3—100 and 140 mg—achieved at best only 20 percent reduction of absolute Aβ in CSF at the maximum drug exposure, and therefore possibly as little as 5-10 percent over a 24-hour period. At this time, this is considered likely too little to make a difference in overall Aβ levels in mild to moderate dementia due to AD.

Furthermore, the drug was up against what is called the biphasic effect, an odd phenomenon where at low doses the inhibitor causes a brief dip followed by an elevation of Aβ. Some scientists believe that low-dose γ-secretase inhibition with a short half-life blocks the enzyme for a few hours, substrate builds up during this time, and once the drug leaves, the unshackled enzyme rattles off more Aβ. On the other hand, substrate dependency and pharmacological manipulation of this effect suggest a more complex mechanism.

Several GSIs of different chemical classes have consistently shown this effect, sometimes called "overshoot," in human, guinea pig, and mouse plasma. There is less evidence yet to conclude that this also occurs in the brain, although it can be seen in in-vitro preparations from peripheral or neuronal cell systems. Eli Lilly and Company reported in their Japanese Phase 1 trial data an initial inhibition followed by an overshoot in plasma. If this phenomenon occurred in the CNS in Phase 3, it could have overpowered any initial Aβ lowering. The group agreed that these results argue that the hypothesis of a Notch-sparing γ-secretase inhibitor, or the amyloid hypothesis for that matter, has still not been tested fully in the clinic; rather, the field is still learning how to do just that.

How much Aβ lowering is enough, anyway? Amyloid-reducing trials using PET imaging may eventually answer this question, at least for its fibrillar forms. For now, data on PDAPP mice crossed to heterozygous BACE knockout mice hint that half of the physiological BACE activity leads to a small decrease of soluble brain Aβ, but a powerful impact on long-term deposition. (For a related discussion, see Alzforum September 29 Webinar.)

The Way Forward—Pre-symptomatic Trials

Over the past five years, the field at large has reached strong consensus that clinical trials should treat people earlier. This should start with defined populations whose genotype, age, and/or biomarker status unquestionably put them at elevated risk. The pharmaceutical and biotech industries have dipped their toes into the water by inching away from the standard mild-to-moderate patient population with a small handful of trials that are testing compounds in mildly impaired people who are positive for an AD biomarker. Three academic initiatives are reaching further back into even subtler cognitive signs, and further still into the asymptomatic phase of AD. Specifically, they are the Dominantly Inherited Alzheimer Network (DIAN; see ARF related news story) of autosomal-dominant familial AD, the Alzheimer's Prevention Initiative (API; see ARF related news story) of the same and of ApoE4 carriers, and the ADCS' Anti-Amyloid Treatment in Asymptomatic AD (A4) trial of amyloid-positive older people from the general population.

Since 2008, the DIAN has enrolled several hundred carriers and non-carrying siblings of a variety of autosomal-dominant pathogenic APP presenilin mutations in Australia, the U.S., and the U.K. In 2010, the API began enrolling what will be several thousand relatives of Colombian families all carrying the E280A presenilin-1 mutation. Using similar assessments, both initiatives are building a body of longitudinal imaging, biomarker, and neuropsychological evidence on the pre-symptomatic and early symptomatic stages of their respective participants' Alzheimer's disease. DIAN proposes to evaluate one to four amyloid-modifying treatments in proof-of-concept biomarker studies before selecting one treatment for a larger trial that will include clinical outcomes. The API proposes trials designed to relate an amyloid-modifying treatment's biomarker effects to clinical outcome, and thus provide evidence needed to help qualify biomarkers for use in the rapid evaluation of pre-symptomatic treatments. A4 is intended to evaluate an anti-amyloid modifying treatment's biomarker and clinical effects in older people with PET or CSF evidence of significant Aβ deposition, but without requiring a particular genetic risk. The proposed studies have complementary roles, and the investigators from each of these groups are working closely together to assist each other in support of their shared goals.

Each initiative is at a different planning stage regarding trial design, drug choice, and funding, with the first trial expected to start in 2012. Innovative designs, including adaptive or combination drug designs, are part of the consideration. Meanwhile, each initiative has been gathering comprehensive longitudinal biomarker information on its prospective participants for some time and is continuing to do so. Their results are generally highly consistent. Some 15 years prior to disease onset by conventional diagnostic criteria, Aβ CSF biochemistry starts to change; some five years prior, CSF tau changes; and subtle cognitive decrements become detectable some two to three years prior. This is roughly as described in Perrin et al., 2009, and Jack et al., 2010 (see Alzforum Webinar). Other emerging data indicate that certain brain imaging abnormalities might even precede these markers in autosomal-dominant cases. The ability to predict when gene carriers will develop symptoms is becoming precise enough to enable clinical trials. Taken together, the growing biomarker and cognitive datasets are making these populations attractive for testing secondary prevention.

For these and indeed all future AD treatment trials, more sensitive tools to assess subtle cognitive changes are needed. Moreover, all such trials should incorporate extensive biomarker measurements so that the field can collect information about how the known biomarkers respond to treatment. The field has adequate data on these markers in the natural history of disease for their use as inclusion criteria. Much less is known about the relative value of each candidate marker as an outcome measure in trials. Will brain volume go up? Down? Will CSF Aβ go up? Down? Will FDG-PET respond first? Is brain amyloid removal necessary for clinical benefit? How much? Which biomarker changes predict subsequent cognitive change?

In this context, it is noteworthy that, as in cognition, mouse models have not so far translated to human AD. For example, MRI volumetry tracks AD progression and continues to be viewed as a potential outcome measure for future trials (even though

responders in the discontinued AN-1792 immunotherapy program initially showed unexpected shrinkage). In contrast, mouse MRI sheds little light on how brain volume might behave in trials. In Tg2576 mice volumetric MRI has been uninformative because developmental expression of the transgene causes the mice's brains to be smaller to begin with, and subsequent MRI does not track disease as amyloid deposits develop.

Rather than relying on mice, then, embedding imaging and CSF and plasma markers in trials across the field is indispensable. This will generate iterative knowledge about their response to different treatments. The U.S. and European regulatory authorities have expressed enthusiasm for the planned DIAN/API/A4 trials. They similarly urge widespread measurement of biomarkers in trials so a body of evidence will emerge to define suitable outcome measures and to bridge the gap between biomarker response and clinical benefit.

In order to obtain highly valued investigational drugs for their trials, the DIAN and API initiatives have to assuage pharma's lingering concerns about whether results in defined genetic populations will translate to the general population with AD. That is where companies see their return on investment. The ADCS A4 trial is a step in this direction. Moreover, as more investigators worldwide are becoming interested in testing drugs in biomarker-positive older people, the methods for how this would be done should be fleshed out in detail and communicated among those groups. By speaking in unison, investigators can argue more persuasively with pharma for giving investigational drugs to people who are still cognitively normal.

The group compiled this wish list to increase the chance of success for future treatment trials:

- An imaging tracer for tau. CSF tau is useful for inclusion, but imaging gives a more direct brain readout with regional information. One candidate, 18F-THK523 (Fodero-Tavoletti et al., 2011), is set to enter first human tests later this year in Australia. In mice, it shows a signal in tau-transgenic strains but not in amyloid-transgenic lines or wild-type.
- Standardize and validate CSF assay for α-synuclein (e.g., Mollenhauer et al., 2011) so it can be added to longitudinal cohorts and clinical trials to capture the contribution of mixed pathology.
- Ditto for emerging CSF assays of Aβ assemblies (e.g., Fukumoto et al., 2010) to complement current CSF measurement of Aβ42 monomer. Much work remains to be done on this important tool.
- Develop brain imaging tracers for α-synuclein, and assays for TDP-43.

Finally, the building of momentum for earlier-stage trials should not marginalize the millions who currently suffer from AD. Negative trials to date have not invalidated the premise of treating diagnosed AD. Researchers recognize a moral obligation to continue to attempt treatment trials in this large population. The negative trials have been disheartening, but they have also been relatively few in number for such a common, expensive disease. The latest information on longitudinal amyloid imaging from the

Australian AIBL study does show a correlation between a person's rate of increase in tracer binding and rate of cognitive decline that persists into the mild AD stage. Particularly in mild AD, the field should attempt to trial anti-amyloid approaches combined with downstream drugs, such as anti-inflammatory, neuroprotective, antioxidant, or anti-tau agents. Combination trials are complex, but the U.S. Food and Drug Administration (FDA) in 2010 published a guidance encouraging such trials for serious diseases, Alzheimer's included.

The group shared the view that the field of AD drug development is too conservative. Patients in high-risk populations want more aggressive trials, and FDA representatives have said on numerous occasions that the agency would tolerate considerable risk of side effects because AD is such a dreaded disease. It is physicians and institutional review boards who remain perhaps overly risk averse. A paternalistic risk aversion ignores the cost of doing nothing. In Alzheimer's, the personal and societal consequences of this stance are exorbitant.

Attendees:
Randall Bateman, Washington University, St. Louis, Missouri
Samantha Budd Haeberlein, AstraZeneca, Sodertalje Sweden
John Collinge, University College, London
Bart De Strooper, Katholieke Universiteit, Leuven, Belgium
Marc Diamond, Washington University, St. Louis, Missouri
Karen Duff, Columbia University, New York City,
Daniel Geschwind, University of California, Los Angeles
Allison Goate, Washington University, St. Louis, Missouri
Jürgen Götz, Sydney University, Australia
Christian Haass, Ludwig Maximilians University, Munich, Germany
John Hardy, University College, London
David Holtzman, Washington University, St. Louis, Missouri
Cynthia Kenyon, University of California, San Francisco
Colin Masters, University of Melbourne
Richard Morimoto, Northwestern University, Chicago
Lennart Mucke, Gladstone Institute, San Francisco
Richard Ransohoff, Lerner Research Institute, Cleveland, Ohio
Eric Reiman, Banner Alzheimer's Institute, Phoenix, Arizona
Christopher Rowe, Austin Hospital, Melbourne
Gabrielle Strobel, Alzheimer Research Forum
Bryce Vissel, Garvan Institute, Sydney
Stacie Weninger, Fidelity Biosciences Research Initiative
Tony Wyss-Coray, Stanford University, Palo Alto, California