Alzheimer’s disease and related forms of dementia affect about 36 million people worldwide, according to the World Health Organization, and this number is only expected to rise, tripling by 2050. Yet patients have few therapeutic options; the US Food and Drug Administration (FDA) has approved the five drugs for Alzheimer’s disease, but even though these medications can help ease certain symptoms they do not alter the trajectory of the disease. This might change thanks to a discovery about two decades ago, when researchers found that a protein called amyloid beta (Aβ) appeared to accumulate in the brain to form plaques and drive the pathology of Alzheimer’s. Since the emergence of Aβ in the field, scientists have been trying to develop drugs to target it and the plaques in which it aggregates.

Eli Lilly and Company entered the field of Alzheimer’s drug research 25 years ago, just before Aβ became the focus of drug development. “Back in 1988, it was still kind of the dark ages of this mysterious disease, but now [there has been] an explosion in the basic knowledge around the diagnostics [and] genetics, and most recently we’ve seen some encouraging results with anti-amyloid treatments,” said Dr. Christer Nordstedt, vice president of Neuroscience Discovery Research and Clinical Investigation at Lilly, when he opened the symposium on 3 December.

Although the so-called “amyloid hypothesis” is by no means conclusive, the idea that a normal protein could accumulate over time and become toxic to brain cells has become more widely accepted in the scientific community. Recent discoveries surrounding the biology and genetics of Alzheimer’s, as well as promising clinical trial data, have created a sense of cautious optimism among researchers in the field.

The pathophysiology of Alzheimer’s

At the start of the meeting, a session on the pathophysiology of Alzheimer’s disease delved into the lingering biological questions surrounding the origin and progression of this cognitive disorder. “Amyloid is both a cause and an effect of Alzheimer’s disease,” said Dr. Dennis Selkoe, a neurologist at Harvard Medical School in Boston, and the symposium’s keynote speaker. Dr. Selkoe’s laboratory discovered through studies of cultured cells that Aβ is produced from amyloid precursor protein (APP) throughout life. Two enzymes, first β-secretase and then γ-secretase, cut APP into fragments. The second cleavage results in Aβ peptides of different sizes. However, as a person ages, something can go wrong in the production pipeline, causing either an overproduction or a failure to clean up the protein from the brain.

Dr. Selkoe began by highlighting a new cell model of APP proteolysis developed in his lab by Allen Chen, and currently under review for publication. The team suggests that instead of acting independently, γ- and β-secretase form a complex with α-secretase, an enzyme that cleaves APP, and by working together to chop it up, thereby preventing Aβ formation. Understanding how these proteins work in concert will inform the development of new drugs that can inhibit the cascade that produces Aβ.

An Aβ monomer on its own is thought to be harmless, but when dimers, trimers and other...
oligomers form, this poses a threat. Some self-aggregate, particularly Aβ42, and form insoluble fibrils and plaques. Small, soluble oligomers of Aβ can lead to synaptic injury and interfere with memory, according to Dr. Selkoe’s work. Plaque cores trap dimers and other oligomers, becoming saturated with age. “They are a parking garage, so to speak, for some of the cars that are bumping into things otherwise. They have a limit, and eventually the garage is full,” explained Selkoe. “Small oligomers are driving around and potentially threatening pedestrians,” he adds, extending the metaphor.

Amyloid also appears to influence signaling pathways for tau, a protein that stabilizes microtubules in the nervous system. Defective or toxic tau may lead to the accumulation of neurofibrillary tangles in the brain and, subsequently, neurodegeneration. “There is a direct connection between the presence of Aβ dimers in brain tissue and the phosphorylation of tau proteins,” Selkoe said.

Importantly, genetics has a hand in determining which individuals are most susceptible to Alzheimer’s. Notable genes linked to early-onset Alzheimer’s disease include the genes for APP and presenilin 1 and 2, which code for γ-secretase. For late onset or sporadic cases, there’s more of an interplay between genes and environment, said Dr. Alison Goate, a geneticist at Washington University in St. Louis. Some studies have shown that environmental enrichment can slow disease progression. The well-known risk variant apolipoprotein E 4 (ApoE4) has a dose-dependent affect: the more alleles you have, the higher your risk.

Dr. Goate’s team discovered a new, rare gene variant that can influence a person’s risk of developing Alzheimer’s later in life. Whole-exome sequencing of 14 families revealed a V232M mutation in the gene for phospholipase-D 3 (PLD3) that tripled the risk of the disease. A further genetic analysis of a larger group linked the variant to a doubled risk of Alzheimer’s. It is unclear exactly what PLD3 does, but Dr. Goate and her colleagues think it might work though a mechanism that depends on either amyloid precursor protein or Aβ itself. Their results were published in a recent paper from the group (Nature 505, 550-554, 2014).

Other researchers are homing in on the effect of individual oligomers. The idea is based on an alternative hypothesis that argues that these oligomers, rather than amyloid plaques, drive progression of the disease. At present, there is a lack of consensus about which oligomers might be the most significant or toxic. In 2006, Dr. Karen Ashe’s team at the University of Minnesota in Minneapolis discovered a rare Aβ oligomer, dubbed Aβ*56. Her team’s recent data from transgenic mouse experiments found that those animals with high levels of Aβ*56 had cognitive impairment, while mice engineered to just produce the types of oligomers more commonly found in plaques did not show cognitive decline.

Dr. Ashe’s recent work is informed by the biophysics of Aβ protein structures. When Aβ oligomers are arranged ‘in-register’ —meaning that their beta sheet protein structure contains aligned amino acid residues—they form fibrils that are more likely to serve as templates and self-aggregate. Crucially, whereas many types of oligomers contained within the plaques do not display in-register conformation, Aβ*56 does.

Biophysical properties may be key in classifying Aβ oligomers into different groups and pinning down whether specific types drive cognitive decline. At the symposium, Dr. Mathias Jucker, a neurologist at the University of Tübingen in Germany, presented research demonstrating that dilute extracts of Aβ plaque material from the brains of human patients, for example, can actually stimulate the induction of Aβ deposition in the brains of young transgenic mice. Dr. Jucker believes that the success of amyloid “seeds” depends on their biophysical conformation, and drew parallels between amyloid and the transmissibility of prions.

Diagnostic advances and strategies
In light of the biological discoveries discussed in the first session, the amyloid cascade remains an attractive target for the drug development community. From β- and γ-secretase to Aβ to plaques themselves, the pathway presents lots of opportunities to intervene. “For a drug developer this is kind of like a wonderland,” said Dr. Richard Mohs, vice president of Neuroscience Early Clinical Development at Lilly. Dr. Mohs kicked off the second session of the day, highlighting advances in diagnostics and clinical trial strategies.

The session highlighted how scientists had revised the diagnostic criteria for the different stages of Alzheimer’s disease in 2011, adding a new category of preclinical disease and expanding the mild cognitive impairment (MCI) stage of the disease. “They recognized...
that Alzheimer’s wasn’t a point in time – that there’s a continuum,” said Dr. Mohs. The guidelines also incorporate evidence from new biomarker data, such as cerebrospinal fluid (CSF) testing, magnetic resonance imaging (MRI), and positron emission tomography (PET) brain scans.

At the symposium, Dr. Mohs and his colleague, Dr. Eric Siemers, senior medical director for Lilly’s Alzheimer’s disease program, provided an overview about how biomarkers have changed the way they think about targeting amyloid and constructing clinical trials. Lilly’s two recent experimental drugs, the γ-secretase inhibitor Semagacestat and the Aβ inhibitor solanezumab (a monoclonal antibody), both advanced to phase 3 trials based on biomarker data from phase 2 trials. Although CSF data from phase 2 trials suggested that Semagacestat hit the target’s central compartment, unfortunately, the drug was associated with worsened cognition at higher doses in a phase 3 trial. In phase 2, solanezumab produced a dose-dependent increase in free Aβ1-42 in CSF, a likely indicator that plaques were releasing amyloid fragments. In 2012, Lilly announced the results of two pivotal Phase 3 trials for solanezumab, called EXPEDITION1 and EXPEDITION2. The results of these trials warranted the initiation of a third Phase 3 trial, called EXPEDITION3, which began recruitment of patients with mild Alzheimer’s disease in 2013.

Advancing the use of new biomarkers can be tricky. Dr. Leslie Shaw, a pathologist at the University of Pennsylvania in Philadelphia, provided a snapshot of the intricacies of biomarker assessment. Over the past 20 years, immunoassay work has established biomarkers such as Aβ levels in CSF, but they still lack formal regulatory approval for use in the clinic for diagnostic purposes, for example. “We’re dealing with a chronic, slowly evolving disease, where the changes in these biomarkers are relatively small,” Shaw said. Slight variation from one laboratory to another and even minuscule human error can impact results. Shaw’s team focuses on determining biomarker cut-off points to separate patients into different clinical groups; they also study longitudinal CSF changes in individual patients over time. Biomarkers can also be used to detect co-pathologies, which Shaw thinks could help determine whether a patient is more likely to progress to dementia.

Going forward, biomarkers will be helpful in determining whether to incorporate patients into trials and in tracking treatment effects. Knowing whether an individual is ‘amyloid positive’, meaning he or she has evidence of abnormal levels of amyloid accumulating in the brain, becomes key with amyloid-targeted treatments, because researchers want the treatment group to display the pathology they are trying to treat. But, not all amyloid-positive patients have initial symptoms of Alzheimer’s or even progress to dementia. Conversely, there are patients who are clinically diagnosed with Alzheimer’s but have no detectable amyloid build-up in the brain—a condition described as suspected non-amyloid pathology (SNAP).

A new focus on treating the disease earlier may untangle some of these inconsistencies, and brain-imaging biomarkers have revealed a clearer picture of the newly defined preclinical phase of the disease in patients. Low levels of accumulating amyloid can appear in the brain 10 to 15 years before any Alzheimer’s symptoms manifest. But, just because amyloid build-up appears in the brain does not mean a patient is destined to develop the disease. “One of the big questions is now that if amyloid is so toxic, how can you walk around with a head full of amyloid early on and not show evidence of cognitive impairment,” asked Dr. Reisa Sperling, a neurologist at Harvard Medical School in Boston. Using PIB-PET scans, Dr. Sperling’s team found that 30-35% of apparently healthy older adults they scanned had amyloid deposits in their brain despite the fact that these individuals showed no symptoms of Alzheimer’s disease. Some of these patients developed symptoms over time, while others didn’t; and among those who did develop the disorder, the speed of its progression varied significantly from one patient to another.

Early insights into the progression of Alzheimer’s through MRI and PET imaging provide an opportunity—and a new focus—for therapeutics. “We need to slow cognitive decline ten years earlier to really make a dent in this disease,” Dr. Sperling said. An upcoming trial named A4, which is based at Harvard and co-designed by Sperling’s team, will attempt to do just that by testing anti-amyloid treatments, including solanezumab as well as other drug candidates, in cognitively normal individuals over the age of 65 who have evidence of brain amyloid pathology indicated by neuroimaging. Patients will be classified on the basis of whether there is evidence of amyloid buildup in their brain scans, in addition to any signs of neurodegeneration or subtle cognitive changes. Dr. Sperling also hopes to incorporate PET scanning for tau or phosphorylated tau to help shed light on the conundrums of its relationship with amyloid and cognitive impairment.

**Translational challenges ahead**

The symposium concluded with a question and answer discussion between the speakers and audience members about the challenges of translating research into new treatments for Alzheimer’s disease. During the course of the discussion, panelists provided a glimpse of where the field might progress over the next decade and how disease paradigms might shift once again.

All of the panelists expressed optimism about the prospect of new therapies, some boldly and others more cautiously. In the future, scientists will have answered some of the pressing questions of surrounding Aβ, tau, and the disease overall. “The biology will
always be complicated,” said Dr. Selkoe. “But, we already know quite a bit about the relationship between Aβ dyshomeostasis and tau dys-homeostasis.”

In the world of clinical trials, Dr. Shaw predicted that that new biomarker tests will be available, and in the next decade many biomarker tests will be automated thanks to next-generation technology. In addition to expressing optimism about new therapies, Dr. Mohs hopes that technology will also move things forward in the clinic, citing electronic readouts of patients’ cognitive abilities as potentially poised to become a new part of assessing older individuals. Additionally, both Dr. Siemers and Dr. Sperling see a potential for combination therapy trials ten years from now. By then, researchers may be giving primary prevention some serious thought as well. Dr. Goate hopes that genetics in turn can give patients more accurate risk assessments and “identify patient populations who are more likely to respond to particular drugs.”

The ultimate hope is that more therapies—either ones that target Aβ across the board or ones that go after specific, highly-toxic versions of Aβ—will become available to patients. Dr. Jucker hypothesized that early treatment procedures aimed at removing amyloid “seeds” (the tiny oligomers that he believes may trigger Aβ accumulation) from the brain might become a reality. Dr. Selkoe in turn said that targeting amyloid at “the spigot” and cutting off production altogether would prove most affective. Lilly’s Dr. Christer Nordstedt summed up symposium and the current state of the Alzheimer’s field with a quote from Sir Winston Churchill: “This is not the end, it is not even the beginning of the end. But it is perhaps the end of the beginning.”

MEETING REPORT