

ALZHEIMER'S DISEASE THERAPEUTICS – ARE WE WINNING OR LOSING?

HIGHLIGHTS FROM THE SOCIETY FOR MEDICINES RESEARCH SYMPOSIUM, HELD ON MARCH 16, 2012, BRUSSELS, BELGIUM

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SUMMARY

Alzheimer's disease (AD) is the major cause of dementia, with a devastating effect both on patients and caregivers. Age is a major risk factor for AD, and with the anticipated demographic change to a more aged population, the prevalence of AD is set to soar to over 100 million by 2050. Unless medicines are found that are able to slow or stop the progression of the disease, the cost to healthcare providers will be unsustainable. Currently, the only medications that are available for patients have a very modest symptomatic effect on cognitive performance for a limited time. The pathognomonic signs of AD were first described by Alois Alzheimer in 1907, and the plaques and tangles that he identified in the brains of patients with AD were subsequently shown to be composed largely of β -amyloid (A β) peptide and hyperphosphorylated tau, respectively. Human genetics has been crucial to our understanding of

the disease and led to the articulation of the "amyloid cascade hypothesis", which has strongly influenced the direction of research for the last 20 years. We are now entering a critical phase where therapeutic approaches that purport to test the amyloid cascade hypothesis by reducing the levels of the A β peptide in the brain are in phase III clinical development. This meeting brought some of the world's leaders in academic research together with pharmaceutical researchers who have made key breakthroughs in developing therapeutic approaches in this most challenging of diseases. The program reviewed the foundational science and then moved on to review therapeutic approaches that target amyloid and tau pathology.

Key words: Alzheimer's disease – γ -Secretase – β -Amyloid – Tau pathology

WILL NEW GENETIC FINDINGS IN AD TRANSLATE TO NEW THERAPIES?

Prof. John Hardy (Institute of Neurology, U.K.) opened the meeting with a brief review of the early data that supported the amyloid cascade hypothesis (ACH) (1). The ACH posits that the deposition of β -amyloid (A β) peptide in the brain parenchyma initiates a cascade of events that includes the formation of intracellular tau deposits, which leads ultimately to the neuronal death that is responsible for the dementia suffered by Alzheimer's disease (AD) patients. In fact, many of the elements of the hypothesis had been articulated, albeit much less robustly, as early as 1984 (2). A key element in the ACH was provided by studies on human genetics, for which having access to AD patients with a familial history of the disease was essential. The genetic linkage studies that were subsequently performed identified three genes in which mutations caused early-onset AD. These genes are the amyloid beta A4 protein (amyloid precursor protein, APP; APP) (3), presenilin-1 (PSEN1) (4) and -2 (PSEN2) (5). These genes encode the substrate (APP) and key parts of the enzyme complex (presenilin-1 and -2) that produce the A β peptide. Since these

initial findings, the advent of new technology and the sequencing of the human genome have enabled genome-wide association (GWA) studies to be performed (6-8). In a GWA study, the common polymorphic variation in the human genome (single nucleotide polymorphisms [SNPs]) is tested to determine whether certain SNPs segregate in a statistically significant manner with cases versus controls. For these to be informative, very large numbers of cases and controls and very high statistical significance are required.

A number of GWA studies have uncovered several SNPs that are associated with an increased risk of late-onset AD. The key issue now is to provide a biological narrative that connects the GWA findings to the disease process. Several of the SNPs are in intergenic regions or within introns that do not affect, in a simple manner, the genes to which they are closest. The major candidates that have been identified as risk genes include: *APOE4* (described much earlier but a highly consistent finding in all GWA studies), *BINI*, *CLU*, *CRT* and *PICALM*. Indeed, several of the proteins encoded by the genes identified by GWA studies also have very low expression in the brain. It could well be the case that these new risk genes represent "response" genes that reflect the physiological response to the disease process, rather than the initial pathological processes.

The ACH also assumes, although this is not often stated explicitly, that the deposition of A β is a wholly pathological process. It could be that the deposition of A β is a physiologically useful process, for example, to prevent microhemorrhage, which goes awry in AD.

TESTING THE AMYLOID CASCADE HYPOTHESIS IN THE CLINIC – A CHALLENGE TO THE CURRENT PARADIGM

Eric Karran (Alzheimers Research UK, U.K.) showed how other genetic findings in neurodegenerative diseases can be accommodated by the ACH. For example, familial British dementia results in an aberrant 34-amino-acid peptide (ABri) being produced that deposits as plaques in the brain, resulting ultimately in tau pathology and neuronal loss (9). Thus, like A β , the ABri peptide is upstream of tau pathology and mutations to the *TAU* gene can cause frontal temporal lobe dementia. The pathological presentation is one of intraneuronal tau-paired helical filament formation as occurs in AD, but without any extracellular plaque deposition. This implies that tau pathology itself can cause neuronal death and also places tau downstream from A β deposition. However, the link between A β deposition and tau pathology is obscure – the term "aggregate stress" was used to suggest that different types of extracellular deposits, either A β or ABri, can bring about intracellular disturbances to tau via an intermediary process that ultimately leads to its aggregation (10). The exact temporal relationship between A β deposition and tau is not known. However, through a combination of post-mortem studies and the imaging of A β deposition in the human brain using a PET ligand (PIB), it is thought that A β deposition occurs many years before patients start to experience cognitive decline (11), and that the deposition of A β in the brain parenchyma occurs prior to robust tau pathology. The role of A β in the AD process is thus unresolved. A β might act to trigger the disease process, after which deposited A β levels are irrelevant; there might be a threshold level of deposition required, above which the disease process is accelerated; or A β might be required to drive the disease process of neuronal loss in a very direct and continuous fashion. In familial AD, where the cause

of the disease is identified and very likely to be due to excessive A β ₄₂ deposition, the major effect is to bring forward the time of disease onset, in some cases very dramatically, but not to accelerate the rate of disease progression thereafter. This disconnection fits with A β having some type of triggering effect after which the disease progresses in much the same way as in sporadic AD. The current phase III clinical trials are being largely conducted in mild to moderate AD patients and are predicated on the premise that affecting A β in this late stage of the disease process will be therapeutically valuable. The success of the current phase III agents solanezumab and bapineuzumab is highly dependent upon the role that A β plays in the disease: as a trigger, a threshold or a driver.

γ -SECRETASE AS A TARGET – IS IT OVER OR JUST BEGINNING?

Bart De Strooper (KULeuven, Belgium) presented data on the γ -secretase complex. γ -Secretase is responsible for releasing the A β peptide after initial cleavage of the APP holoprotein by the γ -secretase enzyme (BACE). As such, this enzyme has been a target for the pharmaceutical industry as they seek therapies to lower the production of A β . The γ -secretase inhibitor and modulator compounds that have entered the clinic were developed prior to the more in-depth knowledge now available of the proteins that constitute the enzyme and its potential mechanism of action. γ -Secretase is comprised of four proteins: APH-1, presenilin, PEN-2 and nicastrin. These proteins assemble in an orderly fashion in the endoplasmic reticulum and Golgi apparatus (12). There are two homologues of APH-1 in humans: APH-1A and APH-1B. In rodents, there is another variant of APH-1B, giving APH-1C. There are two homologues of presenilin: PS-1 and PS-2. There are two apparently intramembranous aspartyls in presenilin that form a catalytic diad to enable proteolysis of substrates. It is likely that this complexity provides γ -secretase with a range of properties that are only now being investigated. The physiological roles of the different complexes can be investigated by knocking out some of the cognate genes in mice and observing their resulting phenotypes. The APH-1A/PS-1 combination is crucial in notch signaling, both during embryogenesis and in adulthood. However, PS-2 and APH-1B knockout animals display only mild phenotypes, with APH-1B knockout animals possessing deficits in pre-pulse inhibition, a gating phenomenon which is also found in patients with schizophrenia. The orphan G-protein coupled receptor 3 (*GPR3*) was found in an expression cloning experiment to increase the production of A β (13) and represents another way in which the activity of γ -secretase can be controlled. The familial AD mutations within presenilin are dispersed throughout the protein, leaving open the question as to how so many mutations can act to give a single phenotype: early-onset AD. In fact, γ -secretase has a complex mechanism of action as a proteinase, acting first as an endoproteinase to make a cleavage at A β amino acids 49/50 or 50/51, and then acting more as an exopeptidase as it progressively cleaves every three amino acids in an N-terminal direction. Using a cell-free assay system, it was shown that the effects of the presenilin familial AD mutations are to act as partial loss-of-function mutations, such that the efficiency of the enzyme is reduced, leading to the early release of longer, more hydrophobic A β peptides. Thus, it would seem that the ratio of the longer to shorter forms of A β is a critical determinant of the propensity of the mutations to bring forward the age of onset of AD.

γ -SECRETASE MODULATORS – CURRENT PROGRESS AND CHALLENGES

Harrie Gijssen (Janssen Pharmaceuticals, Belgium) gave an account of the project running at Janssen to synthesize modulators of the aspartyl protease γ -secretase, which has been the focus of major pharmaceutical company approaches to treat AD since the identification of presenilin. The initial optimism in the field was thrown into question by the failure of semagacestat (Lilly) in phase III trials due to both a worsening of cognition and poor safety, through presumed inhibition of normal notch processing. Dr. Gijssen argued that modulation rather than inhibition of γ -secretase could potentially deliver a safer approach without an adverse effect on cognition, although whether the ultimate goal should be a reduction in the levels of $A\beta_{42}$ or the ratio of $A\beta_{42}/A\beta_{40}$ remains unanswered. To provide the means to address this, potent, CNS-penetrant γ -secretase modulators are required; however, there are major challenges associated with combining the required pharmacology into a molecule with suitable drug-like properties, as modulators are generally large and lipophilic. The Janssen approach, building further on publications from Neurogenetics and Eisai, delivered compound **1** following cycles of optimization, which focused on reducing lipophilicity and improving ligand efficiency parameters to ultimately identify molecules with good CNS drug-like properties to test the hypotheses in vivo (14).

BACE INHIBITION – A TOUGH TARGET STARTS TO YIELD

Andrew Stamford (Merck Research Laboratories, U.S.) gave an overview of the extensive research efforts at Merck to crack one of the initially most promising, but ultimately most challenging, targets the pharmaceutical industry has tackled for the treatment of AD, namely inhibition of BACE. In the widely studied amyloid hypothesis of the disease, accumulation of $A\beta$ fibrils in neuritic plaques plays a key role in the disease pathology and progression, and inhibition of the aspartyl protease enzyme BACE1 could yield a disease-modifying treatment.

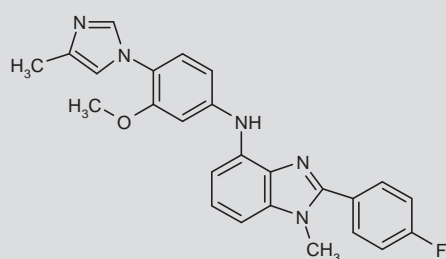
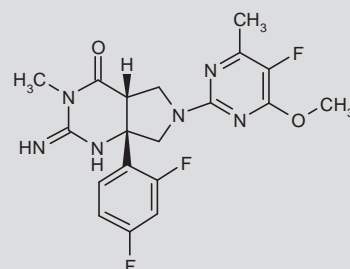
Initial hit finding strategies uncovered potent BACE1 inhibitors which were all P-glycoprotein efflux substrates (15). A subsequent fragment screen using heteronuclear single quantum coherence (HSQC) NMR screening of ^{15}N -BACE1 on 10,000 compounds at 500 μM in

pools of 10 successfully identified a new chemical series (16). Lead optimization led to compound **2**, through a careful balance of maintaining a network of three hydrogen bond donors for interaction with the two active-site Asp residues for potent inhibition, while limiting basicity to pKa 6.9, which led to improved PK properties. The lead compound **2** was advanced to rodent and primate studies to demonstrate its ability to lower the CSF levels of $A\beta$ peptides in the CSF following oral dosing.

TAU PATHOLOGY – OPPORTUNITIES FOR THERAPEUTIC INTERVENTION

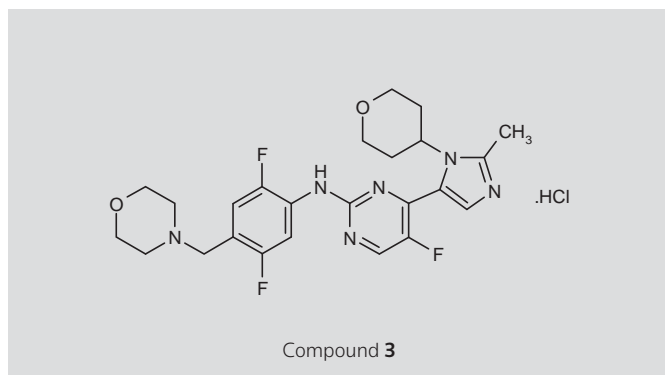
In his talk, Dr. Marc Mercken (Janssen Pharmaceuticals, Belgium) discussed the central role of tau aggregation in AD and other tauopathies, and the possible opportunities for therapeutic intervention. Neuropathological and genetic evidence from the literature was presented, indicating that tau aggregation is essential and sufficient for neurodegeneration. The sequence of events in the progression of tau aggregation at the cellular level suggests a chronic accumulation of tau aggregates in neurons, accompanied by a progressive loss of synapses and neurites. At the regional level in affected brain, a pattern of progression is observed that suggests a spreading of the tau pathology that may be accelerated in sporadic AD versus normal. An aggregate cascade hypothesis for sporadic AD was presented in which tauopathy initiates independently in selected brain areas at an early age and requires the presence of $A\beta$ aggregates for the progression of the tau pathology, leading to synapse loss, neurodegeneration and eventual cognitive decline at a later age. Cellular aggregate stress was hypothesized to be an unspecific but common driver in neurodegenerative diseases.

The lack of well-validated and druggable targets for tau and the unavailability of good cellular and fast animal models have prevented tau aggregation from being a pharmacological priority for many years. In this context, the benefits and risks for the different ways of targeting tau were discussed. The targets discussed included targeting $A\beta$ aggregation, tau post-translational modifications, including phosphorylation, tau and paired helical filament (PHF)-tau proteolysis and other approaches to prevent or alleviate tau aggregate stress. Finally, the rationale behind tau immunization was discussed, as was the presentation of a new fast in vivo screening model and novel anti-PHF-tau monoclonal antibodies.

Compound **1**Compound **2**

GSK-3 BETA INHIBITORS AS A POTENTIAL TARGET TO AMELIORATE TAU PATHOLOGY

Prof. Per I. Arvidsson (iMed Project Director CNS & Pain, AstraZeneca) summarized efforts at AstraZeneca to identify novel inhibitors of glycogen synthase kinase-3 beta (GSK-3 beta) as a means to target AD. GSK-3 beta is the main microtubule-associated protein tau kinase which has been targeted widely due to its role in the phosphorylation of this tau protein, leading to a state of hyperphosphorylation as the precursor to accumulation of neurofibrillary tangles. Screening activities to identify chemical starting points focused on screening against both GSK-3 beta and cyclin-dependent kinase 2 (CDK2), the protein kinase with closest homology, ahead of a functional screen in 3T3 cells overexpressing human tau protein. Chemical starting points were identified from the CDK2 project and the initial project optimized from the research program yielded compound **3**, which was stopped in toxicological evaluation due to cytochrome P450 induction in rats and mice. Further optimization encountered issues with both balancing kinase inhibition versus hERG inhibition and mutagenicity observed in bacterial AMES evaluation. Further optimization is in progress.



DISCLOSURES

R. Davenport is an employee of Takeda Pharmaceuticals UK and G. Macdonald is an employee of Janssen Research & Development. The other authors state no conflicts of interest.

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