MEETING REPORT

New Drug Targets for the Treatment of Alzheimer's Disease October 9–10, 2003, Philadelphia, PA

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The conference on new drug targets for the treatment of Alzheimer's Disease (AD) took place October 9–10 at the Union League in Philadelphia, PA, USA. It consisted of 12 lectures and a discussion session. A pre-conference workshop dealt with the design of clinical trials for anti-Alzheimer drugs. The conference was organized by Pharmaceutical Education Associates (San Diego, CA, USA), chaired by Elliot Mufson (Rush Presbyterian St. Luke's Medical Center, Chicago, IL, USA), and attended primarily by scientists from major pharmaceutical companies.

The pre-conference workshop was chaired by **Paul S. Aisen** (Georgetown University Medical Center, Washington, DC, USA). He outlined the current status of the Alzheimer Disease Cooperative Study (ADCS) that involves a consortium of 35 primary and 50 affiliated sites. ADCS is supported by NIH. Since 1990 17 clinical trials were initiated. Patients in these trials were diagnosed with AD or probable AD. Diagnostic criteria for probable AD include disturbance in memory/learning, deficits in at least one more additional cognitive domain (language, visuospatial and executive functions, motor skills), and impaired daily function. The clinical process of diagnosis involves the patient's history, physical, neurological and mini-mental state examinations (MMSE). MMSE includes assessment of cognitive function, orientation, attention, immediate and delayed recall, visuospatial ability, aphasia, agnosia and apraxia. Additional diagnostic tests may include APOE genotype, cerebrospinal A β , and functional imaging (PET, SPECT, fMRI). At-

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tempts are made to exclude other dementias (vascular, Lewy body, frontal lobe, Creutzfeldt-Jacob's disease, etc.). Minimal Cognitive Impairment (MCI) is viewed by some investigators as prodromal AD, others consider MCI as an independent disease entity. The emerging diagnostic criteria for MCI include: 1) memory test scores one SD below age-adjusted norm, 2) no objective loss of function, 3) diagnostic criteria for dementia are not met. Twelve percent of patients with MCI convert to AD within 4 years. The MMSE scores for MCI are 26–30, for mild AD there are 20–25, for moderate AD 10–19 and for severe AD 0–9. The neuropathological hallmarks of AD (amyloid plaques, neurofibrillary tangles and neuronal degeneration) begin years before the onset of clinical symptoms. Important aspects of neuropathology of AD are: abnormal processing of amyloid precursor protein (APP) and excessive phosphorylation of τ -protein leading to formation of neurofibrillary tangles. The treatment of AD can be either symptomatic or disease modifying. Four cholinesterase inhibitors are approved by the FDA for the treatment of AD and are available by prescription. Antioxidants are available over-the-counter. Preventive effects of NSAIDs and estrogens are supported by epidemiological evidence but not by prospective trials. Under investigation are vaccines, secretase inhibitors, amyloid binding agents, antiinflammatory and neuroprotective drugs. The more recently approved drug is a partial NMDA antagonist, memantine. It protects against amyloid toxicity *in vitro* and was shown to be effective in vascular dementia as well. The clinical measures of drug efficacy include cognition, behavior, function and global scales. Cognition is measured by MMSE and AD Assessment Scale — cognitive subscale (ADAS-cog). ADAS-cog has a scoring range from 0 to 70, higher scores indicate cognitive impairment; efficacy of drugs is measured as the change in ADAS-cog scores from baseline. Behavior is measured with the Neuropsychiatric Inventory (NPI) scale, which is based on responses from a caregiver. Function is measured with an ADAS-Activity of Daily Living (ADL) scale. Functional abilities never recover; maintenance of functional performance is a highly desirable treatment outcome. For global assessment of change Clinician's Interview-Based Impression of Change plus caregiver (CIBIC-plus) is used. In this test seven scores of one to seven are usually assigned; one is the best score, meaning "markedly improved." Clinical trial design issues include parallel groups vs. crossover, placebo vs. standard therapy, randomized withdrawal, open-label extension, co-therapy, and analysis. Two NSAIDs: rofecoxib, 25 mg p.o., q.i.d., and naproxen, 200 mg p.o., b.i.d., were selected for a 12 month-long clinical trial in patients with mild-to-moderate AD; concomitant use of AChE inhibitors, vitamin E, and low dose aspirin was allowed. Endpoints were death, institutionalization, increase in clinical dementia rating (CDR), 15 points decline on ADL and 4 points decline on ADAS. Neither of the two drugs had any significant effect by these criteria. It was concluded that the existent evidence does not support the use of NSAIDs in the treatment of AD. A currently ongoing clinical trial is the homocysteine reduction trial (VITAL) involving the use of folate, 5 mg, and of vitamin B6, 50 mg, to reduce blood levels of homocysteine. Another trial involves collaborative efforts of US and Chinese centers to compare beneficial effects of donepezil and huperzine A in AD patients. Vitamin E is currently being evaluated in patients with AD and Down Syndrome (DS); 400 patients are enrolled in a trial of 3 years duration.

In the main conference **Andrew Plump** (Merck & Co.) lectured on the genetics of AD. The risk for AD increases 2- to 3- fold in first degree relatives of patients with AD. Four Alzheimer's genes have been identified (APOE, APP, PS1, and PS2). Additional foci have been confirmed on chromosomes 6, 9, 10, and 12. Age, gender and ethnicity influence the

risk for AD. Environmental factors favoring the development of AD include head trauma, diet high in saturated fat, and lower level of education. The four identified genes are "risk" genes; they affect the risk of developing AD. PS1, PS2, and APP are causative genes, while APOE is a susceptibility gene. Other genes are likely to influence the expression of the disease. The causative genes lead to the early onset of AD (in patients less than 60 years of age), while APOE is likely to be involved in the late onset AD. Many causative factors are probably still unknown. In 75% of AD patients the disease is sporadic. In the majority of sporadic cases the onset is later than 65 years of age. In 50% of all AD patients the genetic risk is unknown. APOE is likely to be involved in 40% of all AD patients, while less than 2% is attributed to PS1 or PS2. There is a high incidence of AD in patients with Down Syndrome (DS); by 40 years of age virtually all DS patients develop AD. In patients with presenilin genes (PS1 and PS2) the disease is autosomal and dominant. PS1 is located on chromosome 14 and PS2 on chromosome 1. The onset of AD in patients with PS1 is in their 40s, while with PS2 gene the onset may vary from 40 to 80 years of age. The PS2 gene was found only in a very small number of families, mostly Volga Germans.

The APOE gene was identified in 1983; it is the most common genetic determinant of susceptibility to AD. Three isoforms of APOE are known; they differ in aa substitutions at residues 112 and 157 (ϵ_2 cys/sys; ϵ_3 ap/arg and ϵ_4 arg/arg). APOE allele₂ tends to decrease the risk and to increase the age of onset; allele₃ is neutral, while allele₄ increases the risk and decreases the age of AD onset. Lifetime risk of AD increases greatly with the presence of two copies of allele₄ (with ϵ_4/ϵ_4 the risk to develop AD by the age of 80 is 50%). At least 50% of patients with AD have at least one allele₄.

A clinical diagnostic test for presenilin 1 is available (Athena Diagnostics). A test for presenilin 2 is in development by Dr. Echevane (Barcelona, Spain). APOE testing is available from Athena Diagnostics, McGill University and NJ Medical School. Pre-symptomatic APOE testing is highly controversial. It can facilitate family planning and is useful in early diagnosis, but risks are not well defined and no preventive treatment is available. Symptomatic APOE testing may confirm diagnosis but may also cause unnecessary anxiety in family members. Childhood testing is considered not appropriate. Most physicians are aware of APOE testing, but only 18% of physicians ever ordered an APOE test and only 5% ordered it for an asymptomatic individual.

According to recent findings risk of late-onset familial AD is associated with an intronic CYP polymorphism (see *Arch Neurol* 2003;60:29–35). CYP-46 TT was found to be associated with a higher β -amyloid load in brain and with increased CSF levels of β -amyloid and phosphorylated tau protein.

Hank F. King (Univ. of PA, USA) discussed *in vivo* imaging of β amyloid deposits in the brain of AD patients. During the last 10 years King et al. developed a series of ^{123}I labeled imaging agents useful for visualization of brain AD pathology. Many of these agents have been successfully tested in humans. They include tracers for D₁, D₂, and D₃ dopamine receptors and for dopamine transporter (DAT). The D₂ imaging agent (IBZM) is available from Cygne (The Netherlands) and the D₂ imaging agent (IBF) from Nihon Med. Physics (Tokyo, Japan). Recently, a specific DAT imaging agent, (99mTc) TRODAL-1, has been introduced. Phase II clinical studies suggest its usefulness in the diagnosis of Parkinson's and other neurodegenerative diseases.

Elliot Mufson (Rush Presbyterian St. Luke's Medical Center, Chicago, IL, USA) discussed the neurobiology of AD and mild cognitive impairment (MCI). Cholinergic deficits are likely to lead to cognitive, motivational and attentional dysfunction in AD pa-

tients. In severe end-stage AD the cholinergic basal forebrain neurons are selectively vulnerable. The cholinergic degeneration in AD leads to dysfunction of nerve growth factor (NGF) and its receptors (trkA and p75NTR). In end-stage AD there is also a defect in the retrograde transport of NGF, loss of cholinergic basal forebrain function involving cortical projections neurons and loss of cortical choline acetylase (ChAT) activity. In patients with clinically diagnosed MCI the number of TrkA-ir neurons in nucleus basalis is decreased, but ChAT activity is not affected. The hippocampal ChAT activity may even be increased in patients with MCI. It appears that in MCI there is a change in the balance between ACh synthetic activity (an increase in ChAT) and hydrolytic (decrease in acetylcholinesterase, AChE) activities in hippocampal neurons. The occasional increase in ChAT activity in nucleus basalis of MCI patients may represent a compensatory response due to entorhinal cortex hippocampal disconnection.

Putative molecular consequences of cholinotrophic basal forebrain dysfunction in AD and MCI include cell signaling defects and activity of apoptotic genes (BACE, BAD). Since neurotrophic and signal transduction mechanisms are dysfunctional in MCI, while the cholinergic basocortical projection system displays plasticity response, further development of cholinergic therapy appears to be appropriate.

Luc Truyen (Johnson & Johnson) summarized the clinical use of currently available anti-Alzheimer drugs and concluded that the available drugs provide multiple benefits and relevant savings for the patients. According to Truyen AD is currently underdiagnosed and undertreated. Goals in the long-term treatment of dementia include maintenance of cognitive function (ADAS-Cog scores) and of normal daily activity, as well as prolongation of autonomy of patients with a minimal impact on caregivers. Cognitive function is measured with ADAS-Cog scores, without treatment they decline by 5 to 11 points per year. Significant declines in ADAS-cog scores were achieved with rivastigmine (Exelon[®]), donepezil (Aricept[®]), or galantamine (Reminyl[®]). At 12 months after initiation of therapy with galantamine the scores were at their pretreatment level, but still below placebo scores or the projected scores for untreated patients. Galantamine was shown to maintain functional performance for at least 12 months and to delay the emergence of behavioral symptoms. Further studies need to focus on relevant health-economical outcomes as primary endpoints.

Todd E. Golde (Mayo Clinic, Jacksonville, FL, USA) discussed possible mechanisms of action of non-steroidal antiinflammatory drugs (NSAIDs) in the treatment of AD. Golde et al. recently discovered that some NSAIDs selectively lower the levels of A β 42 peptide. Ibuprofen, sulindac sulfide and indomethacin lower A β 42 peptide, while other NSAIDs raise it. The A β 42 lowering effect appears to be independent of the cyclooxygenase (COX) inhibitory effects of NSAIDs. Some NSAIDs appear to target γ secretase. The short-term effects of NSAIDs on A β 42 levels often differ from long-term effects.

The second lecture by **Andrew Plump** (Merck & Co.) was entitled "Targeting β -Amyloid." Familial mutations of β -amyloid precursor (β -APP) are known to cluster around secretase cleavage sites. The mutations affect A β formation and aggregation. The "amyloid cascade" hypothesis of AD postulates that elevation of brain A β (1–42) initiates pathogenetic mechanisms leading to AD and that the reduction of brain A β levels will be beneficial in the treatment of AD. This hypothesis is supported by genetic evidence that all mutations causing familial AD lead to increased production of A β (1–42). Therefore, any treatment that is capable of interfering with A β formation may potentially prevent onset or slow the progression of AD. The primary targets for the development of anti-Alzheimer

drugs are, therefore, BACE or γ -secretase. γ Secretase is a multi-component aspartyl protease. Nicatrin, aph-1 (anterior pharynx defective) and presenilin enhancer-2 are required for γ -secretase activity. Potential inhibitors of γ -secretase can be evaluated *in vitro* in cellular and binding assays. They can also be studied *in vivo* by measuring soluble A β protein in brain, cerebrospinal fluid (CSF), plasma, or plaques.

Some NSAIDs inhibit APP γ cleavage. Sulindac sulfide decreased A β (45) levels *in vitro* with an IC₅₀ of 38 μ M. Use of biomarkers (e.g., plasma or CSF A β) or surrogate outcome measures (PET, sMRI) facilitates the development of γ secretase inhibitors as potential anti-Alzheimer drugs. Neither biomarkers, nor surrogate outcome measures are currently acceptable to the FDA as proof of drug efficacy in AD. Functional cognitive outcome measures are required for clinical evaluation of potential drugs.

According to De Mullis et al. (*PNAS* 2000;98:8850), anti-A β antibody increases clearance of A β protein in CSF. NSAIDs decrease the clearance initially, but increase it chronically.

The development of disease modifying drugs is complicated by difficulties in the interpretation of *in vitro* and *in vivo* animal findings and their relevance to human disease. There is also a need to define meaningful levels of A β reduction and validity of surrogate outcome measures for the clinical situation. The duration of clinical trials can be reduced by proper identification of "at risk" individuals.

The primary prevention trials have the highest probability of success. Such trials should involve a large group of "at risk" individuals and many years of following their cognitive function. Imaging technology can be used to study the progression of the disease in these individuals.

The secondary prevention trials should utilize a large treatable population of patients with clinically diagnosed minimal cognitive impairment (MCI). Each trial should be expected to take several years. A large variability of the disease conversion rate (MCI to AD) among patients is likely to complicate such trials.

The disease progression trials have been used in the development of currently approved anti-AD drugs. The size of such trials is relatively small and the duration can be limited to 12 months. A new drug will have to be tested against placebo or already marketed cholinesterase inhibitor. Cognition function has to be evaluated clinically.

Benjamin Wolozin (Loyola University Chicago Medical Center, Maywood, IL, USA) spoke about the potential role of lipid lowering drugs in the therapy of AD. Cholesterol affects production of A β probably because β and γ secretases are located in the cholesterol-rich domain in the cell membrane. Statins have been shown to reduce A β levels in the cerebrospinal fluid of mice and humans. They also reduce accumulation of A β in transgenic tg2576 mice that overexpress APP. Inhibition of acetyl coenzyme A cholesterol transferase (ACAT) has also been reported to reduce production of A β .

Epidemiological studies suggest a lower incidence of AD in patients treated with statins. The results of progressive studies are, however, mixed. Simvastatin has been reported to slow cognitive decline in patients with mild AD. Pravastatin, however, did not reduce the incidence of dementia in a prospective study involving 6,000 patients.

There is a link between cholesterol metabolism and AD. Cholesterol, its esters and 24(S) hydrocholesterol stimulate 24 cholesterol hydroxylase (Cyp 96) that is present in neurons, plaques and astrocytes. 24 hydroxy cholesterol decreases A β and APP in neurons. The current hypothesis postulates that cholesterol metabolism modulates A β and

is a source of risk factors for AD and that inhibition of cholesterol biosynthesis may conceivably protect from AD.

Peter S. Klein (Univ. of PA, USA) spoke about the role of glycogen synthase kinase-3 α (GSK-3 α) in APP processing. He identified GSK-3 α as the critical regulator of APP processing and demonstrated that at therapeutic concentrations lithium inhibits GSK-3 α and reduces accumulation of β amyloid *in vitro* and *in vivo*. GSK β is required for phosphorylation of tau protein and has been shown to interact with presenilin. Lithium was found to reduce β -amyloid levels in the brain of a mouse model of AD. β -Amyloid production was also reduced by kenpauillone, an inhibitor of cyclin-dependent kinase.

Howard Fillit (Institute for the Study of Aging, ISOA, New York, NY, USA) discussed the role of non-profit organizations in the financing of a search for anti-Alzheimer drugs. There is a particular need for financing of early stage biotechnology programs. While basic research is financed in the USA largely by the government and advanced development of drugs by industry or venture capital, ISOA, a non-profit private organization, primarily supports discovery of leads at academic institutions and start-up biotechnology companies. ISOA committed \$20 million to fund 107 research projects designed to find new leads for the therapy of AD. Fourteen start-up biotechnology companies are supported with grants ranging from \$100,000 to \$500,000. Drug discovery targets funded by ISOA include β -amyloid inhibitors (42% of grants), cognitive enhancers (17%), anti-tangle (2%) and antiinflammatory drugs (2%). Potential applicants can receive additional information on the website of the Institute (www.aging-institute.org).