Role of Cerebrospinal Fluid Biomarkers in Clinical Trials for Alzheimer’s Disease Modifying Therapies

Ju-Hee Kang, Na-Young Ryoo, Dong Wun Shin, John Q Trojanowski, and Leslie M. Shaw

INTRODUCTION

Alzheimer’s disease (AD), a progressive neurodegenerative disorder, is the most common form of irreversible dementia, and it carries with it a considerable human, social, and economic burden. Following the onset of pathological changes in the brain that include the progressive accumulation in the CNS of amyloid beta (Aβ) deposits and neurofibrillary tangles (NFTs) formed by pathological tau, which is thought to begin more than 15 (Aββ) to 10 (NFTs) years before cognitive impairments become clinically manifest, AD patients primarily develop progressive deterioration of episodic memory and a global decline in their cognitive functions. Among several mechanistic and pathological substrates that contribute to the gradual progression of AD over time, the major neuropathological substrates of AD are the aggregation and accumulation of misfolded Aβ and the intracellular deposition of fibrillized and hyperphosphorylated tau proteins (Fig. 1).

Currently, the greatest utility for biochemical cerebrospinal (CSF) biomarkers of AD may be for the early and more reliable diagnosis of AD, which places measures of the levels of CSF Aβ1-42 and tau proteins (total tau and Aβ1-40, amyloid beta 1-42; NFT, neurofibrillary tangle; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; NMDA, N-methyl-D-aspartate; FDA, Food & Drug Administration; APP, amyloid precursor protein; Aβ, amyloid beta oligomer; PPAR-g, peroxisome proliferator-activated receptor gamma; BBB, blood-brain-barrier; RAGE, receptor for advanced glycation end products; LRP-1, low-density lipoprotein receptor-related protein 1; PET, positron emission tomography; IVIG, intravenous immunoglobulin; GSK-3, glycogen synthase kinase 3; ADNI, Alzheimer’s Disease Neuroimaging Initiative; MRI, magnetic resonance imaging; FDG, 2-[18F]-fluoro-2-deoxy-D-glucose; AICD, C-terminal fragment of amyloid precursor protein.

ABBREVIATIONS: AD, Alzheimer’s disease; Aβ, amyloid beta; Aβ1-40, amyloid beta 1-42; NFT, neurofibrillary tangle; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; NMDA, N-methyl-D-aspartate; FDA, Food & Drug Administration; APP, amyloid precursor protein; Aβ, amyloid beta oligomer; PPAR-g, peroxisome proliferator-activated receptor gamma; BBB, blood-brain-barrier; RAGE, receptor for advanced glycation end products; LRP-1, low-density lipoprotein receptor-related protein 1; PET, positron emission tomography; IVIG, intravenous immunoglobulin; GSK-3, glycogen synthase kinase 3; ADNI, Alzheimer’s Disease Neuroimaging Initiative; MRI, magnetic resonance imaging; FDG, 2-[18F]-fluoro-2-deoxy-D-glucose; AICD, C-terminal fragment of amyloid precursor protein.
tau phosphorylated at Thr181) in the revised version of diagnostic criteria for AD research [1-4]. Thus, abundant data from numerous studies carried out in centers across the globe over the past 20 years shows that the levels of Aβ-1-42 in CSF of AD patients are significantly lower than in age-matched healthy elderly controls, whereas the levels of total tau (t-tau) and tau phosphorylated at Thr181 (p-tau181) in AD CSF are significantly higher than those of controls. Moreover, it has been suggested that these CSF biomarkers are useful to differentiate those mild cognitive impairment (MCI) patients who progress to develop AD within the subsequent several years from the stable MCI patients [5-10].

The current standard pharmacotherapy for cognitive improvement in AD patients includes acetylcholinesterase inhibitors and the N-methyl-D-aspartate (NMDA) antagonist memantine. However, the approval of these drugs has not been based on their ability to slow disease progression but to improve the clinical symptomatology. Hence, only symptomatic drugs with transient benefits have been approved for clinical use in AD patients by the US Food and Drug Administration (FDA). Given the fact that genetic and pathological evidence strongly supports the "amyloid cascade hypothesis", several strategies of reducing Aβ accumulation in the brain have been applied to develop "disease-modifying therapy" to cure AD. In addition, a correlation between cognitive dysfunction and neurofibrillary tangle load that is more robust than that between Aβ load and cognitive impairments has led to a parallel strategy to develop tau focused therapies that inhibit and/or block tau aggregate formation, promote the clearance of tau pathology or correct for the loss of tau function when tau is sequestered in NFTs [11]. Other strategies for modifying disease progression include anti-inflammatory, metabolic approaches, neurotrophin-based approaches and mitochondrial targets. It has been estimated that a disease-modifying therapy that could delay both dementia onset and progression by one year would reduce the prevalence by 9.2 million cases of the disease by the year 2050 [12,13]. To reduce the number of future AD cases, a variety of attempts to develop a drug that provides a disease-modifying effect against probable AD have been made; however, there are no approved disease-modifying therapies for AD at this time. Based on lessons from previous clinical trials in probable AD patients, there is a growing consensus that initiation of disease-modifying treatment before the onset or early phase of the disease (before the onset of clinical dementia) may be necessary. To maximize the power of clinical trials in patients without clinical dementia, valid biomarkers for the early detection of patients with AD pathology or the prediction of patients presenting a prodromal phase of AD (commonly referred to as MCI), who will likely develop AD in the future, would be helpful. Currently, Aβ-1-42 and tau proteins (t-tau and p-tau181) in CSF are the most reliable biochemical biomarkers for the early detection of AD, the differentiation of AD from other forms of dementia, and the prediction of MCI progression to AD. In this review, the current development of AD therapeutics, particularly those drugs targeting amyloid and tau pathology, and the clinical performance of CSF biomarkers for AD diagnosis are summarized. The advantages and unresolved issues of CSF biomarkers in clinical trial design are also discussed.

**TARGETS OF DEVELOPING DRUGS IN AD PATHOPHYSIOLOGY**

**Target related to amyloid production, aggregation and clearance**

The molecular chain of events that ultimately results in synaptic and neuronal loss in the brain is complex and remains largely unresolved, even in the well-known pathologic hallmarks of AD, i.e. the deposits of Aβ peptides in amyloid plaques and other types of aggregates and NFTs formed by misfiled and fibrillized tau. A widely accepted hypothesis is that AD might be initiated by the abnormal (amyloidogenic) processing of amyloid precursor protein (APP) followed by the aggregation and accumulation of Aβ in the brain. The strategy to develop anti-Aβ drugs targeting the canonical amyloid cascade can be classified according to the mechanism of action: reduction of Aβ-1-42 production, prevention of Aβ oligomer (Aβ-o) formation, and acceleration of Aβ clearance (Fig. 2). Based on the amyloid hypothesis, several drugs to reduce Aβ burden are being developed for patients with mild-to-moderate AD. Considering that genetic mutations in the APP or PSEN1 genes cause familial AD and that β-secretase (β-site APP cleaving enzyme, BACE1) knock-out mice showed drastically reduced Aβ levels in the brain, the inhibition of β-
and/or β-secretases can be a strategy to block the initiation of the amyloid cascade. Several first-generation γ-secretase inhibitors (e.g., semagacestat) were tested in clinical studies; however, because γ-secretase is also involved in the processing of Notch, a Phase 3 trial for semagacestat in patients with mild-to-moderate AD not only failed to achieve its predetermined end points, but also worsened clinical measures and increased the incidence of skin cancer [14]. Other Notch-sparing, second-generation γ-secretase inhibitors (e.g., begacestar, avagacestat, PF-3804 014 and NIC5-15) are now in the early phase of clinical trials [15-17]. Because amyloidogenic Aβ species are generated by sequential activation of β-secretase and γ-secretase, the inhibition of β-secretase can be the second strategy to suppress the amyloidogenic pathway. However, β-secretase has many endogenous substrates that are not related to APP processing; therefore, no Phase 3 clinical trials of new β-secretase inhibitors are developing, but anti-β-secretase antibodies and the oral compound CTS-21166 are under investigation. Interestingly, previous research found that the thiazolidinedione antidiabetic drugs (e.g., rosiglitazone and pioglitazone) are potentially beneficial in protecting sAPP-secretase activity, leading to upregulation of neuroprotective sAPPα secretion. Several drugs have been tested in early phase clinical trials, but the results are not yet available. Aβ, particularly Aβ1-42, is prone to aggregation and forms toxic Aβo. Given the evidence that the neurotoxic potency of Aβo is higher than the Aβ monomer or insoluble Aβ amyloid fibrils [27,28], compounds inhibiting Aβ aggregation or destabilizing Aβo seem to be promising drug candidates for AD. The initial anti-aggregant is tramiprosate (homotaurine), which binds preferentially to soluble Aβ; however, the outcome of a Phase 3 trial was not significant [29]. Another anti-aggregant that inhibits metal-induced Aβ oligomerization, PBT2, promotes Aβo clearance and improves cognition in an animal model [30]. In a Phase 2a clinical trial in patients with mild AD for 12 weeks, PBT2 was well-tolerated, reduced CSF Aβ1-42 concentrations and improved executive function [31]. However, recent news releases from Prana reported that PBT2 failed to meet its primary endpoint of reducing Aβ plaques in a 12-month phase 2 “IMAGINE” trial (http://pranabiocom/news/prana-biotechnology-announces-top-line-results-phase-2-imagine-trial-pbt2-alzheimers-disease/#.U4OII1Wd0OvAQ). Based on the in vitro and in vivo results of stabilizing Aβo into non-toxic conformers and improving AD-related phenotypes in TgCRND8 transgenic mice [32,33], a cyclohexanexol isomer, ELND005 (Scyllo-inositol), has been tested in a Phase 2 clinical trial [32]. Although the primary endpoints in the Phase 2 trial did not achieve statistical significance, ELND005 (250 mg, bid) demonstrated a biological effect on Aβ in CSF [34]. Currently, the sponsoring companies intend to advance this molecule into Phase 3 studies. To evaluate the biological effects of Aβo or Aβ aggregation inhibitors and to prove their mechanisms of action, a biofluid assay set-up would be a tool to find molecules and test their pharmacodynamic action. Previous research has reported that Aβo concentration measured by in-house ELISA method in CSF of AD patients is significantly higher than healthy controls [35]. Although there is criticism of the measurement of Aβo by single-antibody ELISA methodology, the effect of the anti-aggregant on Aβ oligomerization could be monitored by the
Aβ are two potential targets for enhancement of Aβ plasma and/or Aβ through the blood-brain-barrier (BBB) from the brain to the periphery; the receptor for advanced glycation end products (RAGE) and low-density lipoprotein receptor-related protein 1 (LRP-1) [39,40]. RAGE mediates the influx of Aβ into the brain, while LRP-1 mediates efflux of Aβ from the brain (Fig. 2). In addition to the possible role of RAGE-Aβ interaction for the activation of nuclear factor-κB signaling pathways, which may promote apoptosis and neuroinflammation [41], the RAGE-mediated influx of peripheral Aβ into the brain may increase amyloid load. A RAGE inhibitor or an LRP-1 activator may be a potential candidate for AD treatment based on amyloid clearance. In fact, an oral small molecule inhibiting RAGE activity (PF-04494700) has been tested in Phase 2 trials; however, the development was discontinued [42]. Another strategy to enhance Aβ clearance is the activation of Aβ degradation proteases, including nephrilysin, insulin-degrading enzyme and plasmin [43,44]. However, specific activation of enzymes seems the more challenging approach than inhibition.

**Immunotherapy against amyloid pathology**

Two types of immunotherapy currently exist to enhance antibody-mediated Aβ clearance: active immunotherapy (anti-Aβ vaccine) and passive monoclonal anti-Aβ antibody treatment. The initial active immunotherapy with AN-1792 to induce anti-Aβ antibodies was not tolerable by the activation of cytotoxic T cells and autoimmune reaction followed by meningoencephalitis [45]. Subsequently, the more tolerable active immunotherapies using the improved design of an immunogen-containing the N-terminal, fragment of Aβ1-42 or N-terminal mimic peptide, including ACC-001, CAD106, V950, and Affitope AD02, are currently being tested in the early phases of clinical trials [46]. Passive immunotherapeutic approaches are also currently developing in parallel with active immunotherapy. To date, humanized monoclonal antibodies for passive immunotherapy are in clinical development. For example, an initial early phase clinical trial of bapineuzumab showed good tolerability with mild to moderate adverse events, including symptomatic and asymptomatic amyloid-related imaging abnormalities (ARIA) and improvement of exploratory efficacy measure (minimal mental status examination score; MMSE), when compared to the placebo group. However, the efficacy evaluated by prespecified primary endpoints (Alzheimer’s Disease Assessment Scale-Cognitive; ADAS-Cog, or Disability Assessment for Dementia; DAD) in a Phase 2 trial did not show statistical significance, although a trend in favor of bapineuzumab was observed, particularly in apolipoprotein E4 noncarriers [47]. In addition, there were no observed treatment differences in either CSF Aβ or total tau, but the reduction of p-tau in the bapineuzumab group was significant when compared with the placebo group [48]. Although the clinical efficacy of bapineuzumab in the Phase 2 trial was not dramatic by week 52, amyloid removal from the brain was clearly observed in amyloid (PiB) PET scan [49]. In Phase 3 trial of bapineuzumab for 78 weeks, the clinical efficacy was disappointing although the changes of biomarkers were significant, particularly in ApoE carriers [50]. Another humanized monoclonal anti-Ab antibody, solanezumab (LY2062430) was applied to mild-to-moderate AD patients with an advantage of absence of significant ARIA [51]. Similar to bapineuzumab trial, however, solanezumab failed to have a success of clinical efficacy (phase 3 trial of EXPEDITION 1 and EXPEDITION 2 study) [52]. The disappointing results of intravenous bapineuzumab and solanezumab in phase III trial for mild to moderate AD patients would not necessarily exclude these from AD prevention trial, such as the Dominantly Inherited Alzheimer's Network (DIAN) study [53-55]. For passive immunotherapy using immunoglobulin, three small trials using intravenous immunoglobulin (IVIG) targeting multiple forms of Aβ suggest that IVIG can have favorable efficacy and will be tolerable [56-58]. However, two of these studies were not placebo-controlled studies, and another study was a retrospective case-control analysis. Recently, data of the clinical efficacy of IVIG in a placebo-controlled, multicenter Phase 2 clinical trial and the effect of IVIG on the concentration of plasma and CSF Aβ species have been published [59]. The results of this study showed an acceptable safety profile and a significant difference in plasma Aβ1-40 levels in the highest dose group when compared to the placebo group [60]. Recently, the disappointment of phase 3 clinical trial using IVIG in 390 patients with mild-to-moderate AD was announced, although a dose-dependent reduction in plasma Ab42, increase in plasma, CSF anti-oligomer and anti-fibril antibodies, and reduced brain fibrillar Ab42 was observed [61], and also see http://blog.alz.org/results-of-igiv-study-disappointing-but-not-discouraging.

**Target related to tau hyperphosphorylation**

Intracellular fibrillary NFTs containing hyperphosphorylated and fibrillar species of tau are one of hallmarks of AD pathology, but they are not specific for AD. If tau is abnormally hyperphosphorylated and aggregated, it may be toxic due to gains of deleterious function or loss of the normal function of tau and microtubule instability followed by axonal transport failure (Fig. 3). There may be two approaches to inhibiting tau toxicity [11,62]. The first is the inhibition of abnormal hyperphosphorylation by targeting tau kinase and/or phosphatase. In addition, inhibitors of tau aggregation or disassemblers may be beneficial for protecting neurons from tau aggregate toxicity. There are several candidates of tau kinase and phosphatase related to tau hyperphosphorylation [63]. For example, glycogen synthase kinase 3 (GSK-3) is a well-known tau kinase, which is balanced with a phosphatase, protein phosphatase 2A. Valproic acid and lithium, well-known for the treatment of epilepsy and bipolar disorder, respectively, are GSK-3 inhibitors [64]. However, they didn’t show consistent results for cognitive improvement or changes in CSF tau or p-tau concentration in clinical trials [65-67]. Several new GSK-3 inhibitors are being tested in clinical trials. NP031112 (tideglsib), which is a non-ATP competitive GSK3 inhibitor, reduces tau hyperphosphorylation and amyloid deposition, prevents neuronal death, and improves cognitive function in animal models [68]. This drug showed clinical benefits in a pilot, randomized, double-blind, placebo-controlled clinical trial, and it is currently being confirmed in...
CSF Biomarkers in Clinical Trial of AD

**Clinical Performance of CSF Biomarkers for Early Diagnosis of AD**

By direct contact with extracellular space of the brain, CSF is the most useful biological fluid reflecting molecular events in the brain, which have driven intense research efforts to develop biochemical biomarkers for AD diagnosis in CSF. On the basis of prevailing scientific evidence and reliable clinical performance, CSF biomarkers have been involved in the recently published revision of AD diagnostic criteria for research purposes as supportive evidence for AD pathophysiology [3,4]. When we analyzed the overall clinical performance of CSF AD biomarkers (concentration of Aβ1-42, t-tau and p-tau181) for the diagnosis of AD in previously reported clinical studies, both mean sensitivity and specificity for Aβ1-42 and t-tau are over 80%, while those for p-tau181 are slightly lower than 80%. When Aβ1-42 and t-tau are combined (i.e., Aβ1-42/t-tau ratio or regression model using Aβ1-42 and t-tau concentration), both the mean sensitivity and specificity are higher than 85% [72]. Furthermore, longitudinal follow-up studies showed that CSF biomarker measurement has an ability to predict future development of AD in MCI patients [5-10]. The prediction of progression from MCI to preclinical AD to AD is important not only for the early diagnosis followed by therapeutic intervention but also for the design of clinical trials for developing disease-modifying therapies. In this context, large-scale clinical studies are underway to test the clinical relevance of CSF biomarkers for early diagnosis of AD, even in the preclinical stage of AD development. For example, data from North American Alzheimer’s Disease Neuroimaging Initiative (ADNI) showed the clinically reliable predictive performance of CSF AD biomarkers [8,73], using cut-off values determined by independent non-ADNI autopsies-confirmed samples [7]: the sensitivities of Aβ1-42/t-tau, p-tau181, and Aβ1-42/t-tau ratio are 96.4, 69.6, 67.9, and 85.7%, respectively, and the specificities are 76.9, 92.3, 73.1, and 84.6%, respectively. North American ADNI is a multicenter, prospective, longitudinal, observation study for the evaluation of clinical characteristics, genetics, imaging biomarkers, and CSF AD biomarkers in healthy elderly subjects, MCI, and AD patients [74]. This study (ADNI-1) was completed in 2009, and ADNI-2 is ongoing. In ADNI-2, early amnestic MCI patients were added. The early amnestic MCI was defined as individuals meeting clinical criteria for amnestic MCI, who score between 0.5 and 1.5 standard deviation below the mean of normal healthy controls on delayed paragraph recall performance. Therefore, ADNI studies provided the long-term follow-up data for the changes in the initial diagnosis (e.g., early or late MCI to AD or normal to MCI). In addition to North American, European and Australian ADNI studies in western countries, Japanese, Chinese, and Korean ADNI studies are currently ongoing in Asia. Currently, numerous investigators are supporting the evidence that CSF biomarkers have a good diagnostic performance, particularly in combination with other biomarkers, including genetic biomarkers (e.g., ApoE genotype) and imaging biomarkers (e.g., hippocampal volume determined by MRD) [75-78]. However, there are several limitations for the application of CSF biomarkers for the diagnosis of AD in clinics distributed elsewhere. For example, the cut-off values for AD diagnosis are different across the studies due to interlaboratory variability in the measurement of CSF biomarker concentrations [73]. In other words, the causes of interlaboratory variability of CSF biomarker concentrations measured by immunoassay technologies were not yet completely elucidated (see below). To minimize the interlaboratory variability of CSF biomarker concentrations, global efforts, including the development of standardized protocols, reference materials and reference methods, are underway [79,80].

In addition, the amyloid hypothesis and chronological cascade relationships among amyloid and tau pathology, biomarkers of CSF level of Aβ1-42 and tau and imaging biomarkers, and clinical symptoms, still remain to be proven (Fig. 1), although no clear findings can negate this hypothesis. With the reliable clinical performance of CSF biomarkers, genetic and imaging biomarkers have a diagnostic value for early diagnosis of AD or prediction of disease progression. In particular, it is very important the elucidation of the neurodegenerative process and biomarkers reflecting the pathogenic process in preclinical stage of the disease, since it will accelerate the development of new therapeutics for AD treatment as well as the prevention of the disease [11,81]. To do this, the long-term longitudinal

![Fig. 3. Schematic presentation of tau mediated neurodegeneration. Phosphorylation and dephosphorylation of tau control the stability of microtubule. Hyperphosphorylation of tau induces disassembly of mitrotubules, causing axonal transport failure. Unbound tau produces oligomers or aggregates which congest axonal transport, and the tau pathology is synaptically transmitted. By direct contact with extracellular space of the brain, tau-targeting-drug-davunetide-washes-out-phase-3-trials).](image-url)
study observing the changes of clinical parameters, and CSF and imaging biomarkers in population with various degree of the disease will be required. Currently, ADNI-GO and ADNI-2 studies including early MCI subjects as well as normal healthy elderly subjects, MCI and early AD patients are underway.

CSF BIOMARKERS FOR AD TRIALS

The use of biomarkers in clinical trials depends on the mechanism of action of the developing therapy, goal of the trial, question to be solved, and stages of the trial. There are several purposes to include AD biomarkers in clinical trial design. First, they can be included in clinical trials as surrogate endpoints for the evaluation of the effects of a potential, new, disease-modifying therapy. Unlike cardiovascular or cancer clinical trials, in which the primary endpoint is typically the occurrence of a specific event, the use of mortality as an endpoint is not feasible for AD clinical trials due to the very large required sample size or follow-up period. If the measurement of biomarkers is valid, biomarker outcome measures can provide the evidence for the biochemical and/or molecular effects of a new therapy. In addition, biomarkers can be used as surrogate endpoints in which clinical measurements are not available, e.g., very early pre-symptomatic stage of AD. Although much work remains to be performed for the application of biomarkers as surrogate endpoints, the measurement of valid biomarkers has a potential to decrease the time that is required for evaluating clinical efficacy. To this end, it is necessary to determine whether the measurement of AD CSF or imaging biomarkers correlate with clinical endpoints and predict future clinical benefit or decline. In an AN1792 Aβ immunization Phase 1 trial, a small portion of antibody responders showed greater atrophy and progression of dementia despite the apparent reduction of amyloid load at autopsy [82,83]. However, it was evident in a larger Phase II trial that AN1792 immunization has a long-term functional benefit in antibody responders [84]. Among the current key unknowns are the time frames for affecting CSF biomarker measurement and what degree the biomarker change might relate to the clinical outcome. Therefore, we need to carefully consider the qualification and validation of AD biomarkers before using them as surrogate markers for clinical endpoints.

Second, biomarkers can be used diagnostically with clinical workup for inclusion and exclusion criteria. In particular, in early phase trials aiming to show mechanistic proof of concept, biomarker measurements can be used as a stratification tool for the pharmacodynamic evaluation of a drug. For example, the effects of a drug targeting brain amyloid reduction (e.g., anti-Aβ immunotherapy or secretase inhibitors) can be assessed in subjects with a high amyloid load who are differentiated by CSF or imaging biomarkers. In recent clinical trials, BMS-708163, a Notch-sparing γ-secretase inhibitor, showed a good to lerance and a dose-dependent decrease of Aβ1-42 and elevated t-tau and p-tau181) can differentiate between those patients with underlying AD as the cause of the cognitive impairment from those MCI patients categorized in an early stage of non-AD dementia, patients with stable MCI for long periods of time, or patients in a group who will recover to normal cognition [86]. Disease-modifying drugs are likely to be most effective when they are given early in the pathogenic process of AD. Therefore, if patients in the early reversible stage of the disease can be differentiated from normal subjects by CSF or imaging biomarkers combined with clinical diagnosis, the possibility to have success in a clinical trial of disease-modifying therapy will increase.

Finally, biomarkers can be used for enrichment of study subjects, particularly in Phase 2 and 3 trials, and for increments of statistical power. In fact, AD patients with more extensive cortical atrophy, with low CSF Aβ1-42 level, or with genetic risk factors (e.g., ApoE4 allele) progress more rapidly than others [87]. A placebo-controlled trial including rapidly progressing subjects may enhance the opportunity to observe the drug-placebo difference within the follow-up time frame of the trial. In addition, the involvement of CSF and imaging biomarkers in clinical trial design may minimize the possible clinical variability in subjects who are recruited by clinical diagnosis alone. Furthermore, the statistical power of clinical trial design may increase by inclusion of biomarkers as baseline covariates to assess the treatment effects. As a consequence of low predictability of the progression to AD from MCI by clinical assessment alone, the inclusion of MCI patients in a treatment trial without the benefit of a biomarker-based assessment is likely to obscure a clinical outcome or require an increased sample size and longer observation. The enrichment and stratification strategy for recruitment of patients by inclusion of CSF AD biomarkers and/or other biomarkers will likely improve sample homogeneity and statistical power, and therefore, it would allow for a substantial reduction in sample size and cost-saving in clinical prevention trials or clinical trials of AD-modifying therapy in AD or MCI patients [69,88,89]. There are several prospective studies to evaluate the predictability of CSF AD biomarkers for AD progression from MCI with the realistic sample size and follow-up duration for what might be considered in Phase 2 or 3 clinical trials and with highly standardized protocols [5,7,90]. Despite the difference and lack of formal comparison, they agreed that CSF AD biomarkers showed a good diagnostic performance for MCI due to AD. Therefore, there is a trend to voluntarily include the CSF AD biomarkers in the design of current clinical trial for various purposes.

FUTURE PERSPECTIVES

Given the clinical performance of CSF AD biomarkers, many of the current AD clinical trials are focusing on the beneficial roles of biomarkers in the design of a cost-effective clinical trial in addition to the understanding of disease progression. Although recent results of a solanezumab trial argued the lack of correlation between CSF biomarker changes and treatment effect, biomarkers have the potential to be extremely useful for various purposes in clinical trials. We have learned from the massive amounts of data
provided by ADNI and related projects around the world that there is still much to learn about how well CSF, imaging and genetic biomarkers reflect pathology and clinical manifestation. In addition, it might be important to determine the precision, reproducibility, and the causes of interlaboratory variability in the measurement of CSF AD biomarkers. For standardization, it will be important and consistent with the field to emphasize major standardization efforts like North American ADNI, started in 2004, and world-wide ADNI and other Alzheimer’s Association-sponsored studies. The global collaborative efforts of investigators from academia, industry, and regulatory agencies to minimize the current analytical issues will likely position CSF biomarkers to become a contributing factor for successful clinical trials and development of new AD therapies.

ACKNOWLEDGEMENT

This work was financially supported by Mid-career Researcher Program (2013R1A2A2A01008223) through the National Research Foundation of Korea (NRF) funded by Ministry of Science, ICT and Future Planning for Ju-Hee Kang.

REFERENCES

1. Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberge-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha G, Karp J, O’Brien J, Passquier F, Robert P, Rossor M, Salloway S, Stern Y, Visser PJ, Scheltens P. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol.* 2007;6:734-746.
2. Dubois B, Feldman HH, Jacova C, Cummings J, DL, Dekosky ST, Barberge-Gateau P, Delacourte A, Frisoni G, Fox NC, Galasko D, Gauthier S, Hampel H, Jicha GA, Meguro K, O’Brien J, Passquier F, Robert P, Rossor M, Salloway S, Sarazin M, de Souza LC, Stern Y, Visser PJ, Scheltens P. Revising the definition of Alzheimer’s disease: a new lexicon. *Lancet Neurol.* 2010;9:1118-1127.
3. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 2011;7:270-279.
4. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 2011;7:263-269.
5. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Mithlon L. Association between CSF biomarkers and incident Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol.* 2006;5:228-234.
6. Herukka SK, Helisalmi S, Hallikainen M, Tervo S, Soininen H, Pirttilä T, CSF Abeta42, Tau and phosphorylated Tau, APOE epsilon4 allele and MCI type in progressive MCI. *Alzheimers Dement.* 2011;7:269-274.
7. Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, Blennow K, Soares H, Simon A, Lewczuk P, Dean R, Siemers E, Potter W, Lee VM, Trojanowski JQ. Alzheimer’s Disease Neuroimaging Initiative. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative studies. *Ann Neurol.* 2009;65:403-413.
8. De Meyer G, Shapiro F, Vanderstichele H, Vannmechelen E, Engelborghs S, De Deyn PP, Coart E, Hansson O, Minthon L, Zetterberg H, Blennow K, Shaw L, Trojanowski JQ. Alzheimer's Disease Neuroimaging Initiative. Diagnosis-independent Alzheimer disease biomarker signature in cognitively normal elderly people. *Arch Neurol.* 2010;67:949-956.
9. Buchhave P, Minthon L, Zetterberg H, Wallin AK, Blennow K, Hansson O. CSF cerebrospinal fluid levels of β-amyloid 1-42, but not of tau, are changed already 5 to 10 years before the onset of Alzheimer dementia. *Arch Gen Psychiatry.* 2012;69:98-106.
10. Palmqvist S, Hertzje J, Minthon L, Wattmo C, Zetterberg H, Blennow K, Londos E, Hansson O. Comparison of brief cognitive tests and CSF biomarkers in Alzheimer’s disease in mild cognitive impairment: six-year follow-up study. *PLoS One.* 2012;7:e86598.
11. Yoshiyama Y, Lee VM, Trojanowski JQ. Therapeutic strategies for tau mediated neurodegeneration. *J Neurol Neurosurg Psychiatry.* 2013;84:784-785.
12. Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer’s disease. *Alzheimers Dement.* 2007;3:186-191.
13. Scott Td, O’Connor AC, Link AN, Baulieu TD. Economic analysis of opportunities to accelerate Alzheimer’s disease research and development. *Annals of the New York Academy of Sciences.* 2014;1313:Annals Reports pages 17-34.
14. Doody RS, Raman R, Farlow M, Iwatsubo T, Vellas B, Joffe S, Kieburtz K, He F, Sun X, Thomas RG, Aisen PS. Alzheimer's Disease Cooperative Study Steering Committee, Siemers E, Sethuraman G, Mohs R. Senomagastat Study Group. A phase 3 trial of senomagastat for treatment of Alzheimer’s disease. *N Engl J Med.* 2013;369:341-350.
15. Coric V, van Dyck CH, Salloway S, Andreassen N, Brody M, Richter RW, Soninen H, Thein S, Shiovitz T, Pelcher G, Colby S, Rollin L, Dockens R, Pachai C, Portelius E, Andreasson U, Blennow K, Soares H, Albright C, Feldman HH, BM. Safety and tolerability of the γ-secretase inhibitor avagacestat in a phase 2 study of mild to moderate Alzheimer’s disease. *Arch Neurol.* 2012;69:1430-1440.
16. Dockens R, Wang JS, Castaneda L, Sverdlov O, Huang SP, Blennow R, Gu H, Wong O, Li H, Berman RM, Smith C, Albright CF, Tong G. A placebo-controlled, multiple ascending dose study to evaluate the safety, pharmacokinetics and pharmacodynamics of avagacestat (BMS-708163) in healthy young and elderly subjects. *Clin Pharmacokinet.* 2012;51:681-693.
17. Tong G, Wang JS, Sverdlov O, Huang SP, Blennow R, Groop L, Castaneda L, Gu H, Wong O, Li H, Berman RM, Smith C, Albright CF, Dockens RC, Multicenter, randomized, double-blind, placebo-controlled, single-ascending dose study of the oral γ-secretase inhibitor BMS-708163 (Avagacestat): tolerability profile, pharmacokinetic parameters, and pharmacodynamic markers. *Clin Ther.* 2012;34:684-697.
18. Yan Q, Zhang J, Liu H, Babu-Khan S, Vassar R, Biere AL, Citron M, Landreth G. Anti-inflammatory drug therapy alters beta-amyloid processing and deposition in an animal model of Alzheimer’s disease. *J Neurosci.* 2003;23:7504-7509.
19. Escribano L, Simón AM, Gimeno E, Cuadrado-Tejedor M, López de Matunana R, García-Osta A, Ricozbaraza A, Pérez-Medivar L, Del Río J, Frechilla D. Rosiglitazone rescues memory impairment in Alzheimer's transgenic mice: mechanisms involving a reduced amyloid and tau pathology. *Neuropharmacology.* 2010;53:1003-1004.
20. Landreth G, Jiang Q, Mandrekar S, Heneka M. PPARgamma agonists as therapeutics for the treatment of Alzheimer's disease. *Neurotherapeutics.* 2008;5:481-489.
21. Risner ME, Saunders AM, Altman JM, Ormandy GC, Craft S, Foley DM, Zwartau-Hind ME, Hoxford DA, Rose AD. Rosiglitazone in Alzheimer's Disease Study Group. Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. *Pharmacogenomics J.* 2006;6:246-254.
22. Gold M, Alderton C, Zvartau-Hind M, Eggington S, Saunders AM, Irizarry M, Craft S, Landreth G, Linnamägi U, Sawchek S. Rosiglitazone monotherapy in mild-to-moderate Alzheimer's disease: results from a randomized, double-blind, placebo-controlled phase III study. Dement Geriatr Cogn Disord. 2010;30:131-146.

23. Tzimopoulou S, Cunningham VJ, Nichols TE, Searle G, Bird NP, Mistry P, Dixon IJ, Hallett WA, Whither B, Brown AP, Zvartau-Hind M, Lotay N, Lai RT, Castiglia M, Jeter B, Matthews JC, Chen X, Bundy D, Reiman EM, Gold M, Rahime EA, Matthews PM. A multi-center randomized proof-of-concept clinical trial applying [11F]FDG-PET for evaluation of metabolic therapy with rosiglitazone XR in mild to moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

24. Harrington MN, Sawchek S, Chiang C, Davies J, Donovan C, Saunders AM, Irizarry M, Jeter B, Zvartau-Hind M, van Dyck CH, Gold M. Rosiglitazone does not improve cognition or global function when used as adjunctive therapy to AChE inhibitors in mild-to-moderate Alzheimer's disease: two phase III studies. Curr Alzheimer Res. 2011;8:592-608.

25. Geldmacher DS1, Fritsch T, McClendon MJ, Landreth G. Oligomers are elevated in cerebrospinal fluid of Alzheimer disease. Lancet. 2003;4;68:45-50.

26. Suto T, Hanayu H, Hirao K, Kanetaka H, Sakurai H, Iwamoto T. Efficacy of PPAR-γ agonist pioglitazone in mild Alzheimer disease. Neurobiol Aging. 2011;32:1626-1633.

27. Klein WL, Krafft GA, Finch CE. Are amyloid-beta oligomers the solution to an Alzheimer's disease conundrum? Trends Neurosci. 2001;24:219-224.

28. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002;297:353-365.

29. Aisen PS, Frontera R, Verity SH, Samuelli D, Haine D, Garreau D, Duong A, Suhy J, Oh J, Lau WC, Sampalis J. Tramiprosate in mild-to-moderate Alzheimer's disease - a randomized, double-blind, placebo-controlled, multi-centre study (the Alphase study). Arch Med Sci. 2011;7:102-111.

30. Allsop D, Nakaiga M. High-molecular-weight beta-amyloid structures in vitro improve cognition and pathology in a mouse model of Alzheimer's disease. Neuron. 2014;82:1536-1543.

31. Del Tredici K, Braak H. Oligomers are elevated in cerebrospinal fluid of Alzheimer's disease. Arch Neurol. 2011;68:45-50.

32. Salloway S, Fritsch T, McClendon MJ, Landreth G, Aisen PS. Are amyloid-degrading enzymes viable therapeutic targets in Alzheimer's disease? J Neurophilol Exp Neurol. 2011;70:944-959.

33. Winblad B, Andreasen N, Minthon L, Fossler A, Imbert G, Dumortier T, Maguire RP, Blennow K, Lundmark J, Staufenhmiel M, Orgogozo JM, Graf A. Safety, tolerability, and antibody response assessments of Aβ immunotherapy with CAD106 in patients with Alzheimer's disease: randomised, double-blind, placebo-controlled, first-in-human study. Lancet Neurol. 2012;11:597-604.

34. Salloway S, Fritsch T, Blennow K, Fox NC, Blennow K, Raskind M, Sabbagh M, Honig LS, Doody R, van Dyck CH, Mulnard R, Barakos J, Gregg KM, Liu E, Lieberburg I, Schenk D, Black R, Grundman M; Bapineuzumab 201 Clinical Trial Investigators. A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. Neurology. 2009;73:2061-2070.

35. Blennow K, Zetterberg H, Rinne JO, Salloway S, Wei J, Black R, Grundman M, Liu E; AAR-001 201/202 Investigators. Effect of immunotherapy with bapineuzumab on cerebrospinal fluid amyloid beta-1-42 levels in patients with mild to moderate Alzheimer disease. Arch Neurol. 2012;69:1002-1010.

36. Rinne JO, Brooks DJ, Rossor MN, Fox NC, Bullock R, Klunk WE, Mathis CA, Blennow K, Barakos J, Okello AA, Rodriguez Martinez de Llano S, Liu E, Kollier M, Gregg KM, Schenk D, Black R, Grundman M. 11C-PET assessment of changes in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. Lancet Neurol. 2010;9:363-372.

37. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

38. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

39. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

40. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

41. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

42. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

43. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

44. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

45. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

46. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

47. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

48. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

49. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.

50. Tramiprosate DA, Suhy J, Oh J, Lau WC, Sampalis J. A phase 3b, randomized, placebo-controlled study applying [11F]FDG-PET for evaluation of metabolic therapy with tramiprosate XR in mild-to-moderate Alzheimer's disease. J Alzheimers Dis. 2010;22:1241-1256.
M, Sabbagh M, Honig LS, Porsteinsson AP, Ferris S, Reichert M, Ketter N, Nejadnik B, Guenzler V, Miloslawsky M, Wang D, Lu Y, Lual J, Tudor IC, Liu E, Grundman M, Yuen E, Black R, Brashear HR; Bapineuzumab 301 and 302 Clinical Trial Investigators. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. N Engl J Med. 2014;370:322-333.

51. Farlow M, Arnold SE, van Dyck CH, Aisen PS, Snider BJ, Bapineuzumab 301 and 302 Clinical Trial Investigators. Updated results from phase 3 trial of solanezumab for mild-to-moderate Alzheimer's disease. N Engl J Med. 2014;370:311-321.

52. Bateman RD, Xiong C, Benzinger TL, Fagan AM, Gentz A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, Holtzman DM, Santacruz A, Buchöke V, Oliver A, Moulder K, Aizid P, Gheiti B, Klunk WE, McDade E, Martins RN, Masters CL, Mayeux R, Ringman JM, Rossor MN, Schofield PR, Sperling RA, Salloway S, Morris JC; Dominantly Inherited Alzheimer Network. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 2012;367:798-804.

53. Worley S. After disappointments, Alzheimer's researchers seek out new paths: biomarkers and combination therapies may lead to disease-modifying treatments, experts say. P T. 2014;39:363-374.

54. National Institute on Aging. Preventing Alzheimer's disease; What do we know? http://www.nia.nih.gov/alzheimers/publication/preventing-alzheimers-disease/introduction

55. Dodel RC, Du Y, Depuybou C, Hampael H, Frolich L, Haag A, Bartenstein P, Paulsen S, Teipel SJ, Breitening F, Nölker C, Möller HJ, Wei X, Farlow M, Sommer N, Oertel WH. Intravenous immunoglobulins containing antibodies against beta-amyloid for the treatment of Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2004;75:1472-1474.

56. Fillit H, Hess G, Hill J, Bonnet P, Tao C. IV immunoglobulin is associated with a reduced risk of Alzheimer disease and related disorders. Neurology. 2009;73:180-185.

57. Rökk JJ, Korens M, Toledo JB, Trojanowski JQ, Shaw LM. Clinical utility and analytical challenges in measurement of cerebrospinal fluid amyloid-[beta](1-42) and [tau] proteins as Alzheimer disease biomarkers. Clin Chem. 2013;59:903-916.

58. Weill Cornell Medical College. Updated results from phase 3 trial of IVIG for Alzheimer's disease. http://www.sciencedaily.com/releases/201307301307160992743.htm.

59. Knight EM, Gandy S. Effects of lithium and valproic acid on trophic factor deprivation-induced glycogen synthase kinase-3 activation, c-Jun expression and neuronal cell death. Neuropharmacology. 2005;48:576-583.

60. Profeno LA, Jakimovich I, Holt CJ, Porsteinsson A, Tariot PN. A randomized, double-blind, placebo-controlled pilot trial of safety and tolerability of two doses of divalproex sodium in outpatients with probable Alzheimer's disease. Curr Alzheimer Res. 2005;2:553-558.

61. de Ser T, Steinwachs RC, Gertz BJ, Ayala MV, Gómez-Carrillo R, Medina M, Verica J, Redondo P, Sadowski D, León T. Treatment of Alzheimer's disease with the GSK-3 inhibitor tideglusib: a pilot study. J Alzheimers Dis. 2013;33:205-215.

62. Wischik CM, Bentham P, Wischik DJ, Seng KM. Tau aggregation inhibitor (TAI) therapy with remberTM arrests disease progression in mild and moderate Alzheimer's disease over 50 weeks. Alzheimers Dement. 2008;4 Suppl 2:T176.

63. Schmecheldein DE, Gerard R, Vatalisk N, Harper L, Ross JS, Bari M, Walling D, Stedman M, Winston JL, Morimoto B, Keith JR. A phase 2, double-blind, placebo-controlled study to evaluate the safety, tolerability, and effect on cognitive function of AL-108 after 12 weeks of intranasal administration in subjects with mild cognitive impairment. Alzheimers Dement. 2008;4(4 Suppl 2):T745.

64. Cui Y, Liu B, Luo S, Zhen X, Fan M, Liu T, Zhu W, Park M, Jiang T, Jin JS; Alzheimer's Disease Neuroimaging Initiative. Identification of conversion from mild cognitive impairment to Alzheimer's disease using multivariate predictors. PLoS One. 2011;6:e21896.

65. Hinrichs C, Singh V, Xu G, Johnson SC; Alzheimers Disease Neuroimaging Initiative. Predictive markers for AD in a multi-modality framework: an analysis of MCI progression in the ADNI population. Neuroimage. 2011;55:574-589.

66. Hong D, Shen D; Alzheimer's Disease Neuroimaging Initiative. Predicting future clinical changes of MCI patients using longitudinal and multimodal biomarkers. PLoS One. 2012;7:e33182.

67. Mattson N, Zetterberg H. What is a certified reference material? Biomark Med. 2012;6:369-370.

68. Mattson N, Andreasson U, Persson S, Carrillo MC, Collins S, Chalbot S, Cutler N, Dufour-Rainfray D, Fagan AM, Heegaard NH, Robin Hsiung GY, Hyman B, Iqbal K, Lachn B, Lleo
A. Lewczuk, Molinuevo JL, Parchi P, Regeniter A, Rissman R, Rosenmann H, Sancesario G, Schröder J, Shaw LM, Teunissen CE, Trojanowski JQ, Vanderstichele H, Vandijck M, Verbeek MM, Zetterberg H, Blennow K, Kaiser SA; Alzheimer's Association QC Program Work Group. CSF biomarker variability in the Alzheimer's Association quality control program. *Alzheimers Dement.* 2013;9:251-261.

81. Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* 2010;9:119-128.

82. Fox NC, Black RS, Gilman S, Rossor MN, Griffith SG, Jenkins L, Koller M; AN1792(QS-21)-201 Study. Effects of Abeta immunization (AN1792) on MRI measures of cerebral volume in Alzheimer disease. *Neurology.* 2005;64:1563-1572.

83. Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, Jones RW, Bullock R, Love S, Neal JW, Zotova E, Nicoll JA. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet.* 2008;372:216-223.

84. Vellas B, Black R, Thal LD, Fox NC, Daniels M, McLennan G, Tompkins C, Leibman C, Ponfret M, Grundman M; AN1792 (Q8-21)-251 Study Team. Long-term follow-up of patients immunized with AN1792: reduced functional decline in antibody responders. *Curr Alzheimer Res.* 2009;6:144-151.

85. Ereshefsky L, Jhee SS, Yen M, Moran SV. The role for CSF dynabridging studies in developing new therapies for Alzheimer's disease. *Alzheimer's Dement.* 2009;5 Suppl 4:P414-P415.

86. Jicha GA, Parisi JE, Dickson DW, Johnson K, Cha R, Ivnik RJ, Tangalos EG, Boeve BF, Knopman DS, Braak H, Petersen RC. Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. *Arch Neurol.* 2006;63:674-681.

87. Okonkwo OC, Mielke MM, Griffith HR, Moghekar AR, O'Brien RD, Shaw LM, Trojanowski JQ, Albert MS; Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid profiles and prospective course and outcome in patients with amnestic mild cognitive impairment. *Arch Neurol.* 2011;68:113-119.

88. van Rossum IA, Vos S, Handels R, Visser PJ. Biomarkers as predictors for conversion from mild cognitive impairment to Alzheimer-type dementia: implications for trial design. *J Alzheimers Dis.* 2010;20:881-891.

89. Holland D, McEvoy LK, Desikan RS, Dale AM; Alzheimer's Disease Neuroimaging Initiative. Enrichment and stratification for predementia Alzheimer disease clinical trials. *PLoS One.* 2012;7:e47739.

90. Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M, Herulka SK, van der Flier WM, Blankenstein MA, Ewers M, Rich K, Kaiser E, Verbeek M, Tsolaki M, Mulugueta E, Rosén E, Aarsland D, Visser PJ, Schröder J, Marcussen J, de Leon M, Hampel H, Scheltens P, Fortiili T, Wallin A, Jönhagen ME, Minthon L, Winblad B, Blennow K. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA.* 2009;302:385-393.