CLINICAL INVESTIGATION

Alzheimer's disease clinical trials: past failures and future opportunities

Over a decade has elapsed since the US FDA has approved a medication for Alzheimer's disease (AD) despite clinical trials of numerous agents over a wide array of mechanisms including neurotransmitter modulation and disease modifying therapy targeting amyloid and tau. The failures of clinical trials in AD may be due to inadequate understanding of mechanisms of action and/or poor target engagement; however, other factors could include inadequate study design, stage of AD along the continuum studied, inclusion of participants without Alzheimer's pathology into clinical trials and limited power of endpoint measures. Future studies will need to carefully assess these possible shortcomings in design of upcoming trials, especially as the field moves toward studies of disease modifying agents (as opposed to symptomatic treatment) of AD and to patients that are very early in the disease spectrum.

Keywords: Alzheimer's disease • Alzheimer's disease biomarkers • amyloid • clinical trials • preclinical Alzheimer's disease • tau

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US FDA approved medications

More than three decades ago, the cholinergic hypothesis proposed that degeneration of cholinergic neurons in the basal forebrain and the associated loss of cholinergic neurotransmission in the cerebral cortex, hippocampus and other areas contributed significantly to the deterioration in cognitive function seen in Alzheimer's disease (AD) [1]. In 1993, the first centrally acting cholinesterase inhibitor, tacrine, was approved by the US FDA for treatment of AD based on evidence from three pivotal studies [2–4] that showed statistically significant, dose-related improvements on tests of cognition, clinician- and caregiver-rated global evaluations and quality-of-life measures. It was subsequently approved in several European countries. The adverse event profile of hepatotoxicity, nausea, vomiting, abdominal pain and diarrhea has limited its use and it is no longer in production in the USA. Subsequent cholinesterase inhibitors – donepezil, rivastigmine and galantamine – have been FDA-approved and continue to provide significant, but modest symptomatic benefit [5–7].

The compound memantine introduced a second mechanism for symptomatic treatment of AD into clinical practice. Memantine, an *N*-methyl-D-aspartate (NMDA) partial antagonist that regulates overstimulation of excess glutamate in the CNS, has also shown mild but statistically significant improvement of symptoms in patients with moderate to severe AD [8]; and in 2003, it was the last FDA-approved medication for AD. Approval of new formulations and dosing of these medications has subsequently occurred, but no novel drugs.

Although these medications provide modest benefit for the symptoms of AD in some patients, they do not arrest or reverse the underlying neurodegenerative disorder. Further, not all patients respond to these treatments, or their benefits are limited by intolerable side effects. Additional symptomatic treatment options are necessary, and therapeutic interventions for AD that can decelerate or even prevent disease progression are

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urgently needed. Numerous symptomatic as well as disease-modifying agents have been, and continue to be, actively pursued. Treatments that slow or stop disease progression remain a significant unmet medical need.

Symptomatic therapy

Many investigative compounds have focused on treating the cognitive symptoms of AD by modulating neurotransmission disturbances resulting from neurodegeneration.

Acetylcholine

The deficit of cholinergic function that follows the loss of neurons in the basal forebrain has been extensively studied and helped lead to development of the currently available centrally acting cholinesterase inhibitors. Other molecules designed to enhance cholinergic function have not been successful.

Other cholinesterase inhibitors

In addition to the four cholinesterase inhibitors that were approved by the FDA, several others were investigated but were ultimately unsuccessful due to lack of efficacy, intolerable side effects or impractical/ineffective dosing. These include velnacrine [9], sustained-release physostigmine [10], eptastigmine [11], metrifonate [12] and huperzine A [13]. Although the huperzine A study was negative on its primary endpoint, a trend toward cognitive improvement was found on the higher dose.

Nicotine receptor agonists

Augmentation of cholinergic function by stimulation of cholinergic receptors was also attempted, with little success thus far. Nicotine patches [14] and the partial α7 agonists, ispronicline (AZD3480) [15], GTS-21, TC-5619, ABT-126 and MEM 3454 have been studied [16]. Other nicotinic agonists or modulators currently under investigation are EVP-6124, MT-4666 and MK-7622.

Muscarinic agonists

Development of both full and partial agonists of the M1 muscarinic receptor has been limited due to adverse effects. In 3–6 month Phase II and III studies, study drugs cevimeline (AF102B), milameline, xanomeline, sabcomeline (SB 202026), talsaclidine and alvameline (LU 25–109) generally showed the parasympathomimetic effects of gastrointestinal symptoms, hypersalivation, sweating and frequent urination, rendering them clinically inadequate [17]. ANAVEX 2–73 targets sigma-1 and muscarinic receptors and is currently being studied.

Glutamate-NMDA receptor modulators

In addition to memantine, other NMDA receptor antagonists were tested without success including remacemide, EVT 101, D-cycloserine and neramexane [18–20].

Glutamate-AMPA receptor modulators

Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors, which are another subgroup of glutamate receptors, have also been tested as potential therapeutic targets for AD. Compounds such as Ampalex and LY451395 have not shown success in clinical trials [20].

Serotonin

A number of serotonin receptors have been postulated to be potential therapeutic targets for the cognitive, behavioral and affective symptoms of AD, but have not yielded evidence of significant efficacy in clinical trials thus far. Development of the $5HT_{1A}$ antagonist Lecozotan was discontinued after Phase II due to poor tolerability [21]. The 5-HT_{1A} agonist xaliproden, thought to be neuroprotective, was found to decrease loss of hippocampal volume in two Phase III studies, but it lacked efficacy on cognitive endpoints [22]. The $5HT_4$ agonist PRX-3140, $5HT_4$ partial agonists such as PF04995275 and RQ-9 and 5-HT_6 antagonists including LU-AE58054, PRX-07034, PF-05212365, SR57746 and SB742457 may be in development [21,23].

Histamine

Latrepiridine, an antihistamine that was once used in Russia, was thought to stabilize mitochondria and therefore possibly have neuroprotective effects. Despite robust findings in a Phase II study, it did not meet endpoints in two Phase III studies on primary or secondary endpoints. MK-0249, an H_3 receptor inverse agonist, showed no cognitive benefit in a 4-week, Phase II study in mild-to-moderate AD [24]. In a Phase II, 16-week monotherapy trial in mild-to-moderate AD, GSK239512, an H_3 receptor antagonist, did not meet primary cognitive endpoints [25]. Other agents targeting histamine, including ABT-288 and GSK189254, have been unsuccessful.

Other neurotransmitters

A number of other agents targeting various neurotransmitter systems have been tested but, thus far, have been unsuccessful. These include the GABA-B antagonist SGS-742 and the phosphodiesterase-4 inhibitors MEM 1414 and MK-0952 [23]. Although FDA-approved and widely used for treatment of AD in the past, ergoloid mesylates, a combination of three dehydrogenated ergot alkaloids that may stimulate dopaminergic and seroto-

nergic receptors, were unsuccessful in a pivotal trial [26]. A meta-analysis of 47 studies showed ergoloid mesylates to be very modestly more effective than placebo [27].

Antidiabetic agents

Insulin has been tested due to alterations in cerebral glucose metabolism observed in AD. A pilot study suggested a cognitive benefit of intranasal insulin in both mild cognitive impairment (MCI) due to AD and patients with mild-to-moderate AD [28]. A larger intranasal insulin study is currently enrolling 240 participants with MCI due to AD or mild AD.

Rosiglitazone lowers blood glucose by improving target cell response to insulin without increasing pancreatic insulin secretion. It is an agonist for peroxisome proliferator-activated receptor-γ (PPAR-γ), a nuclear receptor predominantly expressed in adipose tissue. Activation of PPAR-γ receptors influences the expression of a number of genes involved in glucose and lipid metabolism and also produces anti-inflammatory effects. However, two Phase III studies evaluating rosiglitazone in an extended release form showed no efficacy [29].

Two small studies of another PPAR-γ agonist, pioglitazone, demonstrated a cognitive improvement in AD [30,31]. Pioglitazone is currently being tested in a large AD prevention study that will enroll 5800 cognitively normal participants.

Liraglutide is a glucagon-like peptide-1 agonist that increases pancreatic secretion of insulin in the presence of elevated glucose concentrations and is currently recruiting for a Phase II AD study. A small pilot study for Exendin-4, another glucagon-like peptide-1 agonist, is also currently recruiting.

Miscellaneous

Deep brain stimulation of the nucleus basalis of Meynert or fornix has shown favorable results in a very small pilot study. Larger clinical trials are underway. Transcranial magnetic brain stimulation is also being explored as a therapeutic option in clinical trials.

Curcumin, a component of turmeric, is thought to provide benefit in AD through multiple possible mechanisms, but two clinical trials have reported no benefit [32]. Other compounds that failed to show efficacy in AD studies include: estrogen replacement therapy [33,34]; acetyl-L-carnitine [35]; ginkgo biloba [36]; nicergoline [37]; a growth hormone secretagogue, MK-677 [38]; and docosahexaenoic acid, an omega-3 fatty acid [39].

Disease-modifying therapy

The two dominant pathways of disease modifying therapies have been antiamyloid agents or τ-targeted therapies. Although researchers have sharply debated between these two pathways, they may not be mutually exclusive of each other.

The amyloid hypothesis

The amyloid hypothesis posits that excess accumulation of brain amyloid beta (Aβ), the component of neuritic plaques which is one of the hallmark pathologic findings, causes AD. For over two decades, the amyloid hypothesis has been the main target for disease-modifying therapies. This hypothesis is supported by: the presence of Aβ in neuritic plaques; the genetics of dominantly inherited familial AD involving mutations of amyloid precursor protein (*APP*) and presenilin (*PS*) genes (which increase the rate of Aβ production); and the occurrence of Alzheimer-like changes in middle-age patients with Down syndrome (trisomy 21) who have an extra gene copy of *APP* [40].

Alzheimer's pathology develops a decade or more before the appearance of clinical symptoms. The goal of antiamyloid agents has been to decrease production, prevent aggregation or to increase removal of Aβ. This protein is cleaved from APP by the sequential action of β- and γ-secretases, producing Aβ fragments rendering both β-secretase and γ-secretase as potential therapeutic targets.

γ-secretase inhibitors

Molecules that can inhibit γ-secretase also bind to Notch, an important transmembrane receptor involving an extensive signaling pathway implicated in numerous processes in embryonic development, hematopoiesis, cell adhesion and other cell-to-cell contacts. The challenge of developing a γ-secretase inhibitor has been to selectively inhibit the γ-secretase without affecting Notch. A large 18-month study of semagacestat in mild-to-moderate AD using two co-primary endpoints Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog) and Alzheimer's Disease Cooperative Study-Activities of Daily Living scale (ADCS-ADL) was terminated before completion on the recommendation of the data and safety monitoring board, when worsening of ADCS-ADL scores was significantly greater in the higher-dose semagacestat group. Patients treated with semagacestat also presented with greater adverse events of weight loss, skin cancers and infections. Although not statistically significant, worsening of ADAS-Cog scores was also greater in patients treated with semagacestat [41].

Another γ-secretase inhibitor, avagacestat (BMS- 708163), was designed to bypass Notch-related side effects, but 6-month Phase II trial results demonstrated patients with mild-to-moderate AD who took high doses suffered more adverse events (mainly gastrointestinal and dermatologic) and appeared

cognitively worse than those on placebo [42]. Given these results were similar to those of semagacestat, there are concerns regarding γ-secretase inhibitors as a class.

γ-secretase modulators

Clinical trials testing modulators of γ-secretase have also not met with success so far. In a large 18-month Phase III study (n = 1684), tarenflurbil (R-flurbiprofen) did not show efficacy on the co-primary efficacy endpoints of the ADAS-Cog and ADCS-ADL [43]. Two γ-secretase modulators, CHF 5074 and EVP-0962, are currently in development [44].

β-secretase inhibitors

β-site APP cleaving enzyme (BACE) is an enzyme involved in the first step of the pathway leading to production of Aβ; it is postulated that reduction of Aβ production via inhibition of BACE is another potential therapeutic target. The recent discovery of a mutation in the *APP* gene found in approximately 0.5% of the Icelandic population has lent credence to this hypothesis [45]. This mutation was found to reduce BACE's ability to cleave APP and lowers the chance of developing AD in carriers fivefold by age 85.

Potent small molecule inhibitors of BACE with favorable pharmacokinetic characteristics and brain penetration have entered into clinical development. Reported Phase I studies in humans show robust dose-dependent reductions of Aβ concentrations in cerebrospinal fluid (CSF), plasma or both (e.g., molecules MK-8931, LY2886721, E2609, BI1181181/VTP-37948 and AZD3293). Since BACE and a related protein BACE2 have protein substrates beyond APP, there is a potential for toxicity related to on-target or nonspecific BACE inhibition (in addition to any molecule-specific off-target effects.) While LY28886721 was discontinued in Phase II due to hepatotoxicity, an interim safety analysis of 200 patients treated with MK-8931 for 3 months supported progression to Phase III clinical trials [46]. A Phase II/III study for AZD3293 is currently enrolling. The Alzheimer's Prevention Initiative (API) has announced a prevention study with a BACE inhibitor in cognitively normal apolipoprotein E (*APOE*) ε4 homozygotes age 60–75 [47].

α-secretase activators

α-secretase is an enzyme that cleaves APP in a manner that precludes formation of the toxic species of Aβ. Thus, molecules that activate the α-secretase enzyme, such as EHT-0202, are being tested.

Antiaggregants

Monomers of Aβ tend to aggregate spontaneously and form larger soluble molecular species (oligomers/protofibrils). Continued aggregation results in the formation of insoluble fibrils that eventually precipitate in the brain. The levels and distribution of soluble Aβ better correlate with severity of disease than those of insoluble fibrils [48]. Although the equilibria between monomeric Aβ, oligomers or protofibrils and insoluble Aβ fibrils remain poorly understood, oligomeric Aβ has been shown to be toxic to neurons and synapses, suggesting it an appropriate target for AD therapy [49].

Tramiprosate is a patented variant of the amino acid taurine that binds to soluble Aβ to reduce amyloid aggregation and subsequent brain deposition. A large 18-month study in 1052 patients with mild-to-moderate AD with co-primary endpoint measures (ADAS-Cog and Clinical Dementia Rating – Sum of Boxes [CDR-SB]) showed a trend toward slowing of decline that was not statistically significant [50].

A Phase II trial assessing low and high doses of a small molecule inhibitor of the receptor for advanced glycation endproducts (RAGE), PF-04494700/TTP488, was halted at the interim analysis. An interim safety analysis demonstrated worsened confusion, falls and cognitive decline in the high-dose arm, which was discontinued. Later, an interim futility analysis for the remaining low-dose arm showed no benefit, and the trial was stopped. Although no longer receiving study drug, participants were followed through the 18-month endpoint. Patients in the low-dose group who completed the 18 months were found to have improved cognitive scores on the ADAS-Cog (despite no improvement at the 12-month time point), but not on other clinical outcome measures. The lead investigator argued that stopping the study due to the interim futility analysis at 12 months may have prevented seeing positive effects that required a greater amount of time to become evident [51]. A large Phase III study has been announced, but has not yet begun enrollment.

Scylloinositol (ELND005) is an inositol stereoisomer that is thought to prevent Aβ oligomer aggregation. A Phase II trial in 353 patients with mild-to-moderate AD was negative on its primary cognitive and functional endpoints [52]. It is currently being studied as a possible treatment for agitation and aggression in AD. Using amyloid positron emission tomography (PET) imaging as its primary outcome, PBT2, a metal protein-attenuating compound thought to disrupt Aβ aggregation, did not show treatment effect.

Immunotherapy

Immunotherapy in AD treatment is designed to clear Aβ, thereby reducing its toxic effects. Active and passive immunization therapy for AD have been widely studied. In active immunization, an antigen that is designed to induce an antibody-mediated immune response

is administered to the patient. In theory, just a few administrations of antigen could generate a prolonged antibody response. Many elderly patients, however, may not be able to generate therapeutically adequate titers of antibodies and may be more likely to develop side effects which could be persistent. In passive immunization, antibodies are delivered directly to the patient, bypassing the need for the body to create antibodies. Although a repeated administration of antibody is required, rapid clearance of antibodies in passive immunotherapy is an advantage should side effects present [49].

Active immunotherapy

AN1792, a vaccine targeting full-length $A\beta_{1-42}$, was the first immunotherapy agent for AD in clinical trials. A 12-month, Phase II trial of 372 patients with mild-to-moderate AD was terminated early due to a T-cell-mediated aseptic meningoencephalitis in 6% of the vaccinated patients. This was explained by QS-21, the immune adjuvant, stimulating a pro-inflammatory T helper (Th) 1-type immune response [53]. As a result, subsequent vaccine developers have attempted to generate immune responses which involve Th2 stimulation rather than Th1. Another problem in this study was that only approximately 20% of those vaccinated raised sufficient antibody titers [54].

Although clinical outcome measures did not show benefit, AN1792 was shown to have an effect on the biology of the disease. In brain autopsies from study participants who received AN1792, fewer amyloid plaques were present compared with what would be expected in an individual with longstanding AD [55,56]. Additionally, concentrations of total tau protein, a biomarker associated with neuronal loss, were slightly reduced in CSF [54]. Volumetric MRI measured greater rates of brain atrophy in vaccinated patients as compared with placebo. It was hypothesized that the reduction in brain volume was a result of removal of Aβ [57].

Over four years of follow-up data after immunization with AN1792 revealed that patients who developed an immune response in the Phase II study also demonstrated significantly reduced functional decline compared with placebo-treated patients. Long-term data also showed no differences in volume loss between antibody responders and placebo [58].

Subsequent active immunotherapy agents have entered Phase II studies. Vanutide cridificar (ACC-001) was designed to avoid the safety concerns of AN1792 by targeting the N-terminal end of Aβ, amino acids 1 to 7 (autoimmune meningoencephalitis caused by Th1 lymphocyte activation in the AN1792 study was attributed to Aβ residues 15 to 42). Vanutide cridificar was tested in multiple Phase II trials, but results have not been published. It has been reported that this compound has been discontinued [59]. Other active immunotherapy agents currently under investigation include ACI-24, CAD-106 and Affitope AD02. V950 has completed a Phase I study but has not proceeded to Phase II. The API has announced that it will study CAD-106 in cognitively normal *APOE* ε4 homozygotes age 60–75 [47].

Given the decreased immune system response of elderly patients to vaccinations, active immunotherapy may be best implemented in a younger population, possibly as part of a prevention strategy.

Passive immunotherapy: monoclonal

In contrast to active immunotherapy, which requires the patient's immune system to manufacture antibodies in response to administration of antigen, so-called passive immunotherapy consists of delivering the antibody directly to the patient, thereby bypassing the immune system. Monoclonal immunotherapy can potentially be directed against specific targets.

Bapineuzumab (AAB-001), a humanized monoclonal antibody directed against the N-terminal of Aβ, which preferentially binds insoluble amyloid, showed a suggestion of benefit for *APOE* ε4 noncarriers in Phase II studies [60,61]. However, two subsequent large Phase III studies of intravenous bapineuzumab every 13 weeks for 78 weeks for mild-to-moderate AD did not show significant differences in efficacy for either the *APOE* ε4 carriers or noncarriers on primary outcomes [62]. Furthermore, bapineuzumab treatment was associated with amyloid-related imaging abnormalities – edema/effusions (ARIA-E, previously called vasogenic edema) as well as cerebral microhemorrhages (ARIA-H), especially in *APOE* ε4 carriers. ARIA-E describes a signal abnormality on MRI FLAIR sequences thought to represent parenchymal brain edema and/or sulcal effusions thought due to extravasated intravascular fluid from shifts in amyloid, and ARIA-H refers to hemosiderin deposition detected on gradient recalled-echo/T2*-weighted sequences thought to represent blood degradation products, including microhemorrhages (< 10 mm) [63]. The development of bapineuzumab for the treatment of AD has since been halted.

Aβ monomers spontaneously aggregate and form larger soluble species of oligomers and subsequently form the insoluble fibrils that precipitate in the brain. Oligomeric Aβ has demonstrated neurotoxicity *in vitro* and *in vivo* [64], thus implicating soluble Aβ species as an attractive target.

Solanezumab, a humanized monoclonal antibody that preferentially binds soluble monomeric forms of Aβ, also did not meet primary endpoints in two large (n = 2052 total) Phase III studies testing intravenous administration

monthly for 18 months in patients with mild or moderate AD dementia [65]. However, a pooled analysis of the two studies showed a significant slowing of cognitive decline in the mild dementia group. Solanezumab showed a positive safety profile and did not show a significant increase in ARIA-E compared with placebo. A third Phase III study of solanezumab is currently underway testing only patients with mild AD dementia who have demonstrated amyloid pathology on CSF or PET.

Phase II results of intravenous and subcutaneous crenezumab were recently announced (unpublished). Although the study did not meet primary endpoints of ADAS-Cog and CDR-SB, an exploratory analysis demonstrated a significant reduction in cognitive decline in the mild dementia group in patients in the intravenous high-dose arm, echoing results of the solanezumab Phase III studies in mild-to-moderate AD. Subcutaneous crenezumab is currently being tested through the API in cognitively normal *PS-1* mutation carriers in Antioquia, Colombia [47].

A phase III study of gantenerumab in MCI due to AD was discontinued due a futility analysis; however, a separate phase III study in AD dementia continues.

Other monoclonal antibodies currently in clinical trials include ponezumab, BAN2401, BIIB037, and MEDI1814.

Both gantenerumab and solanezumab are being testing in The Dominantly Inherited Alzheimer's Network Trials Unit (DIAN-TU) study, which enrolls people with an autosomal dominantly inherited AD mutation who are cognitive normal (preclinical AD), MCI, or mild stage of AD dementia [66]. Solanezumab is also being tested in a separate preclinical AD study called the Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease (A4 Study) [67].

Passive immunotherapy has advantages over active immunotherapy given the ability to target specific domains of amyloid. Passive therapy also has a lower risk of irreversible autoimmune complications, as was seen in AN1792. However, high costs of production and administration could limit its use.

Passive immunotherapy: polyclonal

Intravenous immunoglobulin (IVIG) is a polyclonal antibody preparation derived from the blood plasma of healthy donors. It is currently used for other medical conditions such as immunodeficiency syndromes and autoimmune disorders. IVIG contains the majority of IgG antibodies in the human repertoire, approximately 0.5% of which bind to Aβ. After promising early phase results, an 18-month, Phase III study in 390 mild-to-moderate AD participants showed no significant effect, and any ongoing studies of IVIG were discontinued [49].

Tau

Even though the tau pathology of AD, neurofibrillary tangles, has been found to better correlate with AD clinical symptoms than amyloid plaques [68], the amyloid cascade hypothesis has been the dominant view regarding the pathogenesis of AD. However, given the failure of antiamyloid agents to reach primary clinical endpoints, the field has increasingly expanded to include other approaches such as tau-targeted therapies. Tau is a microtubule-associated protein that is primarily expressed in the cell bodies and axons of neuronal cells of the CNS. In AD, tau is hyper-phosphorylated, causing it to aggregate. The microtubule structure is subsequently altered resulting in impairment of microtubule function, formation of neurofibrillary tangles and ultimately cell death.

Immunotherapy

Immunotherapy agents targeting tau pathology are only now approaching clinical trials with ACI-35 and AADvac-1, active vaccines that propose to stimulate the patient's immune system to produce antibodies against phosphorylated tau protein.

Phosphokinase inhibitors

Studies targeting kinases involved in phosphorylating the tau protein have been negative, including lithium [69], valproate and tideglusib.

Tau aggregation inhibitors

Methylthioninium chloride (otherwise known as methylene blue) is thought to inhibit tau aggregation. A Phase II study has shown a good safety profile [70], and is now being tested in two Phase III studies as a 'second generation' compound, TRx0237.

Microtubule stabilizers

BMS 241027 is designed to bind and stabilize microtubules and could be beneficial in many tauopathies, including AD. A Phase I study in AD has been completed but results have not been announced. Davunetide, an intranasal neuropeptide derived from a growth factor that is thought to help stabilize microtubules, did not show efficacy. An intravenous formulation, AL-208, has been tested but results have not been published.

Antioxidant & anti-inflammatory agents

Part of the pathogenesis of AD includes microinflammation. Numerous antioxidant, anti-inflammatory and cholesterol-lowering agents have been essentially negative, including selegeline [71], coQ10, idebenone (a coQ10 analog) [72], celecoxib [73], rofecoxib [74], naproxen [75], prednisone [76], HF 0220 and

hydroxychloroquine [77]. Although some clinical trial data supports use of vitamin E (2000 IU/day) [78], a Cochrane Collaboration review concluded that there is insufficient evidence to support its use for treatment of AD [79].

The very large Alzheimer's Disease Anti-Inflammatory Prevention Trial [80] tested whether the anti-inflammatory agents celecoxib or naproxen could delay the onset of dementia in cognitively healthy elderly subjects with a family history of AD. A total of 2528 participants were randomized to three treatment arms (celecoxib, naproxen and placebo), but treatments were stopped about 3.5 years after the first person was randomized due to possible cardiovascular adverse events of this drug class that emerged from other studies. Although initial data suggested possible benefit from naproxen between 2 and 3 years after randomization [81], the report from nearly 7 years of follow-up data did not show dementia delay for either agent [82].

Cholesterol lowering & homocysteine-lowering agents

Cholesterol metabolism has been implicated in AD pathogenesis with an association of excess brain cholesterol and an increase in cerebral Aβ [83]. Clinical trials testing atorvastatin [84] and simvastatin [85] were negative. A meta-analysis of placebo-controlled trials of homocysteine-lowering agents such as vitamins B12, B6 and folic acid alone or in combination did not improve cognitive function in individuals with cognitive impairment [86].

Neuroprotectants

Several compounds thought to be neuroprotective, including propentofylline [87], were not effective. Another compound, T-817MA, is under development [88] and is currently recruiting for a Phase II trial.

Resveratrol is one of many bioactive polyphenols in certain foods, such as red grapes, blueberries, peanuts and dark chocolate that is reported to have neuroprotective effects [89]. A Phase II resveratrol study has completed, but results are pending at the writing of this article.

Considerations for future trials

Unfortunately, AD clinical trials have been a tremendous disappointment. Several possible reasons, or combination of reasons, can explain the failure of success in AD studies.

Mechanism of action

One major limiting factor is that AD occurs only in humans; because of this, animal models that approximate AD can be developed in order to carry out preclinical studies, but these may not necessarily predict the outcome of clinical studies. The mechanisms of action that have been studied thus far in AD trials have been consistent with the findings in the animal studies, but may not be the proper targets for clinical improvement in either disease modifying treatments or symptomatic improvement. For example, despite the vast evidence in support of the amyloid hypothesis, numerous failed antiamyloid studies have challenged its validity. Even within the amyloid hypothesis, it is not clear which form of amyloid will be the most beneficial target – fibrillar forms of Aβ, soluble monomeric Aβ, soluble aggregates or Aβ protofibrils.

Alternatively, even if the targets are correct, it is possible that an investigational agent is not engaging its target or is engaging to an insufficient degree. Advancements in neuroimaging, CSF assays and other biomarkers enable *in vivo* identification of the AD pathophysiological process. The use of these biomarker analyses in disease-modifying therapy studies to assess target engagement is increasingly employed in clinical trials. Biomarkers such as amyloid PET, tau PET, FDG PET, volumetric MRI and CSF markers such as Aβ and tau, as well as possible emerging serum and ocular biomarkers can aid in assessment of target engagement. For example, amyloid PET imaging is often used in antiamyloid clinical trials to observe whether the amount of cerebral amyloid has been reduced from baseline to completion of the study to help test target engagement and utility of the compound. Additionally, the growing study of genetics and AD may help to determine not only those who may progress more rapidly, but also those who may better respond to specific therapies.

It remains possible that targeting one pathologic pathway is not sufficient, and that combination therapy of multiple compounds with different mechanisms of action is required for success. Although the amyloid and tau pathways may not be mutually exclusive, at this time, no trial has studied a combination compound targeting these two modalities. However, ALZT-OP1 is an example of a combination drug program currently entering Phase III, which combines an antiamyloid aggregation and an anti-inflammatory compound. A combination of an antiamyloid monoclonal antibody agent, coupled with LY2811376, a BACE inhibitor, is in very early stages of development.

Stage of disease in AD continuum

The lack of success of AD studies has raised the question as to whether the stage of disease generally targeted (mild-to-moderate dementia stages), may be too late in the disease process for the mechanisms tested so

far to be effective. Phase III solanezumab data support this hypothesis in that a subgroup analysis showed a significant slowing of cognitive decline in subjects with mild AD dementia at baseline, but not moderate AD [65]. Recently announced (but yet unpublished) Phase II results of high-dose intravenous crenezumab also showed significant slowing of cognitive decline in an exploratory mild subgroup, but not in the moderate subgroup. The MCI stage of AD has been studied with no significant results thus far, but clinical trials are trending to include both MCI and mild stage of AD dementia.

The field of AD is also starting to see the first AD prevention studies. The technological advances in the field of *in vivo* biomarkers has allowed identification of AD pathology in cognitively normal individuals, presumably a stage of AD in which cognitive and functional changes are not yet clinically evident. The National Institute of Aging and the Alzheimer's Association has termed this the preclinical stage of AD [90] and the International Working Group for Advancing Research Diagnostic Criteria for AD termed this asymptomatic at risk for AD [91].

Amyloid plaque deposition may begin 10 years or more prior to the onset of cognitive symptoms [92,93], thus making amyloid a viable target for prevention. At this time, there are four studies recruiting patients in preclinical AD studies, and one funded trial preparing for launch:

- API: *PS-1* mutation carriers:
  - The API is currently testing subcutaneous crenezumab in a unique kindred of *PS-1* mutation carriers who are cognitively normal in the world's largest early-onset AD kindred in Antioquia, Colombia.
- API: *APOE* ε4 homozygotes:
  - In a separate trial not yet underway, the API proposes to study both CAD-106 and a BACE inhibitor in cognitively normal (*APOE*) ε4 homozygotes age 60–75 [94].
- DIAN-TU:
  - Currently, the DIAN-TU study is testing both gantenerumab and solanezumab [66] in people with an autosomal dominant inherited AD mutation without cognitive symptoms (preclinical AD) and includes MCI and mild stage of AD dementia as well. This study has a unique design that tests multiple disease-modifying therapies (from multiple pharmaceutical partners) simultaneously.
- TOMMORROW (Pioglitizone):
  - A 5-year multinational pioglitazone prevention trial is enrolling approximately 5800 cognitively normal individuals. This study is evaluating a diagnostic algorithm based on age and variants of the *APOE* and *TOMM40* genes for predicting risk of development of the MCI stage of AD, but also testing the ability of low-dose pioglitazone to delay the onset of the MCI stage of AD [95].
- Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease (A4 study):
  - The A4 trial is testing solanezumab versus placebo in approximately 1150 elderly, cognitively normal participants ages 65–85 who have elevated brain amyloid, as determined by a florbetapir PET scan over a 3-year period [67].

Clinical trial design

Clinical trial design may further impede success in AD studies. Initially AD studies were 3–6 months in duration, which may be sufficient when testing for a symptomatic effect. However, Phase III AD studies for compounds assessing putative disease-modifying treatments must be at least 18 months in duration in order to detect treatment effect in cognitive and functional endpoints due to the gradual nature of disease progression. As opposed to symptomatic agents that could show improvement soon after initiating drug, the efficacy of disease modifying agents that slow the progression of the underlying disease can only be seen after sufficient time has elapsed. As the field moves toward studying populations earlier in the course of AD such as MCI and preclinical disease, the length of the studies will need to increase to allow sufficient time to elapse to measure change; however, this increases trial complexity and cost and adds to participant/informant burden which results in a higher drop-out rate. Increasing the sample size can help increase power to detect effects of disease-modifying drugs, but also adds to the difficulty and cost of conducting the trial. Adaptive clinical trials design has been employed in some AD trials as an alternative to conventional study designs. In adaptive design, an interim analysis of data generated during the trial is used to modify the trial as it proceeds. Modifications may include sample size or dosage of study drug, for example. Another trial design that has had success in other therapeutic areas is the multi-arm, multi-compound study that shares a placebo group; this approach, which can reduce the total number of subjects needed for clinical trials, is being employed by the DIAN-TU study.

Furthermore, both the cognitive and functional endpoints often used in AD studies such as the ADAS-Cog, ADCS-ADL scale and CDR scale may not be sufficiently sensitive to detect difference between study drug and placebo for disease modification studies. In fact, in preclinical AD studies (and possibly MCI due to AD studies), functional scales will likely be of no benefit unless the study is of sufficient length to allow for cognitively normal or mildly impaired people to progress to AD dementia. Certainly, as populations earlier in the continuum of disease are being studied, more sensitive measures will be necessary. For example, the API and A4 studies independently derived their own primary outcome scales in order to find the most sensitive measures of cognitive change.

Inclusion criteria for study entry typically required National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria for probable AD while excluding other diseases that could impair cognition. Using only clinical criteria as inclusion criteria likely introduces non-AD patients into AD studies, thereby decreasing the power. In fact, in the early clinical stages of AD (MCI and mild AD dementia), substantial neurodegeneration is already apparent and cortical amyloid is already nearing peak pathological levels [93]. Thus, biomarkers that identify AD pathology such as elevated cerebral amyloid as determined by CSF analysis or an amyloid PET scan are increasingly used as an adjunct to the clinical diagnosis for entry

Executive summary

US FDA approved & available medications for Alzheimer's disease

- Donepezil
- Rivastigmine
- Galantamine
- Memantine

Symptomatic therapy

- Symptomatic therapies that have been tried with poor success include:
- Other cholinesterase inhibitors
- Nicotinic receptor agonists
- Muscarinic agonists
- *N*-methyl-D-aspartate receptor modulators
- AMPA receptor modulators
- Serotonin antagonists and agonists
- Histamine modulators

Disease modifying

- Potential disease-modifying via antiamyloid agents
- γ-secretase inhibitors/modulators
- β-secretase inhibitors
- α-secretase activators
- Anti-aggregants
- Active immunotherapy
- Passive immunotherapy (monoclonal and polyclonal)
- Potential disease-modifying tau
- Active immunotherapy
- Phosphokinase inhibitors
- Tau aggregation inhibitors
- Microtubule stabilizers
- Neuroprotective agents continue to be developed

Considerations for future trials

- The use of biomarker analyses in disease-modifying therapy studies to assess target engagement is increasingly employed in clinical trials.
- Increasingly, studies are targeting the earlier stages of disease.
- Future clinical trial design including duration of study, population size and primary cognitive and functional endpoints will need to be further optimized.
- Supported by the amyloid hypothesis, the main focus of disease-modifying therapy has been antiamyloid agents.
- Although γ-secretase inhibitors and modulators, antiaggregation agents, β-secretase inhibitors and immunotherapy have yet to show significance on primary endpoints, studies continue to test the amyloid hypothesis. Other therapeutic targets are increasingly tested.

into studies for sample enrichment. The bapineuzumab and solanezumab trials underscore the importance of sample enrichment, as these studies suggest that as many as 30% of enrolled mild subjects may not have had amyloid pathology [49]. Future studies will increasingly use biomarker data as inclusion criteria.

Conclusion

Future research in AD will include targeting earlier stages of the disease, employment of biomarkers for inclusion criteria and for outcome measures, use of combination therapy and refinement of clinical trial methods that will improve the efficiency and success of the drug development process.

Future perspective

The results of the completed crenezumab and solanezumab studies provide optimism for an antiamyloid disease modifying agent in the early stages of disease. Over the coming years, the field will continue to trend toward studying the earlier stages of the AD spectrum such as MCI due to AD (prodromal AD) and preclinical stages of the disease. With several studies entering the domain of primary and secondary prevention, the momentum in the field toward addressing these earliest stages provides further encouragement. Another reason for optimism is the advancing development of BACE inhibitors, which, given the learnings from crenezumab and solanezumab, will likely be studied in these early stages as well. The field will rely further on biomarkers for identification of the appropriate population, especially for these early stages of disease to strengthen the power and increase the likelihood of finding a drug effect. Biomarkers will also be used to help verify target engagement of study drugs and potentially be used as a surrogate to cognitive and functional endpoints in clinical trials. Future research is also likely to include creative approaches such as combination therapy of compounds with different mechanisms of action and nonamyloid targets.

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References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Bartus RT, Dean RL, Beer B *et al.* The cholinergic hypothesis of geriatric memory dysfunction. *Science* 217(4558), 408–417 (1982).

2 Davis KL, Thal LJ, Gamzu ER *et al.* A double-blind, placebo-controlled multicenter study of tacrine for Alzheimer's disease. *N. Engl. J. Med.* 327(18), 1253–1259 (1992).

3 Farlow M, Gracon SI, Hershey LA *et al.* A controlled trial of tacrine in Alzheimer's disease. The Tacrine Study Group. *JAMA* 268(18), 2523–2529 (1992).

4 Knapp JM, Knopman DS, Soloman PR *et al.* A 30-week randomized controlled trial of high-dose tacrine in patients with Alzheimer's disease. The Tacrine Study Group. *JAMA* 271(13), 985–991 (1994).

5 Rogers SL, Farlow MR, Mohs RC *et al.* A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. *Neurology* 50(1), 136–145 (1998).

6 Corey-Bloom J, Anand R, Veach J. A randomized trial evaluating the efficacy and safety of ENA 713 (rivastigmine tartrate), a new acetylcholinesterse inhibitor, in patients with mild to moderately severe Alzheimer's disease. *Int. J. Geriatr. Psychopharmacol.* 1, 55–65 (1998).

7 Tariot PN, Solomon PR, Morris JC, Kershaw P, Lilienfeld S, Ding C. A 5-month, randomized, placebo-controlled trial of galantamine in AD. The Galantamine USA-10 Study Group. *Neurology* 54(12), 2269–2276 (2000).

8 Reisberg B, Doody R, Stöffler A, Schmitt F, Ferris S, Möbius HJ. Memantine Study Group. Memantine in moderate-to-severe Alzheimer's disease. *N. Engl. J. Med.* 348(14), 1333–1341 (2003).

9 Murphy MF, Hardiman ST, Nash RJ *et al.* Evaluation of HP029 (velnacrine maleate) in Alzheimer's disease. *Ann. NY Acad. Sci.* 640, 253–262 (1991).

10 Thal LJ, Ferguson JM, Mintzer J, Raskin A, Targum SD. A 24-week randomized trial of controlled-release physostigmine in patients with Alzheimer's disease. *Neurology* 52(6), 1146–1152 (1999).

11 Imbimbo BPT, Troetel WM, Martelli P, Lucchelli F. The Eptastigmine Study Group. A 6-month double-blind, placebo-controlled trial of eptastigmine in Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 11(1), 17–24 (2000).

12 Lopez-Arrieta JM, Schneider L. Metrifonate for Alzheimer's disease. *Cochrane Database Syst. Rev.* 19(2), CD003155 (2006).

13 Rafii MS, Walsh S, Little JT *et al.* A Phase II trial of huperzine A in mild to moderate Alzheimer disease. *Neurology* 76(16), 1389–1394 (2011).

14 White HK, Levin ED. Four-week nicotine skin patch treatment effects on cognitive performance in Alzheimer's disease. *Psychopharmacology (Berl.)* 143(2), 158–165 (1999).

15 Dunbar GC, Inglis F, Kuchibhatla R, Sharma T, Tomlinson MR, Wamsley J. Effect of ispronicline, a

neuronal nicotinic acetylcholine receptor partial agonist, in subjects with age associated memory impairment (AAMI). *J. Psychopharmacol.* 21(2), 171–178 (2007).

16 Geerts H. α7 Nicotinic receptor modulators for cognitive deficits in schizophrenia and Alzheimer's disease. *Expert Opin. Investig. Drugs* 21(1), 59–65 (2012).

17 McArthur RA, Gray J, Schreiber R. Cognitive effects of muscarinic M1 functional agonists in non-human primates and clinical trials. *Curr. Opin. Investig. Drugs* 11(7), 740–760 (2010).

18 Jones RW, Laake K, Oeksengard AR. D-cycloserine for Alzheimer's disease. *Cochrane Database Syst. Rev.* 2, CD003153 (2002).

19 Sonkusare SK, Kaul CL, Rarao P. Dementia of Alzheimer's disease and other neurodegenerative disorders – memantine, a new hope. *Pharmacol. Res.* 51(1), 1–17 (2006).

20 Mancuso C, Siciliano R, Barone E, Butterfield DA, Preziosi P. Pharmacologists and Alzheimer disease therapy: to boldly go where no scientist has gone before. *Expert Opin. Investig. Drugs* 20(9), 1243–1261 (2011).

21 Ramirez MJ, Lai MKP, Tordera RM, Francis PT. Serotonergic therapies for cognitive symptoms in Alzheimer's disease: rationale and current status. *Drugs* 74(7), 729–736 (2014).

22 Douillet P, Orgogozo JM. What we have learned from the xaliproden Sanofi-Aventis trials. *J. Nutr. Health Aging* 13(4), 365–366 (2009).

23 Ji W, Ha I. Drug development for Alzheimer's disease: recent progress. *Exp. Neurobiol.* 19(3), 120–131 (2010).

24 Egan M, Yaari R, Liu L *et al.* Pilot randomized controlled study of a histamine receptor inverse agonist in the symptomatic treatment of AD. *Curr. Alzheimer Res.* 9(4), 481–490 (2012).

25 Grove RA, Harrington CM, Mahler A *et al.* A randomized, double-blind, placebo-controlled, 16-week study of the H3 receptor antagonist, GSK239512 as a monotherapy in subjects with mild-to-moderate Alzheimer's disease. *Curr. Alzheimer Res.* 11(1), 47–58 (2014).

26 Thompson TL, Filley CM, Mitchell WD, Culig KM, LoVerde M, Byyny RL. Lack of efficacy of hydergine in patients with Alzheimer's disease. *N. Engl. J. Med.* 323(7), 445–448 (1990).

27 Schneider LS, Olin JT. Overview of clinical trials of hydergine in dementia. *Arch. Neurol.* 51(8), 787–798 (1994).

28 Craft S, Baker LD, Montine TJ *et al.* Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. *Arch. Neurol.* 69(1), 29–38 (2012).

29 Harrington C, Sawchak S, Chiang C *et al.* 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 3 studies. *Curr. Alzheimer Res.* 8(5), 592–606 (2011).

30 Hanyu H, Sato T, Kiuchi A, Sakurai H, Iwamoto T. Pioglitazone improved cognition in a pilot study on patients with Alzheimer's disease and mild cognitive impairment with diabetes mellitus. *J. Am. Geriatr. Soc.* 57(1), 177–179, (2009).

31 Sato T, Hanyu H, Hirao K, Kanetaka H, Sakurai H, Iwamoto T. Efficacy of PPAR-γ agonist pioglitazone in mild Alzheimer disease. *Neurobiol. Aging.* 32(9), 1626–1633 (2011).

32 Hamaguchi T, Ono K, Yamada M. Curcumin and Alzheimer's disease. *CNS Neurosci. Ther.* 16(5), 285–297 (2010).

33 Mulnard RA, Cotman CW, Kawas C *et al.* Estrogen replacement therapy for treatment of mild to moderate Alzheimer's disease: a randomized controlled trial. Alzheimer's Disease Cooperative Study. *JAMA* 283(8), 1007–1015 (2000).

34 Henderson VW, Paganini-Hill A, Miller BL *et al.* Estrogen for Alzheimer's disease in women: randomized, double-blind, placebo-controlled trial. *Neurology* 54(2), 295–301 (2000).

35 Thal LJ, Carta A, Clarke WR *et al.* A 1-year multicenter placebo-controlled study of acetyl-L-carnitine in patients with Alzheimer's disease. *Neurology* 47(3), 705–711 (1996).

36 Le Bars PL, Katz MM, Berman N, Itil TM, Freedman 0.AM, Schatzberg AF. A placebo-controlled, double-blind, randomized trial of an extract of ginkgo biloba for dementia. North American EGb Study Group. *JAMA* 278(16), 1327–1332 (1997).

37 Amaducci L, Maurer K, Winblad B *et al.* A long-term, double-blind, placebo-controlled efficacy and safety study of nicergoline in patients with mild to moderate Alzheimer's disease. *Eur. Neuropsychopharmacol.* 9, s323–s324 (1999).

38 Sevigny JJ, Ryan JM, van Dyck CH, Peng Y, Lines CR, Nessly ML. On behalf of the MK-677 Protocol 30 Study Group. Growth hormone secretagogue MK-677: no clinical effect on AD progression in a randomized trial. *Neurology* 71(21), 1702–1708 (2008).

39 Quinn JF, Raman R, Thomas RG *et al.* Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. *JAMA* 304(17), 1903–1911 (2010).

40 Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256(5054), 184–185 (1992).

- **This paper details the amyloid hypothesis which has been central in Alzheimer's disease (AD) drug development.**

41 Doody RS, Raman R, Farlow M *et al.* A Phase 3 trial of semagacestat for treatment of Alzheimer's disease. *N. Engl. J. Med.* 369(4), 341–350 (2013).

42 Coric V, van Dyck CH, Salloway S *et al.* Safety and tolerability of the γ-secretase inhibitor avagacestat in a Phase 2 study of mild to moderate Alzheimer disease. *Arch. Neurol.* 69(11), 1430–1440 (2012).

43 Green RC, Schneider LS, Amato DA *et al.* Effect of tarenflurbil on cognitive decline and activities of daily living in patients with mild Alzheimer disease. *JAMA* 302(23), 2557–2564 (2009).

44 Schneider LS, Mangialasche F, Andreasen N *et al.* Clinical trials and late-stage drug development for Alzheimer's disease: an appraisal from 1984 to 2014. *J. Intern. Med.* 275(3), 251–283 (2014).

- **Reviews the history of Alzheimer's disease (AD) clinical trials from 1984 to the present.**

45 Jonsson T, Atwal J, Steinberg S *et al.* A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* 488(7409), 96–99 (2012).

46 Yan R, Vassar R. Targeting the β secretase BACE1 for Alzheimer's disease therapy. *Lancet Neurol.* 13(3), 319–329 (2014).

47 Banner Alzheimer's Institute (BAI) announces partnership with novartis in new study of Alzheimer's prevention treatments. Two drugs to be studied in North America and Europe in volunteers at high risk for disease [press release]. BAI, Copenhagen, Denmark, July 15, 2014. http://banneralz.org/media/43552/banner__news_release_7_14_14_final.pdf

48 Wang J, Dickson DW, Trojanowski JQ, Lee VM. The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. *Exp. Neurol.* 158(2), 328–337 (1999).

49 Lannfelt L, Relkin NR, Siemers ER. Amyloid-ß-directed immunotherapy for Alzheimer's disease. *J. Intern. Med.* 275(3), 284–295 (2014).

- **Details the history and prior studies of immunotherapy for AD.**

50 Aisen PS, Gauthier S, Ferris SH *et al.* Tramiprosate in mild-to-moderate Alzheimer's disease – a randomized, double-blind, placebo-controlled, multi-centre study (the Alphase Study). *Arch. Med. Sci.* 7(1), 102–111 (2011).

51 Galasko D, Bell J, Mancuso JY, *et al.* Alzheimer's disease cooperative study. Clinical trial of an inhibitor of RAGE-Aβ interactions in Alzheimer disease. *Neurology* 82(17), 1536–1542 (2014).

52 Salloway S, Sperling R, Keren R *et al.* A Phase 2 randomized trial of ELND005, scyllo-inositol, in mild to moderate Alzheimer disease. *Neurology* 77(13), 1253–1262 (2011).

53 Lemere CA. Developing novel immunogens for a safe and effective Alzheimer's disease vaccine. In: *Neurotherapy: Progress in Restorative Neuroscience and Neurology.* Verhaagen J, Hol EM, Huitenga I *et al.* (Eds). Elsevier, Oxford, UK, 83–93 (2009).

54 Gilman S, Koller M, Black RS *et al.* Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology* 64(9), 1553–1562 (2005).

55 Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat. Med.* 9(4), 448–452 (2003).

56 Patton RL, Kalback WM, Esh CL *et al.* Amyloid-beta peptide remnants in AN-1792-immunized Alzheimer's disease patients: a biochemical analysis. *Am. J. Pathol.* 169(3), 1048–1063 (2006).

57 Fox NC, Black RS, Gilman S *et al.* Effects of Ab immunization (AN1792) on MRI measures of cerebral volume in Alzheimer disease. *Neurology* 64(9), 1563–1572 (2005).

58 Vellas B, Black R, Thal LJ *et al.* Long-term follow-up of patients immunized with AN1792: reduced functional decline in antibody responders. *Curr. Alzheimer Res.* 6, 144–151 (2009).

- **Reports the long-term results on the first large-scale immunotherapy study in AD.**

59 Therapeutics. Vanutide-cridificar, ALZFORUM. http://alzforum.org/therapeutics/vanutide-cridificar.

60 Salloway S, Sperling R, Gilman S *et al.* A Phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. *Neurology* 73(24), 2061–2070, (2009).

61 Rinne JO, Brooks MN, Fox NC *et al.* 11C-PiB PET assessment of change in fibrillar amyloid-β load in patients with Alzheimer's disease treated with bapineuzumab: a Phase 2, double-blind, placebo-controlled, ascending-dose study. *Lancet Neurol.* 9(4), 363–372 (2010).

62 Salloway S, Sperling R, Fox NC *et al.* Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N. Engl. J. Med.* 370(4), 322–333 (2014).

63 Barakos J, Sperling R, Salloway S *et al.* MR imaging features of amyloid-related imaging abnormalities. *AJNR Am. J. Neuroradiol.* 34(10), 1958–1965 (2013).

64 Walsh DM, Klyubin I, Fadeeva JV *et al.* Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature* 416(6880), 535–539 (2002).

65 Doody RS, Thomas RG, Farlow M *et al.* Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *N. Engl. J. Med.* 370(4), 311–321 (2014).

66 Mills SM, Mallmann J, Santacruz AM *et al.* Preclinical trials in autosomal dominant AD: implementation of the DIAN-TU trial. *Rev. Neurol. (Paris)* 169(10), 737–743 (2013).

67 Sperling RA, Rentz DM, Johnson KA *et al.* The A4 study: stopping AD before symptoms begin? *Sci. Transl. Med.* 6(228), 228fs13 (2014).

- **Details the A4 trial, one of the four major preclinical AD studies that is currently underway.**

68 Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* 42(3 Pt 1), 631–639 (1992).

69 Hampel H, Ewers M, Burger K *et al.* Lithium trial in Alzheimer's disease: a randomized, single-blind, placebo-controlled, multicenter 10-week study. *J. Clin. Psychiatry* 70(6), 922–931 (2009).

70 Wischik CM, Bentham P, Wischik DJ, Seng KM. O3–04–07: tau aggregation inhibitor (TAI) therapy with rember™ arrests disease progression in mild and moderate Alzheimer's disease over 50 weeks. *Alzheimers Dement.* 4, T167 (2008).

71 Birks J, Flicker L. Selegiline for Alzheimer's disease. *Cochrane Database Syst. Rev.* (1) CD000442 (2003).

72 Thal LJ, Grundman M, Berg J *et al.* Idebenone treatment fails to slow cognitive decline in Alzheimer's disease. *Neurology* 61(11), 1498–1502 (2003).

73 Soininen H, West C, Robbins J, Niculescu L. Long-term efficacy and safety of celecoxib in Alzheimer's disease. *Dement Geriatr. Cogn. Disord.* 23(1), 8–21 (2007).

74 Reines SA, Block GA, Morris JC *et al.* Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. *Neurology* 62(1), 66–71 (2004).

75 Aisen P, Schafer K, Grundman M *et al.* Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial. *JAMA* 289(21), 2819–2826 (2003).

76 Aisen PS, Davis KL, Berg JD *et al.* A randomized controlled trial of prednisone in Alzheimer's disease. Alzheimer's Disease Cooperative Study. *Neurology* 54(3), 588–593 (2000).

77 Van Gool WA, Weinstein HC, Scheltens P, Walstra GJ. Effect of hydroxychloroquine on progression of dementia in early Alzheimer's disease: an 18-month randomised, double-blind, placebo-controlled study. *Lancet* 358(9280), 455–460 (2001).

78 Sano M, Ernesto C, Klauber MR *et al.* Rationale and design of a multicenter study of selegiline and a-tocopherol in the treatment of Alzheimer's disease using novel clinical outcomes. *Alzheimer Dis. Assoc. Disord.* 10(3), 132–140 (1996).

79 Farina N, Isaac MG, Clark AR *et al.* Vitamin E for Alzheimer's dementia and mild cognitive impairment. *Cochrane Database Syst. Rev.* doi:10.1002/14651858 (2012) (Epub ahead of print).

80 Martin BK, Szekely C, Brandt J *et al.* Cognitive function over time in the Alzheimer's disease Anti-inflammatory Prevention Trial (ADAPT): results of a randomized, controlled trial of naproxen and celecoxib. *Arch. Neurol.* 65(7), 896–905 (2008).

81 Breitner JC, Baker LD, Montine TJ, Meinert CL, Lyketsos CG, Ashe KH. Extended results of the Alzheimer's disease anti-inflammatory prevention trial. *Alzheimers Dement.* 7(4), 402–411 (2001).

82 ADAPT-FS Research Group. Alzheimer's disease anti-inflammatory prevention trial research group. Results of a follow-up study to the randomized Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT). *Alzheimers Dement.* 9(6), 714–723 (2013).

83 Shobab LA, Hsiung GY, Feldman HH. Cholesterol in Alzheimer's disease. *Lancet Neurol.* 4(12), 841–852 (2005).

84 Feldman HH, Doody RS, Kivipelto M *et al.* Randomized controlled trial of atorvastatin in mild to moderate Alzheimer disease. *Neurology* 74(12), 956–964 (2010).

85 Sano M, Bell KL, Galasko D *et al.* A randomized, double-blind, placebo-controlled trial of simvastatin to treat Alzheimer disease. *Neurology* 77(6), 556–563 (2011).

86 Ford AH, Almeida OP. Effect of homocysteine lowering treatment on cognitive function: a systematic review and meta-analysis of randomized controlled trials. *J. Alzheimers Dis.* 29(1), 133–149 (2012).

87 Kittner B, Rossner M, Rother M. Clinical trials in dementia with propentofylline. *Ann. NY Acad. Sci.* 826, 307–316 (1997).

88 Schneider LS, Farlow MR, Porsteinsson A, Shimakura A, Nakagawa M, Iwakami N. The neuroprotective and neurotrophic agent T-817MA for Alzheimer disease: randomized, double-blind, placebo-controlled proof of concept trial outcomes (O3–06–06). *Alzheimers Dement.* 9(4), 530–531 (2013).

89 Kim D, Nguyen MD, Dobbin MM *et al.* SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. *EMBO J.* 26(13), 3169–3179 (2007).

90 Sperling RA, Aisen PS, Beckett LA *et al.* Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7(3), 280–292 (2011).

91 Dubois B, Feldman HH, Jacova C *et al.* Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet Neurol.* 13(6), 614–629 (2014).

92 Bateman RJ, Xiong C, Benzinger TLS *et al.* Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N. Engl. J. Med.* 367(9), 795–804 (2012).

•• **Pivotal paper which describes the pathophysiological changes in AD biomarkers and changes in cognition in the earliest states of autosomal dominant AD.**

93 Jack CR Jr, Knopman DS, Jagust WJ *et al.* Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* 12(2), 207–216 (2013).

•• **Hallmark paper describes the changes in biomarkers and cognition throughout the AD spectrum.**

94 Roses AD, Saunders AM, Lutz MW *et al.* New applications of disease genetics and pharmacogenetics to drug development. *Curr. Opin. Pharmacol.* doi:10.1016/j.coph.2013.12.002. (2014).

95 Reiman EM, Langbaum JB, Fleisher AS *et al.* Alzheimer's prevention initiative: a plan to accelerate the evaluation of presymptomatic treatments. *J. Alzheimers Dis.* 26(Suppl. 3), 321–329 (2011).

• **Details the Alzheimer's Prevention Initiative study, one of the four major preclinical AD studies currently underway.**