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

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
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, subcomeline (SB 202026), talsacilidine 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 5HT1A antagonist Lecozotan was discontinued after Phase II due to poor tolerability [21]. The 5-HT1A 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 5HT1 agonist PRX-3140, 5HT1 partial agonists such as PF04995275 and RQ-9 and 5-HT6 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 H1 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 H2 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-
ergic 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 LY2886721 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/TPP488, 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 Aβ1–42, 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 brain atrophy in vaccinated patients as 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 cridifiatic (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 AB. 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 anti-amyloid agents to reach primary clinical endpoints, the field has increasingly expanded to include other approaches such as τ-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 tideglsib.

**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 micro-inflammation. 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:** for launch:
  - 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].

**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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• of interest; ** of considerable interest
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• This paper details the amyloid hypothesis which has been central in Alzheimer’s disease (AD) drug development.


• Reviews the history of Alzheimer’s disease (AD) clinical trials from 1984 to the present.

• Details the history and prior studies of immunotherapy for AD.

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

• Details the A4 trial, one of the four major preclinical AD studies that is currently underway.
Alzheimer’s disease clinical trials: past failures & future opportunities

Clinical Trial Perspective


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


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


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