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## Alzheimer's Disease: Dawn of a New Era?

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## Alzheimer's Disease: Dawn of a New Era?

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**ABSTRACT** - Alzheimer's disease (AD) is an irreversible neurodegenerative disease characterized by a progressive decline in cognition and memory, leading to significant impairment in daily activities and ultimately death. It is the most common cause of dementia, the prevalence of which increases with age; however, age is not the only predisposing factor. The pathology of this cognitive impairing disease is still not completely understood, which has limited the development of valid therapeutic options. Recent years have witnessed a wide range of novel approaches to combat this disease, so that they greatly increased our understanding of the disease and of the unique drug development issues associated with this disease. In this paper, we provide a brief overview of the history, the clinical presentation and diagnosis, and we undertake a comprehensive review of the various approaches that have been brought to clinical trials in recent years, including immunotherapeutic approaches, tau-targeted strategies, neurotransmitter-based therapies, neurotropic and hematopoietic growth factors, and antioxidant therapies, trying to highlight the lessons learned from these approaches.

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### INTRODUCTION

The progressive growth of the elderly population associated with constant increase in life expectancy, our limited understanding of the underlying mechanisms involved in the etiology of the disease, and the lack of reliable and effective preventive and disease-modifying therapies available, has rendered Alzheimer's disease (AD) one of the great health challenges of the 21st century. AD is an irreversible neurological disorder that destroys cognitive function including loss of memory, judgment, reasoning, and behavioral control (1). It has serious impact on social functioning, disruption of quality of life, and impairment of activities of daily living. It is the most common cause of dementia that begins slowly and worsens over time with a prevalence that increases with age (2). A complex set of cellular events, including feedback and feedforward responses of astrocytes, microglia, and vasculature might be involved in the etiology of AD, which has recently been reviewed by Strooper and Karran (3). Increased understanding of the underlying mechanisms involved in the etiology of the disease during past two decades has led to the development of a wide range of novel therapeutic

approaches. In this manuscript, we focus mainly on the therapeutic approaches that have entered clinical trials.

### History

Age-related dementia has been recognized since ancient times. One of the earliest references to dementia occurring with age is attributed to Pythagoras, a 7<sup>th</sup> century BC Greek physician. Pythagoras divided the human life cycle into five stages commencing at ages 7, 21, 49, 63, and 81 years-old, describing the last two stages of life as the "senium", a period characterized by a regression in mental capacity and a return to the "imbecility of the first epoch of infancy" (4). In 500 BC, the Greek Judge Solon recognized the impairments on decision making process that could be brought upon by old age, revised laws regarding inheritances and added the provision that "judgment ... [be] not

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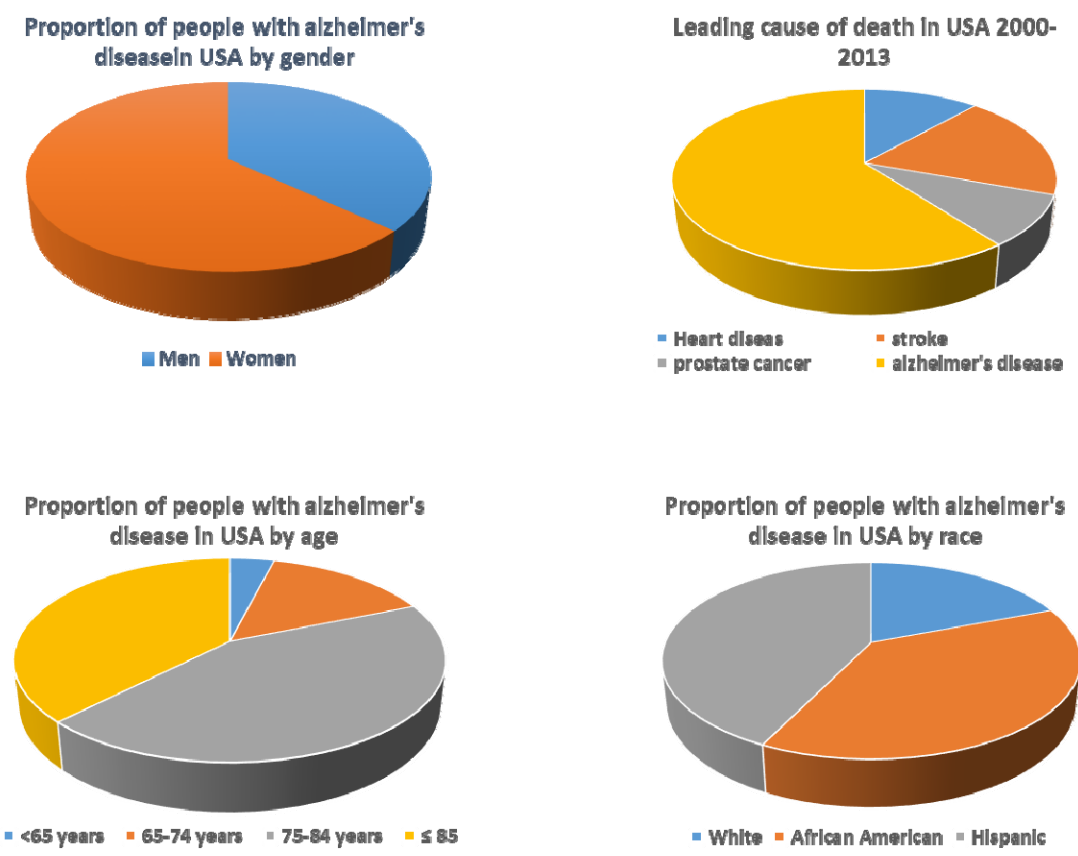
**Corresponding Authors:** Hamidreza Montazeri Aliabadi, PhD, School of Pharmacy Chapman University; #211, 9401 Jeronimo Road; Irvine, CA 92618, USA. E-mail: [montazer@chapman.edu](mailto:montazer@chapman.edu); Viet-Huong V. Nguyen, PharmD, MPH, MS, BCPS, Chapman University School of Pharmacy; 9401 Jeronimo Road Rm 221; Irvine, CA 92618, USA. E-mail: [vinguyen@chapman.edu](mailto:vinguyen@chapman.edu) - The first three authors contributed equally to this work.

impaired by pain, violence, drugs, old age, or the persuasion of a woman” in the making of wills (5). In the 13<sup>th</sup> Century, Friar Roger Bacon recognized that “age is the home of forgetfulness” and that loss of memory and cognitive function was related to a disorder of the brain and not the heart, dispelling common notions of that time (4). However, he further recognized that specific cognitive deficits may be attributed to injuries in different brain areas (6). Dementia, as an abnormal process of aging was first recognized in 1901 by Dr. Alois Alzheimer, after whom Alzheimer’s disease is named. At that time, Dr. Alzheimer began treating a 51-year old woman named Auguste who had symptoms of short-term memory loss and confusion. Dr. Alzheimer followed her case until she died in 1906, after which he studied her brain and determined that her problem was not caused by normal dementia due to aging, but a larger health issue. She was the first confirmed case of Alzheimer’s disease. In 1976, neurologist Dr. Robert Katzman, a pioneer in Alzheimer’s research, identified Alzheimer’s as the most common form of dementia and proposed that it may be a common

cause of death calling it a “major killer” (7). In 1993, apolipoprotein E (*APOE*), which is located on chromosome 19, was the first gene found to be related to the risk of Alzheimer’s. Other genes were subsequently found to be linked to AD (8) identifying possible genetic etiologies for some forms of AD.

**Epidemiology**

It is estimated that approximately 44 million people worldwide are living with Alzheimer's disease. However, only 11 million have been officially diagnosed with AD. Women are more likely to develop Alzheimer's disease than men of the same age. Alzheimer's disease is most common in Western Europe, followed by North America, and is least prevalent in sub-Saharan Africa (9). The global cost of AD and dementia was estimated at 605 billion dollars in 2015. From 2000-2013, AD was the 6<sup>th</sup> leading cause of death in the United States (10), and it is expected to be the third leading cause of death of elderly after cancer and heart disease. AD is also the only disease in the top ten causes of death that cannot be stopped,



**Figure 1.** Statistical data for Alzheimer’s disease in United States, based on the data extracted from [10].

cured, or prevented. Among 5.3 million Americans with AD, 5.1 million are aged 65 and older. **Figure 1** summarizes the distribution of AD based on age, gender, and race.

### Etiology

The etiology of AD is uncertain; however, genetics, environmental, and developmental components are likely to play a role. The greatest risk factor for AD is advancing age, although genetics has also been extensively studied. Mutations in three genes, amyloid precursor protein (APP), presenilin (PS)-1 and PS-2 (both affecting APP processing and production of amyloid-beta peptides), cause early-onset (<60 years) autosomal dominant AD. Early onset-AD, however, accounts for only 1% of AD cases (11). Late-onset AD (>60 years) has most often been genetically linked to apolipoprotein (Apo) E  $\epsilon$ 4. *Apo E4* has a gene-dose effect on increasing the risk and lowering the age of both familial and sporadic forms of late-onset AD. While *Apo E4* is the top gene associated with late onset-AD gene, other genes that may modulate the risk of late-onset AD include *CLU*, *CRI*, *PICALM*, *BIN1*, *SORL1*, *GAB2*, *ABCA7*, *MS4A4/MS4A6E*, *CD2AP*, *CD33*, *APHA1*, and *HLA-DRB1/5*. Other risk factors for AD include head trauma, female gender, learning disabilities, education level, homocysteine levels, socioeconomic status, and previous depression (12). Importantly, vascular risk factors including diabetes and hyperlipidemia are additional risk factors for AD (13, 14). However, age and the *Apo E4* allele continue to be the most significant risk factors for the development of AD along with female sex (15). The greater prevalence of AD in women could be attributed to the higher life expectancy in women, since the incidence of AD in earlier ages is comparable between sexes (16).

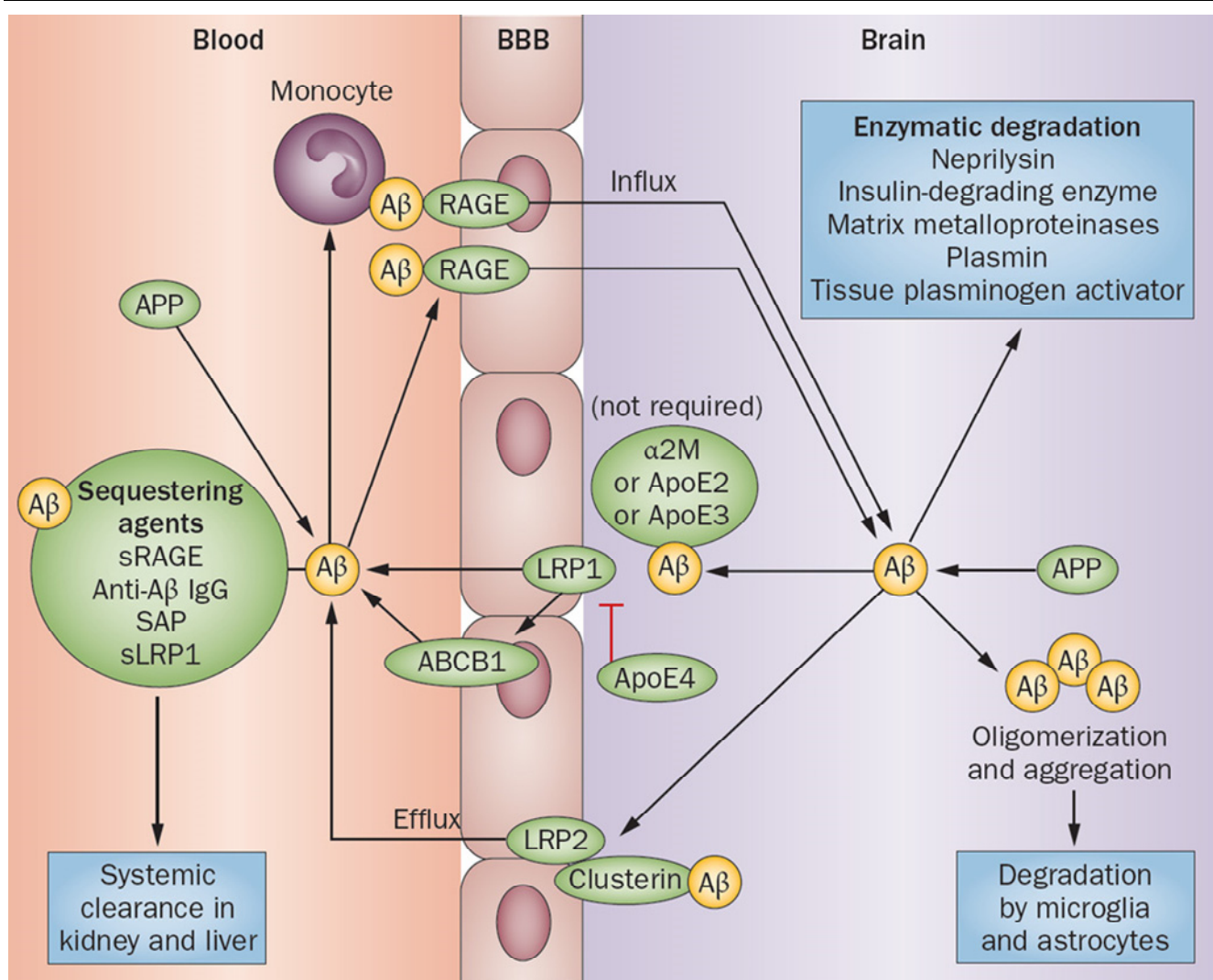
### Pathophysiology of AD

AD is a progressive neurodegenerative brain disorder. At the cellular level, AD is characterized by a progressive loss of cortical neurons most notable in the entorhinal cortex, hippocampus and posterior cingulate cortex (12). The neuronal damage is believed to be related to the deposition of abnormal proteins, which are the hallmark pathological lesions of AD known as the “plaques”, found in the extracellular space, and “tangles”, inside the cells. The neurotic plaques characteristics

of AD include amyloid deposits consisting of amorphous aggregates of misfolded amyloid-beta ( $A\beta$ ) protein (29). The “Amyloid Hypothesis” assumes a central role for  $A\beta$  in the pathogenesis of AD and characterizes AD as a “protein misfolding disease” which leads to aggregation and toxic accumulation of  $A\beta$  in the brain (17, 18). However, although accumulation of  $A\beta$  has been linked to AD; the role of the protein in AD is not entirely clear.

$A\beta$  is produced from a precursor protein known as amyloid precursor protein (APP), a highly conserved membrane protein mainly expressed around the synapse of neuronal tissue (19). APP seems to be involved in neuronal plasticity and synapse formation (20). It is metabolized via two distinct pathways: (i) non-amyloidogenic pathway (due to the non-aggregating nature of the products); and (ii) amyloidogenic pathway responsible for  $A\beta$  synthesis. The non-amyloidogenic metabolic pathway is initiated by  $\alpha$ -secretase and further processed by  $\gamma$ -secretase, which are members of a family of proteolytic proteins with a disintegrin and metalloprotease domain (ADAM) (21). The amyloidogenic pathway is initiated through cleavage of APP by  $\beta$ -secretase (also known as beta-site APP cleaving enzyme; BACE1), and again processed by  $\gamma$ -secretase (22). The  $A\beta$  fragments from the amyloidogenic pathway accumulate to form hard, insoluble plaques that are toxic to neurons, and can lead to neuronal death.

$A\beta$  clearance is also affected by the Apolipoprotein E (ApoE), a lipid binding protein involved in the transportation of cholesterol and triglycerides to different tissues, including brain (23).  $A\beta$  binds to various regions of ApoE, and each  $A\beta$ -ApoE complex then participates in a different metabolic pathway, which includes: (1) clearance by the brain blood barrier; (2) degradation by amyloid degrading enzyme (ADE); and, (3) deposition of  $A\beta$  into the cells. ApoE exists in different isomers, and each  $A\beta$ -ApoE complex is believed to have different rates of catabolism. Catabolism of  $A\beta$ -Apo E4 has been found to be specifically reduced with age, leading to reduced availability of ApoE in brain, which in turn results in accumulation of toxic cholesterol and  $A\beta$  oligomers, increasing the risk of AD (24), while Apo E2 and APO E3 eliminate  $A\beta$  by degradation or transport (25). This mechanism is illustrated in **Figure 2** (reproduced from (26)).



**Figure 2.** LRP1 mediates efflux of unbound Aβ and Aβ bound to ApoE2, ApoE3 or α2M from the brain parenchyma into the blood with the help of ABCB1; ApoE4 inhibits this transport process. Figure reprinted with permission from Tarasoff-Conway JM, et al. *Nat Rev Neurol.* 2015;11(8):457-70 [26].

Although abnormal aggregation and deposition of Aβ has attracted popular attention in the last decade as the primary causative mechanism of AD, it has been proposed that a second mechanism might play an equally important role in the pathophysiology of AD (27) via formation of neurofibrillary tangles. These tangles are insoluble fibers found within the somatodendritic and axons in AD patients (28). Neurofibrillary tangles primarily consist of abnormal aggregation of a protein called tau. Tau was originally recognized as the main microtubule-associated protein in the mammalian brain in 1975 (29), and is mostly expressed in axons, where it stabilizes the

microtubules and promotes transportation of nutrients and other important molecules. Axon deficits observed in AD have been at least partially linked to aberrant hyperphosphorylation of tau, which reduces the capability of Tau in stabilizing the microtubules (27). In AD, hyperphosphorylated tau has been identified as the primary component of the neurofibrillary tangles (30, 31). The formation of these tangles correlates with the severity of AD (32, 33). It has also been shown that ApoE ε4 is associated with increased tau accumulation (28).

**Clinical features of Alzheimer’s Disease**  
AD is characterized by a progressive loss of

memory and other cognitive functions that over time lead to aphasia, apraxia, agnosia, and reduced visual perception. Changes in memory and daily activity are usually the first changes noted at the time of diagnosis, although many patients may also display other subtle symptoms of cognitive impairment (34, 35). These include symptoms such as forgetting words, difficulty remembering names, reduced ability to solve daily problems, and geographic disorientation to the extent that an Alzheimer's patient will usually lose his way home (36). In later stages, neuropsychiatric symptoms appear including depression, agitation and hallucinations with gradual and progressive loss of self-awareness and insight (36). In early stages a patient can care for his/her basic needs and can participate in some activities and hobbies. During the late stages of the disease, patients lose the ability to manage basic activities of daily life, such as personal hygiene, toileting, eating, and dressing. The duration from the onset of the disease to death is approximately 7 years (37).

### Diagnosis of AD

There is no specific test to detect Alzheimer's disease, and the diagnosis is based on clinical history obtained from the patient and the patient's family. Assessment of cognitive function by mental status examination is a necessary component of AD diagnosis. Patients diagnosed with AD are classified based on criteria as probable AD or possible AD with and without evidence of AD pathophysiologic processes. Laboratory and neuroimaging studies are used as an adjunct to the clinical criteria for AD. Brain imaging studies such as MRI and PET scans, can support the diagnosis of AD, only if there is evidence of abnormality such as brain atrophy or decreased fluorodeoxyglucose (FDG) uptake on PET, as biomarkers for neurodegeneration and evidence of the downstream effects of AD pathophysiologic processes (**Figure 3**; (38)). Positive PET amyloid imaging can also contribute to the clinical diagnosis of AD. Laboratory studies such as low CSF AB42, also a biomarker of brain amyloid-beta protein deposition and elevated CSF tau (total and phosphorylated), are also evidence of AD pathophysiologic processes.

Currently, AD can only be diagnosed based on symptoms. However, the pathophysiologic processes of AD begin long before the symptomatic

phases appear. Therefore, literature suggest that AD should be divided into three phases: (a) Pre-clinical AD: Very early signs and symptoms: patient has more memory problems than normal, but symptoms are not severe, and their ability to participate in normal daily activity is not impaired (39); (b) Mild cognitive impairment (MCI): mild changes in thinking and memory, but daily activity is not compromised. Patients are often diagnosed in this stage (40); and (c) Clinical AD: thought, behavior and memory symptoms that impair daily activity and life (41). Use of the aforementioned biomarkers and other biomarkers that measure neuronal integrity, amyloid plaques and neurofibrillary tangles (which are present before cognitive deficits) will help to identify AD patients prior to symptoms and pave the way for early treatment.

### Therapeutic Approaches

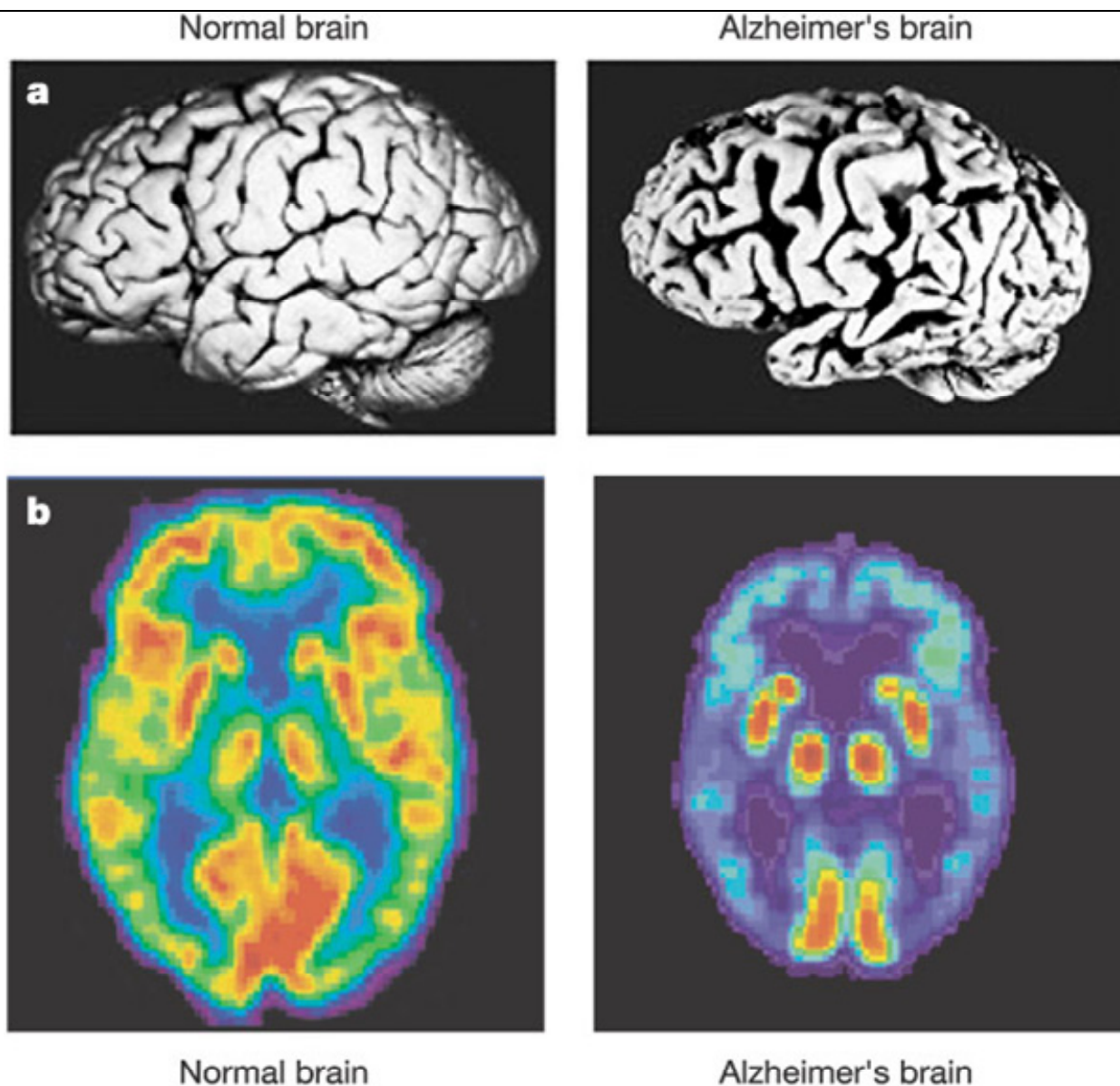
The non-pharmacological approaches to prevent AD include relaxing activities, such as listening to music or cooking that are speculated to improve the neural efficacy of cognitive processing and emotional and behavioral functioning (42). On the other hand, the therapeutic agents currently in the market do not impact the progression of AD and are only expected to alter the cognitive symptoms, which appear in later stages of AD. In the next sections we will review the most recent attempts in search for more effective therapeutic approaches.

### Beta Amyloid Therapy

#### Active immunotherapy

Active immunization (vaccination) against A $\beta$  is one of the therapeutic approaches for AD that has been pursued in the past two decades. Active immunization against A $\beta$ 1-42 (the predominant form of beta-amyloid found in the senile plaques of AD) in mouse models of AD has been successful in prevention and reversal of amyloid plaques, neurotic dystrophy, synaptic loss, and gliosis with correlating improvement of impaired behavioral performance. Active immunization was first attempted in humans in 2000 with the first-generation vaccine AN-1792, a synthetic full-length A $\beta$ 1-42 peptide linked to QS-21, a carrier made of inactivated diphtheria toxin for stimulation of immune system. This first attempt caused an aseptic meningoencephalitis observed in 6% (18/300) of vaccinated patients (43).





### Pet scans (glucose utilization)

**Figure 3.** Neuroimaging studies: a, compared with the normal brain of a healthy person on the right, the brain of an Alzheimer's disease patient exhibits marked atrophy as evidenced by the increased prominence of the sulci and gyri on the surface of the AD brain, particularly in the temporal and frontal lobes; b, Sagittal positron emission tomography (PET) images of a normal and AD brain show glucose uptake in a normal control subject on the left (red and yellow indicate high levels of glucose uptake), while the brain of Alzheimer's patient on the right exhibits large decreases in energy metabolism in the frontal cortex (top of PET image) and temporal lobes (sides of PET image). Reprinted from [38].

This was later attributed to a T-cell mediated response to A $\beta$  residues 15-42 and the phase II trial, which began in AD patients in late 2001 was terminated just a few months into the trial in early 2002 (NCT00021723). Further supporting early termination was data showing that the immunological response to the vaccine was weak,

with only 20% of patients producing a significant antibody response primarily against the N-terminus. However, a later 4.6 year follow-up revealed that vaccinated AD patients reported a functional benefit in the years after vaccination and postmortem pathology examination of vaccinated patient brains found marked clearance of insoluble amyloid



plaques. Phospho-tau analysis showed a reduction of aggregated tau in neuronal processes (44). These findings encouraged further development of other candidates that would avoid the above problems for active vaccination.

Second-generation vaccines have been designed using shorter A $\beta$  peptide segments directed at the N-terminal end of the A $\beta$  protein, which was believed to contain a B-cell epitope while avoiding T-cell activation from A $\beta$  residues 15-42. Many of these second generation vaccines including ACC-001, Affitope AD-02, V-950, and ACI-24 have undergone phase II trials as detailed below. Others, including MER5101, UB-311, Lu AF20513, and CAD 106 are in various stages of preclinical, phase I, II, and phase II/III testing respectively as detailed in **Table 1**. Each of these compounds are associated with unique drug development strategies relayed below.

ACC-001, also known as vanutide cridificar, was designed as a conjugate of multiple short A $\beta$  fragments consisting of amino acids 1-7 again linked to QS-21 (similar to the first generation vaccine AN-1792) for immune stimulation. Vanutide was tested in patients with mild-to-moderate AD in multiple phase II trials in the mid to late 2000s (NCT01284387, NCT00955409, NCT00752232, NCT00498602, NCT01227564, NCT00479557, and NCT00959192). In the trials, more than 90 percent of participants in the active groups responded to the vaccine by making antibodies, which was an improvement over AN-1792 in which only 20% of participants responded by making antibodies. However, the molecule was still plagued by side effects including amyloid-related imaging abnormalities with vasogenic edema (ARIA-E), indicative of potential new microhemorrhages, occurring in two (0.8%) patients. Despite the occurrence of ARIA-E, which would prove to be consistently associated with most if not all of the active vaccines, the molecule was concluded to have an acceptable safety profile. Further exploratory cognitive evaluations, volumetric brain MRI, and CSF biomarkers, however, did not show differences between treatment groups and placebo although some trends were seen including less brain amyloid accumulation in vaccinated patients as assessed by PET imaging and slight reductions in CSF phosphorylated tau (45, 46). The development of this vaccine is not being further pursued and long

term extension studies were terminated in 2013 (NCT0096053, NCT01238991).

Affitope AD-02, directed against a slightly shorter A $\beta$  peptide sequence fragment, A $\beta$ 1-6, was derived from a proprietary method termed AFFITOPE-technology that produces “non-self” proteins and uses aluminum hydroxide as an immunological adjuvant believed to be able to avoid the adverse effects noted with the other active vaccines. This vaccine reached human trials in late 2000s (47, 48) with phase Ia/Ib studies showing favorable safety and tolerability even after 2 years despite some concerns with ARIA-E (NCT00633841, NCT01093664, and NCT00711321). A phase II trial of AD-02 plus an undisclosed immunomodulator was conducted from 2010-2013 and compared AD-02 to placebo and to the immunomodulator itself (NCT01117818) in patients with AD. The trials showed favorable safety and tolerability but primary and secondary outcome measures were not met in the AD-02 group and a subsequent phase II study and observational follow-up study was terminated (NCT02008513, NCT01357629). Surprisingly, however, the company announced in a news release benefit in the placebo group that received the immunomodulator by itself and has renamed this immunomodulator AD-04 and is pursuing development of this agent.

V-950 is an A $\beta$  amino terminal peptide formulated on an aluminum-containing adjuvant along with Iscomatrix™ an improved saponin adjuvant with antigen delivery and presentation properties as well as immunomodulatory capabilities (49). Iscomatrix™ was shown to be able to rapidly traffic antigens into the cytosol of multiple dendritic cell subsets, induce the induction of various cytokines and chemokines, and link innate and adaptive immune responses *in vivo* in a toll-like receptor-independent but MyD99-dependent manner to provide enhanced and accelerated immune responses including a response from a range of subclasses of antibodies as well as CD4<sup>+</sup> and CD8<sup>+</sup> T-cells (50). A phase I, double-blind, randomized, placebo-controlled, dose escalating study to evaluate the safety, tolerability, and immunogenicity of the compound started enrolling 86 patients in 2007 with a planned study completion date of January 2012 (NCT00464334). The authors were unable to find any reports on the results for this trial.

ACI-24 is a liposome vaccine based on the truncated A $\beta$ 1-15 sequence that grew out of work with tetrapalmitoylated preparations of N-terminal A $\beta$  fragments which showed that these preparations rapidly stimulated anti-A $\beta$  antibodies that could dissolve amyloid fibers *in vitro* and *in vivo*. The vaccine consists of an array of A $\beta$ 1-15 sequences that are sandwiched between palmitoylated lysines at either end and are anchored onto the surface of the liposomes so that the peptides adopt an aggregated  $\beta$ -sheet structure, forming a conformational epitope (51). A Phase I/II double-blind, randomized, placebo-controlled, adaptive design study of the safety, tolerability, immunogenicity and efficacy of ACI-24 was initiated in 2009 and enrolled 198 AD patients at five sites in Finland, Sweden, and Denmark (EUCTR2008-006257-40-FI). Patients were treated for one year and followed for two or more years. No results have been announced, although the company has recently launched a phase I trial of ACI-24 in Downs Syndrome patient suggesting an acceptable safety and tolerability profile of ACI-24 (NCT02738450).

MER5101 is a novel conjugate of A $\beta$ 1-15 also conjugated to diphtheria toxoid (DT), and formulated in a nanoparticulate emulsion-based adjuvant. It has currently only been evaluated in animal models but the evidence in these models suggest potential for development. High IgG1 and IgG2b antibody responses have been observed in animal models, suggesting a Th2-biased response. A strong proliferative response was observed when splenocytes were re-stimulated with the A $\beta$ 1-15:DT conjugate, whereas proliferation was absent after re-stimulation with A $\beta$ 1-15 or A $\beta$ 1-40/42 peptides, indicating a cellular immune response against DT while avoiding an A $\beta$ -specific T-cell response. Moreover, significant reductions in cerebral A $\beta$  plaque burden, accompanied by attenuated microglial activation and increased synaptic density, and improvement in cognitive deficits in contextual fear conditioning and the Morris water maze were observed. It is not known whether it will move forward into human trials (52).

LuAF20513 is an engineered protein in which A $\beta$ 1-12 is repeated three times, interspersing each with a stretch of the P30 and P2 Th epitopes from the tetanus toxin conventional vaccine. As most adults have memory T cells that recognize the toxin from prior tetanus inoculation the tetanus toxin

epitopes are believed to stimulate a T helper cell response that induces B cells to make antibodies to A $\beta$ . This approach addresses the challenges seen with prior Alzheimer's vaccines which have been unable to produce successful antibody responses in the immune systems of elderly people whose pool of naïve T cells who can take on a new antigen is low. Patients who carry pre-existing memory T helper cells that recognize tetanus epitopes are expected to be able to mount a strong response to this recombinant epitope vaccine. In animal models, Lu AF20513 was found to induce robust "non-self" T-cell responses and induce production of anti-A $\beta$  antibodies (53). This vaccine is currently recruiting for phase I clinical trials in an open-label, dose-escalation study to assess its safety, tolerability and immunogenicity expected to be completed in 2017 (NCT02388152).

The vaccine, UB-311, is an equimolar mixture of 2 synthetic peptides coupled through an oligonucleotide spacer to A $\beta$ 1-14. In animals, UB-311 produced better antibody responses than did A $\beta$ 1-14 or A $\beta$ 1-28 alone or A $\beta$ 1-14 coupled to keyhole limpet hemocyanin, a very large, immunogenic carrier molecule and also decreased the amount of A $\beta$  deposited in brain with improvement in learning and short-term memory in AD animal models. In a phase I trial and observational extension phase trial conducted in YEAR, 19 Taiwanese AD patients showed cognitive improvement on various measures with an acceptable safety and tolerability profile (54). UB-311 is now in a phase IIa randomized, double-blind, placebo-controlled trials designed as a 3-arm parallel-group, multicenter study to evaluate its safety, tolerability, immunogenicity, and efficacy starting October 2015 with an expected completion date of December 2017 (NCT02551809).

CAD106 combines multiple copies of A $\beta$ 1-6 coupled to a Q $\beta$  virus-like particle. A phase I trial in 58 Swedish patients in 2008 (NCT00411580) concluded that the vaccine was able to induce appropriate A $\beta$  IgG titers and was generally safe and well-tolerated with no reports of meningoencephalitis (55). CAD106 has since been tested in five multicenter phase II trials (NCT01097096, NCT00956410, NCT00795418, NCT01023685, NCT00733863) in the United States and Europe including a 90-week trial, and two 66-week extension trials exploring intramuscular and subcutaneous injections and additional doses of

vaccine and adjuvants with different longer-injection/booster-shot regimens (56). In a unique study design compared to the design of prior active vaccines, a phase II/III trial began in November 2015 and is expected to enroll 1,340 homozygous ApoE4 carriers who are cognitively normal to a 5-year treatment period with an expected completion date in 2023 and will measure the ability to delay diagnosis to MCI or AD dementia (NCT02565511). Besides progressive modifications in the structure and activity of AD molecules, a similar trend towards study designs that target AD at earlier and earlier stages will also be seen in the development of other therapeutic approaches as well.

### Passive immunotherapy

Passive immunization is currently the most widely developed approach in clinical trials, and numerous approaches have completed initial stages and are in phase III clinical trials. The passive immunotherapeutic agents are summarized in **Table 2**.

Bapineuzumab was the first antibody to reach advanced stages of clinical development. Bapineuzumab is a humanized murine monoclonal antibody 3D6, directed against the N-terminal region of A $\beta$ 1-6. Bapineuzumab binds to aggregated A $\beta$  and to a lesser extent soluble A $\beta$  with nanomolar affinity, activating microglial phagocytosis and promoting plaque clearance in animal models. Bapineuzumab was tested in a 12-month phase I study with 80 patients (NCT00397891) completed in 2007 and an 18-month phase II study with 234 patients (NCT00112073) completed in 2009. In these initial safety studies, 3/10 patients on the highest dose in the phase I study and 12 patients in the phase II study suffered amyloid-related imaging abnormalities indicative of potential new microhemorrhages, similar to that seen with active immunization (57, 58). Again, despite the results of the phase II study, bapineuzumab was deemed to be relatively safe and well tolerated as half of the ARIA-E cases were asymptomatic, and half suffered only transient symptoms with just one patient requiring steroid treatment (58). Although no significant difference was seen between treatment groups and placebo in the pre-specified secondary outcome measures of cognition and

function in the phase II study, post-hoc exploratory efficacy analyses hinted at a treatment response in Apo $\epsilon$ 4 non-carriers and some biomarkers did show trends towards improvement, including a trend towards reduction of CSF P-tau and reduction in cortical fibrillar A $\beta$  as assessed by serial amyloid PET (58-60).

Two 18-month phase III trials (NCT00574132, NCT00575055) enrolling a total of 2,452 patients who were Apo $\epsilon$ 4 carriers and non-carriers respectively, were then completed in 2013. Both trials failed to show a treatment effect on cognitive or functional primary outcomes or rate of brain volume loss (61). Reductions were seen in CSF p-tau and amyloid binding as measured by PET was not increased from baseline, but this was seen in APO $\epsilon$ 4 allele carriers only and not in non-carriers. Furthermore, while treatment seemed to prevent increases in plaque buildup, there was no reduction of amyloid binding, indicating that while plaque aggregation may have been prevented, clearance of plaque may not have been stimulated. Additionally, ARIA-E was found to occur with increased bapineuzumab dose and Apo $\epsilon$ 4 allele number, believed to be in part due to increase A $\beta$  load, including vascular A $\beta$  load, in those with increased ApoE4 allele number, which ultimately led to discontinuation of the highest 2 mg/kg dose. Two other phase III trials were terminated on August 6, 2012 because of this lack of clinical benefit, though not necessarily because of any new safety concerns (NCT00667810, NCT00676143). Failure of bapineuzumab to achieve clinical benefit has been attributed to the dose-limiting ARIA-E adverse effect as well as to late-stage treatment (62).

Solanezumab was the second monoclonal antibody to enter advanced clinical development. Solanezumab is targeted against A $\beta$  16–24 and unlike bapineuzumab binds with picomolar affinity only to the monomeric, soluble, toxic species of A $\beta$ . Solanezumab binds soluble A $\beta$  in both the periphery and the brain thereby driving the equilibrium toward the soluble form, putatively resulting in resolution of A $\beta$  plaques (63). Solanezumab may also have reduced frequency of adverse effects such as ARIA compared to bapineuzumab as evidenced even from the very first human trial in which no MRI evidence of

**Table 1** – A summary of the clinical trials based on active immunotherapy approaches (<https://clinicaltrials.gov>)

Drug name	Clinical trial number & Phase	Start & End date	Trial population	Outcome	Special notes
AN-1792 (first generation vaccine)	NCT00021723 Phase II	2001 - 2002	300	Functional benefit and marked clearance of insoluble amyloid plaques with reduction of aggregated tau in neuronal processes.	Made of inactivated diphtheria toxin for immune stimulation that caused aseptic meningoencephalitis observed in 6% (18/300) of vaccinated patients.
ACC-001 or vanutide cridifcar (second generation)	Phase II clinical trials: NCT01284387 NCT00955409 NCT00752232 NCT00498602 NCT01227564 NCT00479557 NCT00959192	2011 - 2014 2009 - 2013 2008 - 2012 2007 - 2013 2011 - 2014 2007 - 2013 2009 - 2013	126 50 40 160 63 86 32	Exploratory cognitive evaluations, volumetric brain MRI, and CSF biomarkers did not show differences between treatment groups and placebo.	Used in combination with an adjuvant immunostimulatory agent (QS-21).
Affitope AD-02 (second generation)	NCT01117818 – phase 2 NCT00633841 – phase 1 NCT01093664 – phase 1 NCT00711321 – phase 1b NCT02008513 – phase 2 NCT01357629 – follow-up	2010 - 2013 2008 - 2009 2009 - 2010 2008 - 2010 2013 - 2014 2011 - 2013	335 24 20 23 194 11	The trials showed favorable safety and tolerability but primary and secondary outcome measures were not met in the AD-02 group and a subsequent.	Uses aluminum hydroxide as an immunological adjuvant.
V-950 (second generation)	NCT00464334 phase I	2007 - 2012	86	No results have been reported for this trial.	An A $\beta$ amino terminal peptide formulated on an aluminum-containing adjuvant with Iscomatrix™ an improved saponin adjuvant with antigen delivery and presentation properties as well as immunomodulatory capabilities.

**Table 1 Continued...**

ACI-24 (second generation)	EUCTR2008- 006257-40-FI, Phase I/II	2009	198	No results have been announced, although the company has recently launched a phase I trial of ACI-24 in Downs Syndrome patient suggesting an acceptable safety and tolerability profile of ACI-24.	
	NCT02738450	2016 - 2016	24		
MER5101 (second generation)				Conjugated to diphtheria toxoid (DT), and formulated in a nanoparticulate emulsion- based adjuvant	
LuAF20513 (second generation)	NCT02388152 Phase I	Currently recruiting (expected to be completed in 2017)	35	Ongoing	Is an engineered protein in which A $\beta$ <sub>1-12</sub> is repeated three times, interspersing each with a stretch of the P30 and P2 Th epitopes from the tetanus toxin conventional vaccine.
UB-311 (second generation)	NCT00965588- Phase I	2009 - 2011	19	To Evaluate Safety, Tolerability and Immunogenicity of Vaccine (UB 311) in Subjects With Alzheimer's Disease	
	NCT01189084- Patients who previously received UB311	2010 - 2011	14		
	NCT02551809- phase II	2015 - 2017	45		
CAD106 (second generations)	NCT00411580- Phase I	2005 - 2008	58	Concluded that the vaccine was able to induce appropriate A $\beta$ IgG titers and was generally safe and well-tolerated with no reports of meningoencephalitis. Phase III: ongoing	
	NCT01097096- Phase II	2010 - 2012	177		
	NCT00956410- Phase II	2009 - 2011	21		
	NCT00795418- Phase II	2008 - 2010	31		
	NCT01023685- Phase II	2009 - 2012	24		
	NCT00733863- Phase II	2008 - 2010	27		
	NCT02565511- phase II/III	2015 - 2023	1340		

inflammation, vasogenic edema, or microhemorrhage was observed in the first 19 patients administered a single dose of solanezumab. Later phase II single dose studies (NCT00329082, NCT00749216) completed in 2008 and 2009 (64, 65) and an additional phase II, randomized, double-blind, placebo-controlled trial evaluating patients exposed to repeated doses completed in 2012 (NCT01148498), confirmed the antibody's safety and tolerability. This later phase II trial showed no benefits in secondary clinical outcome measures and CSF and MRI biomarkers at the end of the 12-week treatment period (66); however, it showed dose-dependent increases in unbound CSF A $\beta$ 1-42 suggesting that solanezumab may shift A $\beta$  equilibria and mobilize A $\beta$ 1-42 from amyloid plaques.

Phase III trials were initiated in 2009 [EXPEDITION1 (NCT00905372), EXPEDITION2 (NCT00904683)] enrolling 2040 AD patients from 16 countries with mild to moderate AD. When all patients were considered, solanezumab failed to show benefit in the primary clinical outcome measure. However, when only patients with mild disease were analyzed in a pre-planned subgroup analysis, improvements in multiple cognitive and functional scales including the 14-item Alzheimer's Disease Assessment Scale (ADAS-Cog14) and the Alzheimer's Disease Cooperative Study-Activities of Daily Living scale (ADCS-ADL) were seen, suggesting that positive therapeutic effects were possible if treatment was given at an early stage of AD (67). Plasma/cerebrospinal fluid biomarker findings also indicated target engagement by solanezumab and the incidence of ARIA with edema or hemorrhage was not significantly different from placebo (67, 68).

Based on these findings, an additional phase III trial [EXPEDITION3 NCT01900665] was initiated in July 2013 and has finished target enrollment of 2100 patients with mild Alzheimer's disease. ADAS-Cog14 and the ADCS-ADL scale are the co-primary outcome measures in this trial with a primary completion date expected in October 2016 and a study completion date expected in October 2018 (NCT01900665). Three other phase III trials in specific subset populations have also recently been initiated including a 48-month trial in patients with dominantly inherited AD initiated in 2012 with an expected completion date in 2019 that has finished actively recruiting (NCT01760005), a 38-

month trial in patients with normal cognitive function who are deemed at risk for AD by florbetapir PET brain imaging initiated in 2014 that is still actively recruiting (NCT02008357), and a 24-month trial in patients with prodromal AD or mild cognitive impairment initiated in June of 2016 (NCT02760602). Of note, in all these ongoing phase III trials, positivity on PET amyloid imaging is an inclusion criterion, as a common finding in both trials with both bapineuzumab and solanezumab was that a quarter of patients with mild Alzheimer's tested negative by means of PET amyloid imaging indicating that a quarter of the patients likely did not have Alzheimer's disease. As such, using positive pet amyloid imaging as an inclusion criterion will greatly increase the potential to show efficacy.

Gantenerumab is another monoclonal antibody being evaluated in AD. It is a fully human IgG1 antibody that preferentially binds to aggregated Ab in the brain and vasculature with subnanomolar affinity and does not alter plasma A $\beta$ . Gantenerumab is a conformational antibody that possesses two binding sites, one in the N-terminus region and another in mid-domain of the A $\beta$  peptide, believed to act centrally to disassemble and degrade amyloid plaques by recruiting microglia and activating phagocytosis (69). Indeed, much initial excitement accompanied the very first in human studies of gantenerumab as treatment resulted in a dose-dependent reduction in brain amyloid level, as assessed by percent change in the ratio of regional carbon 11-labeled Pittsburgh Compound B retention measured by PET. Safety wise, four Phase 1 trials (NCT00531804, NCT01656525, NCT02133937, NCT01636531) conducted internationally in a total of 308 patients suggested that gantenerumab was generally safe and well-tolerated, although ARIA was observed (70).

Phase II/III trials of gantenerumab were initiated in July 2010 and enrolled 799 patients (SCarlet RoAD NCT01224106) and was the first large trial to utilize new diagnostic criteria by the International Working Group to enroll a homogenous population of early symptomatic patients whose memory deficit was likely due to underlying Alzheimer's pathology including patients whose MMSE was above 24 but had a deficit on the FCSRT memory test with CSF evidence of A $\beta$  deposition and positivity on amyloid PET. Dosing in this trial was discontinued



on December 19, 2014, based on an interim futility analysis that suggested no benefit. However, patients continued assessment and in July 2015, Roche reported that in the fastest progressors who also received the highest doses there was a trend towards benefit observed in ADASCog and MMSE scores, brain amyloid load, CSF total tau, and CSF p-tau measures. The main side effect was ARIA which was as high as 14% but was mostly asymptomatic and associated with gantenerumab dose and with the number of ApoE 4 alleles a patient carried. The trial now continues as an open label trial of the higher gantenerumab dose with an expected completion date in 2020. A second phase III trial initiated in 2014 is also ongoing with a higher dose and has completed recruitment of 389 patients with mild AD and is expected to be complete in 2019 (Marguerite Road NCT02051608). Gantenerumab, together with solanezumab, is also being investigated by the Dominantly Inherited Alzheimer Network (DIAN).

Crenezumab (MABT5102A) is another humanized monoclonal antibody under development for AD. Crenezumab is an IgG4 monoclonal antibody which is expected to reduce pro-inflammatory activity and limit risk of vasogenic edema due to reduced effector functions on microglia (71). Crenezumab binds all forms of A $\beta$ , including monomers, oligomers, and fibrils but binds with highest affinity to the oligomers and fibrils inhibiting aggregation and promoting disaggregation. Phase I trials were initiated in 2008 and 2009 in a total of 78 patients (NCT00997919, NCT00736775). No results have been published for phase I trials but safety and tolerability was such that a large multi-center 68-week phase 2 trial enrolling 448 patients in North American and Europe with mild to moderate AD was initiated in 2011 and completed in 2014 (ABBY NCT01343966). In this study both subcutaneous and intravenous administration was assessed. Intravenous administration allowed for almost twice as much delivery of antibody as the subcutaneous administration as dosing was not limited by fears of T-cell mediated inflammation and resulting ARIA. Despite this increased drug exposure, ABBY did not show differences in primary clinical endpoints of cognitive change when assessing the total population of mild-to-moderate AD although further analysis suggested possible efficacy when only mild AD was considered. A 91-patient

biomarker study called BLAZE was also completed in spring 2014 (NCT01397578) and both continue into an open-label extension trial of 361 patients that is expected to be completed in 2017 (NCT01723826). The BLAZE study reported no differences in PET amyloid imaging between groups although differences were observed in CSF A $\beta$ . A phase II trial aiming to evaluate the safety and efficacy of crenezumab in asymptomatic carriers of E280A autosomal-dominant mutation of PSEN1 for which pathological aggregation of A $\beta$  appears at an age of approximately 25 commenced in November 2013 (NCT01998841). A phase III trial was initiated Mar 2016 in 750 patients with prodromal and mild AD (NCT02670083) and is actively recruiting.

Aducanumab (BIIB037) is a monoclonal antibody targeting aggregated forms of A $\beta$  protein, with high-affinity, fully human IgG1 monoclonal antibody against a conformational epitope found on A $\beta$ . A phase Ib study of aducanumab in patients with prodromal or mild Alzheimer's was completed, which showed a statistically significant reduction on amyloid plaque as well as a statistically significant slowing of clinical impairment. A second Phase Ib study however, could not replicate this and also show worsened safety data. In summer of 2012, PRIME, a multicenter, multiple-dose study in 166 people with prodromal or mild AD was initiated and interim results were promising enough that two 18-month phase III studies were initiated in 2015 with an expected completion date in 2022. The ENGAGE and EMERGE studies will enroll 1,350 people with MCI due to AD or mild AD as ascertained by a positive amyloid PET scan each in sites in North America and Europe (NCT02484547, NCT02477800).

Ponezumab (PF-04360365) is a humanized IgG2 $\delta$ A monoclonal antibody that unlike the other monoclonal antibodies binds the free carboxyl terminal of amino acids 33-40 of the A $\beta$  1-40 peptide (72). Five phase I trials showed acceptable safety but two phase II trials concluded in 2011 showed no treatment effect and development of ponezumab for AD is no longer being pursued although its use is being explored in patients with cerebral amyloid angiopathy.

BAN2401 is a humanized IgG1 monoclonal antibody that binds selectively to large, soluble A $\beta$  protofibrils and is thought to lead to their clearance

or neutralize their toxicity based on the discovery of the “artificial mutation” in APP, which leads to a form of Alzheimer's disease with particularly high levels of A $\beta$  protofibrils (73). A phase II, 18-month U.S. trial is enrolling up to 800 people who either have mild cognitive impairment due to AD, or who have probable AD and whose diagnosis is confirmed by a positive amyloid PET scan with the primary outcome being a change in a composite of cognitive tests (NCT01767311).

Another approach closely related to passive immunotherapy of AD is IVIG (Gammagard) administration. IVIG is a mixture of polyclonal antibodies prepared from the blood plasma of thousands of healthy young volunteers. IVIG binds weakly to the monomeric form of A $\beta$  and binds strongly to neurotoxic oligomers and A $\beta$  fibrils. IVIG also has immune-modulating and inflammatory effects which may potentially target other AD mechanisms. A phase I trial in 8 patients showed reduction in cognitive decline in 7 patients that completed the trial and an improvement in cognition six patients. Phase II and III trials in patients with mild-to-moderate ensued but in 2012 a phase III trial of 10% IVIG in 360 patients, while generally safe and well-tolerated was terminated due to lack of efficacy. Some positive results were noted in subgroups, especially among APOE-e4 carriers and moderately impaired AD patients and recruitment is ongoing for further phase III trials (NCT01561053) in these populations (74, 75). Recently, two planned phase III studies (NCT01736579, and NCT01524887) were terminated, since the first Phase III study (NCT00818662) failed to show efficacy and confirmed the safety profile observed in Phase II studies.

### Secretase Inhibitors ( $\gamma$ and $\beta$ )

The  $\beta$ -site APP cleaving enzymes 1 and 2 (BACE1 and BACE2) were initially identified as transmembrane aspartyl proteases cleaving the amyloid precursor protein (APP). BACE1 is a major drug target for Alzheimer's disease because BACE1-mediated cleavage of APP is the first step in the generation of the pathogenic amyloid- $\beta$  peptides. However, BACE1 targeted drug-development has been hindered, because the enzyme has been found to have many substrates with important physiological roles and initial non-selective inhibition of the enzyme was found to

have numerous toxic consequences (76). Furthermore, the active site of BACE1 is relatively large, and many of the bulky compounds that are needed to inhibit BACE1 activity are unlikely to cross the blood-brain barrier. Therefore, much effort has been put into developing non-peptidomimetic  $\beta$ -secretase inhibitors that show high BACE1 selectivity (BACE1/BACE2 selectivity >100) with weak or non-peptide characteristics that favor CNS penetration and oral bioavailability (77). Many BACE inhibitors are still in the preclinical phase and only a few have entered clinical trials to date. Clinical trials involving  $\beta$ -secretase inhibitors are summarized in Table 3.

The BACE1 inhibitor furthest along in development is MK-8931, which is a diaryl amide-substituted 3-imino-1,2,4-thiadiazinane 1,1-dioxide derivative and a small molecule inhibitor of BACE1/2 (with a higher affinity towards BACE1). The first phase I/II studies were completed in 2012 (NCT01496170, NCT01537757) and demonstrated tolerability with dose-proportional increases in plasma and CSF exposure. Importantly CSF A $\beta$  was reported to be reduced by up to 92% (78). An 18-month phase 3 trial (EPOCH, NCT01739348) enrolling 1,960 participants with mild to moderate AD patients was then initiated in 2012 followed by a second phase III trial (APECS, NCT01953601) enrolling 1500 patients with earlier stages of the disease (prodromal AD) in November 2013. The EPOCH trial has finished recruitment and has an estimated primary completion date in 2017 with a final completion date in 2019 and the APECS trial is recruiting with an estimated primary completion date in 2019 and final study completion date in 2021. No preclinical or trial data has yet been published for this molecule with most information on its development being reported by the developing drug company.

AZD3293 is an orally active, potent, highly permeable (able to penetrate BBB) inhibitor of BACE1 with unique slow off-rate kinetics that is hoped to translate into a prolongation of any observed effect beyond the turnover rate of A $\beta$ . It has demonstrated time- and dose-dependent reductions of brain A $\beta_{40}$  in mice, guinea pigs, and dogs as well as other tissue types (79). Reductions of up to 75% in CSF AB levels were reported in initial phase I safety findings announced in December 2013 and in at least ten other phase I studies that further evaluated safety, tolerability,

**Table 2** - A summary of the clinical trials based on passive immunotherapy approaches (<https://clinicaltrials.gov>)

Drug name	Clinical Trial Number & Phase	Start & End date	Trial population	outcome	Special notes
<b>Bapineuzumab</b>	NCT00397891 - Phase I	2006 - 2010	80	Bapineuzumab was deemed to be relatively safe and well tolerated as half of the ARIA cases were asymptomatic, and half suffered only transient symptoms with just one patient requiring steroid treatment. In phase III both trials failed to show an effect on cognitive or functional primary outcomes or rate of brain volume loss.	
	NCT00112073 - Phase II	2005 - 2008	234		
	NCT00574132 - Phase III	2007 - 2012	1331		
	NCT00575055 - Phase III	2007 - 2012	1122		
	NCT00667810 - Phase III	2008 - 2013	901		
	NCT00676143 - Phase III	2008 - 2012	1100		
<b>Solaneuzmab</b>	NCT00329082 - Phase II	2006 - 2008	19	-In phase II confirmed the antibody's safety and tolerability. - Phase III: ongoing	Have reduced frequency of adverse effects such as ARIA compared to bapineuzumab.
	NCT00749216 - Phase II	2008 - 2009	33		
	NCT01148498 - Phase II	2010 - 2012	55		
	NCT00905372 - Phase III	2009 - 2012	1000		
	EXPEDITION1 NCT00904683 - Phase III	2009 - 2012	1040		
	EXPEDITION2 NCT01900665 - Phase III	2013 - 2020	2100		
	EXPEDITION3 NCT01760005 - Phase III				
	NCT02008357 - Phase III	2012 - 2019	210		
	NCT02760602 - Phase III	2014 - 2020	1150		
		2016 - 2021	2450		

<b>Table 2 Continued...</b>					
<b>Gantenerumab</b>	NCT00531804 - Phase I	2006 - 2010	60	It is generally safe and well-tolerated. Phase III: ongoing	It is a fully human IgG1 antibody that preferentially binds to aggregated Ab in the brain and vasculature with subnanomolar affinity and does not alter plasma A $\beta$ . The main side effect was ARIA which was as high as 14% but was mostly asymptomatic and associated with gantenerumab dose and with the number of ApoE 4 alleles a patient carried.
	NCT01656525 - Phase I	2012 - 2014	28		
	NCT02133937 - Phase I	2014 - 2014	31		
	NCT01636531 - Phase I	2012 - 2012	120		
	NCT01224106 - Phase II/III	2010 - 2019	799		
	NCT02051608 - Phase III	2014 - 2019	389		
	<b>Crenezumab (MABT5102A)</b>	NCT00997919 - Phase I	2009 - 2010		
NCT00736775 - Phase I		2008 - 2010	56		
NCT01343966 - Phase II		2011 - 2014	448		
NCT01397578 - Phase II		2011 - 2014	91		
NCT01723826 - Phase II		2012 - 2017	360		
NCT01998841 - Phase II		2013 - 2020	300		
NCT02670083 - Phase III		2016 - 2021	750		
<b>Aducanumab (BIIB037)</b>		NCT01397539 - Phase I	2011 - 2013	53	Statistically significant reduction on amyloid plaque as well as a statistically significant slowing of clinical impairment
	NCT02434718 - Phase I	2015 - 2016	20		
	NCT02782975 - Phase I	2016 - 2016	40		
	NCT01677572 - Phase I	2012 - 2019	197		
	NCT02484547 - Phase III	2015 - 2022	1350		
	NCT02477800 - Phase III	2015 - 2022	1350		

<b>Table 2. Continued...</b>						
<b>Ponezumab (PF-04360365)</b>	NCT00733642 - Phase I	2008 - 2009	15	Acceptable safety Profile	IgG2 $\delta$ A monoclonal antibody that (unlike the other monoclonal antibodies) binds the free carboxyl terminal amino acids 33-40 of the A $\beta$ 1-40 peptide	
	NCT00455000 - Phase I	2007 - 2009	37			
	NCT01125631 - Phase I	2010 - 2011	8			
	NCT01005862 - Phase I	2010 - 2012	17			
	NCT00607308 - Phase I	2008 - 2010	20			
	NCT00722046 - Phase II	2008 - 2011	198			Phase II studies failed to show any significant therapeutic effect. It is no longer pursued for AD treatment.
	NCT00945672 - Phase II	2009 - 2011	36			
	NCT01821118 - Phase II	2013 - 2015	36			
	<b>BAN2401</b>	NCT01230853 - Phase I	2010 - 2013			80
NCT02094729 - Phase I		2013 - 2015	26			
NCT01767311 - Phase II		2012 - 2018	800			
<b>IVIG (Gammagard)</b>	NCT00299988 - Phase II	2006 - 2010	24	Generally safe and well-tolerated; however, efficacy was not observed.	A mixture of polyclonal antibodies prepared from the blood plasma of thousands of healthy young volunteers. IVIG binds weakly to the monomeric form of A $\beta$ and binds strongly to neurotoxic oligomers and A $\beta$ fibrils.	
	NCT00812565 - Phase II	2009 - 2010	58			
	NCT01300728 - Phase II	2011 - 2017	52			
	NCT01561053 - Phase II/III	2012 - 2016	350			
	NCT00818662 - Phase III	2008 - 2012	390			
	NCT01736579 - Phase III	2012	6			Terminated, due to lack of efficiency
	NCT01524887 - Phase III	2012	508			Terminated, due to lack of efficiency

metabolism, and potential drug interactions of the compound (80). A large phase II/III trial began enrollment in September 2014, enrolling 2,202 patients with MCI due to AD or mild AD, to be treated for 2 years and set to run for 5 years (AMARANTH, NCT02245737). In June 2016, a second phase III trial was initiated (DAYBREAK-ALZ, NCT02783573) with target enrollment of 1899 patients with mild AD only, also set to run 5 years with a 3-year treatment period.

JNJ-54861911 is another inhibitor of BACE1 also in phase II/III trials. It was initially optimized through multiple steps by the Shionogi group in Japan from hits with amino-dihydrothiazine or modified versions of the cyclic isothiourea warhead. JNJ-54861911 first began phase I testing in 2013 and has undergone at least 11 phase I and phase II trials. The inhibitor has been reported to be safe and well-tolerated with dose-dependent reductions in A $\beta$ 1-37, A $\beta$ 1-38, A $\beta$ 1-40, and A $\beta$ 1-42 with 90% reduction in AB seen with the highest dose (81). In October 2015, a phase II/III study began in asymptomatic people at risk of developing Alzheimer's dementia (NCT02569398). This trial will enroll 1,650 participants in Europe, Australia, and Mexico. This trial will run until the year 2023. A long-term safety and tolerability study of 100 patients from previous phase I/II trials is ongoing.

E2609 is another BACE inhibitor that is also in development. It was initially developed through several series of bicyclic aminodihydrothiazines fused with unsaturated five- and six-membered rings, and has shown significant reduction in CSF and plasma levels of A $\beta$  in non-human primates. At least eight phase I trials have been completed evaluating the safety and pharmacology of E2609 in nearly 500 healthy volunteers and people with early Alzheimer's disease including evaluation of the compounds effects on the heart and interactions with drugs commonly prescribed to the elderly. The drug shows a reduction of A $\beta$  levels in plasma and a dose-dependent reduction of up to 80 percent in CSF A $\beta$  levels with headache and dizziness being the most common adverse events although several cases of orolabial herpes relapse were observed (82, 83). An 18-month phase II study in 700 people with MCI due to AD or prodromal AD who have a positive amyloid PET scan was initiated in November 2014 and results are expected in January 2018 (NCT02322021).

CNP520 is another oral inhibitor of BACE

developed by Novartis that recently completed phase II trials (March 2016 in 125 healthy patients; NCT02576639). It is also being currently evaluated in a phase II/III prevention study that will measure delay to diagnosis of MCI or AD dementia in 1340 cognitively normal, homozygous ApoE4 carriers aged 60 to 75. This study is somewhat unique in that, half of the participants will be randomized to either CNP250 or matching placebo, and the other half to CAD106 or placebo (NCT02565511). Many other BACE inhibitors have failed phase I/II trials including LY2886721 terminated in phase II in 2013 after four cases of abnormal liver biochemistry values, LY2811376 terminated in late phase I due to damage to the pigment epithelium of the eye found in simultaneous rat toxicology studies and CTS21166, a small transition-state analog inhibitor that was the first to pass a phase I clinical trial in 2008 but whose development is not being continued for unknown reasons. Various BACE inhibitors including, GSK188909, have never gone beyond pre-clinical stage of development.

$\gamma$ -Secretases have also been evaluated in clinical settings. While the amyloidogenic pathway is initiated by BACE1, the  $\gamma$ -secretase enzyme is responsible for the final stage of amyloidogenesis, leading to the generation of A $\beta$ 1-40 and A $\beta$ 1-42 and is consequently a target for AD treatment. However, substrate promiscuity promotes major off-target secondary adverse effects and has hindered development of  $\gamma$ -secretase inhibitors early on. For instance, Notch, a major protein responsible for regulating cell proliferation, development, differentiation, and cellular communication, is one of the targets of  $\gamma$ -secretase. Inhibition of Notch processing is responsible for adverse events seen with early  $\gamma$ -secretase inhibitors including severe gastrointestinal, hematopoietic side effects, and neurodegeneration and have been hampering the development of clinically useful  $\gamma$ -secretase inhibitors so far.

Semagacestat (LY450139) is a  $\gamma$ -secretase inhibitor that reduces A $\beta$ 40 and A $\beta$ 42 production and was the first  $\gamma$ -secretase inhibitor to go into phase III clinical trials. In preclinical animal studies, semagacestat reduced both soluble A $\beta$  and amyloid plaque burden. Phase I evaluated semagacestat in 27 healthy volunteers and showed dose-dependent reduction in AB synthesis in the CSF (84). Phase II trials, showed a greater number of skin-related side effects in the treatment group



**Table 3** - A summary of the clinical trials based on passive  $\beta$ -secretase inhibitors (<https://clinicaltrials.gov>)

Drug name	Clinical Trial Number & Phase	Start & End date	Trial population	Outcome	Special note
<b>MK-8931</b>	NCT01953601 - Phase III	2013 - 2021	1500	ongoing	No preclinical or trial data has been published
<b>AZD3293</b>	NCT02783573 - Phase III	2016 - 2021	1899 patients with mild AD	Ongoing	
<b>JNJ-54861911</b>	NCT02569398 - Phase II/III	2015 - 2023	1650	Ongoing	Long term safety and tolerability study is still ongoing
<b>E2609</b>	NCT02322021 Phase II	2014 - 2018	700	Ongoing	
<b>CNP520</b>	Phase II NCT02565511	November 2015- August 2023	1340	Ongoing	
<b>LY2886721</b>	Phase II	-	-	Terminated in 2013 after four cases of abnormal liver biochemistry values	
<b>LY2811376</b>	Phase I	-	-	Terminated in late phase I due to damage to the pigment epithelium of rat eye	
<b>CTS21166</b>	Phase I	-	-	Passed phase I	The development is not continued for unknown reason(s)
<b>GSK188909</b>	Preclinical	-	-	-	Has never gone beyond preclinical stage of development

including rash and lightening hair color. Despite these side effects, two 21-month phase III trials, IDENTITY-1 and IDENTITY-2, were initiated and enrolled a total of 3036 patients. Both trials were terminated in April 2011 because of an increased risk of skin cancer and infections in the treatment group. Moreover, cognition and function not only did not improve but worsened in all treatment groups (85). Failure of Semagacestat was believed to be due to the

broad inhibition of 40-plus substrates of  $\gamma$ -secretase, particularly Notch, which was previously not well-known. In particular, effects on cognition were believed to be due to the accumulation of the neurotoxic precursor of  $A\beta$  (the C-terminal fragment of APP or CTF $\beta$ ) resulting from the block of the  $\gamma$ -secretase cleavage activity on APP (86).

Avagacestat is another  $\gamma$ -secretase inhibitor which was discontinued as a result of a lack of efficacy (NCT00810147, NCT00890890, NCT00810147, and NCT01079819). Avagacestat was reported to selectively block processing of the enzyme's APP substrate with 137-fold selectivity for APP over Notch in cell culture, and to reduce CSF A $\beta$  levels without causing Notch-related toxicity in animals (87, 88). About a dozen phase I trials evaluated the safety and pharmacology of avagacestat in healthy volunteers and people with Alzheimer's disease. In particular, drug-interaction studies were conducted with cholinesterase inhibitors, blood thinners, and a range of other drugs commonly used in aging populations, including topical antibiotics and disinfectants. In 2009, two Phase 2 trials were started, of which one was completed, and the other was terminated. In the first multinational trial with 209 people with mild to moderate Alzheimer's disease many patients dropped out due to gastrointestinal and dermatological side effects and non-melanoma skin cancers were also seen. The trial generated a dose-dependent pharmacodynamics model for the effects on CSF biomarkers in some patients, but at the two higher doses cognition trended toward a worsening compared with placebo (89). ARIA also occurred in some patients. In the second phase II trial, avagacestat was evaluated in the prodromal population and showed similar results and the trial was terminated in 2012.

Begacestat (GSI-953) was another  $\gamma$ -secretase inhibitor that went through pre-clinical development was, which was 16-times more selective for the inhibition of APP cleavage over Notch. However, phase I trials (NCT00959881, NCT00547560) in healthy human volunteers only affected the plasma concentration of A $\beta$  and not its CSF concentration and development was halted (90). Another compound, NIC5-15 (pinitol) is a naturally occurring cyclic sugar alcohol found in soy and several other plants and fruits. It is also believed to modulate  $\gamma$ -secretase that is selective for APP. A Phase 2a and 2b trial were completed in 15 and 30 people respectively in 2013 and the compound was reported to indicate good tolerability, as well as stabilization of cognition as measured by the ADAS-Cog (NCT01928420). It is unclear if this is being pursued for further development.

Some non-steroidal anti-inflammatory drugs (NSAIDs) such as tarenflurbil, act as  $\gamma$ -secretase modulators and shift the  $\gamma$ -secretase cutting point to produce shorter nontoxic A $\beta$  fragments. A phase II trial with the NSAID tarenflurbil in mild to moderate AD showed a significant improvement in primary outcome of global functioning and activities of daily living and a positive trend towards improved cognition with a statistically significant reduction in newly synthesized A $\beta$  in human volunteers. Phase III trials were completed in 2008, and demonstrated an increased deterioration in cognition and activities of daily living compared to placebo-treated controls and it was discontinued.

Compound CHF-5074, a molecule with a formulation based on the structure of R-flurbiprofen, was being reported as a  $\gamma$ -secretase modulator that affected A $\beta$ - and other amyloid-related outcomes. Subsequent preclinical studies proposed other mechanisms of action, such as restoring neurogenesis, reorganizing the astrocytic cytoskeleton, reducing tau, rescuing synaptic plasticity, or acting on microglia to counteract inflammation (91). Three phase I clinical trials in 144 healthy young men reported CHF-5074 to be safe and tolerable in these subjects; one notable side effect was mild diarrhea. Three phase II trials were conducted and the first reported trends toward improved executive function, were reported for carriers of the ApoE4 risk allele, but not non-carriers (92). It is unclear if the agent is undergoing further development as the company awaits the outcome of licensing negotiations.

There are many other  $\gamma$ -secretase inhibitors in preclinical development. BMS-299897 is another  $\gamma$ -secretase inhibitor that has been tested in animal models. BMS-299897 blocked the increase in A $\beta$ 1-42 content and decreased A $\beta$ 1-40 levels significantly in the A $\beta$ 25-35 mouse model of Alzheimer's disease (93). MRK-560 is also another  $\gamma$ -secretase inhibitor that is potent, orally bioavailable, and can penetrate the brain. MRK-560 caused marked inhibition of both A $\beta$ x-40 and A $\beta$ x-42 in plasma, brain, and cerebrospinal fluid with good correlation between plasma and brain and plasma and CSF (94). Treatment of AD mice/rats with the  $\gamma$ -secretase inhibitor DAPT has also been studied and resulted in decreased A $\beta$  levels in plasma and cerebrospinal fluid (CSF) (95).

**Table 4** - A summary of the clinical trials based on passive  $\gamma$ -secretase inhibitors (<https://clinicaltrials.gov>)

Drug name	Clinical Trial Number & Phase	Start & End date	Trial population	Outcome	Special note
<b>Semagacestat (LY450139)</b>	NCT00762411 - Phase III	2008 - 2011	1111	Terminated in April 2011 Increased risk of skin cancer and infections	Reduces A $\beta$ 40 and A $\beta$ 42 production First $\gamma$ -secretase inhibitor to go into phase III clinical trials
	NCT00594568 - Phase III	2008 - 2011	1537	Cognition and function not only did not improve but worsened in all treatment groups	
	NCT01035138 - Phase III	2009 - 2011	180		
<b>Avagacestat</b>	NCT00810147 - Phase II	2009 - 2010	209	- GI and dermatological side effects and non-melanoma skin cancers	Selectively blocks processing of the enzyme's APP substrate with 137-fold selectivity for APP over Notch
	NCT00890890 - Phase II	2009 - 2013	263	- Terminated, due to lack of efficacy and similar side effects	
<b>Begacestat (GSI-953)</b>	NCT00547560 - Phase I	2007 - 2009	49	Only affected the plasma concentration of A $\beta$ and not its CSF concentration	16-times more selective for the inhibition of APP cleavage over Notch
	NCT00959881 - Phase I	2009 - 2009	47	Its development was halted	
<b>NIC5-15</b>	NCT00470418 - Phase IIa	2007 - 2010	15	Indicated good tolerability and stabilization of cognition as measured by the ADAS-Cog	Naturally occurring cyclic sugar alcohol found in soy and several other plants and fruits
	NCT01928420 - Phase IIb	2007 - 2014	30		
<b>Tarenflurbil</b>	NCT00105547 - Phase III	2005 - 2008	1600	Increased deterioration in cognition and activities – Discontinued	NSAID
<b>CHF-5074</b>	NCT00954252-Phase I	2009 - 2010	84	Improved executive function for carriers of the ApoE4 risk allele but not non-carriers	It is not clear if it is undergoing further development
	NCT01203384-Phase I	2010 - 2010	48		
	NCT01258452-Phase I	2011 - 2011	12		
	NCT01303744-Phase II	2011 - 2012	96		
	NCT01421056-Phase II	2011 - 2012	74		
	NCT01602393-Phase II	2012 - 2013	51		

### Tau Targeted Therapies

Aggregates of abnormally hyperphosphorylated tau are the components of neurofibrillary tangles, the hallmark lesion in AD other than beta-amyloid. Tau has more recently been emerging as potentially an equally important biological target for innovative AD therapies. Potential tau therapies include inhibition of tau phosphorylation, microtubule stabilization, prevention of tau oligomerization, and enhancement of tau degradation as well as tau immunotherapy. Most agents directed against the tau protein are still in preclinical tests, with only a handful having reached clinical trials.

The tau therapy furthest along in development is TRX0237 also known as leucomethylthionium (LMTX). This compound is a derivative of a well-known Methylene Blue dye, i.e. methylthionium chloride but was improved for enhanced bioavailability and tolerability (96). The purported action of both of these compounds is to act as tau aggregation inhibitors by not only inhibiting the formation of new oligomers, but more importantly to release soluble tau from oligomers and paired helical filaments in a monomeric form which is susceptible to proteases (97). In a large 12-month phase II study in 321 subjects, MTC was found to stabilize the progression of AD over 50 weeks in both mild and moderate AD (98). The more stable, reduced version of methylthionium, LMTX, was tested in two parallel phase III studies in AD in 250 centers in 22 countries world-wide enrolling 891 patients with mild-to-moderate AD (NCT01689246) completed at the end of 2015 and in 800 patients with mild AD (NCT01689233) completed in June 2016. Of note, these therapies are also being tested in other phase III trials for another tauopathy, frontotemporal dementia (NCT01626378). The clinical trials based on tau targeted therapies are summarized in **Table 5**.

Because tau is a component of microtubules and hyperphosphorylation of tau results in microtubule destabilization and resulting cytoskeleton degeneration, other agents of interest in AD are agents with microtubule stabilizing effects (99). Agents that have been tested in phase I clinical trials include epothilone D (BMS-241027) and TPI-287. Epothilone D (BMS-241027) is a small molecule taxane drug that has been previously studied in oncology and is capable of penetrating the blood-brain barrier and normalizing tau binding at low doses. In tau transgenic animals,

BMS-241027 restored microtubule dynamics to baseline levels with beneficial effects seen on performance in the Morris water maze deficits, tau pathology, and neurodegeneration (100). However, BMS 241027 has undergone phase I trials in 40 patients which were completed in October 2013 (NCT01492374) and the agent has not been pursued further in AD development. TPI-287 is another tubulin-binding and microtubule stabilizing agent that is also a synthetic derivative of the taxane drugs used in oncology for various brain tumors. It is being tested in phase I trials in 33 patients in November 2013 that is currently recruiting AD patients for phase I study (NCT01966666). The study is set to run until March 2017. A phase I study in another tauopathy, corticobasal degeneration (CBD), or progressive supranuclear palsy (PSP) was also initiated in 2014 (NCT02133846).

Similar to beta-amyloid, immunotherapy directed against the tau protein is also a target for development. Passive immunization with specific monoclonal antibodies directed against phosphorylated tau is being studied and are in various stages of pre-clinical testing (101, 102). Because active immunization against tau is much further along in development. Active immunization using human, recombinant, full-size, non-phosphorylated tau protein has been associated with neurotoxicity in animal models, this has led to the development of modified peptides to actively immunize against Tau. AADvac1 is the first vaccine to undergo clinical trials for active immunization against tau in AD. AADvac1 is directed against shortened, non-native tau which is susceptible to aggregation and is targeted against amino acids 294 to 305 of the tau sequence derived from the regulatory domain driving the oligomerization of tau. An extra N-terminal cysteine residue is added to obtain attachment of the peptide to the keyhole limpet hemocyanin and coupled to using aluminum hydroxide carrier (103). Phase I trials in 30 patients with mild-to-moderate AD were conducted from 2013-2015. Two patients reportedly withdrew due to adverse events, including a viral infection which was followed by a seizure. Safety and tolerability however was deemed such that a 24-month phase II trial in patients with mild-to-moderate AD was initiated in December 2015 with an expected completion date 2018. It intends to enroll 185 patients with mild AD

(ADAMANT, NCT02579252).

Another potential AD therapy in development which actively immunizes against the tau protein is the liposomal-based vaccine ACI-35. The vaccine contains 16 copies of a synthetic tau fragment that is phosphorylated at the protein's pathological phosphorylation residues S396 and S404 and is anchored into a lipid bilayer. It uses the adjuvant MPLA (104). In pre-clinical studies it rapidly generated high titers of polyclonal IgG antibodies specifically directed against phosphorylated tau, rather than non-phosphorylated tau, reducing soluble tau as well as insoluble, aggregated tau in brain extracts (105). In December 2013, a phase 1b multicenter, double-blind, randomized, placebo-controlled study of the safety, tolerability and immunogenicity of ACI-35 was initiated recruiting 24 patients. The trial has been completed and results are pending (ISRCTN13033912). As hyperphosphorylated tau protein is prone to aggregation leading to loss of cytoskeletal microtubule-stabilizing properties and degeneration of neurons, potential compounds preventing tau phosphorylation are also of interest. The most important protein kinase involved in tau phosphorylation is glycogen synthase kinase 3 beta (GSK-3 $\beta$ ) whose enzyme activity is also reportedly up-regulated by neurotoxic A $\beta$ . Multiple GSK-3 $\beta$  inhibitors are under development.

Tideglusib (NP031112, NP-12) is an orally available small molecule drug of the thiadiazolidinone class. It acts as an irreversible GSK-3 $\beta$  inhibitor that has been shown to reverse amyloid in brain and prevent cell loss and reduce spatial memory deficits in preclinical studies (106, 107). Phase IIa studies in 30 patients reported good tolerability except for a transient increase in serum transaminase levels (108). However, the follow-up phase IIb trial was aborted in 201 of 306 patients with mild-to-moderate AD (ARGO, NCT01350362), because primary endpoints were not met. The drug continues to be evaluated in progressive supranuclear palsy which is considered a pure tauopathy.

Insulin is also being studied in the treatment of AD. Insulin has been proposed to play a role in multiple AD disease pathways including against A $\beta$  toxicity, neuro-inflammation, and tau pathology. The role of insulin in tau pathology is believed to be due to its effects on the decreasing the activity of GSK-3, thereby leading to inhibition of tau

phosphorylation. Nasal insulin is being explored specifically because this particular route of administration can effectively deliver insulin directly into the brain without the unwanted effect of increasing systemic insulin levels. Multiple small pilot studies have been conducted with nasal insulin. These studies have reported benefit in cognition including improved verbal memory and attention, although these studies are small and inherently methodologically flawed (109-111). Differential effects of insulin by ApoE genotype has also been proposed (112). Phase II/III clinical trials which were initiated in 2013 are currently ongoing for Humulin R in patients with the mild form of AD and is expected to be completed in 2017 (NCT01767909).

The efficacy of the human insulin analogue glulisine (Apidra<sup>®</sup>) is also being evaluated in patients with AD. A phase II, single center, randomized, double-blind, placebo-controlled of intranasal glulisine enrolling 90 patients with mild cognitive impairment and probable mild AD was initiated in August 2015 and is expected to be completed in September 2017 (NCT02503501). Prior phase I studies showed that glulisine was well tolerated but failed to have an acute impact on cognition in ApoE4 carriers with AD (113). However, these studies were not powered to show effect.

## Neurotransmitter-based therapies

### Muscarinic agonists

The muscarinic acetylcholine receptors are G protein coupled receptors which comprise 5 subtypes, M1 - M5, that play an important role in attention, the sleep-wake cycle and memory (114-116). M1 in particular, plays a pivotal role in cognition and memory and are distributed throughout the cortex and hippocampus. M1 muscarinic receptors are believed to play an important role in the regulation of secretase activities and synthesis of A $\beta$  peptide, and subsequent tau hyperphosphorylation and cognitive dysfunction (117, 118). M1 receptor activation activates  $\alpha$ -secretase and leads to increased secretion of sAPP $\alpha$  and subsequent inhibition  $\alpha$ -secretase which then decreases production of A $\beta$ . M1 receptor activation is also believed to inhibit oxidative stress which leads to cell death. As such M1 receptor agonists have been targets for drug development in AD.

**Table 5** - A summary of the clinical trials based on passive  $\gamma$ -secretase inhibitors (<https://clinicaltrials.gov>)

Drug	Clinical Trial Number & Phase	Start & End Date	Trial Population	Outcome	Special note
<b>Leucomethylthioninium (LMTX) (TRX0237)</b>	NCT01689233 - phase III	2012 - 2016	800		Tau aggregation inhibitor
	NCT01689246 - Phase III	2013 - 2015	891		
<b>Epothilone D (BMS-241027)</b>	NCT01492374 - Phase I	2012 - 2013	40	Not being pursued for AD	A small molecule taxane drug capable of normalizing tau binding
<b>TPI-287</b>	NCT01966666 - phase I	2013 - 2017	33		Synthetic derivative of taxane drugs
<b>Active immunization (AADvac1)</b>	NCT02579252 - phase II	2016 - 2019	185		Active immunization against tau protein
<b>Tideglusib (NPO31112, NP-12)</b>	NCT00948259 - Phase I/II	2008 - 2009	30	Good tolerability (with transient increase in serum transaminase - Aborted in 201 of 306 patients)	Irreversible GSK-3b inhibitor transient increase in serum transaminase levels
	NCT01350362 - Phase II	2011 - 2012	306		
	NCT02586935 - Phase II	2015 - 2017	90		
<b>Insulin</b>	NCT01767909 - Phase II/III	2013 - 2017	240	Humulin® R U-100	Effects on the decreasing the activity of GSK-3
<b>Glulisine (human insulin analog)</b>	NCT01436045 - Phase II	2011-2013	12		Failed to have an acute impact on cognition in ApoE4 carriers in phase I
	NCT02503501 - Phase II	2015-2017	90		

There are three types of M1 mAChR-targeting drugs currently in development, including orthosteric agonists, allosteric agonists, and M1 positive allosteric modulators (M1 PAM).

The first generation of M1 receptor agonists bind to an orthosteric ACh-binding site. Xanomeline was an M1/M4 selective

muscarinic receptor agonist evaluated in patients with probable AD. In phase II studies in 343 patients a significant treatment effect was seen in cognition, global function, and behavioral symptoms but almost 50% of patients in the high-dose arm discontinued treatment due to adverse events which were primarily gastrointestinal in nature



believed to be due from non-selective binding of M1 and M2 (119). The clinical studies pursued based on different neurotransmitter-based therapies are summarized in **Table 6**. Other M1 muscarinic agonists include AF102B, AF150(S), and AF267B which are highly bioavailable, blood-brain barrier penetrant, partial agonists that elevate the non-amyloidogenic amyloid precursor protein *in vitro*, decrease beta-amyloid levels *in vitro* and *in vivo*, and can inhibit beta-amyloid and oxidative-stress-induced cell death in cell lines. These drugs also restored cognitive impairments in several animal models for AD (120). AF102B failed in clinical trials due to gastrointestinal side effects, confusion, and diaphoresis and AF267B failed to show efficacy in clinical trials and was discontinued. LY593093 is a newer M1AChR orthosteric agonist that demonstrates modest to no activity at the other muscarinic subtypes and displays efficacy in the *in vivo* models of cognition (121). However, it is unclear if it is being pursued for clinical trials.

Allosteric compounds bind to allosteric binding sites and have a better selectivity for the M1 subtypes than do the previously described orthosteric agonists. Allosteric compounds can be classified into regular agonists and M1-positive allosteric modulators (PAMs). Allosteric agonists of the M1 receptor activate the receptor at a site distinct from the orthosteric ACh site which contributes to selectivity for M1 receptors. Early allosteric agonists (e.g., AC-42) could not activate M1 receptors in systems such as brain slices and had poor solubility in a physiologic buffer system, which prevented the *in vivo* use. Newer allosteric agonists, such as TBPB, were developed to be highly selective for M1, blood brain barrier penetrant, and also activate NMDA in hippocampal pyramidal neurons. TBPB promotes the processing of APP towards the non-amyloidogenic pathway, decreases A $\beta$  production *in vitro*, and has been shown to improve cognition in animal models (122). 77-LH-28-1 is another potent, selective and CNS penetrant allosteric M1 agonist which was a structural analog of AC-42 that was reported to improve cognition in animal models (123) as did AC-260584 (124). However, it is unclear if any of these agents are being pursued for clinical development. A unique agent is AF710B, which is a highly potent and selective allosteric M1 muscarinic agonist while also being a  $\sigma$ 1 receptor agonist. AF710B was a potent and safe cognitive enhancer

in animal models, while also reportedly decreasing BACE1, GSK3 $\beta$  activity, neuro-inflammation, soluble and insoluble A $\beta$ 40, A $\beta$ 42, plaques and tau pathologies (125). While AF710B is believed to have much promise as a potential therapy in AD, it has not yet entered clinical phases of development. Currently, the only M1 receptor agonist that is in clinical trials is HTL9936 which completed the phase I trials in 2014 (NCT02291783).

The M1 PAMs group cannot activate receptors directly, but can change the receptor conformation after binding, which then leads to a change in the ligand-binding and functional properties of the M1 receptors. These muscarinic agents then have a novel and highly targeted mode of action and act only on a single muscarinic receptor subtype which is functioning sub-optimally and therefore be of use therapeutically in the early stages of AD. Therefore, M1 PAMs are active in the presence of the endogenous neurotransmitter ACh and inactive in its absence, which theoretically aids in the reduction of side effects. Brucine was one of the first PAMs identified and was capable of selectively enhancing the effects of acetylcholine at M1 receptors by an allosteric mechanism (126). There are several other PAMs that act on M1, such as VU0029767 and VU0090157 which were previously in preclinical development (127). ML169 is a M1 PAM which has more recently been in preclinical developed (128). No M1 PAMs are currently in clinical trials.

### Nicotinic agonists

The role of nicotinic receptors in AD is less clear than muscarinic agonists, but it is known that nicotinic acetylcholine receptors (nAChRs) play diverse roles in cognition, memory, and neuro-protection (129). Unlike muscarinic receptors, which are G-protein coupled receptors, nicotinic receptors are ligand-gated ion channels. The therapeutic nicotine effect is secondary to increased release of ACh through the activation of nAChRs and the subsequent activation of M1 receptors by acetylcholine (130). Studies have shown that  $\alpha$ 7-nicotinic agonists attenuate A $\beta$  mediated toxicity but may increase phosphorylation of tau (131, 132). Many novel ligands for  $\alpha$ 7-nicotinic AChR have reached phase III development.

EVP-6124 (also known as encenicline hydrochloride) is a novel selective  $\alpha$ 7-nAChR partial agonist that was evaluated for the treatment of cognitive deficits in both schizophrenia and AD

that very recently failed phase III trials. In AD, an initial ascending-dose phase I/II study showed smaller doses of 0.1 to 1 mg/day of EVP-6124 (0.1 to 1mg/day) to be safe and well-tolerated in 49 people with mild to moderate AD for 28 days. Secondary efficacy endpoints at that time suggested that EVP-6124 could improve attention, verbal fluency, and executive function, when given in conjunction with acetylcholinesterase inhibitors (133). As such, a 24-week phase II trial (NCT01073228) was conducted in 409 people using higher doses in mild to moderate AD patients and met most of its primary and secondary endpoints, including cognitive measures (134). This led to the initiation of two international phase II trials enrolling a total of 958 patients with mild to moderate AD who were already taking an acetylcholinesterase inhibitor in October 2013 (NCT01969123 and NCT01969136). The trial compared two fixed, undisclosed add-on doses of EVP-6124 to placebo and was set to run through 2016 with results being available in 2017. However, in September 2015, the FDA placed a hold on these trials due to reports of rare but serious gastrointestinal side effects. Phase III studies in schizophrenia continued as these patients did not seem to suffer from the same gastrointestinal side effects, but after the drug missed its co-primary endpoints in the schizophrenia phase trials, the development of this compound was discontinued.

ABT-126, also known as nelonidine, is another  $\alpha 7$ -nAChR allosteric modulator that has undergone extensive clinical development. It is similarly being tested in schizophrenia and Alzheimer's disease. Phase 1/2 research for ABT-126 began in 2009. The compound was compared to both placebo and to donepezil and preliminary data was reported to have shown good tolerability for ABT-126, with side effects similar to donepezil (135). In 2012, two 24-week Phase 2b trials in 400 patients each, compared ABT-126 added on to donepezil to placebo, and compared ABT-126 monotherapy to placebo (NCT01549834). However, although ABT-126 was generally well tolerated with the most common adverse events were agitation, constipation, diarrhea, fall, and headache, ABT-126 did not demonstrate significant improvement in the primary efficacy endpoint and a treatment effect was not observed for any secondary efficacy measures of cognition, function, or global improvement (136).

### Serotonin antagonists

Serotonin indirectly affects neurodegenerative processes. 5-HT<sub>6</sub> serotonin receptors in humans are present in areas responsible for memory and cognitive function. Blockade of 5-HT<sub>6</sub> receptors increases synaptic acetylcholine release, improving cholinergic transmission and enhances memory and cognition (137-139). Antagonists of 5-HT<sub>6</sub> are under development for AD. Idalopirdine (Lu AE58054) is a 5-HT<sub>6</sub> that has been reported to be highly selective, aside from some affinity for adrenergic receptors (140). Lu AE58054 has successfully undergone phase I trials in healthy volunteers. Since the results of preclinical tests suggested that the use of 5-HT<sub>6</sub> antagonists in combination with cholinesterase inhibitors may increase the beneficial effects of both drugs on cognition, a phase II study with idalopirdine and donepezil was carried out among 278 patients with moderate AD. Results indicated improvement of cognitive function, although in 14 people taking idalopirdine transient increases in liver transaminase levels were observed (141). Two Phase III studies evaluating the efficacy of LuAE58054 were initiated in 2013 enrolling 932 patients (STARSHINE, NCT01955161) and 858 patients (STARBEAM, NCT02006641) with mild-to-moderate AD also on donepezil therapy. These studies have completed recruitment and are expected to be completed in 2017. An open-label extension phase III study for these trials were initiated in 2014 and this trial will also evaluate psychiatric endpoints (NCT02079246).

Another 5-HT<sub>6</sub> receptor antagonist, SB-742457 also known as RVT-101 has also reached phase III trials. In animals, SB-742457 has been reported to not only have beneficial effects in AD models, but also in age-related cognitive decline in rats (142). SB-742457 was also studied in patients with or without donepezil in phase II clinical trials completed in 2011 (NCT00710684). In the studies evaluating SB-742457 alone, no benefit was seen in cognition, but a significant improvement in global function was seen in those studies that evaluated patients also receiving donepezil (143). A phase III trial was initiated in 2015 enrolling 1150 patients with mild-to-moderate AD who are also stable on donepezil (MINDSET; NCT02585934). This trial is expected to be complete in 2017. Also, a phase II trial in patients with dementia with lewy body disease was initiated in January 2016 (HEADWAY-

DLB; NCT02669433). SUVN-502 is a selective antagonist of the 5-HT<sub>6</sub> serotonin receptor that is currently recruiting for phase II human trials. In September 2015, a phase 2a proof-of-concept trial began enrolling 537 patients with probable Alzheimer's disease to compare SUVN-502 to placebo, all given in addition to donepezil. The treatment period of the trial is to last 26 weeks and the study is expected to be complete in June 2017 (NCT02580305).

Other 5-HT receptor subtypes have been studied in AD but have failed to show benefit. For instance, lecozotan, a 5-HT<sub>1a</sub> receptor antagonist which was shown to produce significant improvement in task performance efficiency and reverse learning deficits in animals (144), went as far as phase II/III studies (NCT00277810); however, it was not pursued further for AD development. Another orally active 5-HT<sub>1a</sub> receptor antagonist that was triggering some interest was called xaliproden, which was reported to counteract A $\beta$ -induced neuronal toxicity and memory deficits in rats (145). However, two 18-month Phase 3 trials in mild-to-moderate Alzheimer's disease, one enrolling 1,455 patients, the other 1,306 completed in 2007, found no beneficial therapeutic effect (NCT00104013 and NCT00103649).

### Histamine antagonists

Histamine H<sub>3</sub> receptors (both auto- and hetero-receptors) are also present in large amounts in the prefrontal cortex, hippocampus, and hypothalamus which are important for memory and cognition. Blockage of the pre-synaptic H<sub>3</sub> receptor leads to increased release of acetylcholine, dopamine, GABA, noradrenaline and histamine into the synaptic cleft, indirectly improving cholinergic neurotransmission (146). ABT-288, a competitive selective H<sub>3</sub> receptor antagonist was a histaminergic antagonist that reached phase II trials in 242 patients with mild to moderate AD completed in 2011. Unfortunately, the trial was terminated early, as no statistical changes in cognitive function was found and because futility criteria were met (147). GSK239512, another H<sub>3</sub> antagonist with an acceptable safety profile, underwent phase II trials in 196 patients with mild or moderate AD (NCT01009255). In this 16-week, double-blind, randomized, parallel group study, GSK239512 improved Episodic Memory; however,

no improvements were observed in any other domains of cognition or other secondary endpoints indicating that H<sub>3</sub> agonists at most, have modest and selective effects on cognitive function in patients with mild-to-moderate AD (148). Finally, dimebon is an antihistamine that has been used in Russia since the 1980s to treat allergic rhinitis, which made it to phase III trials in AD. Dimebon is a pleiotropic drug with activities beyond blocking H<sub>1</sub> histamine receptors. A mechanism of action for cognitive benefit has never been conclusively established; however, a broad spectrum of effects has been proposed including modulation of NMDA glutamate receptors,  $\alpha$ -adrenergic receptors, and serotonergic or dopaminergic receptors (149). Phase I and II trials of a total of 197 Alzheimer's patients in Russia showed Dimebon was safe, well tolerated, and significantly improved the clinical course of patients with mild-to-moderate AD (150). This was then followed by two Phase III trials initiated in 2009 in patients with mild to moderate AD conducted in the Americas, Europe, Australia, and New Zealand, but neither Phase III study detected change in any primary or secondary outcome (NCT00838110, NCT00675623, and NCT00829374; CONNECTION).

### Neurotrophic and Hematopoietic Growth Factors

#### Brain-Derived Neurotrophic Factor

Brain-derived neurotrophic factor (BDNF) is a member of neurotrophins family, which are formed through post-translational modifications to proneurotrophins. It has been suggested that BDNF could play an important role in AD, due to promotion of neuron survival and synaptic regulation via TrkB receptor and PI3K/Akt signaling pathway (151). It has been linked to learning and memory, due to its role in axonal sprouting, dendritic proliferation, and neuronal differentiation (152). Loss of BDNF function has been reported in neurodegenerative and psychiatric diseases (153, 154). Genetic structure and intracellular mechanisms of BDNF was recently reviewed by Adachi et al (155).

Multiple studies have revealed a drop in BDNF levels in AD patients (156-159), while other reports indicate an increase in BDNF expression compared to control (160), which could be a compensatory repair mechanism during early stages of AD.

**Table 6** - A summary of the clinical trials based on neurotransmitter-based therapies (<https://clinicaltrials.gov>)

Drug	Clinical Trial Number & Phase	Start & End Date	Trial Population	Outcome	Special Note
<b>HTL9936</b>	NCT02291783 - Phase I	2013 - 2014	108		M1 receptor agonist
	NCT00766363 - Phase I	2008 - 2009	49	- Well-tolerated - improved attention, verbal fluency	$\alpha$ 7 nAChR partial agonist for the treatment of cognitive deficits in both schizophrenia and AD given in conjunction with acetylcholinesterase inhibitors
<b>EVP-6124</b>	NCT01073228 - Phase II	2010 - 2012	409	- Met most of primary and secondary endpoints	
	NCT01969123 - Phase III	2013 - 2017	474	- Terminated	
	NCT01969136 - Phase III	2013 - 2017	403	- Terminated	
	NCT02327182 - Phase III	2014 - 2015	117	- Terminated	
	NCT02004392 - Phase III	2014 - 2017	348	- Terminated	
<b>ABT-126 nelonicline</b>	NCT00948909 - Phase II	2009 - 2010	274	- Good tolerability	$\alpha$ 7-nAChR allosteric modulator
	NCT01549834 - Phase II	2012 - 2013	434	- No significant improvement	
	NCT01527916 - Phase II	2012 - 2013	438	- No significant improvement	
	NCT01676935 - Phase II	2012 - 2014	349	- Terminated	
	NCT01690195 - Phase II	2012 - 2014	343	- Terminated	
	<b>Idalopirdine (Lu AE58054)</b>	NCT01019421 - Phase II	2009 - 2011	278	
NCT01955161 - Phase III		2013 - 2017	932		
NCT02006641 - Phase III					
NCT02079246 - Phase III		2014 - 2017	858		
NCT02006654 - Phase III		2014 - 2017	1770		
		2014 - 2017	734		

**Table 6. Continued...**

<b>SB 742457 (RVT-101)</b>	NCT00710684 - phase II	2008 – 2011	684	- No benefit alone; significant improvement with donepezil	Beneficial effects in AD models but also in age-related cognitive decline in animals
	NCT02585934 - Phase III	2015 - 2017	1150		
<b>SUVN-502</b>	NCT02580305 - Phase II	2015 - 2017	537	With donepezil	
<b>lecozotan</b>	NCT00277810 - phase II/III	2006 - 2008	250	Not pursued further for AD development	5-HT <sub>1a</sub> receptor antagonist
<b>xaliproden</b>	NCT00104013 - Phase III	2003 - 2007	1455	- No treatment effect	5-HT <sub>1a</sub> receptor antagonist reported to counteract A $\beta$ -induced neuronal toxicity and memory deficits in rats
	NCT00103649 - phase III	2003 - 2007	1306	- No treatment effect	
<b>ABT-288</b>	NCT01018875 - Phase II	2009 - 2011	242	- Terminated; no change in cognitive function	Competitive selective H <sub>3</sub> receptor antagonist
<b>GSK239512</b>	NCT01009255 - phase II	2009 - 2010	196	Modest and selective effects on cognitive function in patients with mild-to-moderate AD	H3 antagonist
<b>Dimebon</b>	NCT00838110 - Phase III	2009 - 2010	742	No change in any primary or secondary outcome	Antihistamine used in Russia since the 1980s to treat allergic rhinitis
	NCT00675623 - Phase III	2008 - 2009	598		
	NCT00829374 - Phase III	2009 - 2011	1003		

Overall, it has been hypothesized that activating BDNF intracellular signaling could be a promising strategy in AD treatment (151). In 2013, Xu et al. reported a positive effect of a variety of flavonoids on expression levels of BDNF (161). Recently, Diaz-Gerevini et al. reported a link between flavonoid resveratrol and enhancement of synthesis and mechanism of BDNF (162). Another possible pharmacological intervention that could positively affect BDNF levels is curcumin. Curcumin has been extensively studied for anticancer, antioxidant, and anti-inflammatory properties (163). However, it has been suggested that curcumin could also inhibit DNA methyltransferase, histone acetyltransferase and histone deacetylase, and modulate miRNA in hippocampus, which are

associated with an increase in BDNF levels (164). Antidepressants have also reported to increase BDNF levels in AD patients (165). However, a recent clinical trial showed no significant effect of donepezil treatment on BDNF levels (166), while a 2009 clinical trial have indicated an increase in BDNF levels in lithium-treated patients with early AD (167). BDNF levels have been analyzed as a biomarker in other clinical trials conducted on AD treatment, especially in trials on the effect of physical activity and exercise on AD (168-170). Beneficial effect of BDNF in several mouse models of AD has also been reported. Delivering the BDNF gene in transgenic mice that constantly produces BDNF, has interfered with loss of synapses, and enhanced the cognitive function, without co-interacting with  $\beta$ -amyloid (171).

### **Nerve Growth Factor**

Another member of neurotrophic factors is nerve growth factor (NGF), which is a glycoprotein synthesized from the inactive form, pro-NGF (172), and exerts its trophic effect via TrkA and P57 receptors (173). NGF plays an important role in maintaining neuronal proliferation and growth of neurons by interacting with other growth factors, and maintenance of cholinergic neurons and memory-related parts of CNS (e.g., hippocampus) (174). A drop in NGF levels with aging has been reported in many animal models (175-177), which could be an indication of the beneficial effects of NGF in AD patients. However, recent studies have not revealed a significant difference in blood, CSF, and brain levels of NGF in AD patients compared to healthy subjects (178). In a small phase I clinical study (8 patients) reported in 2005, transfected fibroblasts with permanent expression of NGF were implanted in the forebrain of the patients, and significant increase in neuronal activity was reported (179). Also, a more recent Phase I clinical study with *ex vivo* gene delivery to AD patients demonstrated enhanced cerebral metabolism and cognitive function without significant adverse effects (180). Positron emission tomographic (PET) scans confirmed the inhibition of the metabolic decline expected in AD patients.

### **Glia-Derived Neurotrophic Factor**

Originally isolated from medium of a rat glioma cell line, glial-derived neurotrophic factor (GDNF) is another important growth factor, especially for survival and maintenance of dopaminergic neurons in midbrain (181). GDNF also seems to play a major role in enhancing the blood brain barrier. However, the neurotrophic effect of GDNF is only observed in the presence of transforming growth factor  $\beta$  (TGF $\beta$ ) (182). The CSF levels of GDNF has shown a significant increase in AD patients (despite a decrease in serum levels of this growth factor), which has been explained as an adaptive process to enhance neurotrophic effect (183). Other researchers have reported an increase in GDNF plasma levels in AD patients (184). It has been indicated that treatment with GDNF could protect rabbits against AD-like symptoms, mainly by increasing the expression level of anti-apoptotic proteins (185). Also, transfection with GDNF-expressing gene using a lentiviral vector has improved cognitive mechanisms in a transgenic

mice model of AD (186). Maybe even more interestingly, this study has also demonstrated an increase in BDNF expression level, which might indicate a link between the two growth factors in their protective role against neuron degeneration.

### **Granulocyte-Colony Stimulating Factor**

A potent growth factor with a stimulating effect on myeloid cells, granulocyte-colony stimulating factor (G-CSF), is also reported to inhibit apoptosis and induce neuronal differentiation *in vitro* (187). A decrease in G-CSF blood levels in early AD patients has been reported by Laske et al, with no data available for the CSF or brain level of this growth factor (188). Tsai et al. have reported a significant cognitive improvement in transgenic mice models of AD after 5 days of subcutaneous injection of G-CSF (189). They also reported that G-CSF administration induced neurogenesis around A $\beta$  aggregates in these acute and chronic animal models of A $\beta$ -driven AD. In 2012, a pilot clinical study in 8 patients with mild to moderate stages of AD did not show a serious adverse effect for five days of subcutaneous G-CSF administration, but did provide evidence for positive changes in hippocampal-dependent cognitive tasks (190).

### **Stem Cell Factor**

Stem cell factor (SCF) is a hematopoietin expressed in CNS that enhances neurogenesis (191). It might also have a role in survival and migration of neural stem cells (192). Along with G-CSF it helps bone marrow-derived progenitor cells differentiate to microglial cells or neuronal stem cells (192). This suggests a protective role towards neurons, which might be exerted via PI3K/Akt or MEK/ERK pathways (193). A decrease in plasma and cerebrovascular fluid levels of SCF has been reported in AD patients (194). It has also been shown that a higher SCF level correlates with slower deterioration of cognition in AD patients (195).

### **Antioxidant Therapies**

There is significant evidence for involvement of oxidative stress in the early stages of AD, as well as activation of signaling pathways that contribute to lesion formation and neuron degeneration (2). In fact, oxidative stress and glycooxidation are closely associated with AD development (196). Also, many preclinical and small clinical studies have shown



that antioxidant drugs have significant effect on the development of Alzheimer's disease. Reports show antioxidants could contribute to breaking down superoxide radicals and hydrogen peroxide compounds, and prevent them from damaging the neurons (197).

### Vitamins

**Vitamin E:** As a free radical scavenger, alpha tocopherol or vitamin E acts as an antioxidant and has been studied in AD treatment. In 1996, Sano et al. reported a randomized double-blind, placebo-controlled clinical study, which showed some benefit for vitamin E administration to AD patients (58% chance of death, institutionalization, change to a Clinical Dementia Rating of three, or loss of two basic activities of daily living within two years for recipients of 2000 IU/day vitamin E compared to 74% for patients who received placebo) (198). In another clinical study published more than a decade later, however, patients who received 800 IU vitamin E per day and did show a reduction in oxidative stress, no significant difference compared to placebo group was observed in terms of Mini-Mental State Examination (MMSE) score after six months (199). However, a recent meta-analysis of different medical and dietary interventions in AD patients has demonstrated a protective effect for vitamin E (200). The potential effect of vitamin E on cognitive function of AD patients was recently reviewed by La Fata et al (201).

**Vitamin C:** Ascorbic acid (vitamin C) is an important antioxidant in intracellular functions, with some of highest concentrations in CNS (202). It easily reacts with reactive oxygen species to neutralize their activity, and become ascorbate free radical, which is then recycled (203). Vitamin C seems to play an important role in neuronal repair, generation of new cells, and expression of many different genes (204). In a longitudinal study on 71-93 years old Japanese-American men, the results suggested that vitamin E and C supplement intake might have a protective effect against vascular dementia and may improve cognitive function in elderly patients (205). More interestingly, the 2004 Cache County study indicates that only the combination of vitamins C and E could reduce the risk of AD (206).

**Vitamin A and Carotenoids:** A lipid-soluble antioxidant, vitamin A is synthesized in the body from structurally more complex carotenoids in our diet. Several studies have indicated a decrease in plasma levels of vitamin A and  $\alpha$ -carotene (but not  $\beta$ -carotene) in AD patients compared to control individuals (207). However, there have been reports on the positive effect of  $\beta$ -carotene on cognitive function (208), and a correlation between higher  $\beta$ -carotene plasma levels and memory in elderly individuals (65-94 years old) (209). In 2004, Ono et al. reported vitamin A and  $\beta$ -carotene would inhibit the formation of  $\beta$ -amyloid fibrils *in vitro*, and even destabilize already formed fibrils in a dose-dependent manner (210). Also, vitamin A deprivation could cause  $\beta$ -amyloid accumulation (211) and memory deficit (212) in adult rodents. The role of carotenoids in prevention of AD symptoms and delaying of AD symptoms was recently reviewed by Obulesu et al. (213).

**Vitamin B12:** Low levels of vitamin B12 are shown to be associated with neurodegenerative disease and cognitive impairment (214). Meta-analysis studies indicate a lower level of vitamin B12 in AD patients (215), and a recent study indicates that administration of vitamin B12, along with other vitamins, selenium, and phospholipids could preserve synaptic function (216). In 2014, Li et al. performed a systematic review and meta-analysis on efficacy of vitamin B supplementation in AD (217). Their results did not confirm the efficacy of vitamin B family in improving cognitive function, and authors concluded that these vitamins are unable to stabilize or slow decline in cognition in AD patients.

### Other Antioxidants

Omega 3 polyunsaturated fatty acids, e.g., eicosapentaenoic acid (ESA) and docosahexaenoic acid (DHA) have been known to improve brain function, and therefore, there has been suggestions on their role in preventing neurodegenerative diseases, including AD. In fact, insufficient DHA intake has been related to brain aging, and reports suggest a positive effect for DHA in delaying the onset of AD (218). DHA is selectively accumulated in synaptic membranes and oligodendrocytes (219). A decrease in DHA brain levels in AD patients is also reported (220). Several clinical studies indicate beneficial effects of omega 3 in AD, which have

been recently reviewed by Thomas et al (221). Alkaloids, including caffeine, have also been studied extensively for their potential effect on AD progression. However, the results of observational studies vary significantly, and are therefore inconclusive (222). Ginkgo biloba, a natural plant containing flavonoids and terpenoids, has both antioxidant and anti-inflammatory properties, as well as free radical scavenging ability, can reduce amyloid precursor protein and inhibit A $\beta$  aggregation (223).

Metal chelators, glutathione peroxidases, SOD enzymes, antioxidant enzyme MnSOD, repair enzymes such as lipases, proteases, and DNA repair enzymes play important roles in neuronal survival and could protect brain tissue against oxidative damage (2). Melatonin, a pineal gland hormone with well-known role in sleep regulation, easily passes BBB, is a scavenger of hydroxyl, carbonate, alkoxyl, peroxy and aryl cation radicals, and stimulates activities of antioxidative enzymes, which suggests a protective role in neurodegenerative disorders (224). It is also hypothesized that the higher AD prevalence in women could be related to reduction of estrogen in postmenopausal stage (225). Xu et al meta-analysis has also shown a protective role for estrogen in AD (200).

## CONCLUSION

It has long been recognized that the anti-Alzheimer's drugs currently available for treatment modify only the late downstream effects of the AD pathophysiologic process and only temporarily reduces symptoms with no effect on disease progression. Drug development scientists have long sought therapeutic approaches that target the etiologic processes of AD which can then ultimately affect disease progression. The past two decades has seen rapid growth in the understanding of the underlying mechanisms involved in the etiology of AD, including the role of A $\beta$  and tau, the role of various neurotransmitters, and the role of growth factors and antioxidants. This has led to the development and testing of hundreds of molecules and numerous approaches targeted within these mechanistic categories including active and passive immunization against A $\beta$  and tau, inhibition of A $\beta$  aggregation through BACE-1 and  $\gamma$ -secretase inhibition, modulation of various neurotransmitter

systems with subsequent effects on A $\beta$  and tau pathology along with symptomatic effects, and finally administration of various growth factors and antioxidant therapy for promotion of neuronal survival against a now-recognized wide-reaching and complex neurodegenerative process. Many of these molecules and approaches have been promising enough to be advanced to human testing in close to two thousand phase I-III clinical trials with the most promising agents in these categories reviewed in this article.

Despite this immense effort, no new drugs have been approved for market, since the approval of meantime in 2003. As reviewed in this article, scientists have faced multiple challenges in this era of failed AD drug development. Some challenges were overcome by strategic drug design, as seen in the development of active A $\beta$  vaccines and the challenge of overcoming cerebral vasogenic edema caused by T-cell immunogenicity ultimately accomplished by targeting smaller non-T-cell immunogenic peptide sequences, or as seen in non-selective NOTCH inhibition in BACE-1 therapeutic approaches which was overcome by design of highly selective BACE-1 inhibitors. However, despite careful honing of drug design, optimization of pharmacodynamics and pharmacokinetics, and significant modifications that introduced reduction in toxicity, no new AD drugs have been found to significantly affect clinical outcomes and delay disease progression.

What are we doing wrong? While several contributing factors may be responsible for the continuous failure of clinical trials, one common challenge that has continued to emerge and that all drug development scientists must consider is the timing of treatment and disease staging. Most clinical trial study designs in the past two decades have focused on administering treatment to patients with mild-to-moderate AD and measuring for improvement in symptoms or clinical outcomes as measured by various cognitive scales, an approach that worked well for acetylcholinesterase inhibitors and memantine. However, these drugs targeted late stage processes contributing to overall cognitive symptoms with no effect on degenerative processes. It is now well known that the etiologic and neurodegenerative processes of AD start at least a decade prior to the appearance of symptoms. At symptom start then, there is likely already significant irreversible neurodegeneration present.

Furthermore, the diagnosis of AD is not made until symptoms observably impair daily activities, at which point even more severe neurodegeneration is likely present. Drug development scientists are increasingly recognizing that administering drug therapy even in mild-to-moderate disease may be too late to affect disease progression. For instance, the EXPEDITION studies, which were the phase III trials of the monoclonal antibody solanezumab, an agent which was highly anticipated to be the most successful of all agents ever in development, failed to show benefit in the primary clinical outcome measure when administered in patients with mild to moderate AD. However, when only early disease was evaluated (e.g mild AD), there was suggestion toward some benefit in patients with only middle disease. As such, we are increasingly seeing study designs similar to that of the active vaccine CAD-106, which is currently enrolling ApoE4 carriers who are cognitively and will measure the ability to delay diagnosis to MCI or AD dementia after 5 years of treatment. Studies of genetic forms of AD are easier to design, conduct, and analyze than studies of non-genetic forms of AD, as patient ascertainment through genotyping provides a reliable biomarker of likely AD development. However, genetic AD is much less common than non-genetic AD and design of clinical trials with early ascertainment of patients with non-genetic AD is absolutely necessary. Early patient ascertainment with non-genetic forms of AD had previously been difficult, due to the lack of reliable biomarkers often leading to on-treatment groups containing people that would never go on to develop the disease anyways. With the development and validation of the PET-ligand florbetapir, PET amyloid imaging can now be utilized as a biomarker in non-genetic forms of AD, allowing more accurate ascertainment of patients who would likely go on to develop AD for inclusion in clinical trials prior to them actually meeting clinical criteria for AD diagnosis. As such, agents such as solanezumab and bapenzumab and most recently the BACE inhibitor XYZ are enrolling or have announced plans to enroll patients in re-designed clinical trials that target patients with normal cognitive function who are deemed at risk for AD by PET amyloid imaging as inclusion criteria. This recent trend in clinical trials shifts the participants towards earlier disease and younger ages, which in

itself can potentially affect the interaction of the agent with intracellular mechanisms.

AD drug development has undergone rapid growth in the past two decades. Despite the lack of positive clinical trial outcomes, the lessons learned with each trial and each failure have brought us closer to a deeper understanding of the disease and the drugs we are using to combat the disease. With an armamentarium that has been finely honed to optimize success, and the ability to now conduct appropriately designed clinical trials, we are at the dawn of a new era in the fight against this deadly disease that has plagued humanity for centuries.

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