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# ALZHEIMER'S DISEASE: THE AMYLOID HYPOTHESIS ON TRIAL

DOES AMYLOID PATHOLOGY LAY DOWNSTREAM OF OTHER CELLULAR EVENTS THAT ARE RESPONSIBLE FOR AD PATHOGENESIS?

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## ABSTRACT

Alzheimer's Disease (AD) is an common, irreversible neurodegenerative condition and is considered as one of the biggest global public health challenges. The amyloid hypothesis of Alzheimer's disease has been the foundation of efforts to understand the disease for almost 25 years. The hypothesis proposes that deposition of beta-amyloid (Aβ) protein is central to the pathogenesis of the disorder. However, recently its validity has been called into question after the failure of Aβ-targeting therapies in clinical trials, suggesting that the amyloid pathology lies downstream of (an)other cellular event(s) that is/are responsible for AD pathogenesis. Nonetheless, there could have been insufficient target engagement or the drugs may have been administered too late in the disease process. Studies to investigate this matter are on their way, until then we can not draw harsh conclusions on giving up on the amyloid hypothesis. However, AD is probably much more complicated and there are probably many roads leading to the disease. Therefore, we need focus on other therapeutic strategies as well.

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# INTRODUCTION

Alzheimer's Disease (AD) is an irreversible neurodegenerative condition and is the most common form of dementia, affecting 4-8% of the elderly population worldwide.[1] Prevalence increases exponentially every 5 years from 65 years of age and will probably rise even further with the increasing longevity.[2] The symptoms of the disease are progressive memory loss, reduced cognitive capacity accompanied by abnormal behaviour and personality changes and eventually dementia.[3] The German physician A. Alzheimer first described this disorder and its underlying brain pathology in 1907 in a paper titled "About a Peculiar Disease of the Cerebral Cortex".[4]

# STAGES OF AD

Alzheimer's disease is a slow progressive disorder. Its continuum is considered as having three stages (Figure 1). In the first stage, the preclinical stage, patients are asymptomatic or early symptomatic, but they do harbour AD neuropathology as indicated by biomarkers. The second stage, the prodromal stage, patients have significant impairment in a single cognitive domain, typically episodic memory. And the third stage, the dementia stage, in which patients have impairment in multiple cognitive domains.[5]

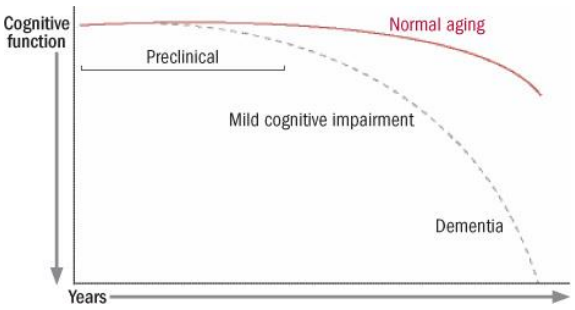


Figure 1 - **Model of the clinical trajectory of Alzheimer's disease (AD).**[6]

## PRECLINICAL

The preclinical or presymptomatic stage is defined by measureable biomarker changes in the brain, which increases the risk for progression to AD dementia. These measureable biomarker changes may occur years before symptoms can be detected. Biomarker changes include CSF Aβ-42, increased amyloid tracer retention on positron emission tomography (PET) imaging, decreased fluorodeoxyglucose18F (FDG) uptake on PET, CSF tau and atrophy of the brain structure seen by using magnetic resonance imaging (MRI). However, they do not establish diagnostic criteria. According to the National Institute on Aging and the Alzheimer's Association, preclinical AD can be classified in three stages. The first stage is characterized by the presence of amyloid accumulation in the brain. The second stage involves both amyloid positivity and signs of neuronal injury as evidences by high cerebrospinal fluid (CSF) tau, regional brain atrophy or hypometabolism. The third stage is defined by amyloid positivity, evidence of neurodegeneration and subtle cognitive impairment. It is important to realize that not every individual progresses beyond stage 1 or stage 2 and that individuals in stage 3 are postulated to be more likely to progress to the prodromal stage and AD dementia.[6]

## PRODROMAL

The prodromal stage or mild cognitive impairment (MCI) is characterized by change in cognition, impairment of one or more abilities (including memory, attention, language and ability to plan), ability to function independently and absence of dementia. If all of the above-mentioned characteristics are present in an individual, other medical problems that could account for the impairment in cognition should be ruled out. However, some disorders (Lewy bodies and vascular disease) can exist in combination with AD. Biomarker testing in individuals with MCI could establish support for the underlying etiology of the

clinical syndrome and determine the likelihood of cognitive and functional progression.[7]

## DEMENTIA

When Alzheimer progresses to a point in which memory, thinking and behaviour are so impaired that an individual is not able to function independently anymore; one has reached the stage of dementia. The diagnosis of AD dementia is probable when there are cognitive or behavioural symptoms which develop gradually, hamper the daily activities, increase over time and involve at least two of the following domains: memory, executive function, visuospatial ability, language, behaviour and personality. In addition, other options of dementia, such as stroke or delirium, should be ruled out. The diagnosis of AD dementia is fundamentally a clinical diagnosis.[8]

## THEORIES ON AD PATHOGENESIS

AD is characterised by the accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs) comprised of hyperphosphorylated tau protein. With AD it is uncertain whether the histopathology drives the disease or the disease drives the histopathology.
In 1991 one found an mutation in the amyloid precursor protein (APP) with caused AD, so AD sometimes represents a primary amyloidosis.[9] The hypothetical model of AD also shows Amyloid beta (Aβ) peptide accumulation as the key early event in the process (Figure 2). However, some data suggest that amyloidosis in AD is a secondary event.[10] Other alterations, such as synaptic, mitochondrial, metabolic, inflammatory, neuronal, cytoskeletal, and other age-related alterations, may play an earlier and more a more central role.[11,12] Accumulation of Aβ is followed by synaptic dysfunction, neurodegeneration and eventual neuronal loss, leading to cognitive decline. Age, genetics, brain and cognitive reserve and other brain diseases are thought to negatively affect the progression of AD.[6]

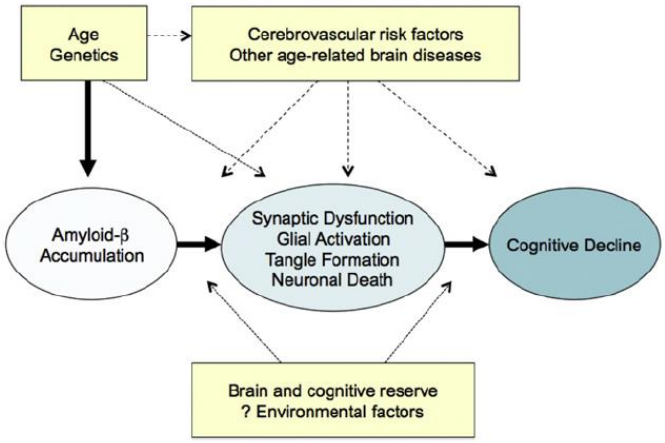


Figure 2 - **Hypothetical model of AD pathophysiological Cascade**.[6]

## Aβ PLAQUES

Amyloid beta (Aβ) is produced by the cleavage of its parent protein, amyloid precursor protein (APP). APP is a transmembrane protein. Its carboxylic terminus is located intracellular and its N-terminus is located extracellular. Enzymes can cleave APP in several places near the C-terminal end. Enzymes with α-secretase activity cleave APP in the middle of the Aβ domain, 83 amino acids from its carboxyl terminus. β-secretases cleave APP 99 amino acids from its C-terminus. γ-secretase cleaves twice; the cut occurs 50 amino acids from the C-terminus or 57, 59 or 61 amino acids from the C-terminus. This creates an amino acid peptide with the APP C-terminus called the amyloid intracellular domain (AICD).
The APP secretases work in combination. In the non-amyloidogenic pathway both α and γ secretases process APP, α-secretase cleaves APP producing a large N-terminal peptide called soluble APPα (sAPPα) and C-terminal fragment CTFα. Sequential γ-secretase activity cleaves CTFα, producing a 3 kD peptide called P3 and the amyloid intracellular domain (AICD). The amyloidogenic pathway is characterised by sequential β-γ secretase activity. Cleavage of APP by β-secretase is producing sAPPβ and C-terminal fragment CTFβ, this is followed by cleavage of CTFβ by γ-secretase producing the Aβ peptide and AICD.

With sequential α-γ secretase activity the γ-secretase cut occurs 50 amino acids from the C-terminus and with sequential β-γ secretase activity the γ-secretase cut-cite varies, producing an Aβ oligomer peptide with a typical length of 38-42 amino acids.[10] The Aβ peptide with a length of 42 amino acids is considered as culprit in causing dementia in AD.[13]
Aβ clearance is also enzymatically mediated by Aβ-degrading enzymes. These enzymes include neprilysin (also known as neutral endopeptidase, NEP) and insulin degrading enzyme (IDE).[10] Some Aβ peptides are cleared from the brain. However, impaired clearance of Aβ-42 can cause fibrils due to paring with other identical molecules. Fibrils meanly consist of Aβ-42, but Aβ-40 might also be included. Subsequently, the fibrils can aggregate to form the characteristic amyloid plaques.[13]

Alterations in transport processes that shuttle Aβ across the blood-brain barrier can also play a role in aggregation and accumulation of Aβ.[14]
Aβ oligomers impair synaptic function by interaction with cell-surface membranes and receptors causing altered signal transduction cascades, change in neuronal activity and triggering release of neurotoxic mediators by microglia (Figure 3). Vascular abnormalities can cause microinfarct due to impairment of the supply of nutrients and removal of metabolic by-products. Subsequently, the activation of astrocytes and microglia is promoted. The lipid-carrier protein apoE4 increases Aβ production and impairs Aβ clearance. ApoE4 is cleaved into neurotoxic fragments that destabilize the cytoskeleton in stressed neurons, causing impairment in mitochondrial functions.[14]

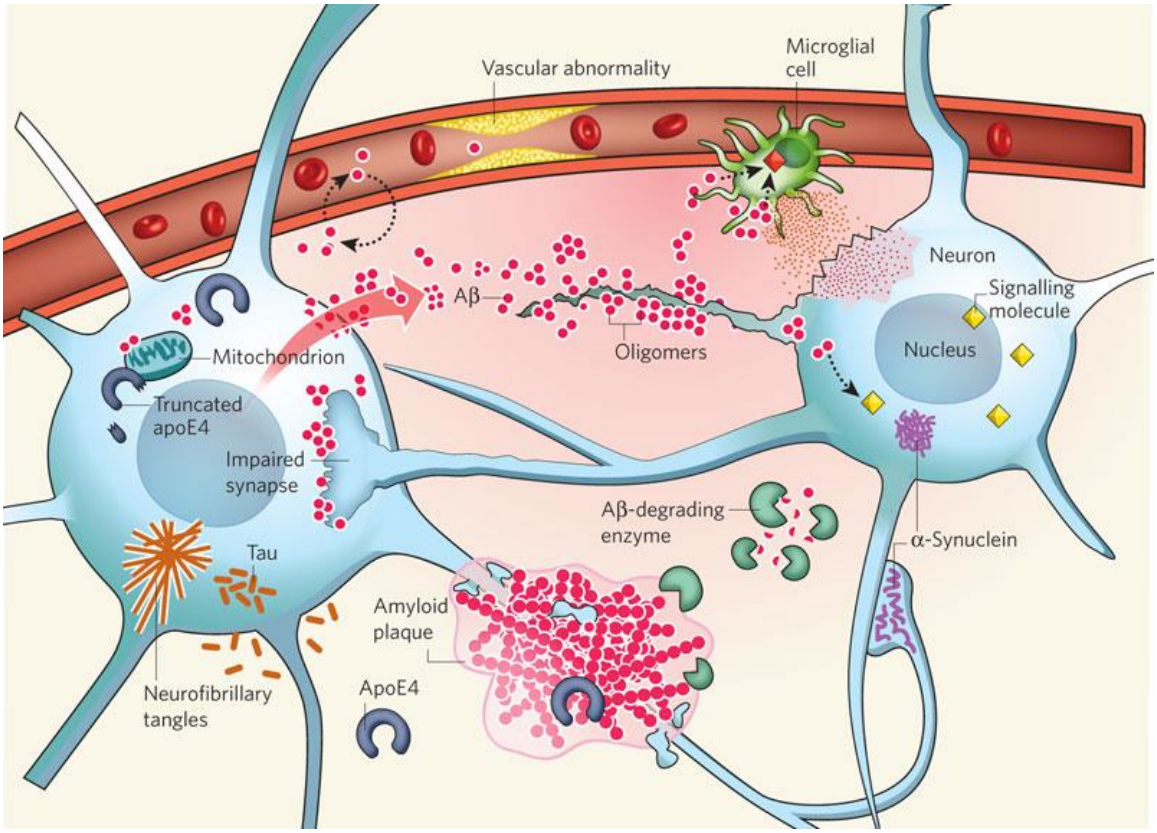


Figure 3 – **Some protagonists in the pathogenesis of AD**. Aβ oligomers cause altered signal transduction cascades, change in neuronal activity and triggering release of neurotoxic mediators by microglia. Vascular abnormalities promote the activation of astrocytes and microglia. The lipid-carrier protein apoE4 increases Aβ production and impairs Aβ clearance. Truncated apoE4 causes mitochondrial dysfunction. Tau and α-synuclein can also form oligomers and aggregates, known as neurofibrillary tangles and Lewy bodies) displacing vital intracellular organelles.[14]

## NEUROFIBRILLARY TANGLES

The second hallmark of AD is neurofibrillary tangles (NFTs). These are intracellular aggregations containing abnormally configured and excessive phosphorylated tau protein. Normally tau is unphosphorylated and associates with microtubule cytoskeleton elements to stabilize them. Hyperphosphorylation of tau causes disengagement from the microtubules and filament formation of tau with other tau proteins. These filaments can become enmeshed with one another, forming intracellular tangles. The microtubules normally help transport nutrients and other cellular components from the cell body to the axon. However, NFTs cause abnormal intracellular trafficking, collapse of the microtubule-based cytoskeleton, and subsequent neuronal death. Consequently, the neuron becomes unable to communicate with other neurons. The released tau filaments also contribute to activation of microglial cells and stimulating the deleterious cycle leading to progressive spread of neuronal degeneration.[10,15]

## CLASSIFICATION OF AD

AD is classified based on age of onset and whether it is developed spontaneously or as a result of genetic mutations. Familial AD (FAD) is caused by hereditary mutations, whereas sporadic AD (SAD) is heterogeneous with risk factors for developing AD, of with aging is considered the principal risk factor. Most individuals develop late-onset AD (>65 years, LOAD), of with the majority is sporadic. A few individuals develop early onset AD (<65 years, EOAD), with is generally familiar. Familial AD (FAD) is associated with mutations in the amyloid precursor protein (APP) located on the long (q) arm of chromosome 21 at position 21.3, presenilin-1 (PSEN1) located on the long (q) arm of chromosome 14 at position 24.2 and presenilin-2 (PSEN2) located on the long (q) arm of chromosome 1 at position 42.13.[16]

## RISK FACTORS OF AD

Aging is considered the principal risk factor of sporadic AD.[16] The risk increases exponentially every 5 years from 65 years of age. Both heredity and environmental factors could increase the risk. Therefore, the risk could increase if more than one family member has the illness.
Besides mutations in APP, PSEN1 and PSEN2, which cause early-onset familial AD, mutations in ApoeE4 can also cause AD. However, mutations in this gene usually cause late-onset AD and are considered sporadic. Inheriting one copy of the ApoeE4 gene variant will cause up to four times the normal risk and inheriting both copies will cause up to ten times the normal risk.[17]
Furthermore, since the APP gene is located on chromosome 21, individuals with Down Syndrome usually develop early-onset AD.[18]
Another important risk factor for AD is increased blood pressure and high serum cholesterol concentration. In particular the combination of both in midlife increase the risk of AD later in life.[19] Stroke is also associated with AD, especially in combination with the presence of known vascular risk factors.[20]
Research also has shown that there is a significant association between depression and AD. The association is the highest when depression symptoms first occur within 1 year before the onset of AD. However, the association was still modest if depression symptoms first occurred more than 25 years before the onset of AD.[21]
Previous head trauma is also associated with an increased risk of developing AD. However, this association was only present in males. The gender difference in the risk might be due to the influence of the female hormones, oestrogen and progesterone.[22]
Diabetes mellitus might be associated with an increased risk of developing AD. A few studies have examined the association, but their results are inconsistent. Some studies found an increased risk in persons with diabetes mellitus, while others did not find this association.[23]

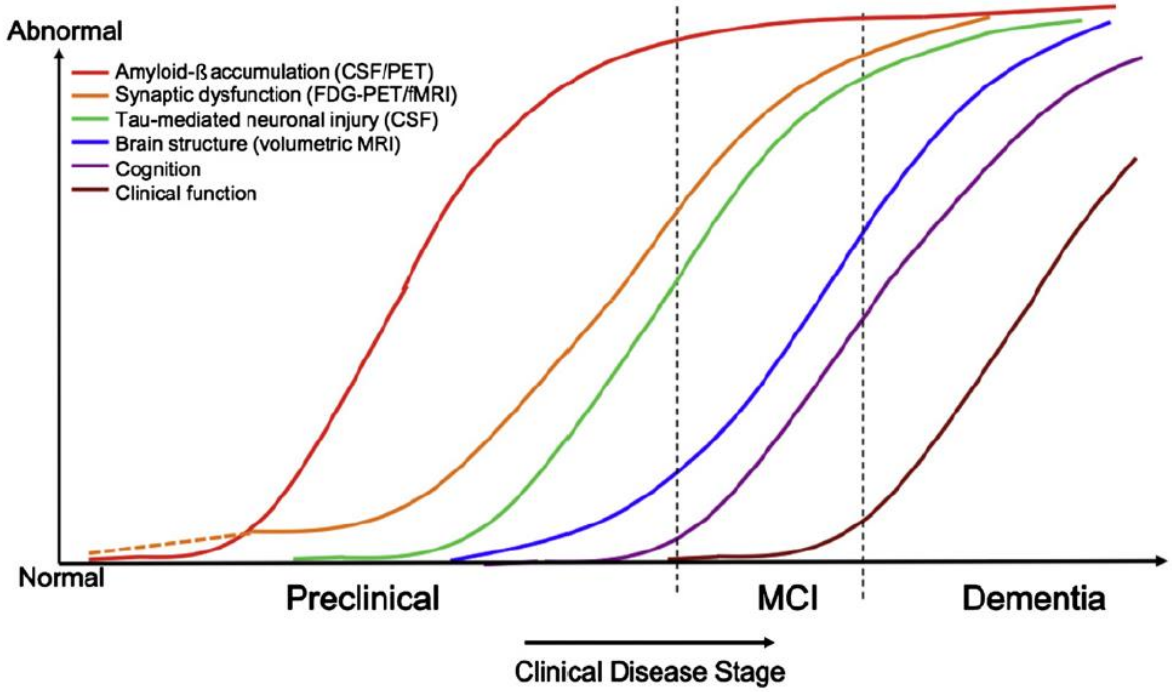


Figure 4 - **Hypothetical model of biomarkers in AD**. The biomarkers change from normal to maximally abnormal (*y*-axis) as a function of disease stage (x-axis). The dashed line of synaptic dysfunction is to indicate that it might be detectable in carriers of the ApoeE4 gene before detectable Aβ deposition.[6]

## BIOMARKERS OF AD OVER TIME

Recently, a biomarker model has been proposed in which the most validated biomarkers become abnormal (Figure 4). Biomarkers of brain Aβ amyloidosis include reduction in CSF Aβ-42 and increased amyloid tracer retention on positron emission tomography (PET) imaging. Synaptic dysfunction is characterised by decreases fluorodeoxyglucose18F (FDG) uptake on PET with a temporoparietal pattern of hypometabolism. CSF tau is thought to be a biomarker of neuronal injury. Atrophy of the brain structure can be seen using magnetic resonance imaging (MRI). The atrophy is characteristically seen in the medial temporal lobes, paralimbic and temporoparietal cortices.
Aβ accumulates before the onset of clinical symptoms. The lag phase in between are different between individuals, probably because of differences in brain reserve, cognitive reserve and the added contributions of coexisting pathologies. Aβ accumulation alone is not sufficient to produce the clinical symptoms of AD. Biomarkers of synaptic dysfunction might be detectable before detectable Aβ deposition in ApoeE4 carriers. Note that the rates of change of each biomarker change over time. The biomarker model provides diagnostic value in a very early stage and measures of disease progress.
Besides biomarkers, behavioural markers can also play a role in early identification of AD. However, both normal ageing and gradual cognitive decline before the onset of AD are difficult to distinguish. Nowadays, researchers are not yet able to assess the risk of progression to AD in individual elders by cognitive measures, despite multiple studies about this subject.[6]

## CHOLINERGIC HYPOTHESIS

The 'cholinergic hypothesis' was the first theory proposed to explain AD. The only current approved drugs for mild to moderate dementia due to AD are also based on this theory.[24,25] The theory is based on degeneration of cholinergic neurons in the nucleus basalis of Meynert (NBM) and the loss of cholinergic inputs to the neocortex and hippocampus. Post-mortem

AD brains are characterised by decreased choline acetyltransferase (ChAT), acetylcholine (ACh) release and reduction of nicotinic and muscarinic receptors in the cerebral cortex and hippocampus.[26] Acetylcholine is important in learning and memory and blocking cholinergic activity leads to memory deficits similar to those seen in aged individuals. However, this could be reversed using a cholinergic agonist.[27] This lead to early clinical studies using acetylcholinesterase inhibitors. There are currently three acetylcholinesterase inhibitors that have been approved by the Food and Drug Administration (FDA) for the treatment of mild to moderate AD, called donepezil, rivastigmine, and galantamine. Memantine can also be prescribed to treat moderate to severe Alzheimer's disease. This is an N-methyl D-aspartate (NMDA) antagonist, which regulates the activity of glutamate by partially blocking the NMDA receptors. The FDA has also approved a combination of memantine and donepezil, for the treatment of moderate to severe Alzheimer's disease. The effectiveness of cholinesterase inhibitors and memantine varies across the population and is largely palliative. However, they have potential to be used in combination with other disease-modifying compounds.[28]

## AMYLOID CASCADE HYPOTHESIS

The second and most prevailing theory of AD is the 'amyloid hypothesis', which asserts that aggregation and deposition of Aβ peptides, especially Aβ-42, are a primary event in AD pathogenesis.[29] The plaque development is followed by increased Tau phosphorylation and the formation of intracellular neurofibrillary tangles (NFTs). The theory is supported by FAD, where mutations have been found within APP leading to early-onset, autosomal-dominant AD. This also suggested novel therapeutics targeting β- and γ-secretase, enhancers of α-secretase activity or through strategies to clear plaques such as anti-Aβ immunotherapy. However, the amyloid cascade hypothesis cannot fully explain the root causes of sporadic AD.[30,31,32]

## TAU HYPOTHESIS

The third hypothesis is the 'tau hypothesis', which postulates that abnormalities in the intracellular protein tau are causative. Evidence of this hypothesis include correlation of severity of dementia with increasing accumulation of NFTs and level of hyperphosphorylated tau in the CSF with the extent of cognitive impairment.[33] Therapies based on this hypothesis aim to inhibit the phosphorylation and/or aggregation of Tau protein. Furthermore, there has been conducted research into microtubule stabilizers and anti-tau immunotherapy.[34,35,36]

## MITOCHONDRIAL CASCADE HYPOTHESIS

The "mitochondrial cascade hypothesis" states that mitochondrial dysfunction is at the apex of the cascade. The genetically determined mitochondrial starting line along with the rate of mitochondrial decline determine the age of onset of AD. The mitochondrial function declines with age and drives a variety of age-associated physiologic changes. The cell physiology initially compensates for this change and during this compensation phase the typical histology changes of AD begin to manifest. However, eventually a point is reached where compensation is no longer possible and other histology changes arise. These histology changes that can either occur during compensation or after the compensation threshold is reached include Aβ production, tau phosphorylation, synaptic loss, cell cycle re-entry and neurodegeneration.[37]
Because mitochondrial DNA is maternally inherited, mothers mostly influence the risk of developing AD. However, mitochondrial decline is not only genetically determined, but also environmentally.[37] Evidence of this hypothesis include elevated mutation levels of mtDNA in AD subject brains.[38] Furthermore, AD neurons have fewer normal appearing mitochondria, many are in various states of disrepair and lysosomal degradation. They also contain higher levels

of the common 5 kb mtDNA deletion.[39] There are also studies, which report association of AβPP and Aβ with mitochondria. However, the opinions about whether mitochondrial function alters Aβ dynamics or Aβ alters mitochondrial function in late-onset AD are divided.[40,41]

### METABOLIC HYPOTHESIS

According to the 'metabolic/signal transduction hypothesis' AD requires a genetic predisposition and one or more environmental factors that affect one or more specific signal transduction pathways. This results in phosphorylation/dephosphorylation imbalance and subsequent abnormal hyperphosphorylation of tau that leads to NFT formation and dementia. The most implicated proteins in the hyperphosphorylation of tau are glycogen synthase kinase-3b (GSK-3b), cyclin-dependent protein kinase 5 (CDK5), cyclic AMP-dependent protein kinase, calcium, calmodulin-dependent protein kinase II and protein phosphatase-2A (PPA2). Environmental factors affecting brain metabolism, such as glucose, cholesterol and reactive oxygen species, might increase the risk of developing AD.[42]
Studies also showed that type II diabetes increased the risk of AD and that SAD patients are also more vulnerable to type II diabetes, indicating a close interaction between both disorders. Insulin seems to play an important role, since insulin treatment increases the incidence of SAD among diabetics. [43] Other proposed mechanisms by which diabetes could increase the risk of AD development are glucose toxicity, oxidative stress, elevated levels of advanced glycation end products and cytokine-mediated neuroinflammation. It is thought that activated dsRNA-dependent protein kinase (PKR) can trigger inflammation and accumulates in the brain and cerebrospinal fluid of AD patients. It is presumed that tumor necrosis factor α (TNF-α) mediates Aβ oligomer-induced PKR activation. However, oxidative stress is also known to be able to activate PKR. Once activated, PKR acts as a pro-apoptotic kinase and negatively controls the protein translation leading to an increase of β-secretase 1 (BACE1) translation. PKR down-regulation also decreases neuroinflammation and Aβ accumulation.[44,45,46]

## RECENT RESEARCH THERAPEUTIC STRATEGIES FOR AD TREATMENT

Recently researched therapeutic strategies for AD treatment are shown in Table 1. Phase I studies are studies that are usually conducted with healthy volunteers and that emphasize safety. The goal is to find out what the drug's most frequent and serious adverse events are and, often, how the drug is metabolized and excreted. Phase II studies are studies that gather preliminary data on effectiveness (whether the drug works in people who have a certain disease or condition). For example, participants receiving the drug may be compared to similar participants receiving a different treatment, usually an inactive substance, called a placebo, or a different drug. Safety continues to be evaluated, and short-term adverse events are studied. Phase III studies gather more information about safety and effectiveness by studying different populations and different dosages and by using the drug in combination with other drugs. Studies in phase IV occur after FDA has approved a drug for marketing. These including postmarket requirement and commitment studies that are required of or agreed to by the study sponsor. These studies gather additional information about a drug's safety, efficacy, or optimal use.

### THERAPIES DIRECTED AGAINST Aβ

Therapies based on the 'amyloid cascade hypothesis' include therapies that are focussed on direct targeting of Aβ, Aβ anti-aggregation and decrease of Aβ production. For direct targeting antibodies are used which are precisely directed against

Table 1 - **Overview recent researched drugs for AD including mechanism of action and phase of development.**[5,47,55]

| Mechanism of action | Drug | Phase |
|---|---|---|
| Anti Aβ vaccine | AN-1792 | II |
| | CAD106 | I/II |
| | ACC-001 | II |
| Humanized monoclonal anti- Aβ antibody | Bapineuzumab | I/II/III |
| | Solanezumab | I/II/III |
| | Gantenerumab | I/II/III |
| | Crenezumab | I/II/III |
| Anti-tau vaccine | AADvac1 | I/II |
| γ-secretase inhibitor | Semagacestat | III |
| γ-secretase modulator | Avagacestat | I/II |
| β-secretase inhibitor | MK-8931 | I/II/III |
| | LY2886721 | I |
| | E2609 | I/II/III |
| GSK-3β inhibitor | Tideglusib | I/II |
| | Intranasal Humulin R | I/II/III/IV |
| 5-HT6 receptor antagonist | Idalopirdine (Lu AE58054) with donepezil | II |
| RAGE inhibitor | Azeliragon (TTP-488) | III |
| α7-nAChR antagonist | Encenicline (EVP-6124) | II |

different forms and carrier proteins of Aβ, APP and transport channels. For the decrease of Aβ production secretase inhibitors and modulators are used.[47]

## IMMUNOTHERAPY FOCUSED ON Aβ

This approach includes both passive immunization and active immunization. Passive immunization consist of an injection of pre-prepared antibodies whereas active immunization is focused on the stimulation of the immune system to produce its own antibodies through administration of a vaccine. Both passive and active immunization have advantages and disadvantages. With passive immunotherapy repeated infusion of antibodies are required while using active immunotherapy only a small number of vaccinations are necessary to generate a prolonged antibody response. However, with passive immunotherapy delivery of a known amount of therapeutic antibodies to the patient is reproducible and antibodies can be cleared rapidly if side effects develop, while with active immunotherapy the aging immune system of elderly patients may generate autoimmune side effects instead of producing the appropriate antibodies.
There is a great variety of mechanisms that can be targeted using immunotherapy, both Aβ species and non-Aβ species. Possible targets are AβPP, the monomeric Aβ molecule, Aβ aggregation intermediates, Aβ carrier proteins and transport channels.
Only a small fraction (approximately 0.1%) of the antibodies which had been introduced in the peripheral circulation will be detected in the brain or cerebrospinal fluid.[48]
However, it is thought that large amounts of antibodies in the peripheral circulation can drive the movement of Aβ out of the CNS by passive diffusion down a concentration gradient.[49]
The transport of antibodies into the CNS is not fully known, but it is presumed that the lymphatic system, perivascular spaces and areas within the CNS in which the blood–brain barrier (BBB) is leaky are involved.
The receptor for advanced glycation

endproducts (RAGE) is thought to be involved in the influx of antibodies into the CNS and efflux is thought to be via the low-density lipoprotein receptor (LPR). However, other channels, carriers and receptors are also involved. RAGE inhibitors can therefore, in theory, prevent the movement of Aβ from the blood into the brain and therefore reduce CSF Aβ levels.[50] The small amount of antibodies that do reach the brain after peripheral administration can exert a variety of effects on Aβ in the CNS. Such as, increasing Aβ clearance, activate microglial cells to reduce plaques, disrupt or promote Aβ aggregation, reduce Aβ toxicity, signal to generate or retard inflammation or interfere with cell-to-cell transmission of Aβ and its aggregates.[51,52]

### DECREASING Aβ PRODUCTION – SECRETASE INHIBITORS & MODULATORS

In order to reduce Aβ production one can interfere with secretase-activity. This can be achieved by using a β-secretase inhibitor, which binds to the enzyme and decreases its activity. Therefore, less APP will be cleaved by β-secretase and less Aβ will be produced. However, the development of β-secretase inhibitors is a challenge since this complex has many more substrates and can therefore lead to significant side effects.[53]
γ-secretase can also be inhibited to reduce Aβ production. However, substrate promiscuity presents similar issues facing *β*-secretase inhibitors. One of the targets of γ-secretase is Notch protein. This protein is responsible for regulating cell proliferation, development, differentiation and cellular communication. The toxic effects of γ-secretase inhibitors are most likely due to inhibition of Notch. However, some non—steroidal anti-inflammatory drugs (NSAIDs) and other small organic molecules can modulate γ-secretase, causing a shift in its cleavage activity, resulting in shorter Aβ species without affecting Notch cleavage.[54]

## STRATEGIES FOCUSED ON TAU PROTEINS

Tau-centered therapies are focussed on the inhibition of the phosphorylation of Tau protein, inhibition of aggregation of Tau protein, microtubule stabilizing and immunomodulation.[55]

### INHIBITORS OF TAU HYPERPHOSPHORYLATION

Kinases known to have increased expression in AD are CDK5, GSK3β, Fyn, JNK, p38, ERK1 and ERK2. All kinases are considered possible targets for AD therapy. However, CDK5, GSK3β and ERK2 are the most strongly linked with tau hyperphosphorylation.[55]
Cyclin-depended kinase 5 (CDK5) requires activation with a p35 and p39.[56] Elevated levels of calcium cause cleavage of p35 and p39 by calcium-dependent protease calpain to the more stable p25 and p29 fragments.[57] These fragments upregulate CDK5 activity.[58] Calpain activation, p25 accumulation, CDK5 co-localization with NFTs and elevation of CDK5 activity have all been observed directly in the AD brain.[59,60,61,62]
Glycogen synthase kinase 3 beta (GSK3β) is a multifunctional kinase involved in various cellular processes.[63] It is highly expressed in the brain and linked to AD and other CNS disease states.[64] Apart from its roles in tau phosphorylation, GSK3β is also interacts with other AD-related mechanisms, such as components of the plaque-producing amyloid system and other Alzheimer's disease-associated proteins.[63]
Extracellular regulated kinase 2 (ERK2) is highly expressed in neurons and, besides tau phosphorylation, it decreases the affinity of tau for microtubules about 10-fold. This results in a reduction of the ability of tau to stabilize microtubules.[65] Activated ERK is a prominent feature in AD and it also establishes a link between abnormal tau phosphorylation and oxidative stress.[66]
Inhibition of each of these kinases is potentially problematic since they are involved in so many other physiological processes.

### INHIBITION OF AGGREGATION OF TAU PROTEIN

Another therapeutic strategy is the inhibition of tau aggregation. This strategy is only applicable before aggregates become irreversibly insoluble through cross-linking.[67] Known inhibitors are rhodamine-based inhibitors, phenylthiazolulhydrazide inhibitors, N-Phenylamines and Anthraquinones, Benzothiazoles, Phenothiazines, Porphyrins, and Polyphenols.[68]
LMTX (TRx 0237) seemed an promising tau protein aggregation inhibitor. The compound is an purified form of the phenothiazine methylene blue, also known as methlythionium chloride.[69] However, phase III trials presented negative results.[70]

### MICROTUBULE STABILIZERS

Microtubule (MT) stabilizers are also considered as possible AD therapeutics. They might compensate for the dysfunction caused by tau aggregates.[55] One MT-stabilizer that seemed promising was the brain-penetrant epothilone D (EpoD), which was evaluated in tau transgenic mice and in AD patients. The microtubule stabilizer improved fast axonal transport, cognitive performance, less forebrain tau pathology and increased hippocampal neuronal integrity.[71] However, research was discontinued in 2013 after a failed clinical trial. Another MT-stabilizing compound, TPI 287, is currently investigated. This compound is also used in cancer research and stabilizes microtubules by binding to tubulin.[55]

### ANTI-TAU IMMUNOTHERAPY

Another possible therapeutic therapy is by using passive and/or active immunotherapy. The most promising compound thus far seems to be the active vaccine AADvac-1. The vaccine consists of a synthetic peptide derived from amino acids 294 to 305 of the tau sequence. AADvac-1 uses aluminum hydroxide as an adjuvant.[72] Currently, the compound is subjected to a 24-month phase II safety trial in mild to moderate AD (NCT02579252). In the study the safety of eight subcutaneous injections of 40 microgram of vaccine with the adjuvant aluminium hydroxide are compared to placebo. The trial is slated to run until February 2019.[70]

## OTHER THERAPEUTIC STRATEGIES

Other therapeutic strategies are influencing the serotonin transmission, affecting the histaminergic neurotransmission, enhancement of acetylcholine response of α-7 nicotinic acetylcholine receptors and repositioning drug development.

### INFLUENCE ON SEROTONIN TRANSMISSION – ANTAGONISTS OF THE 5-$HT_6$ RECEPTOR

The neurotransmitter serotonin directly affects neurodegenerative processes. Receptors for this transmitter are present in the entire brain, especially in the frontal cortex, hippocampus and striatum. These areas play an important role in memory and cognitive function.[73] Compounds affecting 5-$HT_6$ receptors are potential therapeutic targets to compensate for the gradual deterioration of cholinergic activity by degeneration of cholinergic neurons as seen in AD brains.
Idalopiridine (Lu AE58054) seems to be a very promising selective $5HT_6$ receptor antagonist. The compound successfully passed a phase I study. This trial was followed by a phase II study of the compound in combination with donepezil, because $5HT_6$ antagonists in combination with a cholinesterase inhibitor may increase the beneficial effects of both drugs on cognition.[74] The results of this trial were promising. They indicated improvement of cognitive function. However, one also noticed elevated liver enzymes in a few patients in the treatment group and higher dropout than in the placebo group. Subsequently, four phase III trials were carried out. However, in September 2016 it was announced that none of the two doses in the first phase III trial reached the primary endpoint.[70]

## AFFECTING THE HISTAMINERGIC NEUROTRANSMISSION – HISTAMINE $H_3$ RECEPTOR ANTAGONISTS

Histamine $H_3$ receptors are also present in the brain, especially in the prefrontal cortex, hippocampus and hypothalamus. Blockage of this presynaptic receptor leads to increased release of acetylcholine, dopamine, GABA, noradrenaline and histamine into the synaptic cleft. Therefore, $H_3$ receptor antagonists may indirectly improve cholinergic neurotransmission.[75] The competitive selective $H_3$ receptor antagonist, ABT-288, was successful in phase I trials. However, phase II trials revealed no statistical changes in cognitive function.[76] Another $H_3$ antagonist, GSK239512, also had a good safety profile. Phase II trials revealed improved episodic memory, but not other cognitive domains or clinical measures. Furthermore, it caused mild to moderate adverse events.[77] Overall, this suggests that $H_3$ receptor antagonists might have a very limited and selective impact.

## ENHANCEMENT OF THE ACETYLCHOLINE RESPONSE OF α-7 NICOTINIC ACETYLCHOLINE RECEPTORS

A selective α-7 nicotinic acetylcholine receptor (α7-nAChR) agonist may enhance cognition without causing side effects related with overactivation of other nAChRs subunits or muscarinic acetylcholine receptors. The α7-nAChR agonist encenicline has been evaluated for the treatment of AD. The drug binds the α7 subunit of the nicotinic acetylcholine receptor and enhances the receptor's sensitivity to its natural ligand acetylcholine.[78] The α7-nAChR agonist showed promising results in phase I and II trials. However, phase III trials failed because of rare, but severe, side effects.[70]

## REPOSITIONING DRUG DEVELOPMENT

Another approach is to see whether already approved drugs for other purposes can be used as AD therapeutics. This approach allows faster introduction of the drug on the market and reduces cost of research. Since research on new compounds for AD often fail, this is a very tempting alternative.
An example is the compound thalidomide. This compound is used to treat leprosy, immunological and inflammatory disorders and many types of cancer.[79,80,81]
Thalidomide reduces TNF-α levels. This cytokine is elevated in the CSF, hippocampus and cortex of patients with AD and is implicated as an inflammatory mediator of neurodegeneration.[82,83] The results of the 24-week trial of the compound to evaluate the effect of thalidomide and placebo on CSF and plasma biomarkers, including BACE1, in patients with mild to moderate Alzheimer's disease are still unknown.[70] However, anti-TNF therapeutics are known to fail in clinical trials of neurodegenerative diseases. This is probably due to antithetic effects of the TNF receptors in the central nervous system. TNFR1 promotes inflammatory degeneration and TNFR2 neuroprotection, so reduction of TNF levels will reduce inflammatory degeneration but also abrogate neuroprotection. TNFR1-selective antagonist ATROSAB and TNFR2 selective agonist (EHD2-scTNF$_{R2}$) showed to be an promising approach in treating neurodegenerative diseases.[84]
Another currently approved drug, liraglutide, is also evaluated for its potential to treat AD. The drug is an glucagon-like peptide-1 (GLP-1) receptor agonist that stimulated insulin secretion and is approved for type 2 diabetes. The discovery of the correlation between diabetes and the development of AD gave rise to research trying to explain this correlation. Animal studies revealed that GLP-1 reduces the amount of Aβ and improves cognition.[85,86] The drug was tested in a phase II trial. Results showed a significant difference in cerebral glucose metabolism between the placebo and treatment groups. However,

amyloid load increased and cognition remained unchanged in both groups.[70] Nilvadipine (Nivadil) is used for the treatment of hypertension. The dihydropyridine calcium antagonist improves cognition, reduces Aβ levels in the brain and increases Aβ clearance across the BBB.[87] The drug successfully past the phase I and II studies and is currently subjected to a phase III trial.[70]

## DISCUSSION

*Outcomes researched amyloid AD drugs*

AN-1792 was the first active immunotherapy for AD. The phase IIa trial was suspended because 6% of the patients developed aseptic meningoencephalitis. Aβ residues with an length of 15–42 amino acids are thought to have activated Th1 lymphocytes causing the autoimmune meningoencephalitis.[88] Another active vaccine, CAD106, was successful in phase I and II trials. Currently, a phase II/III trial is running.[89,90] AC-001, also known as vanutide cridificar, is an active vaccine designed to avoid the safety concern associated with AN-1792, by using Aβ fragments consisting of 1-7 amino acids. However, this drug caused one serious adverse event and clinical development has been discontinued.[91]

A very promising humanized monoclonal IgG antibody, solanezumab, is directed against the mid-domain of the Aβ peptide. It recognizes soluble monomeric Aβ. In two phase III trials, solanezumab was noted to be safe, but showed no improvement on the primary outcome measures of ADAS-Cog11 (Alzheimer's Disease Assessment Scale–Cognitive subscale 11) and ADCS-ADL (Alzheimer Disease Cooperative Study Activities of Daily Living). However, a prespecified subgroup analysis of one trial showed that solanezumab reduced cognitive decline in mild AD when measured by ADAS-Cog 14. The analysis of the other trial showed a trend of improved cognition with solanezumab in people with mild AD, but it missed statistical significance. Statistically significant benefit was seen in a pooled analysis of patients with mild AD in both trials. The benefit appeared late and grew over time. Therefore, it is thought to be consistent with a small disease modifying effect.[92] In July 2013, Eli Lilly started the third phase III trial. However, on November 23, 2016, Lilly announced that solanezumab had missed the primary endpoint in this trial. Primary and secondary outcome results were small and fell short of statistical significance.[70]

Bapineuzumab, a humanized monoclonal IgG antibody, was also a promising agent in the treatment of AD. The drug is specific to the N-terminus of Aβ and binds fibrillar and soluble Aβ and activates microglial phagocytosis and cytokine production. In a 12-month Phase I trial, dosages of 0.5, 1.5, or 5 mg/kg of bapineuzumab appeared overall safe and well-tolerated in patients with mild to moderate AD. In an 18-month Phase II trial, 124 patients with mild to moderate AD received either 0.15, 0.5, 1, or 2 mg/kg of bapineuzumab and 110 received placebo every 13 weeks. No significant difference was seen in any of the dose cohorts on either ADAS-Cog or DAD (Disability Assessment for Dementia), the two prespecified primary outcomes. Nonetheless, Phase III trials were initiated based on prespecified exploratory analyses on pooled treatment groups versus placebo, and on the subpopulation of patients who completed the trial. However, all phase III trials were terminated on August 6, 2012 because two large Phase III studies showed no clinical benefit.[93]

Another humanized monoclonal IgG antibody, gantenerumab, is designed to bind to a conformational epitope on Aβ fibrils. Both N-terminal and central portions of Aβ are recognized by gantenerumab. By recruiting microglia and activating phagocytosis it disassembles and degrades amyloid plaques. The drug does not alter plasma Aβ.[94] Four phase I trials showed that gantenerumab was generally safe and well-tolerated, but amyloid-related imaging abnormalities (ARIA) are a concern.[95] In 2010, a phase II trial was started and in

2012 the study was expanded to a phase II/III trial. However, on December 19, 2014, Roche discontinued dosing in the trial based on an interim futility analysis. The trial will continue to follow participants through 2019. In March 2014, Roche started a phase III trial of monthly subcutaneous injections of gantenerumab in patients with mild AD. This trial uses the ADAS-cog and ADCD-ADL as co-primary and various biomarkers and clinical/neuropsychiatric measures as secondary outcomes. This study is actively continuing. In 2016, Roche started two new phase I trials to investigating subcutaneous administration of higher doses of gantenerumab.[70]

The monoclonal antibody, crenezumab, recognizes multiple forms of aggregated Aβ, including oligomeric and fibrillar species and amyloid plaques with high affinity, and monomeric Aβ with low affinity. Traditional passive immunization approaches carry the risk of Fcγ receptor-mediated overactivation of microglial cells. This may contribute to an inappropriate proinflammatory response leading to vasogenic edema and cerebral microhemorrhage. Therefore, crenezumab uses an IgG4 backbone, to reduce the risk of Fcγ receptor-mediated overactivation of microglia.[96] Two phase I safety trials produced no evidence of vasogenic edema or cerebral microhemorrhage, allowing phase II trials to use higher doses and achieve higher brain exposure than was possible with previous immunotherapy approaches. This trial missed its primary endpoints of change on ADAS-cog and CDR-SOB (Clinical Dementia Rating Scale Sum of Boxes). Further analysis suggested a possible efficacy signal in mild AD, similarly to solanezumab's results of the two phase II trials. Crenezumab is the first immunotherapy to be evaluated as part of the Alzheimer Prevention Initiative. The five-year phase II trial started in 2013 and expects to recruit 300 participants and is set to run until 2020. In January 2016, a phase III study in 750 people with MCI or prodromal AD with biomarker evidence of Aβ pathology started enrolling. This trial uses change on the CDR-SB as primary outcome and a range of cognitive and functional measures as secondary outcomes. The study is expected to run until 2021. Results of a 72-patient phase I trial lead to the prediction of the company's scientists that a stronger treatment benefit from the higher dose of 60 mg/kg of crenezumab infused once a month for the above-mentioned phase III study.[70]

Semagacestat is a γ-secretase inhibitor that reduces Aβ-40 and -42 production and secretion by the γ-secretase enzyme complex. This drug is the first γ-secretase inhibitor that reached phase III trials. The phase I trial reported a dose-depended reduction in CNS Aβ production in healthy volunteers.[97] A phase II trial showed, on the primary outcome of safety and tolerability, a greater number of skin-related side effects in the treatment group, for example rash and lightening hair colour. It also showed that semagacestat reduced plasma Aβ levels, but failed to reduce CSF Aβ levels. On secondary efficacy endpoints of cognition and function, this study showed no difference between semagacestat and placebo.[98] Phase III trials were halted because of both an increased risk of skin cancer and infections and lack of efficacy. Notably, both cognition and function not only did not improve but worsened in all three treatment groups.[99]

Avagacestat, also known as BMS-708163, is an γ-secretase modulator that was reported to selectively block processing of the enzyme's APP substrate, relatively sparing Notch processing.[100] Phase I trials were successful. In 2009, two phase II trials were started. One of the trials was terminated. In the other trial most patients dropped out due to gastrointestinal and dermatological side effects. The trial also demonstrated dose-dependent effects on CSF biomarkers in some patients, but at the two higher doses cognition trended toward a worsening compared with placebo.[101] In November 2012, Bristol-Myers Squibb terminated this trial and announced its decision to end further development of avagacestat.

Verubecestat, also known as MK-8931, is a small-molecule inhibitor of the β-secretase enzyme. In phase I/II trials the drug was reported to have been generally safe, without any discontinuations due to side effects, and to have reduced the CSF Aβ concentration in AD patients. The 18-month phase II/III trial started in November 2012 passed an interim safety evaluation. The study is running till July 2019. In November 2013, the phase III trial began. This trial is running till 2019. In October 2016, an additional phase I study was started to compare the liver metabolism of verubecestat in 32 people with hepatic insufficiency to people with normal liver function.[70] In November 2016, Merck published data of, among others, verubecestat's safety profile in rats and monkeys and initial phase I data for exposure to drug of up to one week. Verubecestat reduced plasma, CSF, and brain concentrations of Aβ40, Aβ42, and sAPPβ after acute and chronic administration to rats and monkeys. Furthermore, they observed that chronic treatment of rats and monkeys with verubecestat achieved exposures >40-fold higher than those being tested in clinical trials in AD patients yet did not elicit many of adverse effects. However, in rabbits and mice one observed fur hypopigmentation. This was not seen in monkeys. In humans both single and multiple doses were generally well tolerated and produced reductions in Aβ40, Aβ42, and sAPPβ in the CSF of both healthy subjects and AD patients.[102]

LY2886721 was the first β-secretase inhibitor to reach phase II clinical research. Six phase I studies of in total 150 healthy volunteers showed that the compound reduced CSF Aβ40, Aβ42, and sAPPβ concentrations and increased sAPPα. This is consistent with expectations for β-secretase inhibition. No safety concerns were apparent in dosing up to six weeks. In the phase II study, started in March 2012, dosing was halted in June 2013 because of four out of 45 patients showed abnormal liver biochemistry values.[70]

Another β-secretase inhibitor, E2609, showed no clinically significant safety concerns in eight phase I trials. The drug showed acceptable tolerability across all doses, with headache and dizziness the most common adverse events. In November 2014, Eisai started a large phase II trial, which is set to run until January 2018. In August 2016, another phase I trial was started to compare the pharmacokinetics and metabolism of the E2609 in people with normal and impaired liver function. Since October 2016 Eisai started the compound's first phase III trial, which is currently recruiting and expected to run till June 2020.[70]

Azeliragon, also known as PF-04494700 or TTP488, is an small-molecule inhibitor of RAGE. RAGE is thought to mediate amyloid transport into the brain. RAGE inhibitors can therefore, in theory, prevent the movement of Aβ from the blood into the brain and therefore reduce CSF Aβ levels. Phase II trials with the compound showed that it was safe and well tolerated in patients with mild-to-moderate Alzheimer disease. In April 2015, a phase III trials of azeliragon began, which is still recruiting and set to run until January 2019.

*Explanation(s) outcomes*

The presence of polysorbate 80 in the formulation of AN1792 is thought to blame for the causative inflammatory T cell response that lead to the development of meningoencephalitis.[103] ACC-001 was developed in part to circumvent that type of T cell activation.[104] However, one of the patients developed skin vasculitis and skin lesions, indicating malfunction of immune or hypersensitive allergic responses. It is not yet clear what could have caused this. Could it be related to the antigen, the alternative adjuvant QS-21, or something entirely different? The CRM197 carrier protein is used in other vaccines including Wyeth's Prevnar, the world's best-selling vaccine, which is given to infants and toddlers to prevent pneumonia.[105] If CRM197 is related to the skin lesions, then it is most likely an unusual event or related to old age. The adjuvant QS-21 is also an

unusual suspect, since the compound has been used in many trials of in total more than 3,500 people. If QS-21 caused the problem, then it would have shown up in earlier trials.[70] However, QS-21 is capable of inducing a much more proinflammatory Th-1 response than some other adjuvants, and it is possible that it may be related to the adverse reaction.[106]

Bapineuzumab and gantenerumab bind primarily fibrillar, deposited Aβ, not soluble monomeric Aβ as does solanezumab, or a mixture of Aβ species, as do crenezumab. Soluble circulating amyloid species, rather than those deposited in amyloid plaques, are implicated in causing longstanding neuroinflammation and neurotoxicity well before their amyloid is deposited in plaques.[107] Therefore, removal of the soluble form of Aβ may be more beneficial in the treatment of AD patients. However, the results of solanezumab's phase III trials in mild AD showed small, non-significant treatment benefit. Nonetheless, it might be that intervention with amyloid-directed therapies is more likely to be beneficial very early in the disease course, or in the pre-symptomatic stages.
It is thought that semagacestat's failure is due to its broad-based inhibition of all γ-secretase's substrates, particularly Notch. Furthermore, a toxic function for an intermediate product of APP processing called β-CTF, whose concentration rises with semagacestat treatment, has also been proposed.[108] The reasons for the toxicity caused by avagacestat remain uncertain. However, the most likely culprit was inhibition of Notch processing, since Notch inhibition is known to destroy cells lining the gut and immune cells, and cause squamous cell carcinoma, in previous animal experiments.[109,110,111]
The liver toxicity caused by LY2886721 represents off-target effects.

Most drugs that aim to prevent Aβ accumulation have consistently failed in clinical trials. This would suggest that the amyloid pathology lies downstream of (an)other cellular event(s) that is/are responsible for AD pathogenesis. Nonetheless, a harsh conclusion on this matter is premature, since there could have been insufficient target engagement or the drugs may have been administered too late in the disease process. The earliest markers of brain Aβ deposition are reduction in CSF Aβ-42 and increased amyloid tracer retention on PET imaging, which occur in the preclinical phase (Figure 4). Trials of solanezumab in asymptomatic and very mildly symptomatic carriers of autosomal-dominant mutations in the Alzheimer's genes, asymptomatic or very mildly symptomatic elderly and people diagnosed with prodromal AD are underway.[70]

*Other possible future research strategies*
Besides research strategies focussed on tau proteins and the other discussed strategies, a new possible research strategy might be focussed on the microbiota. AD might be closely related to the imbalance of gut microbiota. Therefore, modulation of gut microbiota through personalized diet or beneficial microbiota intervention might become a new treatment for AD.[112]
Another potential future research strategy might be focussed on normalization of the neuronal lipid environment. Several lipid alterations have been described in the brain and in peripheral fluids of patients with AD, suggesting the involvement of lipids in the etiology of this condition. It is thought that normalization of brain membrane lipid levels would revert AD-related pathogenic events.[113]

*Conclusion*
If the above mentioned solanezumab trials don't show significant clinical benefit, the focus on the amyloid hypothesis will likely end. Until then, we need to take more shots on goal. Alzheimer is probably much more complicated and there are probably many roads leading to the disease. Therefore, a one-size-fits-all approach might not bring most benefit. Targeted therapies might need to be adjusted to a person's own biology and genetics.

# REFERENCES


1. Ferri, C. P. *et al.* Global prevalence of dementia: A Delphi consensus study. *Lancet* **366,** 2112–2117 (2005).
2. Prince, M. *et al.* World Alzheimer Report 2015: The Global Impact of Dementia - An analysis of prevalence, incidence, cost and trends. *Alzheimer's Dis. Int.* 84 (2015). doi:10.1111/j.0963-7214.2004.00293.x
3. Wimo, A., Jönsson, L., Bond, J., Prince, M. & Winblad, B. The worldwide economic impact of dementia 2010. *Alzheimer's Dement.* **9,** 1–11 (2013).
4. Strassnig, M. & Ganguli, M. About a peculiar disease of the cerebral cortex: Alzheimer's original case revisited. *Psychiatry (Edgmont).* **2,** 30–3 (2005).
5. Rafii, M. S. & Aisen, P. S. Advances in Alzheimer's disease drug development. *BMC Med.* **13,** 62 (2015).
6. Sperling, R. A. *et al.* Toward defining the preclinical stages of Alzheimer ' s disease : Recommendations from the National Institute on Aging and the Alzheimer ' s Association workgroup. *Alzheimer's Dement.* **7,** 1–13 (2011).
7. Albert, M. S. *et al.* The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's and Dementia* **7,** 270–279 (2011).
8. McKhann, G. M. *et al.* The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement.* **7,** 263–269 (2011).
9. Goate, A. *et al.* Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349,** 704–706 (1991).
10. Swerdlow, R. H. Pathogenesis of Alzheimer's disease. *Clin. Interv. Aging* **2,** 347–59 (2007).
11. Pimplikar, S. W., Nixon, R. A., Robakis, N. K., Shen, J. & Tsai, L.-H. Amyloid-Independent Mechanisms in Alzheimer's Disease Pathogenesis. *J. Neurosci.* **30,** 14946–14954 (2010).
12. Herrup, K. Reimagining Alzheimer's Disease--An Age-Based Hypothesis. *J. Neurosci.* **30,** 16755–16762 (2010).
13. Seeman, P. & Seeman, N. Alzheimer's disease: β-amyloid plaque formation in human brain. *Synapse* **65,** 1289–1297 (2011).
14. Mucke, L. Neuroscience: Alzheimer's disease. *Nature* **461,** 895–897 (2009).
15. Rademakers, R., Cruts, M. & van Broeckhoven, C. The role of tau (MAPT) in frontotemporal dementia and related tauopathies. *Hum. Mutat.* **24,** 277–295 (2004).
16. Hampel, H. & Lista, S. Alzheimer disease: From inherited to sporadic AD—crossing the biomarker bridge. *Nat. Rev. Neurol.* **8,** 598–600 (2012).
17. Roses, M.D, A. D. APOLIPOPROTEIN E ALLELES AS RISK FACTORS IN ALZHEIMER'S DISEASE. *Annu. Rev. Med.* **47,** 387–400 (1996).
18. Lai, F. *et al.* A Prospective Study of Alzheimer Disease in Down Syndrome. *Arch. Neurol.* **46,** 849–853 (1989).
19. Kivipelto, M. *et al.* Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. *BMJ* **322,** (2001).
20. Honig, L. S. *et al.* Stroke and the Risk of Alzheimer Disease. *Arch. Neurol.* **60,** 1707 (2003).
21. Green, R. C. *et al.* Depression as a Risk Factor for Alzheimer Disease. *Arch. Neurol.* **60,** 753 (2003).
22. Fleminger, S., Oliver, D. L., Lovestone, S., Rabe-Hesketh, S. & Giora, A. Head injury as a risk factor for Alzheimer's disease: the evidence 10 years on; a partial replication. *J. Neurol. Neurosurg. Psychiatry* **74,** 857–62 (2003).
23. Arvanitakis, Z. *et al.* Diabetes Mellitus and Risk of Alzheimer Disease and Decline in Cognitive Function. *Arch. Neurol.* **61,** 661 (2004).
24. Bartus, R. T. On Neurodegenerative Diseases, Models, and Treatment Strategies: Lessons Learned and Lessons Forgotten a Generation Following the Cholinergic Hypothesis. *Exp. Neurol.* **163,** 495–529 (2000).
25. Bartus, R., Dean, R., Beer, B. & Lippa, A. The cholinergic hypothesis of geriatric memory dysfunction. *Science (80-. ).* **217,** (1982).
26. Tata, A. M., Velluto, L., D'Angelo, C. & Reale, M. Cholinergic system dysfunction and neurodegenerative diseases: cause or effect? *CNS Neurol. Disord. Drug Targets* **13,** 1294–303 (2014).
27. Bartus, R. T. Evidence for a direct cholinergic involvement in the scopolamine-induced amnesia in

monkeys: Effects of concurrent administration of physostigmine and methylphenidate with scopolamine. *Pharmacol. Biochem. Behav.* **9,** 833–836 (1978).
28. Alzheimer's Disease Medications Fact Sheet | National Institute on Aging. (2016). at <https://www.nia.nih.gov/alzheimers/publication/alzheimers-disease-medications-fact-sheet>
29. Hardy, J. & Selkoe, D. J. The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics. *Science (80-. ).* **297,** 353–356 (2002).
30. Castello, M. A. & Soriano, S. On the origin of Alzheimer's disease. Trials and tribulations of the amyloid hypothesis. *Ageing Res. Rev.* **13,** 10–12 (2014).
31. Drachman, D. A. The amyloid hypothesis, time to move on: Amyloid is the downstream result, not cause, of Alzheimer's disease. *Alzheimer's Dement.* **10,** 372–380 (2014).
32. Nalivaeva, N. N., Beckett, C., Belyaev, N. D. & Turner, A. J. Are amyloid-degrading enzymes viable therapeutic targets in Alzheimer's disease? *J. Neurochem.* **120,** 167–185 (2012).
33. MACCIONI, R. *et al.* Anomalously phosphorylated tau and Aβ fragments in the CSF correlates with cognitive impairment in MCI subjects. *Neurobiol. Aging* **27,** 237–244 (2006).
34. Wischik, C. M. *et al.* Tau aggregation inhibitor therapy: an exploratory phase 2 study in mild or moderate Alzheimer's disease. *J. Alzheimers. Dis.* **44,** 705–20 (2015).
35. Shemesh, O. A. & Spira, M. E. Rescue of neurons from undergoing hallmark tau-induced Alzheimer's disease cell pathologies by the antimitotic drug paclitaxel. *Neurobiol. Dis.* **43,** 163–175 (2011).
36. Wisniewski, T. & Goñi, F. Immunotherapeutic Approaches for Alzheimer's Disease. *Neuron* **85,** 1162–1176 (2015).
37. Swerdlow, R. H., Burns, J. M. & Khan, S. M. The Alzheimer's Disease Mitochondrial Cascade Hypothesis. *J. Alzheimer's Dis.* **20,** 265–279 (2010).
38. Coskun, P. E., Beal, M. F. & Wallace, D. C. Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. *Proc. Natl. Acad. Sci.* **101,** 10726–10731 (2004).
39. Hirai, K. *et al.* Mitochondrial abnormalities in Alzheimer's disease. *J. Neurosci.* **21,** 3017–23 (2001).
40. Anandatheerthavarada, H. K. & Devi, L. Mitochondrial translocation of amyloid precursor protein and its cleaved products: relevance to mitochondrial dysfunction in Alzheimer's disease. *Rev. Neurosci.* **18,** 343–54 (2007).
41. Manczak, M. *et al.* Mitochondria are a direct site of A accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. *Hum. Mol. Genet.* **15,** 1437–1449 (2006).
42. Iqbal, K. & Grundke-Iqbal, I. Metabolic/signal transduction hypothesis of Alzheimer?s disease and other tauopathies. *Acta Neuropathol.* **109,** 25–31 (2005).
43. Ott, A. *et al.* Diabetes mellitus and the risk of dementia: The Rotterdam Study. *Neurology* **53,** 1937–42 (1999).
44. Carret-Rebillat, A.-S. *et al.* Neuroinflammation and Aβ Accumulation Linked To Systemic Inflammation Are Decreased By Genetic PKR Down-Regulation. *Sci. Rep.* **5,** 8489 (2015).
45. Mouton-Liger, F. *et al.* PKR downregulation prevents neurodegeneration and β-amyloid production in a thiamine-deficient model. *Cell Death Dis.* **6,** e1594 (2015).
46. Lourenco, M. V. *et al.* TNF-α Mediates PKR-Dependent Memory Impairment and Brain IRS-1 Inhibition Induced by Alzheimer's β-Amyloid Oligomers in Mice and Monkeys. *Cell Metab.* **18,** 831–843 (2013).
47. Godyn, J., Jonczyk, J., Panek, D. & Malawska, B. Therapeutic strategies for Alzheimer's disease in clinical trials. *Pharmacol. Reports* **68,** 127–138 (2016).
48. Lannfelt, L., Relkin, N. R. & Siemers, E. R. Amyloid-ß-directed immunotherapy for Alzheimer's disease. *J. Intern. Med.* **275,** 284–95 (2014).
49. DeMattos, R. B. *et al.* Peripheral anti-A antibody alters CNS and plasma A clearance and decreases brain A burden in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci.* **98,** 8850–8855 (2001).
50. Deane, R. *et al.* RAGE mediates amyloid-β peptide transport across the blood-brain barrier and accumulation in brain. *Nat.*

*Med.* **9,** 907–913 (2003).
51. Arbel, M., Yacoby, I. & Solomon, B. Inhibition of amyloid precursor protein processing by -secretase through site-directed antibodies. *Proc. Natl. Acad. Sci.* **102,** 7718–7723 (2005).
52. Bard, F. *et al.* Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat. Med.* **6,** 916–919 (2000).
53. Menting, K. W. & Claassen, J. A. H. R. Beta-secretase inhibitor; a promising novel therapeutic drug in Alzheimer's disease. *Front. Aging Neurosci.* **6,** (2014).
54. Imbimbo, B. P. & Giardina, G. A. M. γ-secretase inhibitors and modulators for the treatment of Alzheimer's disease: disappointments and hopes. *Curr. Top. Med. Chem.* **11,** 1555–70 (2011).
55. Folch. J, Petrov. D, Ettcheto. M, Abad. S, Sanchez-Lopez. E, Garcia. M, Olloquequi. J, Beas-Zarate. C, A. C. and C. A. Current Research Therapeutic Strategies for Alzheimer's Disease Treatment. *Neural Plast.* **2016,** 15 (2015).
56. Dhavan, R. & Tsai, L.-H. A decade of CDK5. *Nat. Rev. Mol. Cell Biol.* **2,** 749–759 (2001).
57. Patzke, H. & Tsai, L.-H. Calpain-mediated cleavage of the cyclin-dependent kinase-5 activator p39 to p29. *J. Biol. Chem.* **277,** 8054–60 (2002).
58. Lew, J. *et al.* A brain-specific activator of cyclin-dependent kinase 5. *Nature* **371,** 423–426 (1994).
59. Lee, K.-Y. *et al. Elevated neuronal Cdc2-like kinase activity in the Alzheimer disease brain*. *Neuroscience Research* **34,** (1999).
60. Yamaguchi, H. *et al.* Preferential labeling of Alzheimer neurofibrillary tangles with antisera for tau protein kinase (TPK) I/glycogen synthase kinase-3β and cyclin-dependent kinase 5, a component of TPK II. *Acta Neuropathol.* **92,** 232–241 (1996).
61. Grynspan, F., Griffin, W. ., Cataldo, A., Katayama, S. & Nixon, R. . Active site-directed antibodies identify calpain II as an early-appearing and pervasive component of neurofibrillary pathology in Alzheimer's disease. *Brain Res.* **763,** 145–158 (1997).
62. Tseng, H.-C., Zhou, Y., Shen, Y. & Tsai, L.-H. A survey of Cdk5 activator p35 and p25 levels in Alzheimer's disease brains. *FEBS Lett.* **523,** 58–62 (2002).
63. Grimes, C. A. & Jope, R. S. The multifaceted roles of glycogen synthase kinase 3β in cellular signaling. *Prog. Neurobiol.* **65,** 391–426 (2001).
64. Bhat, R. V., Budd Haeberlein, S. L. & Avila, J. Glycogen synthase kinase 3: a drug target for CNS therapies. *J. Neurochem.* **89,** 1313–1317 (2004).
65. Drechsel, D. N., Hyman, A. A., Cobb, M. H. & Kirschner, M. W. Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. *Mol. Biol. Cell* **3,** 1141–54 (1992).
66. Perry, G. *et al.* Activation of neuronal extracellular receptor kinase (ERK) in Alzheimer disease links oxidative stress to abnormal phosphorylation. *Neuroreport* **10,** 2411–5 (1999).
67. Cisek, K., Cooper, G. L., Huseby, C. J. & Kuret, J. Structure and mechanism of action of tau aggregation inhibitors. *Curr. Alzheimer Res.* **11,** 918–27 (2014).
68. Bulic, B. *et al.* Development of Tau Aggregation Inhibitors for Alzheimer's Disease. *Angew. Chemie Int. Ed.* **48,** 1740–1752 (2009).
69. Grüninger, F. Invited review: Drug development for tauopathies. *Neuropathol. Appl. Neurobiol.* **41,** 81–96 (2015).
70. ClinicalTrials.gov. at <https://clinicaltrials.gov/>
71. Zhang, B. *et al.* The Microtubule-Stabilizing Agent, Epothilone D, Reduces Axonal Dysfunction, Neurotoxicity, Cognitive Deficits, and Alzheimer-Like Pathology in an Interventional Study with Aged Tau Transgenic Mice. *J. Neurosci.* **32,** 3601–3611 (2012).
72. Kontsekova, E., Zilka, N., Kovacech, B., Novak, P. & Novak, M. First-in-man tau vaccine targeting structural determinants essential for pathological tau–tau interaction reduces tau oligomerisation and neurofibrillary degeneration in an Alzheimer's disease model. *Alzheimers. Res. Ther.* **6,** 44 (2014).
73. Marcos, B., Cabero, M., Solas, M., Aisa, B. & Ramirez, M. J. Signalling pathways associated with 5-HT6 receptors: relevance for cognitive effects. *Int. J. Neuropsychopharmacol.* **13,** 775–784 (2010).
74. Marcos, B., Chuang, T. T., Gil-Bea, F. J. & Ramirez, M. J. Effects of 5-HT6 receptor antagonism and cholinesterase inhibition in models of cognitive impairment in the rat. *Br. J. Pharmacol.* **155,** 434–40 (2008).

75. Esbenshade, T. A. *et al.* The histamine H 3 receptor: an attractive target for the treatment of cognitive disorders. *Br. J. Pharmacol.* **154,** 1166–1181 (2008).
76. Haig, G. M. *et al.* A randomized study of H3 antagonist ABT-288 in mild-to-moderate Alzheimer's dementia. *J. Alzheimers. Dis.* **42,** 959–71 (2014).
77. Grove, R. A. *et al.* A randomized, double-blind, placebo-controlled, 16-week study of the H3 receptor antagonist, GSK239512 as a monotherapy in subjects with mild-to-moderate Alzheimer's disease. *Curr. Alzheimer Res.* **11,** 47–58 (2014).
78. Prickaerts, J. *et al.* EVP-6124, a novel and selective α7 nicotinic acetylcholine receptor partial agonist, improves memory performance by potentiating the acetylcholine response of α7 nicotinic acetylcholine receptors. *Neuropharmacology* **62,** 1099–1110 (2012).
79. Walker, S. L., Waters, M. F. R. & Lockwood, D. N. J. The role of thalidomide in the management of erythema nodosum leprosum. *Lepr. Rev.* **78,** 197–215 (2007).
80. Moreau, P., Attal, M. & Facon, T. Frontline therapy of multiple myeloma. *Blood* **125,** 3076–3084 (2015).
81. Kumar, N., Sharma, U., Singh, C. & Singh, B. Thalidomide: chemistry, therapeutic potential and oxidative stress induced teratogenicity. *Curr. Top. Med. Chem.* **12,** 1436–55 (2012).
82. Greig, N. H. *et al.* Thalidomide-based TNF-alpha inhibitors for neurodegenerative diseases. *Acta Neurobiol. Exp. (Wars).* **64,** 1–9 (2004).
83. Gabbita, S. P. *et al.* Early intervention with a small molecule inhibitor for tumor necrosis factor-α prevents cognitive deficits in a triple transgenic mouse model of Alzheimer's disease. *J. Neuroinflammation* **9,** 99 (2012).
84. Dong, Y. *et al.* Essential protective role of tumor necrosis factor receptor 2 in neurodegeneration. *Proc. Natl. Acad. Sci. U. S. A.* **113,** 12304–12309 (2016).
85. During, M. J. *et al.* Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat. Med.* **9,** 1173–1179 (2003).
86. Abbas, T., Faivre, E. & Hölscher, C. Impairment of synaptic plasticity and memory formation in GLP-1 receptor KO mice: Interaction between type 2 diabetes and Alzheimer's disease. *Behav. Brain Res.* **205,** 265–271 (2009).
87. Lawlor, B. *et al.* NILVAD protocol: a European multicentre double-blind placebo-controlled trial of nilvadipine in mild-to-moderate Alzheimer's disease. *BMJ Open* **4,** e006364 (2014).
88. Gilman, S. *et al.* Clinical effects of A immunization (AN1792) in patients with AD in an interrupted trial. *Neurology* **64,** 1553–1562 (2005).
89. Winblad, B. *et al.* Safety, tolerability, and antibody response of active Aβ immunotherapy with CAD106 in patients with Alzheimer's disease: randomised, double-blind, placebo-controlled, first-in-human study. *Lancet Neurol.* **11,** 597–604 (2012).
90. Farlow, M. R. *et al.* Long-term treatment with active Aβ immunotherapy with CAD106 in mild Alzheimer's disease. *Alzheimers. Res. Ther.* **7,** 23 (2015).
91. Arai, H., Suzuki, H. & Yoshiyama, T. Vanutide cridificar and the QS-21 adjuvant in Japanese subjects with mild to moderate Alzheimer's disease: results from two phase 2 studies. *Curr. Alzheimer Res.* **12,** 242–54 (2015).
92. Doody, R. S. *et al.* Phase 3 Trials of Solanezumab for Mild-to-Moderate Alzheimer's Disease. *N. Engl. J. Med.* **370,** 311–321 (2014).
93. Vandenberghe, R. *et al.* Bapineuzumab for mild to moderate Alzheimer's disease in two global, randomized, phase 3 trials. *Alzheimers. Res. Ther.* **8,** 18 (2016).
94. Bohrmann, B. *et al.* Gantenerumab: a novel human anti-Aβ antibody demonstrates sustained cerebral amyloid-β binding and elicits cell-mediated removal of human amyloid-β. *J. Alzheimers. Dis.* **28,** 49–69 (2012).
95. Ostrowitzki, S. *et al.* Mechanism of Amyloid Removal in Patients With Alzheimer Disease Treated With Gantenerumab. *Arch. Neurol.* **69,** 198 (2012).
96. Adolfsson, O. *et al.* An Effector-Reduced Anti- -Amyloid (A ) Antibody with Unique A Binding Properties Promotes Neuroprotection and Glial Engulfment of A. *J. Neurosci.* **32,** 9677–9689 (2012).
97. Bateman, R. J. *et al.* A γ-secretase inhibitor decreases amyloid-β production in the central nervous system. *Ann. Neurol.* **66,** 48–54 (2009).
98. Fleisher, A. S. *et al.* Phase 2 Safety Trial Targeting Amyloid β Production With a γ-Secretase Inhibitor in Alzheimer Disease. *Arch. Neurol.* **65,** 1031–8 (2008).

99. Doody, R. S. *et al.* A Phase 3 Trial of Semagacestat for Treatment of Alzheimer's Disease. *N. Engl. J. Med.* **369,** 341–350 (2013).
100. Crump, C. J. *et al.* BMS-708,163 Targets Presenilin and Lacks Notch-Sparing Activity. *Biochemistry* **51,** 7209–7211 (2012).
101. Coric, V. *et al.* Safety and Tolerability of the γ-Secretase Inhibitor Avagacestat in a Phase 2 Study of Mild to Moderate Alzheimer Disease. *Arch. Neurol.* **69,** 1430 (2012).
102. Kennedy, M. E. *et al.* The BACE1 inhibitor verubecestat (MK-8931) reduces CNS -amyloid in animal models and in Alzheimers disease patients. *Sci. Transl. Med.* **8,** 363ra150–363ra150 (2016).
103. Pride, M. *et al.* Progress in the Active Immunotherapeutic Approach to Alzheimer's Disease: Clinical Investigations into AN1792-Associated Meningoencephalitis. *Neurodegener. Dis.* **5,** 194–196 (2008).
104. Agadjanyan, M. G. *et al.* Prototype Alzheimer's disease vaccine using the immunodominant B cell epitope from beta-amyloid and promiscuous T cell epitope pan HLA DR-binding peptide. *J. Immunol.* **174,** 1580–6 (2005).
105. Center, K. J. & Strauss, A. Safety experience with heptavalent pneumococcal CRM197-conjugate vaccine (Prevenar®) since vaccine introduction. *Vaccine* **27,** 3281–3284 (2009).
106. Cribbs, D. H. *et al.* Adjuvant-dependent modulation of Th1 and Th2 responses to immunization with beta-amyloid. *Int. Immunol.* **15,** 505–14 (2003).
107. Walsh, D. M. & Selkoe, D. J. Aβ oligomers - a decade of discovery. *J. Neurochem.* **101,** 1172–1184 (2007).
108. Mitani, Y. *et al.* Differential Effects between -Secretase Inhibitors and Modulators on Cognitive Function in Amyloid Precursor Protein-Transgenic and Nontransgenic Mice. *J. Neurosci.* **32,** 2037–2050 (2012).
109. Milano, J. *et al.* Modulation of Notch Processing by -Secretase Inhibitors Causes Intestinal Goblet Cell Metaplasia and Induction of Genes Known to Specify Gut Secretory Lineage Differentiation. *Toxicol. Sci.* **82,** 341–358 (2004).
110. Wong, G. T. *et al.* Chronic Treatment with the -Secretase Inhibitor LY-411,575 Inhibits -Amyloid Peptide Production and Alters Lymphopoiesis and Intestinal Cell Differentiation. *J. Biol. Chem.* **279,** 12876–12882 (2004).
111. Proweller, A. *et al.* Impaired Notch Signaling Promotes De novo Squamous Cell Carcinoma Formation. *Cancer Res.* **66,** 7438–7444 (2006).
112. Hu, X., Wang, T. & Jin, F. Alzheimer's disease and gut microbiota. *Sci. China Life Sci.* **59,** 1006–1023 (2016).
113. Torres, M., Busquets, X. & Escribá, P. V. in *Update on Dementia* (InTech, 2016). doi:10.5772/64757