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REVIEW

Review of Advanced Drug Trials Focusing on the Reduction of Brain Beta-Amyloid to Prevent and Treat Dementia

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Abstract: Alzheimer disease (AD) is the most common neurodegenerative disease and typically affects patients older than age 65. Around this age, the number of neurons begins to gradually decrease in healthy brains, but brains of patients with AD show a marked increase in neuron death, often resulting in a significant loss of cognitive abilities. Cognitive skills affected include information retention, recognition capabilities, and language skills. At present, AD can be definitively diagnosed only through postmortem brain biopsies via the detection of extracellular amyloid beta (Aβ) plaques and intracellular hyperphosphorylated tau neurofibrillary tangles. Because the levels of both Aβ plaques and tau tangles are increased, these 2 proteins are thought to be related to disease progression. Although relatively little is known about the cause of AD and its exact pathobiological development, many forms of treatment have been investigated to determine an effective method for managing AD symptoms by targeting Aβ. These treatments include but are not limited to using small molecules to alter the interactions of Aβ monomers, reducing hyperactivation of neuronal circuits altering Aβ's molecular pathway of synthesis, improving degradation of Aβ, employing passive immunity approaches, and stimulating patients' active immunity to target Aβ. This review summarizes the current therapeutic interventions in Phase II/III of clinical development or higher that are capable of reducing abnormal brain Aβ levels to determine which treatments show the greatest likelihood of clinical efficacy. We conclude that, in the near future, the most promising therapeutic interventions for brain Aβ pathology will likely be passive immunotherapies, with aducanumab and donanemab leading the way, and that these drugs may be combined with antidepressants and acetylcholine esterase inhibitors, which can modulate Aβ synthesis.

Keywords: Alzheimer disease, amyloid plaques, clinical trials, immunotherapy, small-molecule drugs

Introduction

No definitive treatments currently reliably combat Alzheimer disease (AD). AD is a terminal and incurable neurodegenerative disease that commonly affects the geriatric population. Symptoms include a gradual irreversible loss of memory and cognitive capabilities, such as the inability to execute common daily tasks.[1] Currently, no effective or reliable method exists to accurately diagnose AD before a postmortem brain autopsy is performed.[2] These postmortem biopsies reveal a combination of pathologies, including brain atrophy that is correlated with both extracellular plaques comprising amyloid beta (Aβ) peptides and intracellular neurofibrillary tangles made up of hyperphosphorylated tau proteins (tau tangles).[2] These 2 proteins are hallmarks of AD and likely play a critical role in the pathological progression of the disease. Despite the increasing prevalence of AD as the most common form of dementia in the general population worldwide,[3] relatively little is known about its cause.[4]

The prominent risk factors for AD are age, genetics, family history, and environmental factors.[5] Among these factors, age is the strongest determining risk factor for developing AD as more than 33% of the general population aged 85 years and older experience AD.[6] Two situations are relevant for genetic risks and family history. First, 1% to 5% of AD cases are "familial AD", in which symptoms are often displayed before the patient reaches the age of 65; in these cases,

331

mutations have been identified in genes associated with Aβ synthesis (ie, the amyloid precursor protein [APP] and the genes for the presenilin 1 and presenilin 2 proteins that are part of the γ-secretase complex).[7] For the rest of AD cases, often referred to as "late onset AD" or "sporadic AD", a family history of AD in a first-degree relative is associated with an increased risk of developing AD.[8] Some of these risks are related to rare and low-penetrance missense variants in specific genes. For example, a mutation resulting in a homozygous *ApoE4* genotype greatly increases the risk of developing AD, likely due to the reduced ability by the body to dispose of Aβ in the brain,[9] which is suspected to result in increased Aβ plaque loads that may participate in neuronal death. Other mutations have been identified in genes, such as *TREM-2, TDP-43, TOMM-40*, and others. In the absence of genetic mutation, environmental factors can be implicated in both familial and sporadic AD. Such factors include a low level of education, low physical activity, unbalanced diet, smoking, and the presence of comorbid conditions, such as cardiovascular disease, hypertension, diabetes, and hepatic disease.[4,5]

Currently, the only standard of care for AD is acetylcholine-esterase inhibitors (AchEIs), such as donepezil, galantamine, and rivastigmine. AchEIs have been correlated with an effect that temporarily stabilizes the progression of AD.[10] However, the benefit of AchEIs in controlling AD progression is unclear due to the insignificant change in conversion rate from mild cognitive impairment (MCI) to AD.[10] Studies indicate that only high doses of AchEIs have proven significantly effective, but efficacy and clinical use have been questionable due to the higher frequency of adverse events associated with higher dosages and longer periods of using such medications.[10] Because of the frequent adverse effects, attempts to investigate other forms of treatment are being pursued.

In the amyloidogenic pathway, Aβ is generated from APP, which is a single-pass transmembrane protein found in many tissues, via the sequential proteolysis by intramembranous proteases, namely β-secretase and the γ-secretase quaternary complex.[11] However, the nonamyloidogenic pathway cleaves APP in the middle of the Aβ sequence via an α-secretase activity, then via the γ-secretase, to produce a small peptide, called p3, of unknown function. The highest levels of APP are expressed in brains with a high expression level in neurons. The predominant theory regarding AD pathogenesis is the "amyloid cascade hypothesis."[12] This hypothesis proposes that the deposition of Aβ is the primary causative agent of AD pathology, with neurofibrillary tangles, cell loss, vascular damage, and dementia following as a consequence of Aβ deposition. Proposed in 1992,[12] this theory continues to be a topic of debate. Effectively, clinical data show a disconnect between Aβ levels in the brain, location of accumulation, and neuronal loss associated with dementia. This issue is illustrated by the fact that most clinical trials exclusively targeting Aβ have failed to improve clinical outcomes in AD patients; these failures have prompted exploration of new therapeutic avenues.[13] A recent proposed amendment to this hypothesis is that Aβ acts as a trigger for tau bundles to result in AD pathology.[14] However, evidence supports Aβ's critical role, as an increased Aβ42/Aβ40 ratio in the cerebrospinal fluid (CSF) predisposes individuals to develop AD.[14] Thus, in addition to the amyloid cascade hypothesis, some authors have formulated an amyloid threshold hypothesis, which proposes that, if Aβ levels are maintained below a certain level, then AD pathology may be reduced and AD symptoms never appear.[15] However, other biochemical and preclinical studies have also supported that the plaques, once formed, will cause pseudo-irreversible locking of Aβ peptides despite changes in CSF Aβ concentrations.[14] Alternatively, non-Aβ-centric hypotheses explain the etiology and progression of AD, which have been thoroughly summarized in a recent review.[16] However, our present review focuses on anti-Aβ therapies, and thus we do not discuss other hypotheses here.

Three main methods are currently the focus of therapeutic interventions addressing the 2 Aβ-centric AD hypotheses indicated above: (i) lowering Aβ production, (ii) augmenting Aβ clearance, and (iii) preventing Aβ aggregation. Among the strategies explored are small-molecule drugs and immunotherapies to remove Aβ and downstream targets from the brain.[17–20] In this article, we review current preclinical and clinical data for brain Aβ reduction using small-molecule drugs and immunotherapies that are being tested in clinical trials or are approved by the US Food and Drug Administration (FDA).

Materials and Methods

Molecular pathways involved in Aβ metabolism were reviewed via a systematic review limited to therapies in Phase II/III trials or that have already received approval by the FDA. Alzforum.com, a forum maintained by the Alzheimer's Association and dedicated to networking medications by allowing academic scientists and private companies to report

initiatives and products, was a major source for finding potential therapies. From this expansive list, our selection criteria included drugs or methods that showed promise due to earlier trials or experiments with similar methodologies. Trial results were then reviewed with in-depth searches on the official websites ClinicalTrials.gov and Clinicaltrialsregister.eu, where the trials and their design were described. Supporting studies completed prior to clinical trials were reviewed in PubMed, when available. Additional scientific publications were reviewed for each drug with regard to their mechanism of action.

Small-Molecule Therapies to Reduce Aβ Loads in the Brain of AD Patients

Small molecule drugs have been heavily employed over the past 50 years as a way of modulating enzymes and receptors.[21] A small-molecule therapy is often a drug that is able to pass through biological barriers (eg, blood-brain barrier [BBB], or cell membranes) easily due to a low molecular weight. It exerts its effect(s) by affecting biological molecules once inside the cell microenvironment. Most often, this type of drug is employed for targeted therapies. Because many enzymes and receptors act as "on and off switches" for cellular activity, small-molecule therapies have been used to activate or deactivate targeted cellular processes.[21]

With accurate chemical design, small-molecule drugs can target enzymes or receptors in a specific manner. This targeting is an efficient way to limit the effects of a drug to a single process, thus reducing the risk of a cascade effect and adverse events. Additionally, quantifying the drug dosage required to avoid toxicity is relatively straightforward because only one molecular pathway is typically affected. Importantly for AD, small molecules historically account for the vast majority of central nervous system (CNS) drugs because of their ability to pass through the BBB by passive or carrier-mediated transport.[22] With the target protein Aβ being inside the brain parenchyma (ie, behind the BBB) when drugs are administered systemically, finding a method to cross the BBB is an important consideration in drug delivery paradigms. The BBB rarely restricts small molecules. Thus, those molecules can reach sites of Aβ protein production and deposition.

However, such therapeutics have drawbacks. Combination therapies are often employed because the specific design of a given small-molecule drug is made to inhibit only a single receptor or enzyme, whereas a biological system often involves several receptor types and intracellular cascades.[21] If one molecular pathway is blocked, another pathway can often take over. Thus, it is difficult to anticipate whether a drug will actually modulate the whole pathobiological process of interest or only part of it, with the latter potentially being insufficient to revert to homeostasis. Additionally, localization to specific tissues is difficult to achieve. With enzymes and receptors expressed in multiple tissues, therapeutic effects may be accompanied by unintended adverse effects caused by changing the metabolism or interacting with other tissues.

Given the potential benefits and drawbacks of small molecules, the ability of small molecules to cross the BBB makes them attractive as therapeutics capable of slowing down the progression of AD; they can be used to affect Aβ metabolism or conformation. Moreover, small molecules can assist in identifying specific pathologies identified by brain imaging methodologies.[23] Aβ structure can be affected by changing the protein's propensity toward its monomeric or oligomeric state. This can be achieved by stabilizing the native state of Aβ, hence preventing conformational changes. Alternatively, small-molecule inhibitors can be used to inhibit the natural aggregate-prone tendency of some peptides and proteins, such as Aβ.[24–27] On the metabolic side, small-molecule drugs can alter enzymatic activities that regulate the anabolism and catabolism of Aβ. Below, we describe small-molecule drugs being tested in clinical trials that can modulate precursor proteins, cellular hyperactivity, and other metabolic dysregulations that are known to lead to increased Aβ production and agglomeration.

Oligomerization Inhibitors

Protein aggregation is critical in the formation of Aβ plaques. For folded proteins with a defined structure, small-molecule drugs have been shown to influence the folding process. However, for aggregation-prone proteins lacking a defined structure, small-molecule inhibitors have largely been limited to screening and detection applications, such as dye-binding assays (eg, florbetapir[18] F-labeled tracer for positron emission tomography [PET][28]). This limitation is due to an inability to create small molecules sensitive enough to individually characterize unique protein subspecies when there is a lack of defined structures in oligomers (eg, Aβ40 vs Aβ42).[24] Nonetheless, new molecules are being developed to attempt to implement small-molecule therapies for abnormal protein aggregates.

If Aβ peptide monomers are prevented from forming oligomers, then fibrils and aggregates, which are suspected to be more toxic, are less likely to form.[29] Rather than using an inhibitor that targets the structure of the oligomeric assembly, the small-molecule drug could target the protein binding domain(s) and thus inhibit multimerization. In such a scenario, the protein would remain in a nonfunctional and aggregate-prone state, which, in the example of AD, reduces the risk that individual peptides form neurotoxic Aβ oligomers. Several small-molecule drugs are currently in Phase III of development and show promise at inhibiting Aβ oligomerization through different mechanisms.

Alzt-Op1

ALZT-OP1 (AZTherapies, Inc.) is a combination drug therapy of cromolyn sodium and ibuprofen, also called Intal. Cromolyn is a prescription drug that suppresses cytokine release and stabilizes mast cells. It is largely used to treat asthma.[30] Ibuprofen's use in AD has been widely studied in preclinical and clinical paradigms.[31,32] One study performed by the Massachusetts General Hospital indicated that cromolyn both inhibited aggregation of Aβ monomers in vitro and decreased soluble Aβ levels in the brains of APP/PS1 mice receiving the drug via intraperitoneal administration.[29] Ibuprofen alone was ineffective at reducing Aβ. However, when ibuprofen was used in synergy with cromolyn, then Aβ deposits were significantly reduced.[33] To date, the drug has completed a Phase I trial (ClinicalTrials.gov identifier NCT04570644) using 24 healthy participants in a 2-day trial and was deemed safe. There were only mild to moderate adverse events in 3 participants while still achieving CSF concentrations sufficient to reduce Aβ production.[34] A Phase III trial (NCT02547818) has been conducted to evaluate changes in clinical dementia rating (CDR) scores with recruitment completed in December 2020. The global CDR rating ranges from 0 to 3, in 0.5-point increments; the score increases as cognitive impairment worsens.[35] A global CDR of 0.5 corresponds most often to mild cognitive impairment, whereas a global score of 1 most often corresponds to mild AD dementia, 2 to moderate, and 3 to severe AD dementia. Most CDRs are tallied by the sum of the boxes (SOB), which summarizes the numerical score derived from each of the 6 domains probed. The trial was an 18-month study set to enroll 600 participants aged 55 to 79 years with confirmed early AD (see published inclusion criteria for this specific trial) and comparing four groups: 1) cromolyn active plus ibuprofen active, 2) cromolyn active plus ibuprofen placebo, 3) cromolyn placebo plus ibuprofen active, and 4) cromolyn placebo plus ibuprofen placebo. To date, no results from the trial have been posted.

ALZ-801/Homotaurine

ALZ-801 (Alzheon, Inc.) is a prodrug of homotaurine, a natural amino acid found in seaweed. The drug has been previously marketed under the names tramiprosate and alzhemed. In vivo, tramiprosate is converted via a hepatic or plasma amidase to homotaurine, which increases absorption and bioavailability.[36] Previous studies have shown that tramiprosate inhibits Aβ42 aggregation into toxic oligomers.[37,38] Moreover, both tramiprosate and homotaurine are metabolized into 3-sulfo propanoic acid (3-SPA). 3-SPA is a naturally occurring molecule in the brain that has also been shown to inhibit Aβ42 aggregation.[36] A series of Phase I randomized, placebo-controlled trials (NCT04585347 and NCT04157712) were performed with 127 healthy adult volunteers. NCT04157712 included a single ascending dose, a 14-day multiple ascending dose, and a single-dose tablet-food effect study that also lasted 14 days.[36] These trials showed that drug concentrations in the brain need to reach levels 5 to 15 times higher than what is required to inhibit Aβ42 aggregation in vitro. However, the interventions also caused mild nausea and vomiting that were not dose related, which resolved after 1 week of receiving the drug.[36] In December 2019, Alzheon analyzed data on the drug and found improved cortical thickness when comparing *ApoE3/3* and *ApoE4/4* carriers with mild AD. Currently in Phase III, the trial began in 2021 (NCT04770220) and will assess 300 *ApoE4* homozygotes with early to mild AD receiving an 18-month course of drug versus placebo. The company will be monitoring CSF concentration of Aβ oligomers, a primary target of ALZ-801, as well as neurofilament light (NfL) and P-tau levels. A secondary outcome will be improvement of cortical thickness. The trial will be conducted at 85 sites in the United States, Canada, and Europe, with completion expected by May 2024.

Inhibition of Amyloid-Induced Neuronal Hyperactivation

A correlation is suspected between hyperactivation of neuronal pathways and Aβ activity. A research team at the University of Munich, Germany, identified that an impaired synaptic glutamate reuptake resulted in Aβ-induced neuronal hyperactivation when hyperactivation was observed in the APP transgenic mouse models of AD used in the study.[39] The mouse models used were both male and female C57Bl/6N wild-type mice and age-matched female APP23×PS45 mice. Upon investigation, they observed that Aβ oligomers-containing brain samples from AD patients induced hyperactivity in hippocampal CA1 neurons from slices prepared from mice. Specifically, at the single-cell level, the degree of hyper-activation induced by Aβ had a positive correlation with baseline neuronal activity.[39] This finding can be interpreted to mean that the greater activity rate there is in neurons, the more Aβ is present to block glutamate reuptake at the synaptic level. The approach is motivating new paradigms in clinical settings.

Levetiracetam

Although clinical trials investigating the correlation between neuronal hyperactivity and Aβ-induced AD are scarce, one small-molecule therapy has made progress. Levetiracetam is an atypical anticonvulsant that modulates synaptic vesicle glycoprotein 2A. The mechanism of action is not fully understood, but it is suspected to inhibit calcium signaling or depolarizing currents in neurons.[40] A study has analyzed the effects of levetiracetam on markers of amyloidogenesis and synaptic proteins in APP knock-in mouse models. It was observed that the chronic administration of the drug lowered the levels of cortical Aβ42, decreased the levels of β-carboxyl-terminal fragment (APP-βCTF) but not full-length APP, reduced plaque burden, and restored the levels of presynaptic endocytic proteins.[41] A 1-year clinical trial administering 500 to 2000 mg levetiracetam to AD patients with reported episodes of seizures (NCT01554683) showed an improved verbal fluency and attention, but it was unclear whether the improvement was a result of reduced seizures or a global cognitive benefit.[42] Adverse effects included sleepiness, headache, and lack of energy. A Phase II trial (NCT04004702) began in January 2020 at the Walter Reed National Military Medical Center in Maryland to evaluate changes on the neuropsychiatric inventory scale, severity of AD, and cognitive abilities in participants with epileptiform activity on electroencephalography who were treated with levetiracetam for 1 year. No data from this study have been published to date, and the assessment of Aβ levels are not indicated in the study design. We suggest that the analysis of Aβ levels (either CSF measures or PET imaging) could be beneficial in determining whether the cognitive benefit from this drug is solely the result of seizure reduction or a global cognitive benefit derived from reduced Aβ loads.

Receptor Inhibitors

Small molecules can also be employed to bind receptors capable of halting the enzymatic process leading to Aβ production. APP is suspected to function as a cell surface receptor and has been implicated in synaptic plasticity.[43] A strong association has been found between Aβ and nicotinic acetylcholine receptors (nAChRs), particularly the α7 subtype. More precisely, Aβ is capable of binding the α7-nAChRs and modulating their function.[44] These nAChRs are found highly expressed in regions of cognition and memory functions, and it has been observed that the expression of α7-nAChRs is reduced in the brain of AD patients.[45] Thus, a possible therapeutic approach would attempt to modulate the binding of Aβ to α7-nAChRs, because this interaction seems to exacerbate AD pathology, such as tau hyperpho-sphorylation (see "Simufilam" below).

Simufilam

One small-molecule drug that has been reported to affect APP synthesis is simufilam (also called PTI-125; Cassava Sciences, Inc.). Simufilam is one potential avenue to prevent Aβ accumulation. When simufilam binds to the intracellular scaffolding protein filamin that regulates the actin cytoskeleton and is involved in cell stability and motility, the protein is unable to stabilize the high-affinity interaction of extracellular soluble Aβ42 to the transmembrane α7-nAChR.[46] This interaction was reported to trigger tau phosphorylation and synaptic dysfunction in several experimental systems.[47,48] A study performed on triple-transgenic AD mice (3xTgAD) treated with simufilam reportedly reduced Aβ and tau deposition, lowered neuroinflammation, and restored synaptic function compared to wild-type mice.[46] A Phase IIb study (NCT04079803) was performed comparing 50 mg and 100 mg simufilam twice daily for 28 days to placebo in 64

participants with mild-to-moderate AD (confirmed by CSF biomarkers), and analyzed CSF phosphorylated-tau 181, total tau, Aβ42, neurofilament light, neurogranin, and chitinase-3-like protein 1 (also called YKL-40). The company reported that all the CSF biomarkers and some plasma biomarkers improved for both doses.[49] This trial is continuing as an open-label extension and is set to run until March 2023. Results on data collected thus far were met with skepticism in August 2021 by whistle blowers who complained of multiple instances of research misconduct involving clinical trial biomarker data. The FDA posted a statement of concern (docket ID# FDA-2021-P-0930) and several independent scientists corroborated the apparent data manipulation. The company denied wrongdoing, stating that figures on their poster had errors, but the underlying data analysis and conclusion were valid. Thus, additional trials will be necessary to get a better assessment of the potential of simufilam to modulate AD-associated pathologies and cognitive decline.

Metabolic Modulators

Metabolic dysfunction has also been heavily tied to the progression of AD.[4,23] One theory is that metabolic dysregulation, such as in diseases like diabetes insipidus, results in an increased load of oxidative stress.[50] Aβ peptides have been studied and shown to inhibit synaptic insulin sensitivity directly in cultured mouse neurons, which can be correlated with the impaired synaptic functioning in AD.[51] These observations have sparked the investigation of several drugs commonly used for diabetes on AD pathologies. At present, FDA-approved metabolic altering drugs include metformin, dichloroacetic acid, and methylene blue. Below we discuss 2 of the small-molecule drugs that are most relevant to cognitive decline and AD.

Metformin

Metformin (also known as glucophage [Merck]) is a generic drug used principally in the treatment of type 2 diabetes mellitus (T2DM). Its main functions are to decrease insulin resistance and blood glucose levels.[52] Insulin resistance is also found in the brain of AD patients and is a reason that AD has been referred to as "type 3 diabetes."[4] Therefore, drugs that reduce insulin resistance may help with the prevention and treatment of AD. Recent studies have shown a correlation between the use of metformin and a reduction in AD-like pathologies in APP/PS1 mice, which seems to be linked to improved microglial autophagy and phagocytosis processes.[53] Metformin also ameliorated cognitive impairment in a diabetic mouse model[54] (diabetes induced via streptozotocin administration) and in the senescence mouse model SAMP8,[55] although the improvement of cognitive functions is not consistent across AD mouse models (reviewed by Craig et al[56]).

Animal data have generated interest in testing metformin in clinical trials. This line of work is spearheaded by Dr. Luchsinger at Columbia University.[57] A Phase II double-blind, placebo-controlled, randomized trial was started in 2008 to assess the efficacy of metformin in 80 MCI patients via the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) and selective reminding test (SRT) scales (NCT00620191). Overall, the group treated with the drug performed significantly better on the SRT scale.[58] However, some toxic events limited the maximum dose tolerated by study subjects, which prompted the design of an improved multisite Phase II/III study for the use of metformin to prevent AD (NCT04098666). This ongoing 24-month dose escalation trial aims to recruit 370 amnestic MCI subjects who are overweight or obese without diabetes and not previously treated with metformin. The primary outcome measure is the free and cued SRT. It is anticipated that half of the participants will undergo amyloid and tau PET imaging at baseline and at the completion of the trial. This trial is expected to end in 2025.

Linagliptin

Linagliptin (also termed Tradjenta; Boehringer Ingelheim Pharmaceuticals, Inc.; generic drug in the United States since August 2021) is an FDA-approved small molecule drug that is used for glycemic control in T2DM. Linagliptin acts as a competitive, reversible inhibitor of circulating dipeptidyl peptidase-4 (DPP-4); thus, it slows down the catabolism and increases the levels of active glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).[59] Higher levels of GLP-1 and GIP stimulate a greater release of insulin from beta cells in the pancreas, while also inhibiting the release of glucagon. Linagliptin has been studied in vitro using human neuroblastoma SH-SY5Y cells challenged with 20 μM of Aβ42 for 24 hours. The drug at 50 nM reduced Aβ42-induced cell death by ~25%.[60]

Furthermore, linagliptin reduced cell death at concentrations ranging from 10 to 50 µM in SK-N-MC neuroblastoma cultures in a dose-dependent manner during Aβ infusion experiments, suggesting that it is protective against Aβ-induced neurotoxicity.[61] The suspected mechanism was the blockade of dipeptidyl peptidase-4, thus elevating GLP-1 levels, which leads to increased insulin release and restoration of insulin signaling. It was thought that these results and those of others showing this neuroprotective effect may be local, but it seems that linagliptin works at the neuronal level to reduce overall oxidative stress loads.[62] In 3xTg-AD mice, linagliptin was shown to attenuate cognitive deficits on the Morris Water Maze and Y-maze. In addition, the drug modulated neuroinflammation and reduced both amyloid and tau pathologies.[63] These data and others suggest that linagliptin has the potential to lower AD progression. Although no clinical trial testing of the drug involving AD subjects has been reported, a cognitive substudy involving 1545 subjects over the age of 50 years was derived from the larger T2DM trial, termed CARMELINA, testing the effects of 5 mg linagliptin on cardiovascular and renal microvascular outcomes in T2DM patients (NCT01897532). The cognitive tests included the Mini–Mental State Examination (MMSE), the trail making test, and the verbal fluency test administered at baseline and after 2.5 years of daily dosing. Overall, the study found no difference in cognitive decline in the drug-treated versus placebo groups, indicating that linagliptin was neither beneficial nor detrimental for cognitive abilities in T2DM patients.[64] Thus, further studies are required to determine the extent of the neuroprotective effects of linagliptin in the context of AD and related dementias.

Amyloid-Beta Degradation Modulation

The body, under physiological circumstances, regulates Aβ levels via disassembly and proteolytic degradation as well as microglial phagocytosis. Although the list is not complete, a growing number of diverse peptidases and proteinases, known collectively as Aβ-degrading proteases, play a role in the metabolism of Aβ, such as neprilysin (NEP), endothelin-converting enzymes 1 and 2, insulin-degrading enzyme, and plasmin.[65,66] There is anticipation that, with further research, understanding the breakdown of Aβ could lead to slowing the progression of AD or developing a possible treatment to stop the disease entirely. Here, we focus our attention on compounds shown to transition back microglial activity from a proinflammatory/neurotoxic to an anti-inflammatory/pro-phagocytic activation state.

Alzt-Op1

Previously discussed under the section on oligomerization inhibitors, ALZT-OP1 also modulates Aβ degradation in vitro in human microglial cells HMG030.[29] Degradation or removal of Aβ and plaques was promoted by modulation of the inflammatory response and microglial phagocytosis both in the human cell line HMC3[67] and in animal experiments, such as Tg2576 mice, with a combination of ibuprofen and cromolyn or with cromolyn alone.[33] BV2 murine microglial cell cultures treated with cromolyn alone or cromolyn plus ibuprofen in the range 10–1000 µM showed enhanced phagocytosis of Aβ42.[33] The effect of cromolyn on Aβ was further increased when paired with drugs such as bromocriptine during in vitro screening on human-induced pluripotent stem cell differentiated into neurons.[68] Whether ALZT-OP1 also regulates microglial activity and the levels and activity of Aβ-degrading enzymes in AD patients remains to be investigated, although it will likely require investigators to analyze fresh postmortem brain tissues, which can only be performed at a few investigative sites.

Sodium Oligomannate

GV-971, also known as sodium oligomannate (Shanghai Green Valley Pharmaceuticals, Inc.), is another drug with the potential to regulate Aβ degradation. Sodium oligomannate is a mixture of acidic linear oligosaccharides isolated from brown algae. Although the mechanism of action is uncertain, one proposed mechanism is that the compound breaks down Aβ aggregates (ie, disassembles plaques).[69] Another proposed mechanism is that this small molecule reduces the inflammatory response exacerbated by astrocytes, thus reduces the oxidative stress applied to neurons, which results in fewer Aβ plaques being formed.[70] A Phase III trial (NCT02293915) was conducted involving 818 subjects in China, which resulted in the treatment group of participants with mild-to-moderate AD having higher ADAS-Cog13 scores than the placebo group at 4, 12, 24, and 36 weeks.[71] However, no biomarkers were measured in this specific trial. Nonetheless, the drug was given conditional marketing approval in China. In August 2020, the manufacturing company

registered another Phase III trial (NCT04520412) that enrolled 2046 participants with mild-to-moderate AD who were not receiving any FDA-approved AD treatment. The study will be performed at multiple sites worldwide. The trial's primary outcomes are changes from baseline on the ADAS-Cog11 and Alzheimer's Disease Cooperative Study–Clinical Global Impression of Change scale total scores after 1 year of drug or placebo. This study should also test biomarker outcomes, such as blood Aβ, P-tau, NfL, inflammation, and microbiome status, plus fecal microbiome. The trial is anticipated to run through 2025.

Repurposing FDA-Approved Small-Molecule Drugs

Although small-molecule drugs are less studied than new therapies, in the past 3 decades several investigations have focused on the modulation of Aβ pathology by FDA-approved small molecules. The main classes of compounds are antidepressants acting as selective serotonin reuptake inhibitors (SSRIs) and inhibitors of acetylcholine degradation. Below, we briefly summarize the current knowledge about these small-molecule drugs with regard to Aβ metabolism.

Repurposing Antidepressants

In both young and old APP/PS1 transgenic mice, a single-dose intraperitoneal administration of 3 SSRIs (fluoxetine, desvenlafaxine, and citalopram injected individually) induced a 25% decrease in brain interstitial fluid (ISF) Aβ levels measurable 18–24 hours after administration.[72,73] Additional experiments in this model have indicated that this reduction in ISF Aβ levels was not due to a change in Aβ elimination, a change in the electric activity of neurons, or a modulation of genes implicated in APP metabolism.[72,74] Chronic administration of citalopram in young APP/PS1 mice for 4 months resulted in the lowering of brain Aβ and plaque burden by 50%.[72] Similar results were obtained in young 3xTgAD mice administered paroxetine for 5 months.[75] In middle-aged APP/PS1 mice (6 months old), daily administration of citalopram for 28 days arrested the growth of preexisting plaques and reduced the appearance of new plaques by 75%.[73] Mechanistic investigations have suggested that the effects on ISF Aβ levels may be mediated by agonists of a subset of serotonin G-protein coupled receptors (5-HTR), namely, 5-HT4R, 5-HT6R, and 5-HT7R, which signal via the protein kinase A/ mitogen-activated ERK kinase/extracellular signal–regulated kinase axis to regulate the APP-processing α-secretase activity at the protein level,[72,74] thus driving APP toward the nonamyloidogenic pathway.

Two indirect studies by Dr. Cirrito's group have suggested that SSRIs have a role in the modulation of Aβ levels in humans. First, using a retrospective analysis paradigm, they reported that cognitively normal elderly patients taking SSRIs (N = 52) within 5 years to treat depressive symptoms displayed significantly less amyloid signals than nontreated patients (N = 134) when assessed by PET imaging with the Pittsburgh Compound B (PIB).[72] A few years later, they reported the effects of an acute dose of citalopram versus placebo on CSF Aβ levels in 23 (11 females, 12 males) normal volunteers aged 18–50 years using the stable isotope labeling kinetics method developed by Dr. Bateman.[73,76] The CSF was sampled hourly starting 8 hours after treatment onset and continued up to 18 hours (N = 10) or 37 hours (N = 13). Overall, the citalopram group had a 35% reduction in CSF Aβ levels compared to the placebo groups for the period of observation, whereas the clearance rate was similar between the 2 groups.[73] These initial observations in humans highlight the pleiotropic effects of SSRIs, not only as serotonin modulators but also as possible inhibitors of Aβ production. Further studies are needed to better elucidate the molecular mechanisms of Aβ metabolism regulated by SSRIs.

Possible Role of Cholinesterase Inhibitors in Aβ Metabolism

As indicated in the Introduction, AChE inhibitors are the main pharmacological line of treatment for AD and relieve cognitive symptoms for several months. In the brain, acetylcholine (ACh) is hydrolyzed by two cholinesterases, AChE and butyrylcholinesterase (BuChE).[77] AChE and BuChE are differentially expressed in the AD brain, with AChE often decreasing during disease progression, whereas BuChE increases. Interestingly, it was recently observed that the 3 FDA-approved AChE drugs for AD also modulate inflammation and amyloidogenesis. Here, we focus on Aβ modulation.

Donepezil is a selective AChE inhibitor.[77] A recent study reported that donepezil reduced Aβ production in primary cortical cultures from rat embryos. At the molecular level, this process was dependent upon the overexpression of sorting nexin protein

33, and, similar to SSRI, an increase in α-secretase activity without increasing the expression of full-length APP.[78] Whether these results can be extended to neurons from the hippocampus and other brain regions remains to be investigated.

Galantamine is also a selective reversible, competitive AChE inhibitor and allosteric nicotinic receptor modulator, but its relative hydrophilicity results in poor crossing of the BBB.[77] In vitro, galantamine infused to newborn rat microglia cultures significantly increased Aβ phagocytosis, which depend upon microglial α7-nAChRs activation and calcium.[79] These results were corroborated by in vivo experiments in adult rats injected with Aβ42 in the hippocampus and treated for 2 weeks with galantamine, and in 9-month-old Tg APdE9[80] mice that received oral treatment for 2 months. In these 2 animal models, galantamine significantly increased brain Aβ clearance.[79] In 5XFAD mice, galantamine was chronically administered via addition in drinking water to 3-month-old animals, an age when plaque deposition is considerable. The treatment was stopped 2 months later, before plaque burden reached saturation levels. With this paradigm, in addition to some behavioral improvements, it was observed that plaque loads were significantly reduced in the cortex and hippocampus of treated Tg mice, irrespective of the sex, when compared to untreated controls.[81] Similarly, 8 weeks of galantamine administration significantly lowered the amyloid plaque loads in 10-month-old APP/PS1 mice, which was accompanied by improved behavioral performance and reduced brain inflammation.[82] Of note, the authors of these last 2 studies did not investigate the molecular mechanisms leading to the amyloid-related histopathological changes.

Rivastigmine is a pseudo-irreversible noncompetitive inhibitor of both AChE and BuChE, although it binds more efficiently to BuChE.[77,83] Dr. Lahiri's group recently published a report on the modulation of Aβ metabolism by rivastigmine in several models and in the human brain.[84] In a previous study, they had shown a reduction by 50% in brain Aβ40 and Aβ42 levels in 5-month-old male APP/PS1 mice treated for 3 weeks with a BuChE.[85] The new study focused on rivastigmine. In vitro, in both differentiated PC12 cells and primary human embryonic brain cells, they observed an increase in byproducts of the nonamyloidogenic pathway (called sAPPα) released in the condition media, accompanied by an increase in the expression of disintegrins and metalloproteinases ADAM-9 and 10, which are suspected to act as α-secretases on APP.[84] By contrast, they observed a significant decrease in byproducts of the amyloidogenic pathway, including in Aβ40 levels. These results were corroborated in hippocampal lysates of female 3xTg-AD mice (on a C57BL/6 background) administered rivastigmine intraperitoneally for 3 weeks.[84] Very elegantly, they also observed an increase in sAPPα in postmortem brain tissues (Brodmann areas 21/22) of AD patients who were treated with rivastigmine compared to non-AD and AD patients who did not receive treatment with AChE/BuChE inhibitors.[84] Notably, the levels of Aβ40 and Aβ42 were not affected by rivastigmine in the human brain, suggesting that the drug was not able to dissolve existing plaques. In a separate project, it was observed that donepezil and rivastigmine were also increasing the clearance of radiolabeled Aβ across the BBB and liver in rats, which could help eliminating Aβ via the sink effect.[86] For the BBB, this effect was accompanied by an increase in the major Aβ transport proteins P-glycoprotein and low-density lipoprotein receptor-related protein 1.

In our opinion, although current data are promising, there is a need for more investigations in human tissues to confirm in vitro and animal data for the modulation of Aβ metabolism by AChE and BuChE inhibitors. If the pleiotropic properties of cholinesterase inhibitors are confirmed, then it will indicate that AChE and BuChE inhibitors should be used during the entire duration of AD treatment in combination with other anti-amyloid and anti-tau therapies to slow the progression of the disease more aggressively than AChE and BuChE inhibitors are capable of alone to have an impact on cognitive symptoms.

Immunotherapies Against Amyloid Beta

Aβ immunotherapy has been a source of research and interest to reduce brain plaque loads since 1999, when it was first reported that immunization of AD mouse models with Aβ may be effective in preventing and treating AD.[87] The basis for Aβ immunotherapy is to use either synthetic peptides to induce active immunization in the host, or to deliver monoclonal anti-Aβ antibodies (mAbs) to provide passive immunization. Both types of immunization aim to decrease Aβ loads in the brain, which, based on the amyloid cascade hypothesis, should slow down the progression of AD.

Active immunization functions by introducing Aβ peptides as the antigen to the host, which then mounts an immune response against Aβ monomers and/or multimers that results in the production of antibodies specifically targeting Aβ.[88,89] The goal of active Aβ immunotherapy is to program the patient's immune system to eliminate endogenous Aβ.[90] Active

immunization for AD in humans was first tested in 2002 in a study where participants were immunized with full-length Aβ42 peptides (named AN1792; Elan Pharmaceuticals) along with the immunological adjuvant QS-21 (NCT00021723). Postmortem brain samples from the participants were then neuropathologically examined and the percentage of plaque formation was assessed to determine effectiveness.[89] It was found that, 6 years after immunization, study subjects who had died in the years following AN1792 administration (N = 7) had fewer brain Aβ aggregates and plaques compared to control subjects who did not receive any treatment, and they experienced slightly less cognitive decline (assessed by ADAS-Cog, MMSE, and the disability assessment for dementia scale[91]), but there was no effect on the progression of neurodegeneration (neuropathological assessment via hematoxylin and eosin, modified Bielschowsky silver impregnation, and immunostaining for Aβ and tau).[89] Importantly, it was found that, despite high levels of serum antibodies to Aβ42, there was no clinical improvement overall after 1 year, and about 6% of participants developed meningoencephalitis, which led to early termination of the study.[92] It is thought that some of the deleterious effects induced by Aβ active immunotherapies in these early studies occurred because they initiated a T-cell reaction similar to deleterious overstimulated Th-1 immune responses.[93–96] Therefore, 2 common concerns for Aβ active immunotherapy treatments are that the therapy induces autoimmune inflammatory toxicity and does not address the full range of AD pathologies, and, therefore, may not consistently produce clinically beneficial results.[97]

Along with the use of synthetic full-length Aβ peptides, other methods of providing active Aβ immunization include the use of Aβ fragments (eg, Aβ1-10) to stimulate the production of antibodies by B cells, which then capture Aβ peptides and clear the complexes from the brain.[90] Active immunotherapy has demonstrated some benefits for AD patients, and ongoing research anticipates identification of improved methods to achieve the desired benefits while mitigating the adverse effects. The current adverse effects and safety of this approach continue to be deterrents for FDA approval. Until recently, UB-311 (United Neuroscience, Inc.) was the only Aβ active immunotherapy investigated in Phase III clinical trials, with no active immunotherapies currently in Phase IV or FDA approved.

Passive immunotherapy for AD has been investigated using humanized mAbs. Passive immunotherapy is the process of injecting pre-made antibodies to provide augmented immunity to the host. These mAbs are usually administered peripherally through intravenous infusions or via subcutaneous injections and must then cross the BBB to reach the brain parenchyma. The current variations in treatment using mAbs derive from differences in selectivity for polymorphic variants, which may recognize epitopes of Aβ based either on the Aβ conformation or a specific sequence of Aβ peptides.[98] A benefit to passive over active immunotherapy is that it overcomes several of the problems encountered with active immunization, including the following.[90]

1. The interaction between Aβ and mAbs causes a decrease in the formation of toxic Aβ aggregates inside the brain.

2. The binding between the Fc domain of the mAb and the Fc-γ receptors found on microglia results in the phagocytosis of the Aβ-mAb complex within the brain.

3. The Aβ-mAb complex activates complement-dependent cytotoxicity, which then lyses the designated target cell of the therapy.

4. mAbs interact with Aβ in the peripheral blood and form a concentration gradient that causes Aβ to flow out from the brain; this process is referred to as the "sink effect."[88,90,99]

This form of therapy allows for direct disassembly of Aβ in the brain. The use of mAbs is also one of the easiest ways to provide anti-Aβ antibodies without increasing the likelihood of an uncontrolled Th-1 mediated antibody response.[97] Another 2 potential benefits to passive versus active immunization are (i) the possibility to target specific conformations of Aβ peptides, which could then be used to remove specific forms of Aβ such as monomers, oligomers, or fibrils; and (ii) the potential ability to rapidly clear administered antibodies in case of adverse reactions.[88]

Problems that have been associated with Aβ passive immunotherapy, which must be addressed in the future, include high costs; effectively crossing the BBB consistently (<0.1%);[100] the risk of hemorrhage; the risk of triggering an immune response against the injected Aβ antibodies; the necessity of repeated infusions or injections over time to maintain a constant amount of therapeutic antibodies; and correctly selecting the appropriate antigens to target toxic forms of Aβ so as to not interfere with physiological Aβ functions, which play an integral role in neuroprotection, modulation of long-term potentiation, and innate immunity.[88,97,101,102] Currently, 4 Aβ passive immunotherapies are in Phase III trials (donanemab, gantenerumab, lecanemab, and solanezumab) and zero are in Phase IV trials, and 1 has been

conditionally FDA approved (aducanumab). Below, we provide more details about both active and passive immunotherapies tested in AD patients.

Active Immunotherapies

Ub-311

One therapy that introduces Aβ peptide as an antigen to the host to elicit an immune response is UB-311 (United Neuroscience, Inc., now Vaxxinity). UB-311 is a synthetic peptide vaccine that consists of 2 Aβ sequences covering amino acids 1–14 linked to different UBITh helper T-cell peptide epitopes,[103] which are introduced with the aim of activating regulatory T-helper type 2 cells (Th-2) more than proinflammatory Th-1 cells,[90] and which are packaged in a proprietary vaccine delivery system. As mentioned above, one difficulty that newer active immunotherapy vaccines must overcome is mitigating the deleterious mounting of autoimmune inflammatory responses.[93–95,97] One of the most plausible reasons that earlier versions of Aβ active immunotherapies developed inflammatory processes is the fact that the Aβ peptides that were introduced as antigens included the Aβ C-terminus region, which is proposed to activate the Th-1 response.[96] Current versions of Aβ active immunotherapies, including UB-311, no longer include this portion of the Aβ peptide for this reason.

UB-311 is specifically designed to avoid any cross-reactivity with similar endogenous antigens.[103] Wang et al found in preclinical studies in guinea pigs (8–12 weeks of age), adult male baboons (8–10 years of age), adult male and female Cynomolgus macaques (>4 years of age), and hAPP751 transgenic mice (mice expressing a mutant human APP with both the Swedish [K670N/M671L] and London [V717I] mutations) and their littermates (14 ± 2 weeks of age) that UB-311 was able to generate N-terminal anti-Aβ antibodies against the sequence 1–10, which neutralized Aβ toxicity and promoted extracellular Aβ plaque clearance from the brain. It was also found that there were no anti-Aβ cellular responses in the hAPP751 mouse model and that acute and chronic dosing were safe and well tolerated.[104] It was later confirmed in Phase I trials that the vaccine formulation appeared to be safe and well-tolerated and that participants could develop a specific antibody response (NCT00965588 and NCT01189084).[103]

Phase IIa testing began in 2015 on 43 patients with mild AD confirmed by amyloid PET, a MMSE score of 20–26, and a CDR of 0.5 or 1 (NCT02551809). In 2017, it was found that UB-311 produced antibodies against Aβ oligomers, fibrils, and plaques but not against monomers, and there were no reported Aβ-related imaging abnormalities after vaccination.[103] By 2020, the sponsor reported during the Clinical Trials on Alzheimer's Disease (CTAD) meeting that 96% of participants developed anti-Aβ antibodies and that those who received 4 boosters declined only half as much on the CDR, Alzheimer's Disease Cooperative Study-Instrumental Activities of Daily Living Inventory (ADCS-iADL), and ADAS-Cog13 scale as those who only received 2 boosters or the placebo.[105] They also reported a modest decrease in Aβ deposits in the brain by the end of the study. A Phase III study was planned after the 2020 CTAD meeting, but no new study was listed in clinical trial registries by the end of 2021, when this review was written. Further studies into the efficacy, dosing, and tolerance of UB-311 are necessary to assess the efficacy of this vaccine to remove Aβ aggregates and plaques as well as its potential to reduce cognitive decline in AD patients.

Passive Immunotherapies

Donanemab

Donanemab (LY3002813, also termed N3pG; Eli Lilly & Co) is one of the latest and most promising mAb passive immunotherapies against Aβ aggregates. It is a humanized IgG1 mAb developed from mouse mE8-IgG2a that targets the pyroglutamate Aβ epitope of (p3-42), which is a common form of Aβ in plaques.[106,107] Thus, donanemab mainly targets Aβ deposited in plaques rather than preventing the growth of existing plaques and the seeding of new plaques, while also avoiding microhemorrhages in the brain, which is common to other plaque-antibody binding therapies.[107]

In a recent study in patients with MCI due to AD performed by Lowe et al (NCT01837641), it was found that donanemab was generally safe and well tolerated up to 10 mg/kg.[108] This was a double-blind, randomized, parallel-group, placebo-controlled, dose-escalation study. Participants with confirmed AD were assigned to 1 of 5 dosing cohorts (with intravenous doses of donanemab ranging from 0.1 to 10 mg/kg) or to placebo, followed by a 12-week follow-up period for each dose level. After the follow-up period, the participants then entered a 5-cohort multiple-ascending dose phase with intravenous doses of donanemab ranging from 0.3 to 10 mg/kg, or placebo, once per month for up to 4 doses depending on the initial dose. This phase ended with

a 12-week follow-up period. Interestingly, only the 10-mg/kg dose led to changes in amyloid PET signals, which indicated a reduction of 40% to 50% in brain Aβ loads.[108] It was also found that around 90% of participants developed antidrug antibodies at 3 months with just a single intravenous dose,[108] which will need to be monitored for possible long-term decrease in drug efficacy.

Donanemab was further analyzed in the Phase II Trailblazer-ALZ trial from 2017 through 2021 (NCT03367403). This study found that mAb treatment correlated better with composite scores for cognition and for the ability to perform daily living tasks than placebo.[109] Mintun et al investigated specifically whether donanemab was beneficial in treating early AD via a randomized, multicenter, double-blind, placebo-controlled paradigm with participants who had early symptomatic AD with Aβ and tau deposition on PET.[109] Participants were then randomly assigned in equal ratios to receive donanemab intravenously (700 mg for the first 3 doses and 1400 mg thereafter) or a placebo every 4 weeks for up to 72 weeks. The primary outcome measure for Trailblazer-ALZ was the change on the Integrated Alzheimer's Disease Rating Scale (iADRS) at 76 weeks versus baseline scores. The iADRS is a composite tool that combines scores from the ADAS-Cog and the ADCS-iADL to better capture disease progression and separate drug versus placebo effects.[110] Secondary outcomes reported changes in scores on the ADAS-Cog13, MMSE, CDR, and ADCS-iADL scales, plus changes in Aβ and tau levels by PET and volumetric MRI assessments. It was found that, at week 76, the donanemab group showed significant improvement on the iARDS scores compared to the placebo group. The trial also demonstrated significant decreases in brain Aβ and tau levels, but improvement was found in regard to most secondary outcomes numerically but failed to reach statistical significance. These outcomes could be considered conflicting, but they are trending in the same direction and the iADRS weights certain elements. Donanemab appeared to contribute to a decrease in brain volume, which may be a result of either Aβ removal or a reduction in central inflammation[111] (similar to a small brain shrinkage observed in multiple sclerosis patients treated with anti-inflammatory drugs).[112] Removal of amyloid as a driver of significant reduction of brain volume has been speculated in studies as far back as AN1792. These results imply that donanemab is a promising treatment to positively tackle the cognitive and functional decline commonly associated with early AD, and potentially in later stages of AD.

Trailblazer-ALZ2 was a follow-up Phase II study (NCT04437511), which was then expanded to a Phase III study. It aims to further evaluate cognitive and memory function changes in participants. The results of this study should be available in the first half of 2023. Donanemab is currently in Phase III of development and tested in parallel to aducanumab (Trailblazer-ALZ4; NCT05108922) with amyloid PET measures after 6 months of treatment, but it has already received an accelerated approval pathway, similar to aducanumab.[113] It was also announced that a 182-week Phase III placebo-controlled prevention trial called Trailblazer-ALZ3 (NCT05026866) will be implemented to evaluate treatment for cognitively normal people who are at high risk for AD based on elevated plasma P-tau217 levels, and the study is expected to run for 3 years.

It is our opinion that further focus should be directed toward donanemab because it is currently showing one of the highest potentials among Aβ-lowering immunotherapies to assist with cognitive improvement. Results from the Trailblazer-ALZ2-4 trials will further elucidate the efficacy of this mAb passive immunotherapy in AD and prodromal patients.

Gantenerumab

Gantenerumab (also termed RG1450; Chugai Pharmaceutical Co., Ltd., and Hoffmann-La Roche) is a humanized IgG1 mAb that binds with a subnanomolar affinity to aggregated Aβ fibrils in the brain parenchyma and vasculature. Its function is to lower Aβ levels by utilizing effector cell-mediated clearance, as demonstrated by AD brain slices cocultured with primary human macrophages.[114] Gantenerumab encompasses both N-terminal and central amino acids of Aβ. The concept for this therapeutic approach is that the mAb will act inside the CNS to disassemble Aβ plaques by recruiting microglia and activating phagocytosis. Gantenerumab has also been found to help prevent new plaque formation without altering plasma Aβ levels in 4-month-old PS2APP (APP751swedish × PS2N141I) mice.[114]

In a Phase I study involving 28 patients with AD (NCT01656525), it was found that gantenerumab was safe and could be considered for at-home injections.[115] By 2019, during Phase II and III trials (NCT01224106 and

NCT02051608), it was reported by Klein et al in an interim analysis that subcutaneous doses of gantenerumab up to 1200 mg once every 4 weeks were correlated with a significant brain Aβ removal in participants with prodromal to moderate AD.[116] This study's primary end point was the change in brain Aβ loads from baseline to weeks 52 and 104. Florbetapir PET was used to evaluate gantenerumab's efficacy. It was later reported that subcutaneous gantenerumab injections of up to 1200 mg also significantly reduced Aβ plaque loads in the brain at 36 months after treatment initiation in the same Phase II and III studies.[117] It was found, however, that clinical benefits were only observed in patients who had fast onset AD, and overall the cognitive benefit was small.[90]

Another Phase II pharmacodynamics study with gantenerumab is currently being performed to evaluate the changes in baseline to week 104 on brain Aβ accumulation (NCT04592341). Participants with a selection criterion of prodromal to mild AD measured by amyloid PET will participate in this study and receive a dose escalation regimen: a single 120-mg subcutaneous injection of gantenerumab every 4 weeks for 12 weeks, followed by 255 mg every 4 weeks for 12 weeks, 255 mg every 2 weeks for another 12 weeks, and then 255 mg once a week until week 104. The primary outcome measure is amyloid PET centiloid levels. The centiloid scaling is a linearization of amyloid PET signals ranging from 0 for no amyloid signals to 100 for a typical AD patient.[112] This scaling system permits a better comparison of amyloid signals when different amyloid PET tracers and scanners are used to assess brain amyloid loads in patients across study sites. The study is expected to be completed in 2024.

A Phase III randomized, double-blind, parallel-group, placebo-controlled study started in March 2017 has been recruiting participants with early AD in order to study the efficacy, safety, and pharmacokinetics of gantenerumab versus placebo on cognition and functioning (NCT03443973). It is expected that this study will finish in 2023. The main evaluation of this study will come in comparing CDR scores from baseline to week 116. The results from this study and the other ongoing trials, along with previous results from completed studies, will further elucidate the potential of gantenerumab in preventing and treating AD. Plus, these data will provide the direction for future investigators to design clinical trials investigating specific parameters, such as efficacy in *ApoE4* versus *ApoE2-3* subjects.

Lecanemab

Lecanemab (BAN2401; Biogen and Eisai Co., Ltd) is a humanized IgG1 mAb version of the mouse mAb158, which has been shown to bind primarily to soluble Aβ protofibrils[118,119] and to target APP which bears the Arctic mutation E693G.[120] Several preclinical studies have shown that lecanemab can help decrease pathogenic Aβ levels, help prevent Aβ deposition, and selectively reduce Aβ protofibrils in the brain of transgenic mice expressing human APP (isoform 695) containing both the Arctic (E693G) and Swedish (KM670/671NL) mutations.[120,121] Phase I (NCT01230853 and NCT02094729) and II (NCT01767311) clinical trial results,[118,122] along with the favorable preclinical findings, have garnered a lot of interest in lecanemab as a possible treatment for AD and have instigated several more trials to evaluate its efficacy.

Interestingly, lecanemab was recently reported to also lower blood P-tau181 levels. This result, combined with the Phase I and II data, motivated the team leading the Dominantly Inherited Alzheimer Network Trial (DIAN-TU) to test the first double adaptive tau-Aβ therapy, with the Eisai's anti-tau antibody E2814 being combined with lecanemab.[123] DIAN-TU is a uniquely designed prevention clinical trial that is testing, in parallel, several promising drugs against Aβ pathology in patients with autosomal-dominant genetic mutations known to induce AD before they develop cognitive symptoms (ie, mutations in the APP, presenilin 1, and presenilin 2 genes that induce the genetic cases of AD, which represent 1% to 5% of AD cases).[124]

A current Phase III randomized, double-blind, placebo-controlled, parallel-group trial called ClarityAD is recruiting participants with MCI due to AD (NCT03887455). In this study, lecanemab's efficacy in changing cognition will be evaluated via the CDR-SOB scale between baseline and month 18. The trial will administer 10 mg/kg of lecanemab intravenously once every 2 weeks for 18 to 45 months and is set to run until 2024.

Another study is the dose escalation Phase III AHEAD3-45 trial, which began in July 2020 (NCT04468659). This prevention-oriented study is a combination of 2 trials run in parallel investigating the efficacy and safety of lecanemab in prodromal AD participants with elevated Aβ levels (A45 Trial) and in prodromal AD with intermediate Aβ levels (A3 Trial).[125] The primary outcome of the A3 Trial is prevention of brain amyloid accumulation at 216 weeks. The main

secondary outcome is brain tau accumulation. For the A45 trial, the primary outcome measure is the Preclinical Alzheimer's Disease Cognitive Composite 5 scale at 216 weeks. Aβ and tau levels are determined by PET with the A3 trial enrolling subjects displaying 20 to 40 centiloids on their screening scan, whereas the A45 trial will enroll subjects with more than 40 centiloids on their screening scan. Participants in this study are receiving 5 mg/kg of lecanemab intravenously once every 2 weeks through week 8, then 10 mg/kg once every 2 weeks through week 96, and finally 10 mg/kg once every 4 weeks through week 216. The results from this uniquely designed parallel trial will be highly indicative as to the clinical value of lecanemab in treating AD. However, additional long-term studies will be required to validate clinical outcomes for patients with early-to-mild AD. Meanwhile, in 2021 Eisai and Biogen began submitting data to the FDA to support the accelerated approval of lecanemab.[126]

Solanezumab

Solanezumab (LY2062430; Eli Lilly & Co.) is the humanized IgG1version of the mouse m266.2 mAb that targets the central sequence of Aβ peptides, residues 16–24,[127] and has a higher affinity to Aβ monomers than to the soluble and toxic conformations.[99] Solanezumab reduces the brain Aβ burden by enhancing CNS and plasma Aβ clearance.[99,128] This is accomplished by solanezumab sequestering all plasma Aβ, which results in an efflux of toxic Aβ out of the CNS into the blood, which confirms that the theory of the sink effect is applicable to Aβ.[129] Initial results from the Expedition 1 (NCT00905372) and Expedition 2 (NCT00904683) Phase III trials did not show significant clinical improvements in participants treated for 18 months with solanezumab.[130]

However, a later complementary data analysis discovered a slowing in cognitive and functional deterioration in participants with AD on the ADAS-Cog14, ADAS-Cog11, and MMSE scales, although the ADCS-ADL and CDR-SOB data did not show any difference between the solanezumab and placebo groups.[131] This motivated the sponsor to initiate the Phase III trial Expedition 3 (NCT01900665), which had an improved design to attempt to demonstrate efficacy of solanezumab on cognition. However, Expedition 3 was discontinued in 2016 due to a lack of evidence to support a reduction in cognitive decline in AD participants at week 80.[132] Similarly, ExpeditionPRO (NCT02760602), which enrolled prodromal AD subjects, was terminated in 2017 because of a lack of evidence that solanezumab would slow cognitive decline.

The mixed results on the efficacy of solanezumab have discouraged many projects to continue forward with solanezumab, and Eli Lilly discontinued their pursuit to gain FDA approval. However, one trial is currently enrolling participants to help definitively determine its efficacy. In the DIAN-TU trial, one arm is evaluating whether intravenous infusions of solanezumab slow the rate of cognitive decline in participants with AD and could improve disease-related biomarkers in subjects with dominantly inherited AD. Solanezumab is administered once every 4 weeks at increasing doses, and this arm is compared to participants receiving gantenerumab. Efficacy will then be measured by evaluating the changes from baseline using 4 measures: the International Shopping List Task, MMSE, Wechsler Memory Scale-Revised Logical Memory Delayed Recall Test, and the Wechsler Adult Intelligence Scale Digit Symbol Substitution Test. It is expected that this study will be completed by the end of 2022.

It is our opinion that DIAN-TU will help determine the eventual efficacy of solanezumab, but, in case of positive results, more studies will be required to determine adequate dosing, toxicity, tolerability, and overall clinical effectiveness in reducing cognitive decline in AD patients.

FDA Conditionally Approved Anti-Aβ Immunotherapies
Aducanumab

Aducanumab (BIIB037, also called Aduhelm; Biogen) is a high-affinity, completely humanized IgG1 mAb against a conformational epitope of Aβ. It binds to Aβ in the N-terminal region (residues 3–7) and is capable of targeting both the oligomeric and fibrillar forms of Aβ found in patients with prodromal and mild AD.[98,133,134] Furthermore, aducanumab captures both soluble and insoluble Aβ aggregates over monomers with extreme selectivity, and it shows a distinct preference to parenchymal Aβ as opposed to vascular Aβ.[98] This mAb was identified in a healthy donor population of elderly subjects who experienced an unusually slow cognitive decline and lacked symptoms of cognitive impairment, therefore indicating these donors might have resisted developing AD.[98,134] Aducanumab is an Aβ passive

immunotherapy and is currently the first and only FDA approved biological treatment for targeting, reducing, and removing Aβ in the brain.

Preclinical experiments on Tg2576 mice demonstrated reduced Aβ plaque size consistent with a dose-dependent distribution, but only in young (9-month-old) and not in old (22-month-old) mice.[133,134] This indicates that aducanumab may be more beneficial in preventing Aβ aggregation than in eliminating already formed Aβ plaques. Similar to other immunotherapies, the reduced Aβ plaque loads observed in this study did not significantly correlate with improved cognition.[133]

A Phase I safety, tolerability, and pharmacokinetics study was then started in 2011 and completed by 2015 (NCT01397539). The study design was a single-ascending dose of 0.3 to 30 mg/kg of aducanumab in participants with mild-to-moderate AD. Overall, the dosing was safe with only mild-to-moderate adverse events that included headache, diarrhea, and dizziness (some possibly related to aducanumab but not to the dose). Thus, the dosage was increased up to 60 mg/kg.[135] Patients who received the 60 mg/kg dose experienced severe adverse events of symptomatic amyloid-related imaging abnormalities (ARIAs), which completely resolved by weeks 8–15.[136] Surprisingly, Aβ40 and Aβ42 levels increased in the plasma for about 3 weeks with the 60 mg/kg dose, which suggested that high levels of aducanumab may bind to soluble monomeric Aβ in humans.[135] Then, the exposure changed in a linear pattern across the doses with little variability between participants. Upon completion of 24 weeks of treatment, there was no significant difference in cognitive abilities compared to the placebo group when measured by the ADAS-Cog13 scale.[135] The results with regard to safety were consistent with preclinical results in that there was no aducanumab-generated plasma spike in recipients, and it positively demonstrated the absence of toxicity on cognitive measures. The Phase Ib study termed PRIME (NCT01677572) also indicated a profound reduction in brain Aβ levels via florbetapir PET in prodromal and mild AD patients receiving monthly aducanumab infusions for 12 months.[134]

Two large Phase III clinical trials named Engage (NCT02477800; N = 1638 subjects) and Emerge (NCT02484547; N = 1647) were developed to assess 2 doses of aducanumab efficacy over 76 weeks of dosing versus placebo. Both studies focused on individuals aged 50 to 85 years who displayed signs of MCI or mild dementia related to AD with confirmed amyloid pathology via PET and used CDR-SOB at week 78 as their primary outcome measure.[137] Both trials were stopped in March 2019 due to an interim futility analysis after 50% enrollment, which indicated that both trials would miss their primary end points because it was assumed that the 2 studies would produce similar results and that all patients would respond to treatment the same way, independently of time of enrollment.[138] This was readjusted in October 2019 with a new report finding that the interim analysis was incorrect and that the trials indicated that the high dose of 10 mg/kg induced a significant reduction in the primary end point of a significant difference in CDR-SOB scores compared to placebo controls, when each trial's data were analyzed independently. This was accompanied by a lessened decline in secondary end points that included MMSE, ADAS-Cog13, and ADCS-ADL scores, with the low-dose group showing some slowing of AD progression but not enough to be statistically significant from the placebo group.[137,139] Aducanumab data were then submitted for FDA review in 2020, and it was approved in June 2021 under the FDA's accelerated approval pathway.[140] This process requires substantial evidence of effect on an intermediate marker (in this case, Aβ removal), a reasonable likelihood of a meaningful clinical benefit, and Phase IV evidence that this benefit can be gathered in a subsequent trial.

After receiving FDA approval, a Phase I study into aducanumab's bioavailability after a single subcutaneous injection versus intravenous infusion in healthy volunteers in the age range 40–70 years was begun in June 2021 and was completed in October 2021 (NCT04924140). Biogen should report on this study in 2022. A Phase IV postmarketing study to obtain real-world effectiveness and safety measures was also begun. In this observational study (NCT05097131), 6000 patients receiving aducanumab throughout the United States will receive follow-ups for at least 5 years to collect data on changes in cognition, function, neuropsychiatric symptoms, caregiver burden, quality of life, cost of care, safety, and ARIA. It is expected that the study will require up to 10 years for completion. However, in the United States, most health insurance providers refuse to reimburse for aducanumab treatments following a decision by the Centers for Medicare and Medicaid Services. In addition, in recent discussions among the NIH-funded Alzheimer's Disease Research Center (ADRC) sites, it was suggested that patients taking this biologic should be followed at regular intervals for MRI scanning to detect the emergence of ARIA. However, very few hospitals in the United States have the capacity

to detect such imaging abnormalities in a timely manner. Thus, the recommendation would be that aducanumab be administered exclusively at hospitals associated with an ADRC so that MRI scans could be analyzed promptly by neurologists with research expertise who can quickly recognize ARIAs (unpublished data). Moreover, in December 2021, the European Medicines Agency stated that it would not approve this mAb in Europe with the data provided for review, citing a high risk of up to 41% for patients with early AD to develop ARIA compared to 10% in placebo groups in the Emerge and Engage trials.[141,142]

It is our opinion that further research should be conducted to verify the safety and efficacy of aducanumab, although the current results are relatively positive, and it is reasonable to expect that aducanumab will become a viable treatment option in the future for lessening the deleterious effects of AD and reducing excess Aβ aggregates in the brain.

Pending FDA Reviews

Following aducanumab's FDA approval, donanemab and lecanemab are currently being reviewed by the FDA to determine whether they should also receive accelerated pathway approval. In addition, and as indicated above, a Phase III trial is currently underway to compare donanemab and aducanumab (Trailbazer-ALZ4) in 200 early AD subjects identified by amyloid PET with florbetapir. In our opinion, this effort is very important to compare immunotherapies that already received FDA approval versus biologics seeking such approval in the near future. We anticipate that further studies will be developed to compare promising new anti-Aβ mAb to aducanumab and determine similar or improved efficacy.

Discussion

In total, we reviewed 13 separate promising AD treatments: 1 FDA-approved immunotherapy, 4 passive immunotherapies, 1 active immunotherapy, and 7 small molecules as well as existing treatments. These potential treatments show the variety of pharmacological approaches attempted to treat a disease process that is not yet fully understood: brain Aβ aggregation. By virtue of the amyloid cascade and amyloid threshold hypotheses, a treatment that would target the production, degradation, or aggregation of Aβ should be strongly considered as a future treatment or preventative paradigm for AD.

Among the therapies listed in the section on small molecules, ALZT-OP1 and ALZ-801 show the most promise. ALZT-OP1's role as a molecule to break down the oligomers of Aβ and degrade the protein addresses the amyloid cascade hypothesis's role of Aβ in AD. This role also allows for a mechanism to remove existing plaques, thus addressing a previous report that suggested that plaque formation made Aβ levels unrelated to AD pathology.[14] This finding is particularly important in preventative paradigms and implies that, if Aβ aggregates are removed before large deposits form, then it could be possible to prevent the appearance of cognitive deficits. As a combination drug of 2 FDA-approved medications and with adverse effects shown to be only mild in a small minority of participants, this drug shows promise to better understand and treat AD. With a third trial completed in December 2020, the results of the study are currently under review and have yet to be published. With the promising early data and recent ending of trials, the results are eagerly anticipated. On the other hand, ALZ-801 was clinically reported to induce only mild adverse effects. With it being fast tracked for treatment of AD by the FDA in 2017 and data already indicating cortical thickness improvement, this medication has received funding from the US National Institute on Aging for further testing. Similar to ALZT-OP1, its mechanism of action shows promise for controlling Aβ aggregation.

In general, mAb treatments have garnered much interest in recent years as potential new effective disease-modifying interventions for AD. Aducanumab is strongly favored as a current therapeutic option because it is the first drug since 2003 to be approved by the FDA for the treatment of AD, and its most recent trials suggest very positive results in markers that would predict effective AD prevention and treatment, such as a significant slowing of decline in CDR-SOB scores, as well as significant positive results favoring treatment over placebo in measurements such as CDR, ADAS-Cog13, and ADCS-ADL.[137,139] Therefore, aducanumab has been found to be both relatively safe and effective, although more efforts need to be invested in preventing ARIA events. We feel that further research should be planned to determine the long-term effects of treatment with aducanumab and to determine whether there is a lasting clinical benefit to

treatment. For example, it is known that biologics often induce an adaptive immune response in the host that could diminish drug efficacy over time.

Donanemab is another viable passive immunotherapy option for Aβ pathology management. A large body of evidence has shown its efficacy, safety, and potential for improving cognitive functions in AD patients. The results from the Trailblazer-ALZ2 and 3 studies will more adeptly determine the future efficacy, applicability, and ultimately the potential for FDA approval of donanemab.

The approval and clinical use of small molecules and immunotherapies in the ever-growing geriatric patient population, which is more prevalently affected by AD, are becoming a continually growing source of interest and need. However, further testing is required before they can be widely deployed for clinical use. Although clinical trial data show promise, efficacy in patients outside of experimental settings has yet to be established. If these drugs are rejected by the FDA, then this avenue for possible exploration, understanding, and treatment for dementia will be delayed. Each of these drugs has been shown to be safe overall, with only mild to moderate or manageable adverse effects. As these trials reach completion and as more drugs with similar, but safer, mechanisms of action continue to be developed and tested, the evidence of small-molecule therapy and passive immunotherapy's ability to modify, prevent, and treat AD becomes increasingly stronger. We emphasize that further research should be performed to specify the benefit of lowering Aβ oligomerization or plaque removal in long-term treatment designs to further understand AD pathogenesis. Moreover, as with the DIAN-TU trial, we suggest that combination therapies targeting Aβ, tau, and inflammation should be tested in patients displaying prodromal and early stages of AD.

Conclusion

The amyloid hypothesis in AD is frequently called into question. What is germane is not whether amyloid is relevant but whether removal of amyloid is a predictor of clinical efficacy because amyloid levels have not correlated well with clinical progression. It might be that removal of amyloid has downstream beneficial effects, such as lowering tau and inflammation. The amyloid cascade and amyloid threshold hypotheses suggest that a treatment targeting the production, degradation, or aggregation of Aβ should be strongly considered as a future treatment or preventative paradigm for AD.

By providing several angles of attack for the different forms of Aβ (ie, soluble and insoluble monomers, oligomers, plaques), physicians will have more options in finding an effective treatment to successfully diminish AD progression using a personalized medicine approach for each individual patient. Passive immunotherapies, which also include lecanemab and gantenerumab, are relatively safe in humans, are at the forefront in pioneering new treatments for AD, and have contributed to an ever-growing mass of evidence indicating their efficacy in preventing and treating neurodegeneration linked to Aβ accumulation. Further studies should focus on identifying the most specific Aβ immunization regimen that targets pathologic and toxic disease processes in AD. Moreover, bidirectional studies that explore the relationship between physical and psychological or cognitive health may be useful in helping determine the specific mechanisms by which these immunotherapies generate improvement in AD in the near future.

Abbreviations

3-SPA, 3-sulfo propanoic acid; Aβ, amyloid beta; ACh, acetylcholine; AChEI, acetylcholine-esterase inhibitor; AD, Alzheimer disease; ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale; ADCS-iADL, Alzheimer's Disease Cooperative Study-Instrumental Activities of Daily Living Inventory; APP, amyloid precursor protein; ARIA, amyloid-related imaging abnormality; BBB, blood–brain barrier; BuChE, butyrylcholinesterase; CDR, clinical dementia rating; CNS, central nervous system; CSF, cerebrospinal fluid; DIAN-TU, Dominantly Inherited Alzheimer Network Trial; FDA, US Food and Drug Administration; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; iADRS, Integrated Alzheimer's Disease Rating Scale; ISF, interstitial fluid; mAb, monoclonal anti-Aβ antibody; MCI, mild cognitive impairment; MMSE, Mini–Mental State Examination; nAChR, nicotinic acetylcholine receptor; NfL, neurofilament light; PET, positron emission tomography; SOB, sum of the boxes; SRT selective reminding test; SSRI, selective serotonin reuptake inhibitor; T2DM, type II diabetes mellitus.

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