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Emerging Novel Therapeutic Approaches for the Treatment of Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is caused by synaptic failure and the excessive accumulation of misfolded proteins especially Aβ and tau, and associated with memory loss and cognitive impairment. Treatment of AD mainly consists of symptomatic therapy and disease-modifying therapy (DMT). Several monotherapies including small molecules or antibodies have been evaluated through multiple clinical trials, but a very few have been approved by the USFDA to intervene the disease's pathogenesis. Past research has shown multifactorial nature of AD, therefore, multi-target drugs were proposed to target different pathways at the same time, however, currently no rationally designed multi-target directed ligand (MTDL) has been clinically approved. Different combinations and bispecific antibodies are also under development. Novel approaches like stem cell-based therapies, microRNAs, peptides, ADCs and vaccines cast a new hope for AD treatment, however, a number of questions remained to be answered prior to their safe and effective clinical translation. This review explores the small molecules, MTDL, and antibodies (monospecific and bispecific) for the treatment of AD. Finally, future perspectives (stem cell therapy, PROTAC approaches, microRNAs, ADC, peptides and vaccines) are also discussed with regard to their clinical applications and feasibility.

Keywords

Alzheimer's Disease (AD), Disease-Modifying Therapy (DMT), MicroRNAs, PROTAC

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by extracellular plaques containing tau protein and β-amyloid (Aβ) containing intracellular neurofibrillary tangles. AD interferes with the performance of daily activities and

is associated with apathy, depression, impaired communication, disorientation, poor judgment, difficulty in swallowing and walking, and behavioral changes [1].

Aβ is derived from the sequential cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase, and aggregation of Aβ leads to formation of oligomers which are toxic to neurons [2]. On the other hand, tau protein is resulted from the microtubule-associated protein tau (MAPT) gene by alternative splicing to form soluble protein isoforms [3]. Evidence has suggested that Alzheimer's related brain changes, including dementia, are resulted from a complex interplay among abnormal tau and Aβ proteins and several other factors [4]. Dementia is the most common form of AD, accounting for 60% - 80% of cases, with less than half considered to be pure AD and the majority to be mixed dementia. Dementia is known to affect more than 47 million people worldwide, and is expected to affect more than 131 million people by 2050 [5].

Many potential risk factors are associated with AD disease progression *i.e.* age, genetic factors, metabolic factors (diabetes mellitus, hypertension, obesity, and low HDL cholesterol), hearing loss, traumatic brain injury and alcohol abuse. Smoking, depression, reduced physical activity, social isolation, and air pollution are risk factors of similar magnitude for dementia, but others such as reduced physical activity, social isolation, and depression are bidirectional and may be part of the prodromal phase of dementia. Several hypotheses have been proposed as well as discovered to improve our understanding of disease etiology. One of the oldest is the cholinergic hypothesis, which is based on the fact that AD patients exhibit reduced activity of choline acetyltransferase and acetylcholinesterase in the cerebral cortex compared to normal brains [6]. Decreased neurotransmitter activity has been reported in postmortem brain tissue of AD patients. Degeneration of cholinergic neurons and loss of cholinergic neurotransmission contributed significantly to the cognitive deficits observed in AD patients [7].

The tau hypothesis is proposed based on AD histopathology of intraneuronal neurofibrillary lesions composed of tau protein [8]. Tau protein becomes pathological when hyper-phosphorylates and polymerizes into filaments and neurofibrillary tangles. This leads to abnormal neuronal morphology, axonal transport, faulty synaptic function and finally neurodegeneration [6]. Most widely accepted hypothesis is the amyloid cascade hypothesis. This theory attributes the clinical consequences of overproduction and/or decreased clearance of amyloid-beta (Aβ) peptides, which lead to increased deposition of Aβ and subsequent neuronal damage. Two main types of Aβ polymers (Aβ-40 and Aβ-42) play a direct role in the pathology of AD.

Researchers have identified two distinct forms of AD, Familial Alzheimer's disease (FAD) and sporadic Alzheimer's disease (SAD). FAD is resulted from autosomal dominant mutations in the genes APP, PSEN1, and PSEN2 [8] [9]. SAD is linked to e4-type allele on chromosome 19, encoding apolipoprotein E (APOE). APOE is present in approximately 50% - 60% of AD patients with no family history of AD. APOE is associated with approximately three times the risk of

developing AD if one copy is present and approximately eight times if two copies are present [10].

Treatment of AD mainly consists of symptomatic therapy and disease-modifying therapy (DMT). A very few number of treatment options have been approved by the US Food and Drug Administration (FDA), which can prevent, slow or halt disease progression and modify the underlying pathology of the disease. Alzheimer's association has mentioned all FDA approved drugs for both symptomatic therapy and DMT of Alzheimer's disease [11]. The conventional approach is symptomatic treatment and clinical management of Alzheimer's disease by acetylcholinesterase inhibition through the use of FDA-approved cholinesterase inhibitors (ChEIs) such as donepezil, rivastigmine, tacrine, galantamine [12], and N-methyl-D-aspartate (NMDA) receptor antagonist memantine [5], although, these drugs have limited efficacy. Current drug development programs aim to ameliorate the disease with agents that prevent or delay the onset of Alzheimer's disease or slowdown its progression [13]. After nearly two decades of rigorous pharmacological research and drug development with no new therapeutic breakthroughs, recent advancements have led to the development and approval of disease-modifying therapies (DMTs) and symptomatic treatments for the neuropsychiatric syndromes associated with AD. Two anti-amyloid monoclonal antibodies (AA-MABs), aducanumab and lecanemab, have been approved for their ability to slow cognitive decline in AD patients, while a third, donanemab, is currently under review. Additionally, brexpiprazole has been approved for the treatment of agitation in dementia related to AD. Moreover, several anti-tau monoclonal antibodies are currently in clinical trials for AD [14].

Inadequate understanding and multifactorial nature of AD led to limited success and limited approval of novel drugs by FDA. More specific strategies are needed to develop new drug molecules or new combinations for different pathways to treat AD. Therefore, this review is mainly focused on disease modifying therapy and we have summarized currently available treatments, including monotherapies (small molecules and Mabs), combination approaches and novel emerging therapies *i.e.* stem cell therapy, antibody conjugates, miRNA, PROTAC technology and vaccines.

2. Novel Small Molecule Based Approaches for Alzheimer's Disease

Growing understanding about AD led the researchers to intervene by developing disease modifying therapies to decline or even reverse the progression of the disease. The most promising approach is preventing early oligomerization through direct interference with monomeric Aβ/Aβ interactions or by promoting non-toxic oligomerization [15]. An additional approach is to prevent the interaction between toxic oligomers of Aβ and target receptors or proteins [16]. One more promising therapeutic approach is based on the modulation of APP/Aβ pathway and can be widely elucidated based on three types of approaches: Modulation of Aβ synthesis, preventing Aβ aggregation, promoting Aβ clearance

[16] [17]. Effective strategies investigated for the past few decades, are discussed below.

2.1. α-Secretases

α-secretases are known as ADAMs (A Disintegrin and Metalloproteases), a family of integral membrane proteins which drive the ectodomain shedding of key transmembrane proteins such as the Notch receptor, APP, TNF-α, ErbB2, and ErbB48. Activation of the α-secretase led to formation of a soluble APP, which has a neuroprotective role as well as synaptogenesis stimulating role [18].

Several small molecules have been investigated in preclinical and clinical studies for AD treatment to enhance α-secretase activity such as agonists of muscarinic acetylcholine, glutamatergic and serotonergic receptors, activators of protein kinase C as well as PI3K/Akt, or GABAA receptor modulators [19]. Etazolate (EHT-0202) is a GABAA receptor modulator and phosphodiesterase 4 (PDE4) inhibitor which has been completed Phase II/III in mild to moderate AD patients. EHT-0202 has altered the course of the disease by inducing α-secretase's remyelination, neurotrophic effect as well as promoting the production of soluble APP (sAPPα) [20].

Acitretin is a synthetic vitamin A analogue used in a treatment of psoriasis, has completed Phase II clinical trial for mild to moderate AD patients [21]. It is a neuroprotective molecule that interacts with the cellular retinoic acid-binding protein (CRABP) to be transported in the nucleus, where it modulates α-secretase activity by enhancing expression of ADAM10 (most relevant α-secretase for the non-amyloidogenic pathway). In preclinical and clinical studies, acitretin was well tolerated and activated the non-amyloidogenic pathway by reducing Aβ levels, and increasing sAPPα levels in CSF [18].

Bryostatin 1 has induced α-secretase activity and improved cognitive impairment in AD mice as well as prevented synaptic loss and increased synaptic maturation. In Phase II clinical studies for mild to moderate AD, Bryostatin 1 was well tolerated and showed potential efficacy with improved cognitive functions [19] [22] [23]. An ongoing phase II study in AD patients is evaluating the effect of APH-1105, a potent analog of bryostatin. The safety and tolerability of intranasally APH-1105 will be evaluated for 12 weeks in 60 patients with AD (NCT03806478) [24].

Rivastigmine is another small molecule, which promotes α-secretase processing by upregulating levels of ADAM-9, -10, and -17 as a redundant family of α-secretases [25]. This increase accompanies by a decline in levels of sAPPβ, Aβ40 and Aβ42. Preclinical and clinical outcomes for α-secretase activation as an anti-Aβ therapeutic approach are still unsatisfying and discouraging, since there is no success yet.

2.2. γ-Secretase

γ-Secretase is a protease complex comprising four subunits: nicastrin, presenilin (PS-1), anterior pharynx-defective 1 (APH-1), and presenilin enhancer 2 (PEN-

2) [26]. γ-secretase inhibitors (GSI) and modulators (GSM) have been developed as potential disease modifying agents in AD to decrease levels of toxic Aβ peptides or to modulate Aβ composition. Aβ40 and Aβ42 are major fragments produced by the γ-secretase enzyme, which aggregate extracellularly to form Aβ plaques.

GSIs were the first class of compounds investigated to reduce the levels of Aβ formation, among several GSIs, avagacestat (BMS-708163) and semagacestat (LY-450149) have been reached in phase-II and phase-III trials, respectively. However, non-selective inhibition and a number of adverse effects led to the withdrawal of both compounds from clinical studies [17] [27].

With the failure of GSIs in clinical trials, other classes of compounds called γ-secretase modulators (GSMs) were discovered, this approach directed to the discovery of selective allosteric negative γ-secretase modulators (GSMs). A subset of NSAIDs such as ibuprofen, indomethacin, flurbiprofen were characterized as first-generation γ-secretase modulators (GSM-I), selectively reduce levels of the pathogenic Aβ42 in mice [18]. However, these NSAIDs were discontinued due to weak *in-vitro* potency and poor brain penetration. Another GSM-1, Tarenfurbil (R-furbiprofen) slowed significant cognitive decline in patients with mild AD in phase II, but failed in phase III due to its poor brain penetration [28].

The second-generation γ-secretase modulators (GSM-II) are modified molecules based on NSAIDs with greater brain penetration, and divided into two categories: 1) carboxylic acid NSAID-derived GSMs and 2) heterocyclic non-NSAID derived GSMs. These modulators reduced the Aβ42 production without affecting Aβ40 levels [27] [28]. Several GSM-II candidate small molecules have been investigated in preclinical and clinical studies. PF-06648671, derived from bicyclic pyridinones was investigated in three phase I trials. PF-06648671 dose-dependently reduced the level of Aβ40 and Aβ42 in CSF and induced Aβ37 and Aβ38 with no change in total CSF Aβ [28]. EVP-0962 is a cyclobutyl group containing analogue of (R)-flurbiprofen which were reached in Phase II for mild AD/cognitive impairment, but it was discontinued due to safety reasons [18].

NGP 555 is only one first in class small molecule modulator of γ-secretase enlisted in phase III of clinical trial for the AD therapy (NeuroGenetic Pharmaceuticals). The linking of NGP 555 to the γ-secretase enzyme complex occurred instantly via Pen-2/PS1-NTFs, allowing ε-site proteolysis of amyloid precursor protein (APP), Notch, or E-cadherin without interference. This process led the production of shorter non-toxic forms of Aβ, demonstrating the effects of NGP 555. NGP555 efficiently crossed the blood brain barrier (BBB) and potentially prevented the production of Aβ42 and Aβ40 in the Tg2576 mouse model of AD [29]. The compound NGP555 [30] demonstrated moderate pharmacodynamic effects in Phase I studies with a significant increase in Aβ38 and a tendency to lower Aβ42 only.

2.3. β-Secretase or β-Site APP Cleaving Enzyme 1 (BACE1)

β-secretase (BACE1) is responsible for initiating the Aβ production and represents

the rate-limiting enzyme in the amyloidogenic pathway of APP processing. APP is cleaved by BACE1 into a membrane bound C-terminal fragment and a soluble APPβ fragment. C-terminal fragment is further cleaved by γ-secretase to generate the Aβ fragment that subsequently aggregates to form plaques. Several studies demonstrated that genetic or pharmacological reduction in β-secretase activity could be a potential strategy in decreasing Aβ brain concentrations and eventually preventing the progression of AD. LY2811376 is the first reported oral BACE1 inhibitor which led to profound Aβ-lowering effects in preclinical and clinical studies [31]. However, nonclinical retinal toxicity due to long-term dosing led to termination of LY2811376 in Phase I trial. Thereafter, a second-generation orally available BACE1 inhibitor LY2886721, reached phase 2 clinical trials in AD [31], but terminated since liver enzymes were abnormally elevated [32]. The third-generation BACE1 inhibitor, LY3202626 decreased Aβ-40 and Aβ-42 concentrations in plasma and CSF fluid. Although, LY3202626 was characterized as a highly potent, CNS penetrant, and low-dose BACE1 inhibitor, but it did not show a clinically significant change in cerebral tau burden and cognitive decline compared to placebo in Phase II, hence discontinued [32] [33]. More BACE1 inhibitors are under clinical investigation in Phases I to III, and all of them demonstrated a favorable ADME profile. Surprisingly, results of phase I evaluation of new small molecule BACE1 inhibitors have demonstrated significant reduction (45% - 95%) in CSF Aβ levels, which has not been previously reported with other anti-amyloid therapies [17].

ALZ-801 is an orally available prodrug of tramiprosate. Tramiprosate exhibited inhibition of β-amyloid oligomer formation by a multi-ligand enveloping mechanism of action, resulting in the inhibition of oligomers production and subsequent aggregation by stabilization of Aβ42 monomers [34]. In Phase 3 studies, tramiprosate exhibited nausea and vomiting and inter-subject variability in plasma levels. ALZ-801 was developed with significantly improved PK properties and to avoid the side effects of parent compound, tramiprosate [35]. ALZ-801 is in Phase 3 development in ApoE4/4 AD patients as a potential disease modifying treatment [35] [36].

Blarcamesine (ANAVEX 2-73) is an oral small-molecule activator of the sigma-1 receptor (SIGMAR1), which is currently in Phase IIa/III clinical trial for Alzheimer's disease (NCT03790709). ANAVEX 2-73 significantly blocked an increase in Aβ1-42 levels in hippocampus and efficiently decreased tau hyperphosphorylation by targeting GSK-3β kinase activity [37].

Buntanetap is another orally bioavailable small molecule which was discovered at the National Institutes of Aging (Bethesda, Maryland). Buntanetap targets neurodegeneration by preventing the formation of multiple neurotoxic proteins, including amyloid beta, tau, alpha-synuclein, and TDP43, by selectively inhibiting translation of these neurotoxic proteins. Buntanetap phase II/III clinical trials expected to be completed in 2024 in mild to moderate AD patients (NCT05686044) [38] [39].

3. Multi-Target Directed Ligands (MTDLs) or Hybrid Molecules

It is well accepted that neurodegeneration is a consequence of several detrimental processes, such as protein aggregation, oxidative stress and neuroinflammation, ultimately resulting in the loss of neuronal functions. The failure of "one drug-one target" strategy and the multifunctional nature of AD inspired the scientific community to investigate another drug design strategy called multitarget-directed ligands (MTDLs). In this context, a number of MTDLs were designed and tested. Ladostigil, a MTDL drug was synthesized by combining pharmacophores from rivastigmine (an AChE inhibitor) and rasagiline (a MAO-B inhibitor). It has been progressed to phase III trials after exhibiting a capacity to slow neurodegenerative decline in patients with mild cognitive impairment. Ladostigil was reported to retain similar levels of inhibitory activity against each target as its parent compounds, with a reduction in the activity of AChE by 25% - 40%, and MAO-B by 70% - 90% in rodents. Several hypothetical multi-target drugs were proposed for the development on the basis of hybrid molecules, however, currently no rationally designed MTDL has been clinically approved.

4. Novel Antibodies Based Approaches for Alzheimer's Disease

Over the past few years, many drugs have been evaluated for their ability to reduce Aβ production and to inhibit Aβ aggregation. Most of the drug molecules have failed, but few are in advanced clinical trials prompted reconsideration of Aβ hypothesis as an important therapeutic target. Therefore, immunotherapy has become the focus of exploration to promote Aβ clearance and has greatly inspired research on anti-Aβ therapies [40]. In this section, we summarize the immunotherapeutic strategies (monoclonal antibodies targeting Aβ aggregation and Tau protein) that have already been approved or are under clinical trials, as well as discussed the bispecific antibodies targeting multiple targets.

4.1. Aβ-Based Immunotherapy

Aducanumab (BIIB037) is a human immunoglobulin 1 (IgG1) monoclonal antibody that binds to the N terminus of Aβ in an extended conformation [41]. It selectively targeted Aβ aggregates including neuritic Aβ plaques and high molecular weight ABOs, but not Aβ monomers and led to slowing down of cognitive impairment in prodromal or mild AD [42] [43].

In a double-blind randomized and placebo-controlled phase 1 trial (NCT01677572), the brain amyloid burden was reduced by Aducanumab in a dose and time dependent manner in patients with prodromal or mild AD. 10 mg/kg aducanumab for 54 weeks showed significant reductions in amyloid positron emission tomography (PET) standard uptake value ratio (SUVRr) composite score in the treated patients [42]. Two different phase III studies ENGAGE (NCT02477800) and EMERGE (NCT02484547) were conducted, but both were terminated due to

unlikely therapeutic effect of aducanumab [44]. It was later revealed that patients receiving high dose of aducanumab showed reduction in both trials, EMERGE met its primary endpoint, where patients in the high-dose group showed a statistically significant reduction of clinical decline from baseline in CDR-SB scores by 22% at 78 weeks. ENGAGE did not meet its primary endpoint, but data from patients receiving high-dose aducanumab were consistent with EMERGE results [45]. Even after getting controversial phase 3 results, Aducanumab was approved by the United States Food and Drug Administration (FDA) as the first DMT for AD in June of 2021. It was given official approval to treat AD based on the endpoint, *i.e.*, removal of amyloid plaques from the brain [46].

Donanemab (LY3002813) is a human immunoglobulin IgG1 antibody that binds specifically to the N-terminal pyroglutamate Aβ epitope, which is present merely in deposited Aβ. In the phase II trial (NCT03367403), donanemab induced a smaller reduction of integrated Alzheimer's disease rating scale score in early stage AD patients. In addition, PET results showed that donanemab-treated patients had significantly reduced amyloid plaques at 76 weeks, and 54.7% of participants had an amyloid-negative status at 52 weeks. A follow-up study of patients enrolled (NCT04640077), is ongoing and two different phase III studies NCT04437511 and NCT05026866 have been started to further determine the safety and efficacy of donanemab [47].

Lecanemab (BAN2401) is a mouse mab158 human immunoglobulin IgG1 that is selectively bound to large soluble Aβ protofibrils. It preferentially targets soluble aggregated Aβ and possessing activity across oligomers, protofibrils, and insoluble fibrils [48]. In phase II study (NCT01767311) it did not meet its primary 12-month endpoint, but reduced brain amyloid plaques and showed sustained clinical remission at highest dose of 10 mg/kg biweekly. Currently two different phase III trial are undergoing. A Phase 3 study (NCT03887455), is ongoing to evaluate efficacy, long-term safety and tolerability of lecanemab in early AD. Another phase III trial (NCT04468659), is being conducted to evaluate the efficacy and safety of lecanemab in preclinical AD patients [49]. Because of more promising results in clinical trials Lecanemab has been given the accelerated approval in January, 2023 by FDA.

Solanezumab (LY2062430) is a humanized monoclonal antibody that targets the middle domain of the Aβ peptide (Aβ13-28) thereby increases Aβ clearance [50]. Among four different completed Phase III clinical trials, EXPEDITION 1 (NCT00905372) and EXPEDITION 2 (NCT00904683) failed to demonstrate the efficacy of solanezumab in slowing cognitive decline and improving function in patients with mild to moderate AD [50] [51]. In addition, two other separate phase III clinical trials, Expedition 3 (NCT01900665) and ExpeditionPRO (NCT02760602), were also terminated due to failure in improving cognitive decline [50] [51]. Currently a phase III clinical trial, A4 (NCT02008357), is ongoing to evaluate the efficacy of solanezumab in asymptomatic or very mild patients with amyloid plaques in the brain.

Crenezumab (RG7412) is a human immunoglobulin IgG1 monoclonal antibody that targets multiple forms of Aβ, including monomers and aggregates [52]. Three different Phase III clinical trials, CREAD (NCT02670083), CREAD2 (NCT03114657), CREAD OLE (NCT03491150) were terminated because a pre-planned interim analysis which found out unlikeliness to hit the primary endpoint of improving CDR-SB scores [53].

Gantenerumab (RO4909832) is a human immunoglobulin IgG1 monoclonal antibody that binds aggregated Aβ with high affinity and promotes Aβ clearance through Fc receptor-mediated phagocytosis [54]. In February 2020, it was announced that gantenerumab did not meet its primary endpoint in a Phase 2 trial (DIAN-TU, NCT04623242) in patients with hereditary AD. Two randomized, double-blind, placebo-controlled, parallel-group Phase III trials, GRADUATE 1 (NCT03444870) and GRADUATE 2 (NCT03443973), are currently underway to evaluate the safety of gantenerumab in broader AD patients to evaluate effectiveness and efficacy. Two other Phase III studies (NCT04339413 and NCT04374253) are ongoing to evaluate the safety and tolerability of long-term administration [55].

Bapineuzumab (AAB-001), a human immunoglobulin IgG1 anti-Aβ mAb, which binds to the five N-terminal residues and clears both fibrillar and soluble Aβ [56]. In two different Phase III trials (NCT00575055) and (NCT00574132), bapinezumab did not improve clinical outcomes in patients with AD. There were no significant differences between the bapineuzumab groups and the placebo groups with respect to the primary end points (scores on the ADAS-cog11 and DAD) [57].

4.2. Tau-Based Immunotherapy

Semorinemab (RO705705) is a humanized anti-tau monoclonal antibody against extracellular tau with an immunoglobulin isotype-binding backbone that can bind to all six human tau isoforms and protect neurons [58]. In a phase 2 randomized, double-blind, placebo-controlled, parallel-group clinical trial (NCT03289143), Semorinemab treatment did not slow the rate of cerebral tau accumulation or clinical decline in prodromal to mild Alzheimer disease [59]. Currently another Phase 2 study of semorinemab in patients with moderate AD is ongoing (NCT03828747).

Gosuranemab (BIIB092) is a humanized IgG4 monoclonal antibody directed against the N-terminus of tau, therefore able to recognize tau isoforms with an intact N-terminus including full length and N-terminal tau fragments [60]. A phase II (NCT03352557) study to assess the safety profile, tolerability and clinical efficacy of gosuranemab in participants with early AD was terminated due to the lack of efficacy in slowing cognitive and functional impairment in comparison to placebo. BIIB076, another monoclonal IgG1 targeting the mid-domain of tau, has completed a phase I trial (NCT03056729).

Tilavonemab (ABBV-8E12) is an antibody that recognizes aggregated extracellular

forms of pathological tau and binds to the N-terminus of tau [61]. Two different phase II trials, (NCT02880956 and NCT03712787) evaluating the efficacy and safety of tilavonemab, did not get expected results and now tilavonemab is discontinued in AD treatment.

Bepranemab (UCB0107) is a humanized monoclonal IgG4 antibody which targets amino acids 235-250 of tau protein. Mid-region antibodies appear to be more likely to interfere with intercellular proliferation of pathogenic and aggregated tau than N-terminally directed anti-tau antibodies [62]. Currently, Phase 2 Trial to test the efficacy, safety and tolerability of Beplanemab in patients with mild Alzheimer's disease is ongoing (NCT04867616).

Zagotenemab (LY3303560) is a humanized anti-tau antibody targeting a conformational epitope of tau, MC1, which is an early pathological conformation of tau [63]. In a Phase 2 trial (NCT03518073), zagotenemab did not meet its primary endpoint and development of zagotenemab was terminated.

JNJ-63733657 is a humanized IgG1 monoclonal antibody, it can recognize the microtubule-binding region of tau with high affinity for pThr217 and its treatment has been shown to reduce pTau in the CSF in a dose-dependent manner [64]. JNJ-63733657 is currently in a Phase 2 (NCT04619420) trial to evaluate its effect on cognitive decline in early AD patients.

E2814 is a humanized, monoclonal IgG1 antibody, that recognizes the HVPGG epitope in the microtubule-binding domain near the central domain of tau [65]. It is currently being tested in a phase I/II trial to assess its safety and target engagement in mild AD participants (NCT04971733).

Lu AF87908 is a humanized, monoclonal IgG1 antibody, which targets pSer396 and pSer404 of tau protein. Currently, Lu AF87908 is in a Phase 1 (NCT04149860) safety and tolerability testing in healthy individuals and patients with AD [66].

PNT001, a monoclonal antibody which targets cis-isomer of tau, phosphorylated at threonine 231. It has completed a phase I trial (NCT04096287) in healthy adults in 2021, with unpublished results.

G7345 (RO6926496) is another antibody against pSer422 of tau, but development for this antibody has been terminated because of the inflammatory response (NCT02281786).

5. Combination Therapy

AD pathogenesis has many critical processes occurring in parallel with extracellular Aβ deposition and intercellular accumulation of hyper-phosphorylated tau [67]. As discussed earlier, involvement of multiple pathways and progressive research outcomes directed the focus of researchers beyond Aβ and tau accumulation, hence, different drug combinations have been tested and different strategies were designed to target AD.

5.1. Antibody and Small Molecule Combinations

Numerous studies have shown that formation of certain N-truncated Aβ is

followed by glutaminyl cyclase (QC) catalyzed conversion of glutamic acid into pyroglutamic acid to form pGlu-Aβ [68]-[70]. Inhibition of QC has been shown to diminish AD like symptoms in mice [71]. Varoglutamstat, a first in-class glutaminyl cyclase (QC) inhibitor, has been proven safe and showed signs of efficacy in clinical phase 1 and 2 studies [72] [73]. Clinical candidate PBD-C06, a humanized pGlu3-Aβ-specific antibody binds with high specificity to pGlu3-Aβ monomers, oligomers and fibrils, including mixed aggregates of unmodified Aβ and pGlu3-Aβ peptides. A novel combination approach of varoglutamstat (PQ912) and a pGlu3-Aβ-specific antibody (m6) (PBD-C06) was tested in pre-clinical AD mice model [74]. Combination of both treatments resulted in significant reductions of Aβ by 45% - 65% compared to monotherapies. PQ912 prevented the formation of pGlu3-Aβ in different compartments, while PBD-C06 antibody was able to clear existing pGlu3-Aβ deposits.

5.2. Antibody and Scanning Ultrasound (SUS) Combination

Therapeutic ultrasound is an alternative strategy for clearing Aβ peptides by transiently opening the blood-brain barrier (BBB) for the uptake of blood borne factors as well as therapeutic agents. Repeated opening of the BBB with the scanning ultrasound (SUS) approach without using any therapeutic agent in 12 and 22 months old APP23 mice has been shown to reduce Aβ and improve memory performance [75] [76].

Another research group showed that the combination of Aducanumab (Adu) and Scanning ultrasound (SUS) treatment yielded a statistically significant decrease in total plaque area in APP23 mouse model of AD. Robust improvement was found in spatial memory for the SUS + Adu group only [77].

5.3. Bispecific Antibodies

We already discussed a number of monoclonal antibodies targeting different species of Aβ and entered clinical trials for AD [78]. Antibodies are large molecules and therefore display very limited passage across the blood-brain barrier (BBB) [79]-[81].

As a strategy, antibodies fused to an additional binding moiety have been designed to increase the fraction of administered antibody that can pass the BBB. One such approach is targeting Transferrin receptor (TfR), expressed by the endothelial cells of the BBB. Various proteins bind to TfR shuttled into the brain by receptor-mediated transcytosis. Thus, bispecific antibodies which can bind to both TfR and Aβ, displayed 10 to 100-fold higher brain concentrations compared to unmodified antibodies [82]-[86]. One such bispecific antibody gantenerumab has already entered phase I (NCT04639050) clinical trials.

Another bispecific antibody has been developed by Denali Therapeutics that targets both Aβ and tau in the brain. This antibody was engineered to bind to transferrin receptors on endothelial cells and thus traverse the BBB more efficiently. Using this antibody transport vehicle (ATV), researchers have demon-

strated the ability to reduce Aβ levels and plaque formation in APP transgenic mice by delivering anti-BACE1 antibodies [87], and have separately demonstrated the ability to decrease tau pathology in a tau-transgenic mouse by delivering antibodies against tau [88].

In a different study [89] HexaRmAb158, a multivalent antibody was developed with additional Aβ-binding sites in the form of single-chain fragment variables (scFv) on the N-terminal ends of Aβ protofibril selective antibody (RmAb158). Due to the additional binding sites, HexaRmAb158 displayed a slow dissociation from protofibrils and strong binding to oligomers *in-vitro*. Transferrin receptor (TfR) binding moiety (scFv8D3) was added to enhance BBB delivery, that led to formation of a bispecific-multivalent antibody (HexaRmAb158-scFv8D3). In an *in-vivo* study, intravenously administration of HexaRmAb158-scFv8D3 was actively transported through the BBB into the brain.

6. Future Prospective and New Approaches

6.1. Stem Cell Therapy for Treatment and Management of Alzheimer's Disease

In recent years, stem cell therapy has demonstrated substantial progress in treating AD, as seen by multiple improvements in clinical studies [90]. This therapy raised the amount of acetylcholine in the brain and led to improved memory and cognitive abilities in AD patients [91]. Stem cell therapy primarily can be split into two categories: endogenous and exogenous mechanisms of action. Stem cells now being employed in AD research are neuronal stem cells (NSCs), embryonic stem cells (ESCs), mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) [92]-[96].

It has been demonstrated that transplanting stem cells from the human umbilical cord, amniotic membrane-derived epithelial cells, and mesenchymal stem cells into the brains of transgenic Alzheimer's rats can help to reduce the symptoms of the disease by reducing the levels of Aβ, APP production, and microglia activation. Cognitive and memory capacities were improved due to the treatment and neuron lifespan was extended [95]. Another study claimed that injecting stromal cell-derived factor 1 into Alzheimer's transgenic mice had a therapeutic effect on lowering the amount of amyloid production by peripheral mononuclear cells, which were then converted into microglia [97].

Human amniotic epithelial cells (HAEC) transplantation improved contiguous memory deficits in transgenic mice while simultaneously boosting acetylcholine levels and cholinergic neuritis in the hippocampus [95]. An experiment was conducted using mESCs (mouse embryonic stem cells) and hESCs (human embryonic stem cells) injected into mature BFCNs (Cholinergic basal forebrain neurons). After the cells were transplanted into AD mice, researchers discovered that the animals' memory and learning efficiency were altered [90]. Hence, identifying the most effective stem cell and transplantation technique for achieving massive transformation is critical for achieving significant transformation.

6.2. MicroRNAs Based Approaches

Several diseases display abnormal expression of miRNAs, such as breast cancer, leukemia, hepatocellular carcinoma, cardiovascular and neurodegenerative diseases. More than 2000 miRNAs have been identified having key roles in differentiating neurons. As reported in literature, miRNAs are implicated in different pathways which are directly involved in the progression of neurodegenerative diseases (**Figure 1(A)**).

Downregulation of miR-212 and miR-132 was noticed in neurally derived plasma exosomes of patients of AD [98]. Hence, the assessment of the levels of miR-212 and miR-132 in extracellular vesicles is considered a promising diagnostic tool for AD. In addition, miR-455-3p demonstrated protective activity against amyloid aggregation through regulation of mitochondrial fission proteins expression and mitochondrial dynamics [99].

As reported, BACE-1 is the secretase mainly involved in the production of amyloid-β peptides in human brain. MiR-298 has been recently identified as a repressor of BACE-1 in human neuronal cell culture model, revealing its therapeutic potential for AD [100]. Moreover, BACE-1 expression is directly regulated by miR-15b, which functions through targeting BACE1 mRNA 3'-UTR [101].

Targeting disease-causing miRNAs with small molecules has shown promising outcome *in-vitro* as well as *in-vivo*. Inforna and Inforna 2.0 are tools developed to identify small molecules to target structured RNAs. Inforna has recently identified compound 1 (**Figure 1(B)**) as an inhibitor of α-synuclein expression [102]. Compound 1 targets the α-synuclein mRNA 5′ UTR and decreases α-synuclein expression via reducing the amount of SNCA mRNA loaded into polysomes.

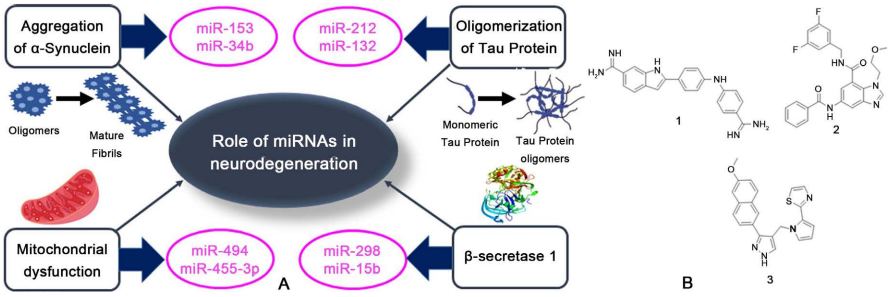

Figure 1. (A) The involvement of miRNAs in various pathways contributing to the advancement of neurodegenerative diseases. (B) Molecular structures of compounds 1 - 3.

Compound 2 (**Figure 1(B)**), a dual inhibitor of acetylcholinesterase and biogenesis of miR-15b, was found more effective than donepezil and anti-miR-15, in protecting SH-SY5Y neuroblastoma cells from amyloid-beta induced cytotoxicity. Also, dual inhibition of tau oligomerization and biogenesis of miRNAs associated with progression of neurological disorders (e.g. miRNA-146a) has been reported as a promising strategy for the design of MTDLs [103]. Compound 3 (**Figure 1(B)**), an inhibitor of miRNA-146a biogenesis in combination with MK-886 (an inhibitor of tau aggregation) showed superior neuroprotective activity. These

studies demonstrated that MTDLs focused on inhibition of the biogenesis of miRNAs has superior therapeutic profile in comparison to mono-targeted therapeutics and further studies in *in-vivo* models of neurodegenerative diseases will be critical for future optimizations.

6.3. PROTACs Technology for Treatment of Alzheimer's Disease

The proteolysis targeting chimeras (PROTACs) are heterobifunctional molecules which degrade a target protein by taking over cellular degradation machinery [104]-[106]. PROTACs degrades proteins through the ubiquitin-proteasome system (UPS). As shown in **Figure 2**, E1 activates ubiquitin first and transfers it to the E2 binding enzyme. At the same time, E3 ligase binds to the target protein (TP) and E2 enzymes enabled ubiquitin to be delivered to the target protein. Hereafter, the proteasome recognizes ubiquitin on the protein and degrades it. PROTACs takeover the inherent intracellular UPS for TP ubiquitination and subsequent proteasome degradation, through TP-PROTAC-E3 ligase complex. These PROTACs are comprised of three parts: 1) a moiety for recognition of target protein (TP), 2) a moiety for binding to the E3 ligase, and 3) the linker for connecting these ligands.

Chu and Lu et al. reported that tau proteins could be degraded by using peptide form of PROTACs [107] [108]. Remarkably, peptidic PROTAC named as TH006 degraded tau in CA3 region of hippocampus *in-vivo*. Silva et al. created a set of unique PROTACs using a tau PET tracer [109] for targeting tau in human differentiated frontotemporal dementia (FTD) neurons. Wild-type and mutant tau variants (A152T and P301L) were sufficiently degraded in neurons using PROTAC T807 with the Kd value of 1.8, 2.1 and 1.7 μM, respectively. C004019, a PROTAC was developed using a linker that could connect tau to a Von Hippel Lindau (VHL) ligand [110] and this PROTAC cleared tau protein in both physiological and pathological conditions *in-vitro* and *in-vivo*. Amazingly, once weekly single dose of the PROTAC could sustain tau reduction and alleviate Aβ-induced neurotoxicity in the brain of 3xTg-AD mouse model without showing noticeable abnormalities. Robust tau clearance was found in hippocampus and cortex of mice along with improvement of synaptic and cognitive functions. Liang et al. has designed and synthesized a series of molecules containing bifunctional groups to recognize the tou-protein and the E3 ligase. Their data showed effective degradation of tou-protein and reduced Aβ-induced cytotoxicity in PC12 cells [111].

Recently, Kargbo et al developed bifunctional PROTAC compound which targets αSyn protein. This compound was developed using a VHL moiety to target protein of interest. The linker could direct VHL E3 ubiquitin ligase in vicinity to target protein for degradation in the UPS system. This PROTAC could prevent the accumulation and aberrant aggregation of αSyn protein in HEK293 cells stably expressing TREX αSyn A53T [112].

The PROTAC technology enabled researchers to develop new therapeutic agents with unique capability for degradation of "undruggable" target proteins

instead of inhibition. However, the toxicity issues related to PROTACs could limit future drug development.

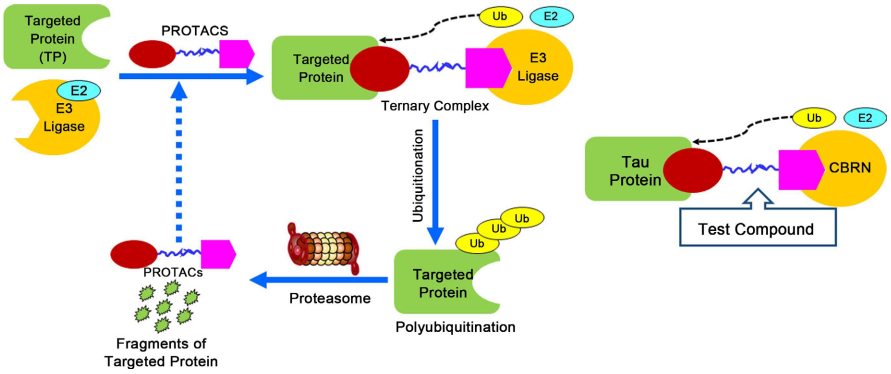

Figure 2. A schematic representation of PROTAC mechanism(s) of actions.

6.4. Antibody Drug Conjugates in Alzheimer's Disease

Antibody drug conjugates (ADCs) contain a monoclonal antibody component, a linker and a drug component. ADC has the capability to release its drug at the target site, intracellular or extracellular, hence better efficacy is expected with minimal toxicity due to limited systemic exposure. ADCs have a monoclonal antibody specific for a particular protein, in AD pathology it is either Aβ or tau proteins. Small molecules having good efficacy in *in-vitro* models can be conjugated with antibodies to enable BBB passage, if cannot penetrate through BBB [113]. Although, ADCs have got popularity in oncology, but there is a great scope for ADCs in AD treatment. Antibodies developed to target Aβ or tau or other pathologic proteins in AD can be developed into an ADC by conjugating appropriate small-molecule drug.

6.5. Peptides Based Approaches in AD

Similar to small molecules, synthetic peptides offer the possibility to modulate Aβ pathway by interacting with endogenous peptides or interfering with protein aggregation. Some oligopeptides have already shown significant effects in animal models of dementia and even progressed into clinical studies. Catania et al. reported the discovery of a naturally-occurring variant of Aβ (AβA2V) that has anti-amyloidogenic properties, and intranasal delivery of this hexamer peptide (Aβ1-6A2V) retained the anti-amyloidogenic abilities of the full-length AβA2V variant [114]. This approach was found effective in preventing the aggregation of wild type Aβ and preventing the synaptic damage associated with amyloidogenesis in a mouse model of AD.

6.6. Vaccines as Potential Therapeutic Agents for AD

The adaptive immune system is fundamental to the pathogenesis and progression of AD. Microglia astroglia and the brain-resident immune cells are potential regulators of neuroinflammatory responses in AD. In 1999, Elan pharmaceuticals

reported that active immunization against Aβ1-42 could reduce Aβ pathology in APP transgenic mice and this vaccine was named as AN1792. Immunization with aggregated Aβ1-42 formulated in strong adjuvants caused reduction in plaque burden and later studies also confirmed improved mental performance in immunized mice [115] [116]. Similar results were attained in additional transgenic mouse models of AD using active and passive immunization [116]. As passive immunization with anti-Aβ antibodies was able to clear the Aβ plaques, therefore, it was clear that antibodies were the relevant effector molecules induced by vaccination.

A number of targets have been evaluated for an active vaccine therapy for AD (**Figure 3**). About 140 immunization procedures against Aβ deposition and 25 against tau have been reported, but no AD vaccine has got FDA approval yet [117]. A DNA vaccine, AV-1959D, targeting the N-terminal epitope of the Aβ peptide was found immunogenic in mice, rabbits, and non-human primates. Also, AV-1959D vaccine was found effective in mouse models of AD (**Table 1**). Repeated dose safety assessment of vaccine did not find any adverse short- or long-term effects in mice. Mice treated with the vaccine confirmed elevation in anti-Aβ antibodies over time [118].

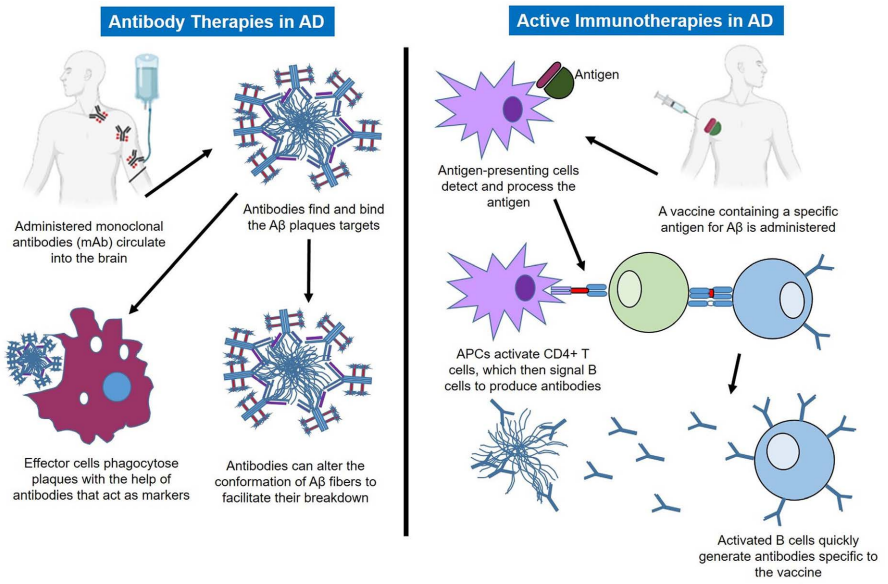

Figure 3. The general theory of immunotherapy for Alzheimer's disease, focusing on Aβ (amyloid-beta) pathophysiology. Antibody therapy involves the administering antibodies directly to the patient, while active immunotherapy involves using vaccines to stimulate the patient's immune system to produce their own antibodies.

More recently, a vaccine developed by modifying yeast cells to express Aβ1-15 on their cell wall (named Y-5A15), has shown to improve cognitive function and decreased plaque formation in animal models [119]. Phase I clinical trial of another vaccine Protollin has been reported in 2021 [120]. Neisseria meningitidis outer membrane proteins were complexed with Shigella flexneri 2a lipopolys-

accharide to generate Protollin vaccine. This combination worked by activating Toll-like receptors (TLRs) 2 and 4, within the nasal cavity [121]. This vaccine was delivered intranasally and displayed efficacy in the removal of Aβ plaques in transgenic mouse models of AD [122] [123].

In light with positive preclinical results several clinical studies were initiated to assess safety and efficacy of vaccines against AD. A vaccine developed by Elan and Wyeth was based on aggregated human Aβ1-42 (AN1792). In phase I clinical study, AD patients were immunized with AN1792 formulated in the adjuvant QS21. Aβ specific antibodies were found in more than half of the immunized AD patients in phase I trials without notable adverse events [115]. Encouraged by the positive safety data, AN1792 vaccine was tested in a relatively larger phase II trial with 372 AD patients to assess its safety as well as efficacy. This clinical trial was discontinued prematurely since 6% of the vaccinated patients developed aseptic meningo-encephalitis [115]. T cells were thought to be the pathological culprit, as mAbs specific for Aβ1-42 have caused no signs of meningoencephalitis [115]. In addition, infiltrating T-cells were found in the brain of diseased patients, suggested Aβ specific T-cells to be the cause of the observed side effects [124] [125].

Table 1. Research information for several active vaccine treatments for Alzheimer's disease and their findings. All demonstrate potential in eliciting immune responses but must be monitored carefully to prevent adverse events from occurring.

Compound	Target	Type	Phase	Participants	Findings	Ref.
AV-1959D	Aβ	DNA Vaccine	Pre-clinical	60	The vaccine elicited an immune response in the antibody production specific to Aβ42 and no short of long-term toxicities was observed.	118
AN1792	Aβ	Vaccine	IIa	375	Reduced Aβ load in the brain, terminated due to development of adverse events resulting from the treatment.	115
Y-5a15	Aβ	Vaccine	Pre-clinical	N/A	Treatment reduced levels of Aβ, and improved cognitive function in mice through eliciting the significant levels of Aβ antibodies.	119
Protollin	Aβ	Vaccine	Pre-clinical	N/A	Significant reduction in Aβ in mice, cognitive function improved following treatment. Adjuvant was not observed in brain tissue.	118, 121

Two major strategies were adopted by the industry to overcome this issue: 1) introduction of Th cell epitopes to be able to bypass Th cell tolerance and 2) shortening of the Aβ1-42-derived peptide. Indeed, peptides of smaller sizes < 8 amino

acids cannot induce T cell responses as they cannot bind to MHC molecules for presentation to T cells.

The next generation candidate for AN1792 consisted of N-terminal seven amino acids of Aβ1-42 covalently linked to the diphtheria toxin cross reactive mutant (CRM197) protein carrier and is named ACC-001. In line with strategies discussed above, this peptide was below the minimum length-requirements for any T-cell epitope to bind to MHC molecules, and strong Aβ1-42-specific antibody responses were induced in the transgenic mice. Additionally, Plaque development was blocked efficiently and cognitive functions were improved.

Similarly, Novartis has developed an Alzheimer vaccine (CAD106) using bacteriophage based VLP technology to target amyloid β. This vaccine consists of the bacteriophage Qβ VLP (Qβ) displaying Aβ1-6 on its surface with the help of chemical coupling. CAD106 was found highly immunogenic for the induction of antibody in transgenic mice as well as rabbits and primates, and did not induce measurable Aβ1-42-specific T cells responses [126]. As reported, immunization with CAD106 inhibited Aβ plaques deposition in transgenic mouse models. According to available information from first in human studies with CAD106 showed that vaccine was well tolerated and induced Aβ-specific immune response in the majority of the patients without showing any signs of meningoencephalitis [127]-[129].

A combinatorial vaccination approach was also reported in literature, where a mixture of two MultiTEP epitope vaccines: AV-1959R and AV-1980R, targeting Aβ and tau were tested in Tau22/5xFAD (T5x) bigenic mice (having pathological Aβ and tau aggregates). T5x mice immunized with a mixture of Aβ- and tau-targeting vaccines generated high Aβ- and tau-specific antibody titers, and significantly reduced hyperphosphorylated tau as well as insoluble Aβ42 within the mice brains [130].

Vaccines are preventative rather than therapeutic modality and it is obvious that prevention of plaque accumulation is far superior over removal of existing plaques. However, none of the currently tested vaccines has clinical POC demonstrating delayed disease progression.

7. Conclusion and Author's Opinion

It has been long time since researchers started working on AD, but due to the complexity of AD, still the treatment of patients remained challenging. Currently approved treatments for AD are limited to cholinesterase inhibitors and memantine or the combination of these agents, although, combining cholinesterase inhibitors and memantine has had limited success in the treatment of AD. In-spite of the early promise of several new drugs, many have failed larger phase III trials, because of not meeting efficacy endpoints. Two main neuropathological hallmarks of AD are amyloid-β plaques (Aβ) and neurofibrillary tangles formed by intracellular accumulation of hyper-phosphorylated tau protein [131]-[133]. Current methods for AD treatment are mostly symptomatic, while some new effective

pathogenesis-relevant therapies that would block the disease course and restore all the compromised functions are demanded. Amyloid hypothesis is considered to be related to the etiology of AD, but nearly all small molecules targeting Aβ or Tau, as monotherapy have failed in the clinic during the past about 20 years.

Meeting continuous disappointments with small molecules, researchers started to develop immunotherapeutic approaches (monoclonal antibodies, vaccines etc.) as DMT. Despite numerous problems regarding immunotherapy for AD, the studies have still progressed in developing anti-Aβ monoclonal antibodies for the treatment of AD. Recently, anti-Aβ monoclonal antibodies (mabs) have been investigated as a treatment for AD, including aducanumab, bapineuzumab, gantenerumab, solanezumab, donanemab and lecanemab. These mabs are distinct in selectivity for polymorphic variants and recognize epitopes based on the specific portion and conformations of Aβ [41]. Aducanumab is the first disease-modifying therapy (DMT) to be approved for AD [134]. It became available in the market for those with MCI (Mild Cognitive Impairment) due to AD and mild AD dementia in 2021. One more monoclonal antibody lecanemab has been approved recently in January 2023 and another mab donanemab is under review by the US Food and Drug Administration (FDA). There were a series of clinical trial failures with applying these mabs, which is still a question on further development of Aβ-targeting drugs. Above mentioned mabs can reduce the levels of Aβ peptides, Aβ-40 and Aβ-42 in cerebrospinal fluid (CSF), or plasma at various degrees with different doses, but the effects of the mabs on p181-tau level differed. Several tau monoclonal antibodies have failed to establish efficacy in comparison to placebo in recent AD trials including semorinemab, zagotenemab, gosuranemab, and ABBV-8E12 [135].

Another immunological approach tested in preclinical and clinical studies is development of vaccines targeting Aβ and tau proteins. Despite of great efforts, none of the currently tested vaccines got approved by FDA for AD.

Limited success as monotherapy and multifactorial nature of AD, led the scientific community to look for logical combinations of therapeutic agents or synthesizing one compound-with-multiple targets (polypharmacological) for targeting different pathways together to get more successful treatment of AD [136]. Combination of ≥2 agents that target separate pathways offers an opportunity for treating the disease by synergistic effects, and very few drug combinations are in clinical trials. Combining therapeutic agents may allow for lower doses of the individual agents, resulting reduced side effects. Lots of efforts have been put forward in this regard, but such therapeutic agents are still in preclinical or early clinical phase.

We think combination of approved drugs for disease modifying therapy *i.e.* one mab combination to other mab and mabs combination to small molecules, could be a useful strategy to treat AD, since monotherapies showed limited efficacies. Also, efforts are still going on to develop bispecific antibodies targeting different pathways, although these studies are at their budding stages. Similar to small

molecules combinations targeting multiple pathways, bispecific antibodies could be better alternative for the treatment of AD, because of more specificity for target proteins. Another approach, we already have discussed is Antibody-Drug-Conjugate generation. As reported in literature, there are numerous small molecules in late stages of their clinical studies. Approval of mabs (Aducanumab and Lecanemab) has opened new avenues to conjugate small molecules to these mabs. ADCs developed with this strategy could have more beneficial effects, since antibodies will direct the small molecules to the site of action. In the same time small molecules related toxicities will be less because of minimum systemic exposure.

Also, innovative ideas like stem cell therapy, microRNA approaches, peptides and vaccines could have fruitful outcomes as monotherapy and in combinations to other therapies; however, these advanced approaches are still in their budding stages with limited success.

Taken together, the challenges of treating AD have steered the current treatment landscape toward investigating new drugs as disease-modifying therapy through multiple strategic approaches to target different pathways in parallel. However, there is still room for novel futuristic ideas and strategies.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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