Journal of Alzheimer's Disease 71 (2019) 715–732
DOI 10.3233/JAD-190507
IOS Press

Review

Developing Effective Alzheimer's Disease Therapies: Clinical Experience and Future Directions

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Handling Associate Editor: Carmela Abraham

Accepted 24 July 2019

Abstract. Alzheimer's disease (AD) clinical trials, focused on disease modifying drugs and conducted in patients with mild to moderate AD, as well as prodromal (early) AD, have failed to reach efficacy endpoints in improving cognitive function in most cases to date or have been terminated due to adverse events. Drugs that have reached clinical stage were reviewed using web resources (such as clinicaltrials.gov, alzforum.org, company press releases, and peer reviewed literature) to identify late stage (Phase II and Phase III) efficacy clinical trials and summarize reasons for their failure. For each drug, only the latest clinical trials and ongoing trials that aimed at improving cognitive function were included in the analysis. Here we highlight the potential reasons that have hindered clinical success, including clinical trial design and choice of outcome measures, heterogeneity of patient populations, difficulties in diagnosing and staging the disease, drug design, mechanism of action, and toxicity related to the long-term use. We review and suggest approaches for AD clinical trial design aimed at improving our ability to identify novel therapies for this devastating disease.

Keywords: Alzheimer's disease, amyloid-β, biomarkers, clinical trial design, combined modality therapy, inflammation, selection of subjects, tau protein, treatment outcomes

BACKGROUND

Treatments to prevent or slow down cognitive decline in Alzheimer's disease (AD) remain an unmet therapeutic need. The drugs that have been approved to date for treatment of mild-to-moderate

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AD are acetylcholine esterase inhibitors, including tacrine (first approved treatment, withdrawn soon after because of reports of liver toxicity), donepezil (also approved for severe AD), rivastigmine (also approved for severe AD), and galantamine [1, 2]. These drugs increase levels of available acetylcholine, associated with memory and learning, during synaptic transmission and thus compensate for the diminished function of cholinergic neurons. Though the established mechanism is symptomatic, Dubois et al. recently reported a reasonably powered multicenter trial of donepezil versus placebo in mild cognitive impairment (MCI) that demonstrated significant effect of the drug in preserving hippocampal and whole brain volume at one year versus placebo [3]. In addition, memantine, an uncompetitive (open-channel) N-methyl-D-aspartate (NMDA) antagonist with neuroprotective properties [4], is approved for treatment of moderate to severe AD.

Numerous clinical trials have explored agents that could potentially not only interfere with the physiology of the disease but also provide short-term symptomatic improvement. However, to date none of the AD modifying treatments has reached regulatory approval.

While the mechanism of the onset of AD is still not fully understood, it is recognized that a strong genetic risk component is usually involved [5–11]. In addition to subjects' genetic make up, multiple other lifestyle factors including physical and mental exercise, heart disease, diabetes, lower education, and mental diseases are thought to accelerate AD progression [12, 13].

Two primary neuropathologies in the brains of AD patients have been recognized and studied extensively over the last two decades, including amyloid plaques formation comprising aggregated amyloid-β (Aβ) and neurofibrillary tangles (NFTs) formed by aggregation of hyperphosphorylated tau protein. The aggregation and extracellular deposition of Aβ oligomers, drives neuronal death and AD pathogenesis [14, 15]. While pathologic concentrations of Aβ (nanomolar and micromolar) are considered neurotoxic, soluble Aβ protein present in low (picomolar) concentrations in normal brains, is thought to have multiple functions including modulating synaptic activity, memory formation, neuronal survival, antioxidant activity, effects on Ca transport, and maintenance of blood-brain barrier (BBB) integrity [16]. The sequential cleavage of native transmembrane amyloid-β protein precursor (AβPP), thought to have a role in neurodevelopment, synaptogenesis, cell adhesion, and memory formation [17–20], is known to be the critical step in formation of Aβ peptides. It is believed that accumulation of Aβ oligomers hampers synaptic transmission and causes irreversible AD progression through an imbalance in production and clearance in neuronal synapses [21].

Several approaches to AD treatment have been tested in clinical trials, including agonists and antagonists of neurotransmitter receptors, β-secretase (β-site AβPP cleaving enzyme - BACE) or γ-secretase inhibitors, therapies targeting Aβ clearance, prevention of Aβ aggregation, modulation of phosphorylation and clearance of tau protein, as well as anti-inflammation compounds and multiple immunotherapy agents directed against Aβ and tau (Fig. 1). Among those, the BACE inhibitors, thought to improve cognitive and functional performance by suppressing Aβ production, are the widely chosen targets.

However, Aβ deposits in the brain start to accumulate years before cognitive symptoms appear [22]. While prevailing opinion supported by the stronger association of Aβ with genetic predisposition is that deposition of Aβ drives the pathology of the disease, NFTs are more closely associated with the cognitive decline [23, 24]. While transient and reversible hyperphosphorylation of tau has been reported to occur in normal brains during fetal development, anesthesia, and hypothermia, it is believed that in AD, the irreversible hyperphosphorylation of tau leads to neuronal loss and consequently to cognitive impairment [25].

Recent studies with a three-dimensional (3D) *in vitro* model of AD showed that *APP* and *PSEN1* gene mutations induce extracellular deposition of Aβ, and plaque formation, as well as tau pathology [26]. Further, these studies suggest that phosphorylated tau (p-tau) accumulations are induced by Aβ accumulation. Other studies of Aβ and tau pathology in the course of AD in human samples showed that soluble Aβ oligomers were abundantly present in early stages of AD, while p-tau did not increase until late stages of the disease [27]. Tau imaging studies using ^{18}F T807 PET in patients with MCI and AD dementia, reported high levels of tau in neocortex correlate with high Aβ burden [28]. It has been reported that NFTs may form independently of Aβ burden due to other neural death pathways [25, 29].

Therapeutic strategies postulate that preventing tau hyperphosphorylation and aggregation can decrease formation of NFTs. Research has identified several potential therapeutic approaches: modulation of

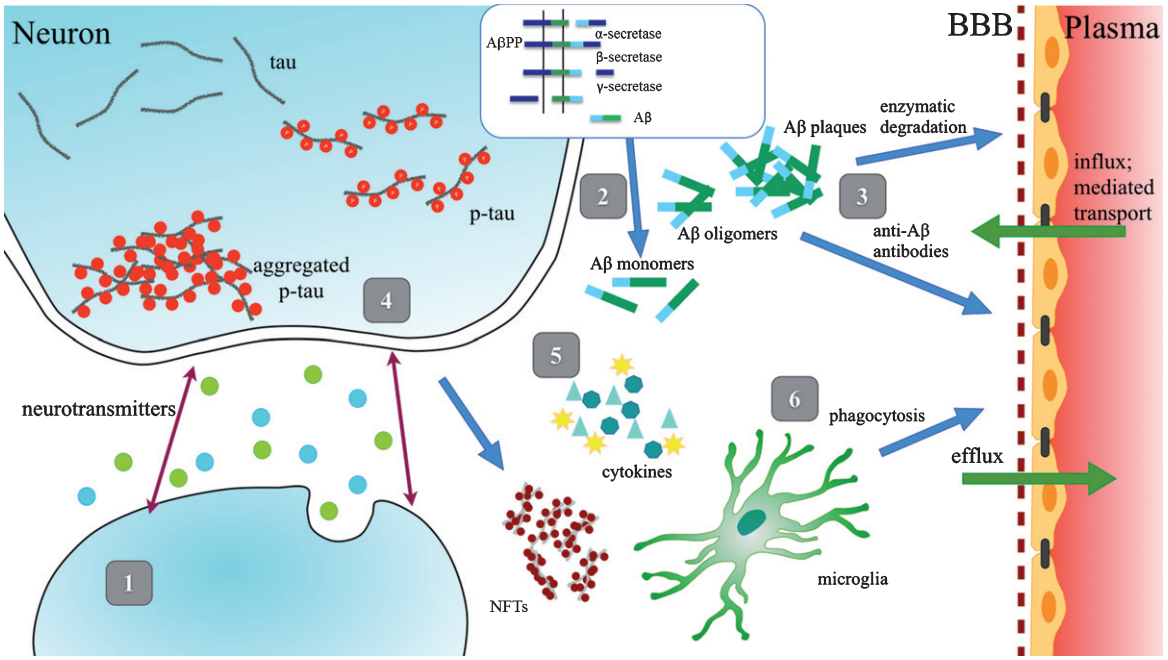


Fig. 1. Schematic Representation of Therapeutic Strategies. 1) Enhancement of neurotransmission; 2) Reduction of Aβ production and aggregation; 3) Enhancement of Aβ clearance; 4) Prevention of tau aggregation; 5) Anti-inflammatory agents; 6) Enhancement of microglial phagocytosis.

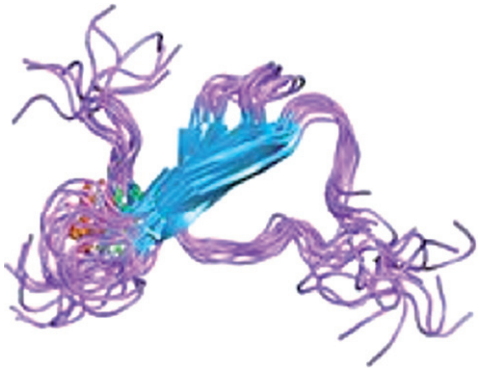
tau phosphorylation, prevention of tau aggregation, and promotion of tau clearance by intracellular and extracellular proteolysis and phagocytosis, as well as anti-tau directed immunotherapies [30, 31]. Only a few drugs that target tau phosphorylation and aggregation have reached late stage clinical trials. In part, this may be due to differences in structure, conformation, and complexity of changes during AD of tau protein compared to Aβ (Table 1). While Aβ consists of 36–42 amino acids, the human central nervous system expresses six tau isoforms that comprise from 352 to 441 amino acids with four sequence repeats in normal as compared to three sequence repeats in AD [32, 33]. Further, changes in Aβ and tau during the progression of AD are very different. Extracellular Aβ modifications during AD progression involve slow polymerization into oligomers that further aggregate. Initial tau modifications in AD progression are intracellular. Therefore, targeting tau protein as the therapeutic approach poses more complex challenges than targeting Aβ.

Inflammation triggered by innate immunity has also been reported to play an important role during both the early and late stages of the disease and contribution of glia to AD pathology and Aβ metabolism has been widely studied [34–38]. Microglia are implicated in propagation of hyperphosphorylated tau between neurons *via* exosomes [39]. In addition to pro-inflammatory response, microglial activation to phagocytic states is believed to have neuroprotective properties [37, 38]. Katsel et al. showed using genetic and protein expression studies that development and progression of dementia depend on the age at onset and are different in demented younger and old aging populations; these features highlight the importance of the immune system in preventing cognitive decline [40].

Therefore, it is hypothesized that neuroinflammation plays a major role in AD progression and that activation and modulation of the innate immune system may lead to new approaches to treatment and prevention of cognitive decline in progression of AD.

While a number of anti-inflammatory drugs have been tested in therapeutic controlled clinical trials, none have been shown to slow the progression of cognitive symptoms in patients with mild to moderate AD [41–50]. For example, early epidemiological studies of NSAIDs, such as ibuprofen, reported lower rates of AD among individuals who had been taking these drugs for chronic treatment of inflammatory conditions [51, 52]. However, controlled clinical trials of ibuprofen at a dose of 400 mg/day showed no cognitive improvement and had known side effects [53].

Table 1
Comparison of properties of Tau and Aβ proteins

	Tau	Aβ
Structure		
Role	Tabular stabilizer	Signal transduction /other
Size (MW)	55,000–62,000	$A\beta_{42} = 45$
# amino acids	352–441	36–43
Normal structures	Six isoforms, 4 sequence repeats	Soluble/single Slow polymerization Normal washout
Structures in AD	3 sequence repeats Phosphorylation & tangles	Oligomers and tangles
AD tangles	Intra neuron	Synapse/interneuron

Clinical trials that targeted Aβ plaque clearance in mild to moderate AD were potentially doomed to fail because at the onset of cognitive symptoms the brain has already been compromised with massive neuronal death. There is a general agreement that Aβ plaque removal cannot compensate for neural dysfunction and death. For mild to moderate AD, stabilizing AD progression by slowing down or inhibiting its pathology is the only viable treatment option. Novel approaches to treat AD at the prodromal stage, before significant neural damage has occurred, attempting to slow down and prevent disease progression are being explored.

Other pathogenic mechanisms have been reported to be associated with the progression of AD [54, 55], and these potential treatments have been studied in animal models and clinical trials. These approaches include antioxidants, drugs that target oxidative stress damage and mitochondrial dysfunction, iron deregulation, and abnormal cholesterol metabolism.

Given the complexity of AD progression and associated immune response, new approaches targeting multiple AD pathologies are being studied. Further, new understanding of the multiple roles of microglia and factors that affect their function in the progression of AD [10, 11, 35, 56, 57], has potential to open vastly new therapeutic options and targets.

SYMPTOMATIC AND DISEASE MODIFYING APPROACHES: CLINICAL EXPERIENCE

The drugs currently used to treat cognitive decline (i.e., cholinesterase inhibitors, NMDA receptor antagonist), and drugs that treat behavioral and psychological symptoms (such as antidepressants and antipsychotic drugs, despite the controversies surrounding their use) have limited therapeutic value. However, despite the large number of approaches tested in clinical trials, cholinesterase inhibitor and NMDA receptor antagonist remain the only approved treatments for AD. Several reviews already provide comprehensive summaries of ongoing clinical and pre-clinical efforts to treatment of AD [54, 55, 58, 59]. Here, we focus on reviewing late stage clinical trials that did not reach efficacy endpoints and ongoing clinical trials, highlighting possible reasons for failures based on the information that has emerged from the body of clinical research.

As we noted earlier all approaches to treat AD targeted one mechanism of action associated with disease pathology and, further, the majority of research of validation of new targets evolved around Aβ. Expanding treatment options to combination therapy addressing several mechanisms is an important

direction with potential to impact treatment as has been shown to be successful for other complex diseases (e.g., HIV and cancer treatment). This multifaceted approach is even more reasonable given the complex aging brain, where immune surveillance and increased vulnerability to inflammatory response can accelerate disease progression. Recently, Eli Lilly started a Phase II study of LY3202626, a small molecule BACE inhibitor, administered together with monoclonal antibody LY3002813 targeting Aβ(p3-42), a pyroglutamate form of Aβ localized to aggregated Aβ in amyloid plaques. However, the combination arm in this trial was halted due to concerns about impact of the BACE inhibition on general cognition.

Other largely unsuccessful attempts to improve cognitive decline by enhancing neurotransmission are shown in Table 2 [60–71]. Despite encouraging preclinical and Phase II clinical results, the number of agents thought to improve cognitive decline by enhancing cholinergic neurotransmission, using serotonin 6 (5-HT6) receptor antagonists, Intepirdine [60], Idalopirdine [61], and PF-05212377 [62], failed to reach efficacy endpoints. Similarly, previous attempts to enhance acetylcholine response using H3 receptor antagonists, ABT-288, GSK239512, and S 38093, did not show sufficient cognitive improvement [64–66]. Other clinical trials discontinued due to the lack of efficacy include Xaliproden (5HT1-A receptor antagonist) [63], Atomoxetine (norepinephrine uptake inhibitor approved for treatment of ADHD) [68], Dimebon (anti-histamine) [69], S47445 (agonist of AMPA receptors for glutamate) [70], and Sembragiline [71]. Encenicline (α7nAChR agonist) Phase III trial was discontinued after adverse events were noted [67].

The majority of drugs in development are focused on Aβ hypothesis, attempting to improve cognitive function through modulation of Aβ and tau levels. (Table 3) [72–87]. The γ-secretase inhibitors, i.e., Avagacestat and Semagacestat, as well as some BACE inhibitors (i.e., Atabecestat) induced serious side effects [73, 77, 79]. Development of other BACE inhibitors, such as Lanabecestat and Verubecestat, has been suspended due to lack of efficacy [74, 76].

Several passive immunotherapy drugs are undergoing clinical testing with mixed results. Bapineuzumab development was terminated because of the lack of efficacy and risk of adverse effects including microhemorrhages in the brain [82]. Intravenous immunoglobulin (IVIg) showed significant reductions in plasma $A\beta_{42}$ levels compared with placebo but no benefits for improving cognition in patients

Table 2
Late stage clinical trials: Neurotransmitters

Drug	Treatment/Target	Clinical Trial Information	Results
Intepirdine	Antagonist of the serotonin 6 (5-HT6) receptor	Phase III (mild to moderate AD)	Lack of efficacy [60]
Idalopirdine	Antagonist of the serotonin 6 (5-HT6) receptor	Phase II (mild to moderate AD)	Lack of efficacy [61]
PF-05212377	Antagonist of the serotonin 6 (5-HT6) receptor	Phase II (mild to moderate AD)	Lack of efficacy [62]
Xaliproden	Serotonin 1A (5HT1-A) receptor antagonist	Phase III (mild to moderate AD)	Lack of efficacy [63]
ABT-288	Histamine H3 receptor antagonist	Phase II (mild to moderate AD)	Lack of efficacy [64]
GSK239512	Histamine H3 receptor antagonist	Phase II (mild to moderate AD)	Lack of efficacy [65]
S 38093	Histamine H3 receptor antagonist	Phase II (mild to moderate AD)	Lack of efficacy [66]
Encenicline	α7 nicotinic acetylcholine receptor (α7nAChR) agonist	Phase III (mild to moderate AD)	Gastrointestinal side effects [67]
Atomoxetine	Norepinephrine uptake inhibitor approved for Attention Deficit/Hyperactivity Disorder	Phase II/III (mild to moderate AD)	Lack of efficacy [68]
BI 425809	Glycine transporter inhibitor; Modulate NMDA receptor function	Phase II (early AD)	Ongoing
Latrepirdine (Dimebon)	Anti-histamine	Phase III (mild to moderate AD)	Lack of efficacy [69]
S47445	AMPARs positive allosteric modulator	Phase II (mild to moderate AD)	Lack of efficacy [70]
Sembragiline	Monoamine oxidase B (MAOB) inhibitor	Phase II (moderate to severe AD)	Lack of efficacy [71]

Table 3
Late stage clinical trials: Disease modifying treatments targeting Aβ and tau proteins

Drug	Treatment/Target	Clinical Trial Information	Results
Etazolate	α-secretase	Phase II (mild to moderate AD)	No significant differences between treatment groups during 3 months of treatment [72]
Atabecestat	BACE inhibitor	Phase II/III (asymptomatic people at risk of developing Alzheimer's dementia)	Liver toxicity [73]
CNP520	BACE inhibitor	Phase II/III (cognitively normal, homozygous *ApoE4* carriers)	Ongoing
Elenbecestat	BACE inhibitor	Two Phase III trials (biomarker-confirmed MCI due to AD/prodromal AD)	Ongoing
Lanabecestat	BACE inhibitor	Phase III (early AD; mild dementia)	Lack of efficacy [74]
LY3202626	BACE inhibitor	Phase II (mild AD)	Lack of efficacy [75]
		Phase II (early AD) in combination with LY3002813	Terminated (results not published)
Verubecestat	BACE inhibitor	Phase III (mild to moderate AD)	Lack of efficacy in both mild to moderate AD and in prodromal patients; Dermatological and behavioral side effects [76]
		Phase III (prodromal AD)	
Scyllo-inositol (ELND005)	Aβ aggregation	Phase II (mild to moderate AD)	Serious adverse events at higher doses [55]
Avagacestat	γ-secretase inhibitor	Phase II (mild to moderate AD)	Serious side effects [77]
r-flurbiprofen (Flurizan)	γ-secretase inhibitor	Phase III (mild to moderate AD)	Lack of efficacy; Poor bioavailability [78]
Semagacestat	γ-secretase inhibitor	Phase III (mild to moderate)	Increased risk of skin cancer and infections; Lack of efficacy [79]
Aducanumab	Aggregated Aβ	Two Phase III (MCI due to AD or mild AD ascertained by a positive amyloid PET scan)	Terminated due to lack of efficacy [80].
BAN2401	Aβ clearance	Phase III (MCI due to AD)	Did achieve primary endpoint at 18 months, but question regarding *APOE* genotype difference between placebo and drug groups [81]
Bapineuzumab	Aβ clearance	Phase III (mild to moderate AD)	Lack of efficacy; ARIA vasogenic edema in *APOE4* carriers [82]
Crenezumab	Aggregated Aβ	Phase III (prodromal to mild AD)	Terminated due to lack of efficacy [83]
		Phase II (prevention trial in presymptomatic *PSEN1* E280A mutation carriers)	Ongoing
IVIg (Gammagard)	Aβ clearance	Phase III (mild to moderate AD)	Lack of efficacy [46]
Gantenerumab	Aβ clearance	Phase II/III (prodromal AD)	Lack of efficacy; Dose and *APOE ε4* -dependent ARIA [84]
		Phase II/III (asymptomatic and very mildly symptomatic carriers of *PSEN1*, and *PSEN2*)	Higher dose Phase III trial in mild AD and prevention trial are ongoing
		Phase III (mild AD)	
LY3002813	Aggregated Aβ	Phase II (early AD; mild dementia)	Ongoing
Solanezumab	Soluble Aβ	Three Phase III (mild AD)	Lack of efficacy [85]
		Phase III (prodromal AD)	Lack of efficacy [86]
		Two Phase II/III prevention trials (asymptomatic or very mildly symptomatic people and asymptomatic and very mildly symptomatic carriers of *PSEN1*, and *PSEN2*)	A4 trial completed (results not published)

(*Continued*)

Table 3
(*Continued*)

Drug	Treatment/Target	Clinical Trial Information	Results
CAD106	Aβ	Phase III (asymptomatic *ApoE4* carriers)	Ongoing
ACI-24	Aβ	Phase I/II	Ongoing
BIIB092	Tau	Phase II (mild cognitive impairment due to AD or mild AD)	Ongoing
C2N 8E12	Tau	Phase II (early AD)	Ongoing
AADvac-1	Tau	Phase II (mild to moderate AD)	Ongoing
LY3303560	Tau	Phase II (early AD)	Ongoing
Methylene Blue (LMTM)	Tau	Phase III (mild to moderate AD)	Lack of efficacy [87]
RO7105705	Tau	Phase II (prodromal to mild AD)	Ongoing

with mild-to-moderate AD dementia, according to the results of a Phase III trial [46].

Tau-targeting approaches to date have been sparse (Table 3). LMTM (Methylene Blue, Tau aggregation inhibitor) was tested in a Phase III study in patients with mild to moderate AD, and failed to show clinical improvement [87]. Similarly, late stage clinical trials, aiming to treat inflammation related to AD including a number of NSAIDs have also had poor efficacy results (Table 4).

The ongoing trials generally target earlier stages of the disease and use in part available biomarkers. BI 425809, a glycine transporter inhibitor thought to modulate NMDA receptor function, is currently in Phase II clinical trial in early AD patients. The next generation of small molecule BACE inhibitors (CNP520, Elenbecestat) are still in ongoing clinical trials in early AD patients and asymptomatic patients. Elenbecestat is being evaluated in a Phase III study in early AD patients with confirmed brain amyloid using positron emission tomography (PET) and/or cerebrospinal fluid (CSF) assessment. CNP520 is being tested in subjects who are otherwise healthy but at increased risk of developing AD based on their age, genotype, and amyloid levels.

Several passive immunotherapies advanced to Phase III and prevention trials after mixed Phase II efficacy results in mild to moderate AD. Crenezumab Phase III trials in patients with MCI or prodromal AD with PET and CSF evidence of Aβ pathology using higher dose, were terminated due to lack of efficacy [83]. Solanezumab missed on the primary endpoint in a Phase III trials in patients with mild AD and prodromal AD [85, 86]. It is also tested in the A4 prevention trial in asymptomatic or mildly symptomatic patients at risk of developing AD-related cognitive impairment, with amyloid plaque buildup as evidenced by florbetapir PET scan, with the goal of slowing down cognitive and memory decline and AD progression. The trial uses Preclinical Alzheimer Cognitive Composite (PACC) as a primary outcome measure as well as number of cognitive and functional scales, imaging and CSF biomarkers as secondary outcome measures. The results of the A4 prevention trial have not been reported to date. Gantenerumab is also being investigated in a Phase II/III trial aimed at preventing dementia in subjects with an inherited autosomal-dominant mutation in *APP*, *PSEN1*, or *PSEN2* [84]. Aducanumab, a human IgG1 monoclonal antibody targeting aggregated Aβ, failed Phase III trials in patients with MCI and mild AD confirmed by a positive amyloid PET scan [80].

Table 4
Late stage clinical trials: Disease modifying treatments targeting inflammation

Drug	Description/Target	Clinical Trial Information	Results
Cromolyn sodium and ibuprofen (ALZT-OP1)	Aβ aggregation and inflammation	Phase III (early AD)	Ongoing
Azeliragon	RAGE inhibitor (Aβ and inflammation)	Phase III (mild to moderate AD)	Lack of efficacy; Tolerance issues at higher doses in a Phase II trial [41]
CHF 5074	γ-secretase	Two Phase II studies (amnestic or non-amnestic MCI)	Discontinued
Celecoxib (Celebrex)	NSAID	Prevention trial (70 years and older with family history of dementia)	Risk of cardiovascular side effects; Lack of efficacy [42]
Etanercept (Enbrel)	TNF-α inhibitor	Phase II (mild to moderate)	Known side effects (gastroenteritis, respiratory and urinary-tract infections, pharyngitis, and cellulitis; Lack of efficacy [43]
r-flurbiprofen (Flurizan)	NSAID	Phase II/III (probable AD)	Lack of efficacy [44]
Sargramostim	Hematopoietic growth factor	Phase II (mild to moderate AD)	Lack of efficacy [45]
GRF6019	Young adult plasma	Phase II (mild and moderate AD)	Ongoing
IVIg (Gammagard)	Human plasma antibodies	Phase III (mild to moderate AD)	Lack of efficacy [46]
Ibuprofen	NSAID	Phase II (mild to moderate AD)	Known side effects at high chronic dose. Lack of efficacy [53]
Lornoxicam	NSAID	Phase II (mild to moderate AD)	Terminated; data not available.
Minocycline	Inflammation and tau	Phase II (MCI and AD)	Data not available
Naproxen	NSAID	Prevention trial (people 70 with family history of dementia) Phase III (mild to moderate AD)	Risk of cardiovascular side effects; Lack of efficacy [48]
Neflamapimod	Inflammation	Phase II (MCI and mild AD)	Ongoing
IVIg (Octagam®10%)	Inflammation	Phase II (amnestic MCI due to AD)	Lack of efficacy; Transient cognitive improvement reported [49]
Pioglitazone	Inflammation	Phase III (cognitively normal participants with genetic predisposition)	Ongoing
Prednisone	Corticosteroid	Phase II (patients with AD)	Lack of efficacy; Behavioral symptoms and an increase in blood glucose levels reported [47]
Rofecoxib	NSAID	Phase II (mild to moderate AD)	Lack of efficacy [48] Withdrawn from market due to increased risk of heart attack and stroke associated with long-term, high-dosage use
Thalidomide (Thalomid)	Aβ and inflammation	Phase II/III (mild to moderate AD)	High therapeutic dose known to cause serious birth defects. Poor safety and tolerability in AD patients [50]

Recently, positive results of the Phase II testing of BAN2401 have been reported at 18 months despite the fact that the results of interim analysis at 12 months missed the primary endpoint [81]. The Phase II trial results reported statistically significant slowing of the course of AD symptoms, as measured by a combination of cognitive assessments and dementia ratings (ADCOMS). However, the changes in the Clinical Dementia Rating Sum of Boxes (CDR-SB) were not statistically significant. Results were further compounded by exclusion of *APOE4* patients form the highest dose group party way through the trial. The concern of the differences between the treatment and control groups remains. In addition, while the safety profile was reported to be acceptable (incidence of amyloid-related edema lower than 10% at any dose, and under 15% in patients with *APOE4* taking the highest dose), the concern of effects of chronic administration remain and the reported results warrant periodical repeated monitoring of patients making disease management even more complex.

Active immunotherapy trials include CAD106, vaccine that induces immunity to Aβ without eliciting an inflammatory response, currently in a Phase II/III prevention trial in homozygous *APOE4* subjects who are cognitively normal. Tau targeting approaches through passive (BIIB092, C2N 8E12, LY3303560, RO7105705) and active (AADvac-1, ACI-24) immunotherapy are currently in Phase II clinical trials.

Few anti-inflammatory agents remain in clinical testing: Pioglitazone, an insulin sensitizer that is approved for treatment of Type 2 diabetes mellitus, GRF6019 (young adult plasma), and Neflamapimod, which is a small molecule believed to shift microglial pro-inflammatory state to a phagocytic state.

AZTherapies reported research results of beneficial effects of a combination treatment, currently in Phase III clinical testing, that studies modified cromolyn sodium, an asthma therapeutic agent, in combination with newly formulated low dose ibuprofen. It was shown that the combination treatment may simultaneously affect Aβ aggregation and inflammation associated with AD in animal models of the disease [88–90].

DISCUSSION

A large number of AD clinical trials have failed despite significant advances in scientific understanding of the disease. The fact that over 400 trials testing over 200 therapeutics have been performed to date with a 99.6% failure rate (i.e., a success rate of only 0.4%) clearly illustrates the importance of the issue [2, 91]. In Tables 2–4, we highlight the late stage clinical trials, some of which are still ongoing, using various disease modifying and symptomatic approaches. An overview of recent clinical trial reports indicates that potential flaws include overall clinical trial design with utilized statistical measures that are perhaps not aligned with the studies' objectives, heterogeneity of patient populations due to deficient inclusion criteria, and difficulties in diagnosing early disease for both treatment and placebo groups. Other flaws include single target mechanism, drug bioavailability, genetic making, and toxicity from chronic administration, which alone or in combination may have contributed to recent failures. These potential shortcomings are explored in detail below.

Study design

Most studies to date were performed in mild and moderate AD patients, with neuronal damage already present to a considerable extent. At this stage, the disease progression path and rate are uncertain and show individual variability in part due to variability in dementia rating and patient populations [92, 93]. In mild and moderate AD patient populations, it becomes apparent that the use of amyloid and tau modulation agents to inhibit generation and/or increase clearance may not compensate for the neuronal damage that is already present, thus challenging the clinical trial's odds of success. Some published results show no or very slow cognitive decline in their placebo group, strongly suggesting a failure in clinical trial design [2]. Other trials have suffered from lack of sensitivity of standard measures of cognitive performance (i.e., CDR-SB) at early and prodromal stages of the disease [93, 94].

In addition, the widely used CDR-SB scale, while clinically meaningful, was not designed to be used in traditional statistical analysis of efficacy of treatment. Because the evaluation is subjective, additional variability may be introduced in multicenter trials, further hampering success. When used as a measure of disease progression in the evaluation of the primary endpoints, unequal size of the change between stabilization and/or improvement in the treatment group (a small change of a few points in this population) as compared to disease progression in the placebo

group (a larger change). Since the typical annual change in CDR-SB reported for early AD patients is between 1-2 points [95, 96], the changes are small and require large number of subjects to reach statistical significance, even in cases when the drug is clinically effective in stabilizing cognitive decline.

As mentioned previously, to overcome the issues with measuring cognitive change early in AD progression, Phase II a clinical trial of BAN2401 used the combination measures, ADCOMS, which at 18 months showed statistically significant changes compared to placebo. However, the changes in CDR-SB were not statistically significant, which could be, in part, attributed to the selected patient population and disease staging in the trial. In prevention trials, the time to diagnosis of dementia has been used as an endpoint and it is likely to show similar large variability. Further, multiple trials have attempted to conduct subgroup analysis after failures to report a clinical benefit. These attempts have been criticized to provide inaccurate information as subgroups are often not properly randomized and do not have significant sample size [91].

Patient population

The majority of clinical trials conducted to date included patients aged from 50 to 90 years old, leading to large variability in the cognitive impairment and disease progression. Thus, heterogeneous mild to moderate AD patient populations require a large number of subjects to accommodate for age, gender, disease stage, genetic predisposition, dementia type (such as Lewy body, senile and vascular, alcohol abuse, injury, or a result of other diseases) and inevitably lead to obscuring observed treatment effects and to failure to show clinical efficacy. The need to identify homogeneous patient populations at risk of developing AD brain pathology requires the use of imaging, CSF, and blood for disease staging, in addition to cognition and functional measures.

Bioavailability and toxicity from chronic administration

It has been noted previously that many drugs in development suffer from inadequate brain bioavailability and appropriate dose selection to show clinical benefit due to toxicity from chronic administration [91]. This is true for small molecules that may be transported from the central nervous system, even after crossing the BBB to target enzyme inhibition or a biochemical pathway, and it is particularly important for large molecules, such as monoclonal antibodies and other biologic drugs. Data on clinical trials that failed due to toxicity issues are shown in Table 4 (drugs targeting inflammation) and Table 5 (drugs targeting Aβ [73, 77, 79, 97–102]) and illustrate the problem in development of disease modifying treatments. While the clinical development of most of these drugs was halted before reaching Phase III, some drugs (such as Bapineuzumab, Azeliragon, Verubecestat) advanced into Phase III with doses that were not sufficient to show benefit in cognitive function. The Atabecestat Phase II/III trial was recently discontinued due to issues with liver toxicity. Similarly, a number of anti-inflammatory agents (Table 4) failed due to increased risk of adverse events with chronic administration. The need to administer the treatment frequently for several years, with high risk of systemic toxicity, requires that an adequate dose is selected early in the drug development phase [91].

Table 5
Discontinued clinical trails targeting Aβ: Adverse events and toxicity

Drug	Description/Target	Results
AAB-003	Aβ	ARIA and microhemorrhages [97]
AN-1792	Aβ	Brain inflammation [98]
Atabecestat	BACE inhibitor	Elevated liver enzymes [73]
Avagacestat	γ-secretase inhibitor	Gastrointestinal and dermatological side effects; Nonmelanoma skin cancers reported; ARIA [77]
Azeliragon	RAGE inhibitor	High dose was associated with confusion, falls, and greater ADAS-cog decline; Low dose was not effective in subsequent trials [99]
LY2886721	BACE inhibitor	Abnormal liver biochemistry values [100]
Semagacestat	γ-secretase inhibitor	Increased risk of skin cancer and infections [79]
Bexarotene	Retinoid (Aβ)	Increased blood lipid levels, risk of cardiovascular side effects [101]
Bapineuzumab	Aβ clearance	Reversible vasogenic edema *APOE ε4* carriers [102]

Intervention is too late for disease modifying agents

Consistent with the observation that Aβ deposits in the brain start to accumulate years before cognitive symptoms appear [22], the clinical results from trials in mild to moderate AD patients, which attempt to modulate Aβ production and clearance, indicate that removing plaques will not reverse the neuronal damage or stop the AD. This limits the treatment targeting Aβ production and clearance to be effective only at early stages of the disease. Limiting Aβ production and aggregation may have limited benefit in mild to moderate AD. Similarly, stage-dependent efficacy of anti-inflammatory agents, suggests that these treatments may be useful early in AD [90, 103]. It is hypothesized, that once Aβ-induced inflammation initiates tauopathy, neurodegeneration progresses and leads to cognitive decline.

Biomarkers

The need to focus interventions on a homogeneous patient population at an appropriate disease stage, as well as the need to diagnose the disease before cognitive symptoms occur, has led to a renewed focus on identifying and validating biomarkers, including structural MRI signs of hippocampal and global brain atrophy, CSF, and blood analysis biomarkers as well as the usage of PET imaging agents. The ability to diagnose the disease at earlier stages while preserving normal brain function would significantly contribute to the potential to develop disease-modifying therapies. So far, no biomarker proposed for early diagnosis and monitoring treatments has been validated.

Currently, the most widely used CSF biomarkers, obtained through lumbar puncture, include those targeting amyloid-β ($A\beta_{42}$ or $A\beta_{42}/A\beta_{40}$), total tau protein (T-tau) and phosphorylated tau ($P\text{-}tau_{181}$) [104]. It has been shown that CSF $A\beta_{42}$ is an indicator of early stages of the disease, while CSF tau indicates the extent of cognitive decline at later stages [23, 24]. CSF and blood levels of neurofilament light chain protein have been shown to correlate well with neurodegeneration.

The use of CSF biomarkers in research and clinical trials, in addition to clinical criteria is detailed in the revised diagnostic criteria of AD [24, 105], suggesting the use of CSF biomarkers in differentiating between AD and other types of dementia. The utility of neuro-inflammatory biomarkers collected from blood, may have limited use when patients are suffering from additional systemic inflammation. Burchell and Panegyres reviewed several other biomarkers and assays including those involving BBB integrity, mitochondrial DNA, vascular endothelial growth factor, as well as immunological factors involved in AD pathogenesis [106]. However, these are still in early development and have not been validated.

With the renewed interest in targeting multiple disease hallmarks, inclusion of other exploratory biomarkers in clinical trials may be a critical component of advancing the understanding of the role of neuroinflammation in AD and developing additional tools for early diagnosis and follow up of disease progression. The correlation of multiple indicators with disease progression, that may help to design improved patient selection related to brain pathophysiology in the future.

Several imaging techniques, including structural magnetic resonance imaging (MRI) and ^{18}F-fluorodeoxyglucose (FDG) are commonly used in clinical research to assist in early diagnosis. There has been significant development in the PET imaging tracers binding to Aβ with the approval of Amyvid (^{18}F-florbetapir), Vizamyl (^{18}F-flutametamol), and Neuraceq (^{18}F-florbetaben).

Similarly, recent results suggest that buildup of tau pathology could better predict future cognitive impairment than Aβ, and subsequently driving research toward new tau imaging agents. For example, increased flortaucipir binding has been shown to correlate with increased cognitive impairment in patients with Aβ plaque [107]. Other tau imaging tracers, including ^{18}F- PI-2620 are currently in clinical testing.

While early clinical trials rarely used imaging and CSF biomarkers, recent clinical work includes more frequent use of PET imaging and CSF biomarkers, either as a secondary outcome measure and/or as inclusion criteria. However, in most of these studies, biomarkers are used in sub-studies with a much smaller number of subjects (i.e., clinical trials for Verubecestat, Lanabecestat, Bapineuzumab, Gantenerumab, and Semagacestat).

Genetic testing is particularly useful in identifying individuals at risk of developing AD and has gained momentum with the number of prevention trials. Some of the genetic testing targets genes with rare variations that cause inherited AD or those that are associated with increased risk of developing AD, such as *APOE ε4*.

New biomarkers and imaging agents as well as potentially combinations of biomarkers, that target specific AD pathology in asymptomatic patients at risk of developing AD, could potentially assist in identifying homogeneous patient populations and provide additional tools for monitoring efficacy of clinical trials.

Combination treatments

Experience to date points to the fact that AD is multi-target disease, and that approaches using one drug, focused on one target may not be sufficient to achieve improvement of clinical symptoms [55].

Azeliragon, a small-molecule RAGE inhibitor, has been thought to provide a combined treatment by lowering Aβ plaque deposition and inducing anti-inflammatory response, but it failed to reach efficacy endpoints in Phase III [41]. Other treatments proposed to mediate amyloid beta clearance and provide anti-inflammatory properties either failed later stage testing due to poor efficacy (e.g., GM-CSF Leukine, Intravenous immunoglobulin) or are still in ongoing clinical trials (e.g., Pioglitazone, GC 021109, GRF6019).

Other approaches targeting both Aβ accumulation and inflammation associated with AD may be needed to slow disease progression before symptoms occur.

CONCLUSIONS AND RECOMMENDATIONS

The number of clinical trials focused on early, mild, and moderate AD that did not achieve primary endpoints and failed to achieve cognitive improvement keeps growing. We believe that the scientific rationale behind all potential AD therapies tested is valid and supported by *in vitro* and *in vivo* animal model data and that this is not the main reason for these clinical trial failures. Trial design and analytical outcome measures are becoming especially important when attempting to treat a chronically progressive disease as AD. Brain neurons and synapses expand the brain network in early years and with aging, network remodeling shifts toward deterioration. This life-long process is associated with genetic predisposition, education, lifestyle, and the environment. It is unequivocally important that new potential therapies address the unique irreversible nature of the brain aging process. Unlike treatments of other diseases such as cancer, where short term aggressive treatments are used in doses that induce systemic toxicity, destroy normal cells, and/or impact the immune system, treatment of AD requires approaches that preserve the balance of Aβ and tau proteins that is essential for neuronal function and further stabilize the brain's innate immune system.

To address multiple parameters affecting complex AD clinical trial design, we highlight some recommendations and potential new approaches.

Experience to date shows that clinical trial design and outcome measures have to be adjusted to allow monitoring of disease progression in the pre-symptomatic stage of AD with sufficient minimum clinically important differences. To eliminate other factors that were associated with failure, clinical trials should include in the study design adequate outcome measures and endpoint selections (see Early Alzheimer's Disease: Developing Drugs for Treatment-Guidance for Industry). The challenge remains to devise methods to reliably identify patients with preclinical AD and to ensure that the methods have predictive value. As the duration, complexity, and cost of clinical trials increase, the clinical research would benefit from introducing appropriate interim analyses and adaptive clinical trial designs.

The need to eliminate heterogeneity of patient population, and especially to identify homogeneous patient populations at risk of developing AD will require the use of imaging, CSF, and blood biomarkers for disease staging, in addition to cognitive and functional measures. It will be necessary to minimize variations due to age (i.e., limit the age spread of the study population to patients between 55 to 70, to avoid younger patients that are far from AD onset and older patients who show faster AD progression), define proper cutoffs for cognitive performance (done in most cases with varying rationale, mainly based on published data), and limit the use of concomitant AD medication that affects brain function (such as strong antipsychotic drugs) at entry. Limiting the use of approved medications (i.e., for a period of more than six months or a year) during the trial, unless the test drug is an adjuvant therapy, should also contribute to smaller variation in cognitive performance during the trial. In addition, it will be necessary to reflect underlying AD pathology when selecting patient population using validated CSF and/or blood biomarkers as well as proper imaging techniques with adequate ranges for the targeted disease stage.

Because the treatment is expected to last many years, systemic toxicity is an expected outcome with aggressive therapies at high doses. To address this

concern, new preclinical methods need to be explored to adequately evaluate potential *in vitro* or *in vivo* toxicity, not only for small molecules but also for biologic treatments, where effect of chronic treatment are typically not easily estimated. In slow progressing AD, the risk of chronic toxicity when using high doses of drugs does not provide benefit rationale as in treatment of other diseases (i.e., cancer). The lowest effective dose needs to be determined and validated based on brain pathophysiology, to provide treatment at Aβ and tau levels typically present in the brains of patients with early AD. Prolonged brain titration with the lowest effective doses, sufficient to treat small daily Aβ and tau changes (reported at pico and nano molar levels) should provide measurable changes that can affect species production and clearance as well as treat the associated neuroinflammation. These changes are not easily predictable and are hard to achieve even with low doses of potentially effective but highly toxic drugs.

AD is a neurodegenerative aging-related disease with no known cure, and neuronal damage cannot be fully reversed even if the mechanisms underlying disease progression are targeted. As a strategy, the goal of AD treatment should be slowing down neural degeneration. Therefore, early treatment options, before the symptoms of AD occur, appear to be the most viable current approach. However, early diagnosis of AD, before cognitive symptoms occur, is still a challenge, because of the lack of appropriate biomarkers and diagnostic criteria for this pre-symptomatic stage of AD. As the focus of current research moves toward prevention trials, the aim of intervention becomes delaying the symptoms and slowing down progression of the disease, especially in subjects who are potentially vulnerable to early disease onset, due to genetic profile, environmental conditions associated with lifestyle, and other contributing diseases.

It is clear that new approaches to treatment of AD will require the identification and validation of new targets and will need to target multiple mechanisms of action that may slow disease progression, when used before clinical symptoms appear (Fig. 1). In addition, performance of drugs that showed limited efficacy and failed to achieve statistical significance when used as a single treatment, may be improved by exploring their combination with anti-inflammatory mechanisms of action. As an example, Solanezumab, an Aβ removal antibody with some degree of success in clinical studies, could be combined with low dose anti-inflammatory agent, a mast cell stabilizer as part of innate immune system, or with an inhibitor of amyloid peptide oligomerization and polymerization.

Identifying a suitable pool of asymptomatic patients and following the rate of progression of the disease is likely to require the use of multiple biomarkers in addition to effective cognition and function measures. Thus, the development and validation of new CSF and blood biomarkers, as well as highly specific PET agents, becomes critical for future clinical research. To ensure sufficient brain bioavailability, brain or CSF drug uptake should be evaluated in the CSF and blood by labeled molecules or their close analogs using biodistribution and pharmacokinetics studies in experimental animals or by brain imaging in humans. This approach should provide proof of bioavailability and has potential to increase success of clinical studies and to reduce the cost and waste of resources associated with unsuccessful clinical trials.

Proposed selection of homogeneous patient population, with narrow age range and with early stages of the disease, as well as the use of CSF and blood biomarkers in addition to stricter cognition and functional measures, would likely increase the number of screening failures resulting in higher recruitment cost and prolong duration of the trials. To address these issues, we recommend the use of adaptive and interim analyses, per FDA guidelines, and additional measures of clinical utility of the treatment. Cognitive and functional performance scales (such as CDR-SB), are fairly acceptable scales recommended by the FDA (see Early Alzheimer's Disease: Developing Drugs for Treatment-Guidance for Industry). While useful in diagnosis and staging of the disease, they have limited value as a measure of disease progression. The CDR-SB scale (as well as other cognition and function tests) was not designed to be used in traditional statistical analysis of efficacy of treatment. The evaluation of the primary endpoints using CDR-SB mean change from baseline in early AD may potentially be challenged by the unequal size of the change between stabilization and improvement (a small change of a few points in this population) as compared to disease progression (a larger change). Due to these potential challenges, we believe that an exploratory responder analysis may alleviate the issues and provide additional information on the efficacy of clinical trials.

Adding CDR-SB analysis at the end of a study for determining response to drug treatment (stable or improvement; responder or non-responder) may be an additional useful tool as an efficacy measure.

Adaptive and interim analyses, per FDA guidelines, as well as, exploring responder analysis in the interim and final analysis, should be considered. Additionally, validated biomarkers may provide more accurate correlation with the progression of the disease, especially when used in combination with cognitive and functional outcomes.

Advances in understanding the role of the brain's innate immune system in the development of AD, and specifically its genetic regulation, have potential to open new therapeutic solutions. Experience to date points out that AD is a multi-target disease, and that approaches using one drug, focused on one target may not be sufficient to achieve improvement of clinical symptoms. Combination treatments with multiple targets may potentially lead to effective therapies.

ACKNOWLEDGMENTS

We acknowledge AZTherapies for their support.

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/19-0507r2).

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