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Title: Reasons for failed trials of disease-modifying treatments for Alzheimer Disease and their contribution in recent research

Article type: review

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ABSTRACT

Despite all scientific efforts and many protracted and expensive clinical trials, no new drug has been approved by FDA for treatment of Alzheimer disease (AD) since 2003. Indeed, more than 200 investigational programs have failed or have been abandoned in the last decade. The most probable explanations for failures of disease-modifying treatments (DMTs) for AD may include late initiation of treatments during the course of AD development, inappropriate drug dosages, erroneous selection of treatment targets, and mainly an inadequate understanding of the complex pathophysiology of AD, which may necessitate combination treatments rather than monotherapy. Clinical trials' methodological issues have also been criticized.

Current drug-development research for AD is aimed to overcome these drawbacks. Preclinical and prodromal AD populations, as well as traditionally investigated populations representing all the clinical stages of AD, are included in recent trials. Systematic use of biomarkers in staging preclinical and prodromal AD and of a single primary outcome in trials of prodromal AD are regularly integrated. The application of amyloid, tau, and neurodegeneration biomarkers, including new biomarkers—such as Tau positron emission tomography, neurofilament light chain (blood and CSF biomarker of axonal degeneration) and neurogranin (CSF biomarker of synaptic functioning)—to clinical trials allows more precise staging of AD. Additionally, use of the Bayesian statistics, modifiable clinical trial designs, and clinical trial simulators enrich the trial methodology. Besides, combination therapy regimens are currently assessed in clinical trials.

The abovementioned diagnostic and statistical advances, which have been recently integrated in clinical trials, are consequential to the recent failures of studies of disease-modifying treatments. Their experiential rather than theoretical origins may
better equip potentially successful drug-development strategies.

Keywords: Alzheimer's disease, clinical trial fails, disease-modifying treatments, Alzheimer's disease biomarkers, combination treatment, clinical trial designs

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Introduction

Given the fact that AD concerns mostly people older than 65 years, the increasing expansion of life span, leads to a fast growing number of AD patients [1]. Consequently, the research focused on AD modifying treatments has become intensively growing. However, despite all arduous research efforts, at the moment there are no effective treatment options for the disease [2,3]. Indeed, no new drug has been approved by FDA for treatment of AD since 2003, although more than 200 therapeutic agents have been assessed in failed or abandoned investigational programs [4,5].

Many explanations for failures of candidate DMTs for AD have been proposed. The most prominent include late initiation of treatments during the course of AD development, inappropriate drug dosages, wrong selection of main treatment targets, and mainly an inadequate understanding of the complex pathophysiology of AD [6]. A novel approach to the treatment problems seems to necessitate combination treatments rather than monotherapy [7]. Clinical trials' methodological issues have also been criticized [4].

Current drug-development research for AD is aimed to overcome these drawbacks. Preclinical and prodromal AD populations, as well as patients representing all the clinical stages of AD, are included in recent trials [8]. Current guidance provided by the US Food and Drug Administration (FDA) for clinical trials in AD further includes use of biomarkers in staging preclinical and prodromal AD and of a single primary outcome in trials of prodromal AD, and additionally the use of Bayesian statistics and modifiable clinical trial designs [3]. Besides, combination therapy regimens are currently assessed in clinical trials [7].

This review aims to further contribute to the understanding of different reasons that are responsible for the failures of clinical trials for AD. It will also try to investigate how the lessons of these failures are integrated as knowledge and gained experience in new trials.

Explanations for failures of candidate DMTs for AD and the consequent shift in current clinical trials

a. Inadequate understanding of the complex pathophysiology of AD: wrong selection of main treatment target and inappropriate drug dosages

Since lack of efficacy of all agents that were studied in phase 3 trials cannot be accurately explained at this time, it is obvious that the current science is not sufficiently advanced and investigators need to recognize the possibility of deficiency of our knowledge.

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Multiple phase 3 failures of agents that aim to reduce beta-amyloid plaques made researchers to abandon the singular focus on amyloid cascade model. Indeed, if patients in anti-amyloid trials are initially positive for high levels of amyloid and although the anti-amyloid drug clears amyloid while finally there is no cognitive benefit, it is reasonable to suggest that pathology or pathophysiology other than singular amyloid needs to be selected as a target [9, 10].

Some of the recent failures in phase 3 studies of anti- amyloid agents in patients with early stage, mild or mild to moderate Alzheimer's disease involved Semagacestat [11], Bapineuzumab [12] and Solanezumab [13].

Further examples of agents targeting beta- amyloid that failed due to lack of efficacy include the β secretase inhibitors (BACE) Lanabecestat [14], Lerubecestat [15] and Atabecestat [16]. These drugs target the β site amyloid-precursor-protein-cleaving enzyme-1 (BACE-1), and although they demonstrated proof of mechanism of action by lowering the plasma and CSF biomarkers Aβ40 and Aβ42, they failed to prove clinical benefit. The clinical trial of Verubecestat in mild to moderate AD was terminated early due to lack of efficacy. A more recent Verubecestat trial targeting patients with prodromal AD showed even more disappointing results. Adverse events, cognition and daily function worsening were more common in the Verubecestat groups than in the placebo group [15].

In current research a more equipotential conceptualization of AD has been adopted. Amyloid pathology is still targeted, but tau pathology seems maybe more firmly and earlier associated with cognitive decline. At the same time, other pathologies such as arteriolosclerosis, blood–brain barrier dysfunction or α-synuclein are also investigated [9]. Ongoing clinical trials assess DMTs with amyloid-related mechanisms, Tau-related mechanisms, and DMTs with other mechanisms such as neuroprotection, anti-inflammatory effects, growth factor promotion, metabolic effects, stem cell therapies [3,5].

b. Inadequate understanding of the complex pathophysiology of AD: late initiation of treatments during the course of AD development

Abnormal deposits of amyloid β and tau tangles and the consequence damage to the brain is believed to start a decade or more before apparent cognitive decline [17].

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The lack of efficacy observed in previous phase 3 trials raised the question whether treating AD patients once they become symptomatic may be too late to reverse the progress of neurodegeneration. It is hypothesized that presymptomatic intervention may halt or delay the progression of the disease [10].

The ongoing development of amyloid targeted agents is a direct result of the previous hypothesis. Although all of the previous agents of this category failed in clinical trials, most of the new agents among them are studied in asymptomatic subjects at risk of developing AD [3, 18]. Ongoing clinical trials with active or passive immunotherapy agents [19], with agents that reduce the Aβ-plaque burden [20, 21], with α-Secretase Modulators [22] and with BACE inhibitors [18] are enrolling prodromal or mild AD patients to test the hypothesis of early interception [3, 10].

Hence, the challenge of DMT development for AD has become more complicated as trial populations include also preclinical and prodromal AD, besides AD dementia patients [23]. Accurate classification of stages of AD, especially preclinical stages, demand a new research framework for the diagnosis of AD that may serve clinical trials of DMTs. in AD [23]. Such a framework based on amyloid, tau, and neurodegeneration biomarkers was introduced by the National Institute on Aging (NIA) and the Alzheimer's Association [24]. Consequently, most of the current clinical trials have integrated the use of fluid [25] or imaging [26] biomarkers. Cerebrospinal fluid (CSF) or blood fluid biomarkers lead the effort to enable more effective DMTs development in AD. Their context of use in clinical trials includes patient selection, patient's classification in a disease state, clarification of therapeutic agent's mechanism of action, appropriate dose selection and measurement of treatment response [27].

CSF biomarkers that are currently used in clinical trials are amyloid beta 42 (Aβ42), total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau) identifying subjects at risk of developing AD [26]. The combination of these biomarkers displays sensitivity of 95% and specificity of 83% in detecting subjects that will develop AD[28], hence it is the main patient-selection tool in trials [25, 29].

An ongoing effort for identification of additional fluid biomarkers is remarked. Current research need them for subject enrichment, drug efficiency monitoring, risk classification and prognosis. Several novel biomarkers have been investigated with the aim to be integrated into drug development programmes [30]:

Novel fluid biomarkers of Aβ metabolism and aggregation

CSF Aβ38: It has the promise to be used for patient selection and to demonstrate target engagement of γ-secretase modulators. Commercial assays are already available for this biomarker [25, 31].

Plasma BACE1 activity shows ability for prognosis and patient selection. Commercial assays are already available for both BACE1 protein levels and BACE1 activity [32].

- *Vascular system's novel fluid biomarkers*

CSF and serum Heart-type fatty acid-binding protein (hFABP) could attribute to patient selection and prognosis. Commercial assays are available for hFABP [33].

Novel fluid biomarkers of inflammation and glial activation

CSF and peripheral blood Triggering receptor expressed on myeloid cells 2 (TREM2) has been observed in increased levels in AD, supporting possible use in patient selection. Commercial assays are available for the measurement of this biomarker [34,35].

CSF and blood chitinase-3-like protein 1 (YKL-40) is expressed in astrocytes besides Aβ plaques and is connected with tau pathology. CSF YKL-40 is regarded as a biomarker of neuroinflammation or astrogliosis in AD and probably can help in patient selection and prognosis. Commercial assays are already available [25, 36].

Novel fluid biomarkers for synaptic dysfunction

CSF Neurogranin is mainly found in dendritic spines and its function is expressed in post-synaptic signaling pathways. It has been shown to predict disease progression in several studies, even in cognitively normal controls [37, 38]. Its levels are correlated with brain atrophy in subjects with Aβ pathology. It is regarded that it could be useful as an AD biomarker for patient selection and prognosis. Commercial assays are already available [37].

CSF SNAP-25 and synaptotagmin are synaptic proteins that take part in the mediation of exocytosis of synaptic vesicles for neurotransmitter release. The levels of these proteins are elevated in AD and MCI. They are suggested as potential AD biomarkers for patient selection. Commercial assays are already available for both of them [39].

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- *Novel fluid biomarkers for α-Synuclein pathology*

CSF α-synuclein levels may be useful for identifying Lewy Body pathology among AD patients, thus this molecule could be used for patient selection [40].

- *Novel fluid biomarkers* ***for TDP-43 pathology***

Plasma TDP-43 has been found elevated in AD and in pre-MCI patients who progressed to AD. Since commercial assays are already available, **TDP-43** may serve as an AD biomarker for patient selection and prognosis, [41].

Iron metabolism associated novel fluid biomarkers

Since Ferritin plays a major role in brain iron homeostasis, **plasma and CSF Ferritin** may be used as AD biomarkers. CSF Ferritin may become a prognostic biomarker while plasma ferritin could be used for the screening of preclinical AD. Commercial assays are available for both plasma and CSF Ferritin detection [42].

Other neuronal proteins as novel fluid biomarkers

CSF Visinin-like protein 1 (VILIP-1) levels have been proved to be elevated in AD patients in many studies and may be used as prognostic biomarker of incipient cognitive decline, of cognitive decline's and brain atrophy's rates, of progression from MCI to AD and of AD differentiation from other dementias [43]. VILIP-1 is a neuronal calcium sensor protein related to synaptic plasticity in signaling pathways [25]. Commercial assays are already available [25].
CSF and plasma NF-L (Neurofilament lights) are promising biomarkers. NF-Ls are expressed in neurons and particularly in axons, where are partly responsible for the transmission of electrical impulses and for normal synaptic function [44]. CSF NF-L levels have been shown to be elevated in AD and MCI patients and to have a linear correlation with cognitive impairment and survival time in AD patients [43]. Plasma NF-L have been found to be increased in pre-symptomatic subjects known to be carriers of AD-causing gene mutations and patients with MCI or AD [45, 46]. They seem also to be correlated with brain atrophy [47]. CSF NF-L could be useful as biomarkers for prognosis, and plasma NF-L could be useful as a non-invasive biomarker for patient selection and prognosis. Commercial and in vitro assays are available [25].

In addition to fluid biomarkers, imaging biomarkers contribute to stratification of patients in disease stages and measurement of disease progression in DMT clinical trials even in the absence of noticeable cognitive impairment [26]. Volumetric MRI, T1-weighted MRI, T2-weighted MRI are useful in structural imaging and quantitative analysis of atrophy in MCI and AD patients. The hippocampus and entorhinal cortex may be the first regions affected by atrophy in MCI and AD. Functional MRIs are used for detecting disease-specific alterations in cognitive functions. Structural and functional MRI findings can predict AD onset in patients with MCI [48, 49, 50]. Amyloid positron emission tomography (PET) is used for detecting the amyloid deposits in preclinical AD and MCI patients and for monitoring the progressive amyloid burden in the brain [51].

The most promising imaging biomarkers seem to be the tau-targeted PET tracers such as Fluortaucipir, which are explored in numerous studies [52, 53]. The advent of these tracers enables researchers to investigate the sequence of accumulation of tau and Aβ in correlation with age and with development of cognitive impairment due to AD. Recent results show that elevated Flortaucipir tau binding is associated with an increased prevalence of cognitive impairment and support further evaluation of tau PET imaging as a possible biomarker for diagnosis, patient staging, and monitoring effects in AD DMT clinical trials [54].

c. Inadequate understanding of the complex pathophysiology of AD: Combination treatments

The complex pathophysiology of AD may be probably unaffected by monotherapy treatments, since all single-agent DMTs have failed to halt the disease progression. Consequently, combination treatments rather than monotherapy may be necessary to delay or halt the multiple cognitive and functional deterioration induced by the disease [55].

Combination trials are different from add-on trials, which are typically used for new therapies in AD comparing a new agent with placebo in patients who are already receiving a background treatment. In combination trials, two drugs are assessed separately, in combination and in comparison with placebo in a 2x2 trial design. Following this methodology investigators can assess the synergistic and individual effects of every drug [56]. The main benefit of this method is that two or more main targets of the disease can be simultaneously addressed (e.g, amyloid and tau) or a single target (e.g. amyloid) can be addressed by two complementary mechanisms of action [55].

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Two combination DMTs are currently in phase 3 clinical trials: The ALZT-OPT1 clinical trial, assessing Cromolyn, an antiamyloid regimen in combination with Ibuprofen, an anti-inflammatory agent in patients with early AD [57]. The second phase 3 combination trial for Gamunex, delivers human albumin through plasma exchange in combination with infusion of intravenous immunoglobulin [56]. This trial targets amyloid in two ways. First, removing and replacing the albumin bound to pathogenic elements of Ab that cross the blood brain barrier by plasma exchange will allow further transfer of Aβ out of the central nervous system. Second, intravenous immunoglobulin may further increase amyloid clearance by combining plasma exchange with binding (bound?) amyloid [58].

The multiple challenges of treating AD have shifted the current investigational landscape toward combining two agents that target different pathways in parallel, or one long pathway in different points, following the models of successful treatment combinations for other serious diseases, such as HIV and cancer [59].

d. Methodological issues

In addition to the proposed reasons for the failures of trials of DMTs for AD disease-modifying drugs discussed above, issues with clinical trial design and methodology should also be considered [4]. Indeed, new innovative study designs are currently applied.

In placebo-controlled 2x2 trials design, patients are randomized to agent A plus placebo, agent B plus placebo, A plus B and placebo plus placebo [60]. This type of study design is used in combination treatment trials and in add-on studies with some modifications, enabling the simultaneous assessment of different agents and of their combination.

Another trial design which is currently used is the 3-arm study, where patients are randomized to treatment agent A, B, or A plus B [56]. The benefits of this design are that no patient remains untreated and that smaller samples of patients are needed. However this design is less appropriate for later stages of drug development, when every different aspect of every drug must be assessed [56].

Furthermore, several adaptive trials are under way for AD. Adaptive endpoints include drug effects on cognitive and clinical measures, a dose escalation algorithm, novel imaging biomarkers, early disease core and novel fluid biomarkers and later disease cognitive

assessments [61]. Most of novel trials use an adaptive Bayesian design to predict effectiveness or fail of individual agents and adaptively randomize patients to the most successful drugs [62]. Additionally, the interim analyses permit stopping since a predefined signal is detected, hence decision making is accelerated. Adaptive randomization and interim analyses can reduce the size and duration of the trial and prevent advancing to phase 3 whenever data demonstrating clinical efficacy are not detected [63].

Discussion - Conclusion

Given the complexity of AD and the high failure rate of the DMTs in development for AD, treatment of patients remains challenging. The complex pathologic pathways of AD in combination with our incomplete understanding of the relationships among the numerous mechanisms involved in the development of the disease (and the factors accelerating the disease progress?), seem to be mainly responsible for the failure of clinical trials. Targeting well selected or multiple pathologic pathways, earlier initiation of treatment, integration of fluid and imaging biomarkers for patient selection, prognosis, monitoring treatment efficacy, as well as implementation of new innovative study designs are fundamental for the development of effective treatments.

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