Brazilian Journal of Psychiatry
bjp
Revista Brasileira de Psiquiatria

Braz J Psychiatry. 2020 Jul-Aug;42(4):431-441
doi:10.1590/1516-4446-2019-0735
Brazilian Psychiatric Association

SPECIAL ARTICLE

Early diagnosis and treatment of Alzheimer's disease: new definitions and challenges

Marcos **Pais**,[1] Luana **Martinez**,[1] Octávio **Ribeiro**,[1] Júlia **Loureiro**,[1] Romel **Fernandez**,[1] Leandro **Valiengo**,[1] Paulo **Canineu**,[1,2] Florindo **Stella**,[1,3] Leda **Talib**,[1] Marcia **Radanovic**,[1] Orestes V. **Forlenza**[1]

[1]*Laboratório de Neurociências (LIM27), Departamento e Instituto de Psiquiatria, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo (HCFMUSP), São Paulo, SP, Brazil.* [2]*Programa de Gerontologia, Pontifícia Universidade Católica de São Paulo (PUC-SP), São Paulo, SP, Brazil.* [3]*Instituto de Biociências, Universidade Estadual Paulista (UNESP), Rio Claro, SP, Brazil.*

The prevalence of Alzheimer's disease (AD), a progressive neurodegenerative disorder, is expected to more than double by 2050. Studies on the pathophysiology of AD have been changing our understanding of this disorder and setting a new scenario for drug development and other therapies. Concepts like the ''amyloid cascade'' and the ''continuum of AD,'' discussed in this article, are now well established. From updated classifications and recommendations to advances in biomarkers of AD, we aim to critically assess the literature on AD, addressing new definitions and challenges that emerged from recent studies on the subject. Updates on the status of major clinical trials are also given, and future perspectives are discussed.

Keywords: Alzheimer disease; amyloid; tau; dementia

Background

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with high epidemiological relevance and significant social impact. The number of cases of dementia is expected to increase two- to three-fold by the year 2050, the majority of them caused by AD.[1-3] In Latin America, as in other low-income regions, the challenge faced is not only the increasing number of people with dementia, but also the lack of investment in training of health professionals and epidemiologic research, which reinforces chronic barriers regarding resources, culture, and stigmas.[4,5]

Alois Alzheimer first described a syndrome of progressive dementia and identified the neuropathological changes associated with its clinical presentation in 1906, publishing his findings in the following year.[6,7] These neuropathological changes are extracellular amyloid plaques formed by amyloid-β (Aβ) peptide deposits, derived from the cleavage of amyloid precursor protein (APP), and intracellular neurofibrillary tangles (NFT) composed of tau protein (microtubule-associated protein).[8] The body of knowledge produced on the subject since then has led to significant advances.

Genetics was a leading front of many discoveries that helped researchers better understand AD. Autosomal dominant mutations in three genes – the *APP* gene, presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) – cause the presenile form or early-onset AD (before age 65). The senile form of the disease, or late-onset AD, is more common, and the crucial susceptibility allele in this form involves apolipoprotein E4 (ApoEε4); homozygosity causes an eight-fold increase in the risk of developing AD.[9,10] A significant shift in the study of dementia occurred in 1976, when Robert Katzman showed that the senile and presenile forms of AD were histopathologically identical.[11] Since then, studies on the pathophysiology of presenile AD have contributed to our understanding of the senile form. The two AD presentations together represent the sixth leading cause of death in the United States alone.[3]

The diagnosis of AD is still based on clinical findings. However, there is a growing understanding that biomarkers could play an important role. Expert consensus on the topic has recognized that identification of the pathogenic process of AD through laboratory tests of blood-based and cerebrospinal fluid (CSF) biomarkers or molecular imaging methods makes it possible to infer the etiology of the underlying disease.[12] The role of biomarkers also differs somewhat at each of the disease stages, establishing the pathological alterations of AD in the preclinical stage and as complementary resources to clinical assessment at the mild cognitive impairment (MCI) and dementia stages.[13] Biomarkers will be particularly

Correspondence: Orestes V. Forlenza, Laboratório de Neurociências (LIM27), Departamento e Instituto de Psiquiatria, Faculdade de Medicina, Universidade de São Paulo, Hospital das Clínicas, Rua Doutor Ovídio Pires de Campos, 785, CEP 05403-010, São Paulo, SP, Brazil.
E-mail: forlenza@usp.br
Submitted Sep 30 2019, accepted Nov 01 2019, Epub Jan 24 2020.

How to cite this article: Pais M, Martinez L, Ribeiro O, Loureiro J, Fernandez R, Valiengo L, et al. Early diagnosis and treatment of Alzheimer's disease: new definitions and challenges. Braz J Psychiatry. 2020;42:431-441. http://dx.doi.org/10.1590/1516-4446-2019-0735

important for the diagnosis of AD in the early stages due to oligosymptomatic clinical presentations or absence of symptoms in cognitively healthy subjects that might benefit from disease-modifying interventions when these become available.

The current generation of AD biomarkers cannot be considered robust when compared with the biomarkers used in other areas of medicine. Nevertheless, research on biomarkers has dramatically increased the accuracy with which AD pathology can be detected in the brain. Recent dementia recommendations stress the importance of biomarker approaches, together with systematic and exhaustive clinical and cognitive investigations.[5]

In contrast, success in the development of drugs to treat AD has not yet been achieved. All approved drugs are symptomatic, with no consistent evidence that any is able to affect disease progression.[14-16] Recently, we have witnessed the failure of almost all anti-amyloid clinical trials. This research line was based on the premise that the removal of Aβ peptide deposits from brain tissue should provide benefits in the cognition and functional capacity of patients with clinical symptoms of AD.[17-19] It is clear now that most clinical trials have been testing potential disease-modifying therapeutic agents too late in the pathophysiological course of AD.[20,21] The next strategies to achieve success should focus on even earlier interventions and other targets.

New definitions

After the establishment of a research workgroup, the first criteria for the clinical diagnosis of AD were defined in 1984.[13,22] Interestingly, Aβ peptide was first identified in brain amyloid plaque in the same year.[23] Although considerable advances in AD research have been made since, the 1984 criteria were largely used without any modification for over 25 years; only in 2011 were they revised. The National Institute on Aging (NIA) and the Alzheimer's Association (AA) workgroups presented recommendations for the diagnosis of AD defining separate entities (and separate guidelines) to characterize the stages of AD: preclinical AD, MCI, and dementia.[13]

The neurobiological changes of AD start long before the onset of the first symptoms.[24-26] The transition between healthy cognitive aging and the earliest manifestations of dementia has been an area of major interest. Petersen et al. established the characterization of the early stages of AD in the late 1990s, as the first to introduce the concept of MCI and use the current diagnostic framework.[27,28] Defining the initial points of each step of the clinical deterioration process is challenging. In this characterization, MCI was considered a stage that precedes the impairment of capacity to perform the activities of daily living.[29] The importance of this stage lies in the growing understanding of a continuum of AD, which ranges from a very oligosymptomatic phase to the full-blown dementia syndrome.[30,31]

Preclinical AD, in turn, is the long, silent stage of the disease which precedes MCI.[32] Criteria for preclinical AD were developed to identify at-risk individuals at a stage when they were still asymptomatic, at which time interventions could delay or ultimately prevent the onset of cognitive impairment and dementia.[13,21,33] This classification integrated the existing knowledge on preclinical AD and consolidated the concept, defining three stages: stage 1, when Aβ accumulation occurs; stage 2, with cerebral amyloidosis co-occurring with evidence of neurodegeneration; and stage 3, with both amyloidosis and neurodegeneration occurring with evidence of very subtle cognitive decline that does not yet meet the criteria for MCI.[13]

The A/T/N classification

Many advances in the characterization of AD biomarkers have been made since 2011. In 2018, Jack et al. proposed an update of the 2011 NIA-AA guidelines unifying the three entities, and created recommendations for the diagnosis of AD grounded on a biomarker-based definition.[34] Known as the A/T/N classification – A (amyloid biomarkers), T (tau biomarkers), and N (biomarkers of neurodegeneration or neuronal injury) –, this system uses biomarkers to support the diagnosis of AD in research settings. This new classification system groups all key AD biomarkers by the pathologic process each one represents, rated as positive or negative. These recommendations create a common language with which researchers can characterize the pathological changes seen in research subjects diagnosed with AD and facilitate subject selection for interventional trials.

It is noteworthy that this framework proposed for research purposes should raise the importance of biomarker assessment in clinical settings. In low-income countries, the demand for affordable assessment creates a challenge for implementation of the latest advances in clinical practice, and simultaneously increases the need for development of more accessible technology, such as plasma biomarkers.

Advances in biomarker studies

The main roles of biomarkers in clinical practice are to support early identification of patients with AD, to monitor therapeutic response, and to aid in differential diagnosis.[20,21,34] Box 1 summarizes the contribution of biomarkers in clinical and research settings.[35,36]

The neuropathological signature of AD in CSF

The three core CSF biomarkers of AD are Aβ peptide, total tau protein (T-tau), and phosphorylated tau (P-tau)

Box 1 Role of biomarkers in clinical and research settings

Diagnosis (clinical settings, subject selection for clinical trials).
Assess disease state, staging, and prognosis.
Assess and monitor the pharmacodynamic effects of candidate compounds.
Demonstrate target engagement.
Aid in dose selection/optimization.
Assess response to and efficacy of therapies.
Identify and mitigate toxicity and adverse effects.
Personalize interventions according to stage and patient characteristics (personalized medicine approach).

protein. Although these biomarkers have been studied for more than two decades, only recently has the pathological signature of AD been elucidated. This signature consists of reduced concentrations of the 42 amino acid Aβ peptide ($A\beta_{1-42}$) combined with increased concentrations of T-tau and of the serine residue 181 of P-tau (^{181}P-Tau).[37-39] Most clinical trials use scales of cognitive enhancement as an endpoint. As noted above, these are not robust enough when compared with biochemical or physiological measures. The use of pharmacodynamic endpoints, such as the AD signature or individual concentrations of Aβ in the CSF, is an alternative for assessing compounds that inhibit enzymes that generate Aβ.[10] However, there is variability in measurements among clinical laboratories, and the lack of standardization makes it difficult to determine valid cutoff values.[40,41]

Genetic mutations associated with AD cause neuropathological changes in the following order: increased $A\beta_{1-42}$, brain amyloidosis, tauopathy, brain atrophy, and decreased glucose metabolism.[42] At least one biomarker has been established for each of these core pathological features. Using the A/T/N system, the principal biomarkers can be divided into three categories: biomarkers of Aβ metabolism and accumulation, biomarkers of tau pathology, and biomarkers of neurodegeneration or neuronal injury.[34] These biomarkers are validated and widely used. In the first category are CSF levels of $A\beta_{1-42}$ and molecular amyloid imaging, such as Pittsburgh compound-B ([^{11}C]-PIB) positron emission tomography (PET), florbetapir (^{18}F) PET, and flutemetabol (^{18}F) PET, which confirm cerebral retention of Aβ. The second category includes elevated CSF levels of ^{181}P-tau and molecular tau imaging, such as flortaucipir (^{18}F) PET. The last category includes decreased fluorodeoxyglucose (FDG) uptake on PET in a specific topographic pattern involving the temporoparietal cortex, mesial temporal, and parietal regions on structural magnetic resonance imaging (MRI), and increased T-tau in CSF.[20,34] Recent developments in CSF and imaging biomarkers are promising for early diagnosis of AD, but their availability is still limited to research settings.

Biomarkers of amyloid accumulation

The amyloid-related or molecular biomarkers of AD are CSF $A\beta_{1-42}$ and amyloid PET. Low $A\beta_{1-42}$ reflects brain amyloid deposition and shows very high concordance with amyloid PET. This pathological change is found in AD and prodromal AD with a sensitivity exceeding 90%.[18] The most widely used amyloid imaging agent is [^{11}C]-PiB. It binds to Aβ aggregates with high affinity, and is capable of differentiating individuals with AD from those with normal cognition. Researchers found comparable sensitivity of CSF $A\beta_{1-42}$ levels and PiB PET for the detection of AD pathology.[43]

Biomarkers of tau pathology and neuronal degeneration

P-tau levels in CSF are taken to represent the presence of tau pathology, including NFT, while CSF T-tau more likely represents neuronal injury or neurodegeneration and reflects disease progression. High levels of P-tau do not occur in other dementias.[41,44] Aβ precedes tau pathology but, unlike amyloid deposition, NFT correlates better with cognitive decline. Even ApoEε4 status correlates with different patterns of tau deposition.[43,45] Additionally, measurements of tau deposition in specific regions are more closely related to early degeneration, atrophy measures, and cognitive decline[46,47]; hence, the importance of developing tau-specific tracers for imaging studies. Although the accuracy and reliability of the technique are still under investigation, the development of tau tracers started almost two decades ago,[48] with flortaucipir (^{18}F) being the most studied.[49]

MRI and FDG-PET are well-established imaging techniques for AD diagnosis and follow-up. FDG-PET measures glucose uptake in neurons and glial cells and is sensitive to synaptic dysfunction. The typical pattern of altered FDG-PET in AD is a temporoparietal and posterior cingulate hypometabolism.[50] Changes in MRI are seen later in the disease process. Cerebral atrophy is believed to spread from within the mesial temporal lobe (MTL), with changes in hippocampal volume and entorhinal cortex thickness, to the parietal, occipital, and frontal lobes over the years; individuals with MCI show the highest rates of atrophy.[43]

Plasma biomarkers of AD

Advances in the area of blood-based AD biomarkers are of the utmost importance. Developing blood biomarkers would increase screening possibilities, adding a much more accessible tool to clinical diagnosis. Plasma examination would allow more frequent sampling in clinical trials and other studies, and would minimize the necessity of lumbar puncture, given its invasive nature. Prescreening with blood biomarkers would also lower the costs of further workup, avoiding unnecessary amyloid PET scans. Candidate blood biomarkers include plasma levels of Aβ, tau protein, and neurofilament light (NFL) chain protein. Assessing these biomarkers can provide sufficiently reliable estimates of brain amyloid positivity and neurodegeneration,[35,41,51] and the use of fully automated immunoassays to measure them can add great accuracy to the detection of their plasma levels.[52]

Just as for CSF Aβ, there are still no worldwide unified cutoff values established for plasma Aβ. Nevertheless, there is a reliable correlation between amyloid status in plasma as measured by the $A\beta_{1-42}/_{1-40}$ ratio and future positivity on amyloid PET.[53] A recent study with a representative number of subjects (n=842) revealed high accuracy in the detection of altered Aβ levels in the brain, correlating with plasma levels of $A\beta_{1-42}$ and $A\beta_{1-40}$; adding APOE genotype, plasma tau, and NFL levels further increased accuracy.[52] Optimized blood Aβ assessment with fully automated immunoassays may improve screening capacity for clinical trials. Although present in other disorders, tau has been reported to be elevated in the plasma of individuals with AD, with the ^{181}P-tau form showing higher specificity.[35,54]

Recent studies have reported promising results of serum and plasma measurement of NFL chain protein.

NFL is the light subunit of neurofilament, the dominant axonal cytoskeleton protein.[55] The presence of NFL chain protein in the CSF indicates axonal damage. Recent studies showed a positive correlation between serum/plasma levels of NFL chain protein and CSF levels, with accuracy comparable to that of CSF biomarkers, but only for neurodegeneration.[56] These correlations have raised interest in NFL as a blood-based biomarker of neurodegeneration and disease progression. A recent meta-analysis showed that NFL chain protein levels both in CSF and in plasma had high diagnostic sensitivity for AD and other neurodegenerative dementias.[57] A cross-sectional, longitudinal data analysis of the Dominantly Inherited Alzheimer Network (DIAN) cohort found that CSF levels of NFL were significantly increased in mutation carriers compared to non-carriers at an estimated 6.8 years before symptom onset. Subsequent longitudinal analyses confirmed the cross-sectional findings. These changes are sensitive enough to pick up early regional brain atrophy and to predict conversion to symptomatic AD. It is noteworthy that serial NFL measurements are a better tool than absolute NFL levels measured in a cross-sectional fashion.[58]

Other approaches using genomics, transcriptomics, metabolomics, lipidomics, and proteomics have been used to generate different AD biomarkers. One study showed that altered microRNAs resulting from the failure of synaptic function are potential plasma biomarkers of AD.[59] Another study comparing AD patients with healthy controls showed decreased platelet levels of one member of the a disintegrin and metalloproteinase (ADAM) family: ADAM10, the primary α-secretase of APP, which plays an important role in reducing generation of Aβ peptide. The same study showed decreased presenilin levels in platelets and leukocytes. Presenilin is the catalytic site of γ-secretase, one enzyme in the reaction that generates Aβ peptide. Levels of the β-site APP-cleaving enzyme 1 (*BACE1*), also known as β-secretase, were also decreased in leukocytes and presented no differences in platelets.[60]

Treatment

Pharmacotherapeutic approaches to AD can be divided into two categories: symptomatic and disease-modifying therapies (DMTs). Symptomatic treatments have a significant impact not only on cognition, but also in symptoms such as agitation, psychosis, and sleep disturbance, which are present in up to 90% of patients with dementia.[61]

The search for DMTs has focused mainly on interventions based on the amyloid cascade hypothesis and tau biology.[10] It is still unknown which, amyloid or tau, is the best drug target. However, Aβ accumulation was the main target of most drugs tested for AD in the past 20 years.[19] A combination of therapies targeting both amyloid and tau may represent a promising alternative.

The amyloid cascade hypothesis

The deposition of Aβ peptide in neuritic plaques induces the neurotoxic events which are followed by NFT formation, resulting in cell loss and vascular damage.[62] This sequence constitutes the amyloid cascade hypothesis, formally proposed in 1992.[63] Many studies have suggested that the amyloid pathway is a very early event in the disease, starting in the hippocampus and entorhinal cortex.[19,63-66]

The amyloid cascade is still a widely accepted model. However, the recent failure of anti-amyloid therapies has been calling the temporal sequence of pathological events in AD into question. Indeed, some evidence has defied the notion that amyloidosis is necessary to define AD.[67] Focusing on this single target could explain failures in clinical trials, as a significant number of studies show conflicting neuropathological findings,[68] suggesting that biomarker development in preclinical or prodromal AD does not follow the timeline proposed by the amyloid cascade hypothesis. Additionally, researchers have reported that 14 to 25% of individuals clinically diagnosed with mild to moderate AD have only sparse neuritic amyloid plaques on postmortem examination.[69-71]

In contrast, a group of researchers still defines the neuropathological entity of AD necessarily by the presence of pathologic amyloid, supporting a central role of the amyloid cascade hypothesis. The definition of AD would require a signature that involves amyloid abnormalities alone (Alzheimer's pathologic change) or in combination with pathologic tau (AD).[34] These definitions would represent stages of the AD continuum, not separate entities.

The need for uniform criteria to guide therapeutic trials makes these conflicting findings an obstacle to recruiting subjects, as the clinical diagnosis may not be accompanied by the expected neuropathological changes, resulting in failure of interventions. The A/T/N classification should represent a solution, since AD stages can be classified by their different neuropathological alterations without risk of overlap.

This controversy also raises questions on how non-AD diagnoses can be suspected. The classic clinical syndrome of AD, i.e., amnestic cognitive impairment, has been associated with multiple neuropathological changes. Some non-AD entities may mimic this clinical syndrome, including primary age-related tauopathy (PART), suspected non-Alzheimer pathology (SNAP), and, especially, limbic-predominant age-related TDP-43 encephalopathy (LATE). LATE is a proteinopathy preferably affecting limbic brain structures commonly observed in the oldest old individuals (past 80 years of age).[72] Other autopsy studies have shown that 10 to 30% of patients clinically diagnosed with AD have no neuropathological changes.[73] One particular study found 47% specificity and 61% accuracy in the clinical diagnosis of AD compared with neuropathological diagnosis; some of the neuropathological findings actually represented TDP-43 proteinopathies.[74]

Symptomatic treatment

Neuropsychiatric symptoms (NPS) are a key clinical feature of AD and may appear in all stages.[61,75] In the preclinical stage, these symptoms may represent an

increased risk of progression to dementia.[76] During the MCI stage, the syndrome of NPS is called mild behavioral impairment (MBI).[77] Psychotropic drugs for the treatment of behavioral and psychological symptoms of dementia (BPSD) are generally prescribed, following guidelines and clinical experience in the treatment of primary psychiatric disorders.[75] Treating BPSD is a very complex task, as there is insufficient evidence on the neurobiological mechanisms of many of the behavioral syndromes seen in clinical practice. Therapeutic alternatives are restricted; however, a combination of non-pharmacological interventions and safe pharmacological options remains the best therapeutic approach.[78]

Acetylcholinesterase (AChE) inhibitors

The cholinergic system is implicated in cognition. Diminished cholinergic synaptic activity caused by reduction in the activity of choline acetyltransferase, an enzyme responsible for acetylcholine synthesis in the nucleus basalis of Meynert, is the basis for the use of AChE inhibitors.[68,79] These medications inhibit the catabolic enzyme AChE, delaying the decrease of acetylcholine levels, and, when used alone, are recommended for the treatment of patients with mild to moderate AD.[2] Three AChE inhibitors are available for AD treatment: donepezil, galantamine, and rivastigmine. Rivastigmine has a transdermal patch presentation with evidence of impact on treatment adherence.[80] When used in combination with the uncompetitive glutamatergic receptor antagonist memantine, these medications are also used to treat severe stages of the disease.[2,19] The failure of AChE inhibitors to delay the onset of AD from MCI in some studies[14-16] has given rise to the question of whether these drugs are capable of disease modification, with little evidence of significant impact on the disease course. Although possible benefits were found in subsamples of patients (such as ApoEε4 carriers)[14] and on AD-like neuropsychiatric symptoms at baseline,[15] the long-term benefits of these drugs are arguable. One study from our group showed that long-term treatment with donepezil was associated with a significant reduction in BACE1 expression in the platelets of patients with AD,[81] pointing to a possible disease-modifying effect of AChE inhibitors.

Disease-modifying therapies

Disease-modification in AD requires accurate diagnosis at the pre-dementia and preclinical stages, justifying the need to understand critical aspects of the neuropathological changes of AD.[82] How Aβ peptides (soluble, oligomeric, or plaque) lead to cell death, how tau tangles affect neuronal function, the relationship between Aβ and tau tangles, and the apparent interneuronal spread of tau are gray areas in research that need to be addressed. Developing DMTs might then be able to attenuate decline and preserve cognitive and functional capacity. In parallel, researchers should tackle not only AD pathogenesis, but also its risk factors (discussed below).

During the last decade, research has identified many candidate molecular targets for earlier, more specific AD therapies. In the amyloid cascade, the main targets are the senile or neuritic plaques and fibrillary Aβ or Aβ oligomers at the pre-dementia and dementia stages. In preclinical AD, preventing Aβ accumulation would be the main objective, with overproduction of Aβ, abnormal APP metabolism, and reduced Aβ clearance being the targets of intervention. In cytoskeletal degeneration, i.e., tau pathology, NFTs would be the primary target. However, upstream alterations responsible for NFT formation would be better targets at earlier stages. Finally, other mechanisms that lead to secondary toxicity include inflammation, oxidative stress, glial activation, among others.[83] Molecular targets in this respect, besides the Aβ peptide, include BACE, tau protein, markers of inflammation, and even the 5-HT2A receptor.[10,18,84,85]

Drug development for AD has consistently shown a high failure rate.[10] In 2014, Cummings et al. examined 413 AD trials testing 244 drugs carried out between 2002 and 2012. Almost all clinical trials failed, with the exception being the successful completion of the memantine trial.[86] At the beginning of 2019, 28 agents were being studied in 42 phase III trials; 17 of them were DMTs.[18] Nonetheless, there are still no new DMTs available for AD. Table 1 shows a summary of an annual update on AD drug development. Clinical trials with the purpose of disease modification, cognitive enhancement, and control of NPS are included.[18]

Several high-profile phase III clinical trials recently failed to explore the amyloid cascade hypothesis. Although preliminary results were promising, these clinical trials failed to demonstrate cognitive enhancement or clinical improvement occurring together with the observed neuropathological changes.[18] However, these trials showed positive results in drug-target engagement, with reported Aβ clearance from the brain.[10,87]

Amyloid-based therapies

Anti-amyloid immunotherapy

The amyloid cascade hypothesis of AD pathology implicates one possible pathway – the amyloidogenic

Table 1 2019 update on drug development

Trials	156
Agents tested	132
Main primary mechanisms of action and objectives of trials	
Cognitive enhancement	19 (14)
Treatment of NPS and BPSD	14 (11)
Disease modification	96 (73)
Main primary targets	
Amyloid	38 (40)
Tau	17 (18)
Types of agents	
Disease-modifying biologics	
Disease-modifying small molecules	
Symptomatic (system-reducing small molecules)	

Data presented as n or n (%).
BPSD = behavioral and psychological symptoms of dementia; NPS = neuropsychiatric symptoms.

pathway – in which APP is sequentially cleaved until the Aβ peptide is released, $A\beta_{1-42}$ being the form most prone to aggregation and most neurotoxic.[59,63,88] Schenk et al. first reported that immunization of PDAPP transgenic mice which overexpress mutant human APP prevented and reduced AD-like neuropathologies.[89] The anti-amyloid compounds developed since then aim at clearing Aβ peptide from the brain parenchyma or reducing its aggregation.[2] Active and passive immunotherapies have been tested over the years. The first compound tested, AN-1792, an Aβ antigen, failed to demonstrate efficacy in mild to moderate AD, and was also toxic. The trial was discontinued in 2002.[90] These interventions represent secondary prevention actions, since the compounds were tested after the disease process had already begun.

In general, immunotherapies targeting Aβ were well tolerated. Nevertheless, risks have been described with passive immunotherapies, including amyloid-related imaging abnormalities (ARIA) appearing as vasogenic edema (ARIA-E) or cerebral microhemorrhages (ARIA-H), which represent increased vascular permeability due to an immune-inflammatory response against vascular deposition of Aβ.[91]

Convergent findings suggest that therapeutic interventions targeting amyloid should be prophylactic, tested years before amyloid deposition. Ongoing clinical trials are focused on earlier stages of AD and asymptomatic at-risk subjects. The only ongoing active immunotherapy, CAD106, an anti-amyloid vaccine, is now in a phase III clinical trial (Generation S1) of a preventive paradigm.[19] CAD106 stimulates a B-cell and carrier-induced T-cell helper response without activating an Aβ-specific T-cell response.[92] Four passive immunotherapy agents, all anti-amyloid monoclonal antibodies, are now in development in phase III clinical trials of patients with early and preclinical AD and asymptomatic subjects at risk for AD.[19]

The antibody solanezumab, which binds to the central region of Aβ, with evidence of a preference for soluble monomeric Aβ,[93] is being tested in a preventive paradigm. The Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease Study (A4) is testing solanezumab in asymptomatic or mildly symptomatic older adults with biomarker evidence of brain amyloid deposition,[94] and the Dominantly Inherited Alzheimer Network – Trials Unit Study (DIAN-TU) is testing the compound in asymptomatic or mildly symptomatic carriers of autosomal dominant mutations in *APP*, *PSEN1*, or *PSEN2*.[95]

Crenezumab, a compound that binds to multiple species of Aβ (mostly fibrils and oligomers),[96] is being evaluated in two phase III clinical trials enrolling patients with prodromal to mild AD: A Study of Crenezumab Versus Placebo to Evaluate the Efficacy and Safety in Participants With Prodromal to Mild Alzheimer's Disease (CREAD) and CREAD2, with expected completion in 2020 and 2021, respectively.[19]

Gantenerumab binds to both the N-terminal and central regions of Aβ, with higher affinity for oligomers and fibrils than for Aβ monomers.[97] The aforementioned DIAN-TU study includes a gantenerumab arm, and two other phase III studies – Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Efficacy, and Safety Study of Gantenerumab in Patients with Early Alzheimer's Disease (GRADUATE 1) and GRADUATE 2 – are ongoing, both enrolling patients with early AD and biomarker evidence of brain Aβ deposition.[19]

Finally, aducanumab, an antibody that binds to soluble and insoluble Aβ and with a higher selectivity for monomers, was the first compound to show both a decrease in Aβ load in the brain and positive effects on cognition and global clinical status, although limitations from dropout rates occurred.[98] Aducanumab was under investigation in two phase III clinical trials, ENGAGE and EMERGE, in patients with prodromal AD and positive amyloid PET scans[99]; however, in March 2019, the studies were discontinued based on the results of a futility analysis.[100] Table 2 summarizes the remaining and active phase III clinical trials of immunotherapies for AD.

BACE inhibitors

Defective BACE activity is involved in the accumulation of amyloid in the brain parenchyma.[66,101] BACE inhibition would hypothetically decrease Aβ production. Some BACE inhibitors were tested in recent clinical trials, but failed to slow the progression of AD.[19,85,102] Two phase II/III studies (Generation S1 and Generation S2) tested CNP520 (umibecestat), an oral, long-acting, selective BACE1 inhibitor. However, as of July 2019 (results yet to be published), they were discontinued due to worsening of cognitive measures, and side effects like weight loss.[19] Although the strategy was unsuccessful, these clinical trials showed interesting changes in biomarkers, with reductions in concentrations of toxic Aβ species in the brain, CSF, and plasma.[10] Only two phase III clinical trials testing BACE inhibitors in early AD are still active. Elenbecestat is currently being tested in two double-blind, placebo-controlled phase III studies (MISSION AD1 and MISSION AD2).

Tau-based therapies

Findings such as neurodegeneration occurring before amyloidosis,[67,103-106] evidence of neurodegeneration in face of normal amyloid levels,[107,108] axonal injury,[104] and tau lesions in late myelinating regions predating amyloid deposition in prodromal AD[109] may explain the failure of trials targeting amyloid. Other explanations might be problems with patient selection, subjects at different stages of the disease or inappropriate time of intervention, inadequate dose, target engagement, choice of clinical assessment scales, gaps in the understanding of AD pathophysiology.[18,19]

There is compelling evidence that tau-altering pharmacologic interventions would be worthwhile. Tau pathology is more firmly associated with clinical and cognitive decline than is amyloid pathology, and tau may accumulate in susceptible regions earlier than amyloid.[21,110]

Tau pathology is seen in the brain most prominently as NFTs, not only in AD but also in other neurodegenerative illnesses. The insoluble forms of tau protein are the main component of NFTs. Tau-directed immunotherapies have been developed based on the recognition that NFTs,

Table 2 Anti-amyloid active and passive immunotherapy compounds in phase III secondary prevention trials for AD, with status updated as of late 2019 (only active trials)

Agent	Mechanism of action	Mild to moderate AD	Early, preclinical or prodromal
CAD106	Active immunotherapy (Aβ antigen)	One trial discontinued (no efficacy)	One preclinical AD trial ongoing – Generation S1
Solanezumab	Passive immunotherapy (anti-Aβ monoclonal antibody)	Two trials discontinued (no efficacy)	One prodromal AD trial discontinued (strategic) Two preclinical AD trials ongoing – A4 and DIAN-TU
Crenezumab	Passive immunotherapy (anti-Aβ monoclonal antibody)	One trial discontinued (no efficacy)	Two preclinical AD trials ongoing – CREAD and CREAD2
Gantenerumab	Passive immunotherapy (anti-Aβ monoclonal antibody)	One trial discontinued (no efficacy) One trial ongoing	One prodromal AD trial discontinued (no efficacy) Two early AD trials ongoing – GRADUATE 1 and GRADUATE 2 One preclinical AD trial ongoing – DIAN-TU
Aducanumab	Passive immunotherapy (anti-Aβ monoclonal antibody)	No trials at these AD stages	Two early AD trials discontinued (no efficacy in lower doses) – ENGAGE and EMERGE Reduced clinical decline with longer exposure to higher doses (results yet to be published)

A4 = Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease Study; AD = Alzheimer's disease; Aβ = amyloid-β; CREAD = A Study of Crenezumab Versus Placebo to Evaluate the Efficacy and Safety in Participants with Prodromal to Mild Alzheimer's Disease; DIAN-TU = Dominantly Inherited Alzheimer Network – Trials Unit; ENGAGE and EMERGE = A Phase III, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Efficacy, and Safety Study of Aducanumab in Patients with Early Alzheimer's Disease; Generation S1 = A Study of CAD106 and CNP520 Versus Placebo in Participants at Risk for the Onset of Clinical Symptoms of Alzheimer's Disease; GRADUATE = A Phase III, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Efficacy, and Safety Study of Gantenerumab in Patients with Early Alzheimer's Disease.

synapse loss, and neuronal death are associated with clinical deterioration in AD.[111] Studies of tau-based therapies have involved anti-tau antibodies and active immunization, tau antiaggregants, tau kinase inhibitors, and gene therapy.[112] The main objectives of these strategies are the reduction of tau oligomer levels, prevention of tau aggregation, and blockage of hyperphosphorylation or microtubule destabilization.[113]

Many issues that emerged from anti-amyloid drug development justify investment in tau-based therapies. It is clear now that amyloid-based therapies may be more effective in the preclinical stages of the illness, taking a long time to show significant results and requiring more subjects than in trials of prodromal and mild AD.[114,115] As in the early debates of anti-amyloid therapies, many questions have yet to be answered in the development of tau-based therapies.[116] The hypothesized characteristics of tau-based approaches have fueled the discussion and given impulse to this line of investigation. These interventions are supposed to be more effective in symptomatic patients and more likely to show benefits in patients at more advanced stages of the disease. Thus, clinical trials could require smaller samples, at lower cost, and less time to show results. More importantly, anti-tau approaches are not restricted to AD treatment, but may be employed in other neurodegenerative diseases in which tau deposition occurs, such as progressive supranuclear palsy (PSP) and frontotemporal dementia (FTD).[116]

Lithium

Convergent findings have confirmed the ability of lithium to modulate neurotrophic and protective responses in the brain. Lithium is implicated in critical intracellular mechanisms of neurotrophic responses and neurodegeneration.[117-119] Inhibition of the enzymatic activity of glycogen synthase kinase 3 beta (GSK3β) is the hypothesized mechanism for prevention of tau phosphorylation and, thus, a neuroprotective effect of lithium in AD.[120]

The body of evidence from studies of bipolar disorder, in addition to a few trials in AD, supports the potential use of lithium as a DMT for AD.[121-123] A previous report of a randomized controlled trial has shown that long-term treatment with lithium in amnestic MCI reduced P-tau levels in the CSF, with patients showing cognitive and functional stabilization during treatment.[124] Lithium carbonate was then compared with placebo to determine benefits in MCI.[125] Patients received lithium or placebo for 2 years and were followed-up for another year, with target lithium levels defined at a subtherapeutic window between 0.25 and 0.5 mEq/L. Lithium-treated patients remained stable over 2 years, showed better performance on cognitive tests, and had a significant increase in CSF $A\beta_{1-42}$ during follow-up. Comparable positive outcomes were not observed in the placebo group. The long-term use of low-dose lithium period may be protective against cognitive decline and preserve functional capacity. However, only a few controlled intervention trials have tested the benefits of lithium in this setting, and additional research is needed.[124,126-129]

Other mechanisms explored in clinical trials failed to demonstrate efficacy. Receptor for advanced glycation end products (RAGE) inhibition would address neuroinflammation and oxidative stress. A phase III study was terminated in mid-2018.[10,130,131] Increased insulin resistance promotes both Aβ deposition and tau phosphorylation. A trial with pioglitazone failed an interim futility analysis and was terminated, with results not yet published. Idalopirdine, a serotonin 5-hydroxytryptamine-6

(5-HT6) antagonist, was tested to establish its efficacy as adjunctive therapy to AChE inhibitors for symptomatic treatment of patients with mild-moderate AD. No improvement in cognition occurred.[10]

Rehabilitation and cognitive training

Some NPS seem to respond better to nonpharmacological interventions.[132,133] Studies have shown that the engagement of persons with dementia in rehabilitation and cognitive training activities is more efficacious when interventions are tailor-made.[134] A randomized, double-blind clinical trial to evaluate activity programs tested the outpatient version of an occupational therapy intervention, the tailored activity program (TAP). Preliminary results were promising on both NPS and caregiver burden.[78] Recently, studies of information technology-based cognitive intervention programs have been conducted. Randomized controlled trials of these approaches showed significant enhancement in cognition and functional capacity, with persistent results.[135] The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) combined comprehensive intervention with technology to enhance cognitive function.[136]

Prevention of dementia

The number of people with dementia is rising worldwide. However, in some countries, there has been a decline in dementia incidence in specific age groups.[1,137,138] This decline has not been associated with a single risk or protective factor, but rather with major societal changes over the years, particularly improvement in living conditions due to improved access to education and healthcare.[139] Primary prevention of dementia, i.e., controlling risk factors, has a major impact on incidence. For almost two decades, there has been evidence that a reduction in the prevalence of risk factors has a potential impact on dementia prevalence.[140,141] One-third of cases are probably preventable by addressing nine major modifiable risk factors: midlife hypertension, midlife obesity and diabetes, late-life depression, physical inactivity, smoking, social isolation, and 11 to 12 years of formal education. Peripheral hearing loss was recognize as a significant and modifiable risk factor after the results of a meta-analysis.[2] Preventive interventions in midlife (from age 45 to 65) include addressing hearing loss, hypertension, and obesity. Interventions in late life (after age 65) include smoking cessation, treating depression, physical activity, avoiding social isolation, and treating diabetes. Lower early-life education increases the risk of dementia, and there is no evidence of additional protection after secondary school.[2]

Conclusion

The high prevalence of AD and its great impact on the functional capacity of affected individuals emphasize the need to develop more effective therapies capable of halting or slowing the progression of the degenerative process and improving the symptoms of the disease. Population aging and the burden of AD on public services reinforce the need for early diagnosis.

A new comprehension of the neuropathological changes of AD is emerging. The biomarker-base classification system proposed in 2018 is evidence of a broader concept of the disease's pathological process, and the impact of this new perception on biomarker and drug development studies is already evident. However, clinical trials still face many challenges. Identifying the best molecular target or combination thereof and developing better protocols to assess intervention outcomes using biochemical and physiological measures (e.g., concentrations of $A\beta_{1\text{-}42}$ in CSF, amyloid, or tau visualization on PET) as endpoints are necessary strategies to solve these challenges. Finally, the main objective of detection of AD in its preclinical stages is to facilitate early therapeutic intervention, which is the premise underlying most ongoing efforts to find new therapies.

Acknowledgements

The Laboratório de Neurociências (LIM27), USP, receives financial support from the Alzira Denise Hertzog Silva Association (ABADHS), Instituto Nacional de Ciência e Tecnologia (INCT) program for Instituto Nacional de Biomarcadores em Neuropsiquiatria (INBION), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; grant 14/50873-3), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant 465412/2014-9).

Disclosure

The authors report no conflicts of interest.

References

1 Prince M, Wimo A, Guerchet M, Ali GC, Wu YT, Prina M. World Alzheimer Report 2015: the global impact of dementia. London: Alzheimer's Disease International (ADI); 2015.
2 Livingston G, Sommerlad A, Orgeta V, Costafreda SG, Huntley J, Ames D, et al. Dementia prevention, intervention, and care. Lancet. 2017;390:2673-734.
3 Alzheimer's Association. 2018 Alzheimer's disease facts and figures. Alzheimers Dement. 2018;14:367-429.
4 Nitrini R, Bottino CM, Albala C, Custodio Capuñay NS, Ketzoian C, Llibre Rodriguez JJ, et al. Prevalence of dementia in Latin America: a collaborative study of population-based cohorts. Int Psychogeriatr. 2009;21:622-30.
5 Parra MA, Baez S, Allegri R, Nitrini R, Lopera F, Slachevsky A, et al. Dementia in Latin America: assessing the present and envisioning the future. Neurology. 2018;90:222-31.
6 Zilka N, Novak M. The tangled story of Alois Alzheimer. Bratisl Lek Listy. 2006;107:343-5.
7 Cipriani G, Dolciotti C, Picchi L, Bonuccelli U. Alzheimer and his disease: a brief history. Neurol Sci. 2011;32:275-9.
8 Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. Lancet. 2006;368:387-403.
9 Loy CT, Schofield PR, Turner AM, Kwok JB. Genetics of dementia. Lancet. 2014;383:828-40.
10 Fish PV, Steadman D, Bayle ED, Whiting P. New approaches for the treatment of Alzheimer's disease. Bioorg Med Chem Lett. 2019;29:125-33.
11 Lijtmaer H, Fuld PA, Katzman R. Letter: Prevalence and malignancy of Alzheimer disease. Arch Neurol. 1976;33:304.
12 Frisoni GB, Boccardi M, Barkhof F, Blennow K, Cappa S, Chiotis K, et al. Strategic roadmap for an early diagnosis of Alzheimer's disease based on biomarkers. Lancet Neurol. 2017;16:661-76.

13 Jack CR Jr, Albert MS, Knopman DS, McKhann GM, Sperling RA, Carillo MC, et al. Introduction to revised criteria for the diagnosis of Alzheimer's disease: national institute on aging and the Alzheimer association workgroups. Alzheimers Dement. 2011;7:257-62.
14 Petersen RC, Parisi JE, Dickson DW, Johnson KA, Knopman DS, Boeve BF, et al. Neuropathologic features of amnestic mild cognitive impairment. Arch Neurol. 2006;63:665-72.
15 Feldman HH, Lane R. Study 304 Group. Rivastigmine: a placebo controlled trial of twice daily and three times daily regimens in patients with Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2007;78:1056-63.
16 Winblad B, Machado JC. Use of rivastigmine transdermal patch in the treatment of Alzheimer's disease. Expert Opin Drug Deliv. 2008;5:1377-86.
17 Sarazin M, Dorothée G, de Souza L, Aucouturier P. Immunotherapy in Alzheimer's disease: do we have all the pieces of the puzzle? Biol Psychiatry. 2013;74:329-32.
18 Cummings J, Lee G, Ritter A, Sabbagh M, Zhong K. Alzheimer's disease drug development pipeline: 2019. Alzheimers Dement (N Y). 2019;5:272-93.
19 Panza F, Lozupone M, Logroscino G, Imbimbo BP. A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease. Nat Rev Neurol. 2019;15:73-88.
20 Hampel H, Frank R, Broich K, Teipel SJ, Katz RG, Hardy J, et al. Biomarkers for Alzheimer's disease: academic, industry and regulatory perspectives. Nat Rev Drug Discov. 2010;9:560-74.
21 Bondi MW, Edmonds EC, Salmon DP. Alzheimer's disease: past, present, and future. J Int Neuropsychol Soc. 2017;23:818-31.
22 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on Alzheimer's disease. Neurology. 1984;34:939-44.
23 Huang YM, Shen J, Zhao HL. Major clinical trials failed the amyloid hypothesis of Alzheimer's disease. J Am Geriatr Soc. 2019;67:841-4.
24 Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, et al. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. Lancet Neurol. 2013;12:357-67.
25 Sperling RA, Karlawish J, Johnson KA. Preclinical Alzheimer disease-the challenges ahead. Nat Rev Neurol. 2013;9:54-8.
26 Dubois B. The emergence of a new conceptual framework for Alzheimer's disease. J Alzheimers Dis. 2018;62:1059-66.
27 Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol. 1999;56:303-8.
28 Forlenza OV, Diniz BS, Teixeira AL, Stella F, Gattaz W. Mild cognitive impairment. Part 1: clinical characteristics and predictors of dementia. Braz J Psychiatry. 2013;35:178-85.
29 Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, et al. Current concepts in mild cognitive impairment. Arch Neurol. 2001;58:1985-92.
30 Diniz BS, Nunes PV, Yassuda MS, Forlenza OV. Diagnosis of mild cognitive impairment revisited after one year. Preliminary results of a prospective study. Dement Geriatr Cogn Disord. 2009;27:224-31.
31 Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7:280-92.
32 Mormino EC, Sperling RA, Holmes AJ, Buckner RL, De Jager PL, Smoller JW, et al. Polygenic risk of Alzheimer disease is associated with early- and late-life processes. Neurology. 2016;87:481-8.
33 Jessen F, Amariglio RE, van Boxtel M, Breteler M, Ceccaldi M, Chételat G, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimers Dement. 2014;10:844-52.
34 Jack CR Jr, Bennett DA, Blennow K, Carillo MC, Dunn B, Haeberlein SB, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14:535-62.
35 Bateman RJ, Blennow K, Doody R, Hendrix S, Lovestone S, Salloway S, et al. Plasma biomarkers of AD emerging as essential tools for drug development: an EU/US CTAD task force report. J Prev Alzheimers Dis. 2019;6:169-73.
36 Cummings J. The role of biomarkers in Alzheimer's disease drug development. Adv Exp Med Biol. 2019;1118:29-61.
37 Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol. 2006;5:228-34.
38 Diniz BS, Pinto Júnior JA, Forlenza OV. Do CSF total tau, phosphorylated tau, and beta-amyloid 42 help to predict progression of mild cognitive impairment to Alzheimer's disease? A systematic review and meta-analysis of the literature. World J Biol Psychiatry. 2008;9:172-82.
39 Forlenza OV, Radanovic M, Talib LL, Aprahamian I, Diniz BS, Zetterberg H, et al. Cerebrospinal fluid biomarkers in Alzheimer's disease: diagnostic accuracy and prediction of dementia. Alzheimer Dement (Amst). 2015;1:455-63.
40 De-Paula VJ, Radanovic M, Diniz BS, Forlenza OV. Alzheimer's disease. Subcell Biochem. 2012;65:329-52.
41 Blennow K. A review of fluid biomarkers for Alzheimer's disease: moving from CSF to blood. Neurol Ther. 2017;6:15-24.
42 Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 2012;367:795-804.
43 Counts SE, Ikonomovic MD, Mercado N, Vega IE, Mufson EJ. Biomarkers for the early detection and progression of Alzheimer's disease. Neurotherapeutics. 2017;14:35-53.
44 Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, et al. A/T/N: an unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology. 2016;87:539-47.
45 Matsuda H, Shigemoto Y, Sato N. Neuroimaging of Alzheimer's disease: focus on amyloid and tau PET. Jpn J Radiol. 2019;37:735-49.
46 Wang L, Benzinger TL, Su Y, Christensen J, Friedrichsen K, Aldea P, et al. Evaluation of tau imaging in staging Alzheimer disease and revealing interactions between β-amyloid and tauopathy. JAMA Neurol. 2016;73:1070-7.
47 Cho H, Choi JY, Hwang MS, Lee JH, Kim YJ, Lee HM, et al. Tau PET in Alzheimer disease and mild cognitive impairment. Neurology. 2016;87:375-83.
48 Márquez F, Yassa MA. Neuroimaging biomarkers for Alzheimer's disease. Mol Neurodegener. 2019;14:21.
49 Marquié M, Normandin MD, Vanderburg CR, Costantino IM, Bien EA, Rycyna LG, et al. Validating novel tau positron emission tomography tracer [F-18]-AV-1451 (T807) on postmortem brain tissue. Ann Neurol. 2015;78:787-800.
50 Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S, et al. Alzheimer's disease. Lancet. 2016;388:505-17.
51 Henriksen K, O'Bryant SE, Hampel H, Trojanowski JQ, Montine TJ, Jeromin A, et al. The future of blood-based biomarkers for Alzheimer's disease. Alzheimers Dement. 2014;10:115-31.
52 Palmqvist S, Janelidze S, Stomrud E, Zetterberg H, Karl J, Zink K, et al. Performance of fully automated plasma assays as screening tests for Alzheimer disease-related β-amyloid status. JAMA Neurol; 2019 Jun 24. doi: 10.1001/jamaneurol.2019.1632. [Epub ahead of print]
53 Ovod V, Ramsey KN, Mawuenyega KG, Bollinger JG, Hicks T, Schneider T, et al. Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. Alzheimers Dement. 2017;13:841-9.
54 Zetterberg H, Wilson D, Andreasson U, Minthon L, Blennow K, Randall J, et al. Plasma tau levels in Alzheimer's disease. Alzheimers Res Ther. 2013;5:9.
55 Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci. 2005;233:183-98.
56 Mattsson N, Andreasson U, Zetterberg H, Blennow K; Alzheimer's Disease Neuroimaging Initiative. Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. JAMA Neurol. 2017;74:557-66.
57 Zhao Y, Xin Y, Meng S, He Z, Hu W. Neurofilament light chain protein in neurodegenerative dementia: a systematic review and network meta-analysis. Neurosci Biobehav Rev. 2019;102:123-38.
58 Preische O, Schultz SA, Apel A, Kuhle J, Kaeser SA, Barro C, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. Nat Med. 2019;25:277-83.

59 Siedlecki-Wullich D, Català-Solsona J, Fábregas C, Hernández I, Clarimon J, Lleó A, et al. Altered microRNAs related to synaptic function as potential plasma biomarkers for Alzheimer's disease. Alzheimers Res Ther. 2019;11:46.
60 Bram JM, Talib LL, Joaquim HP, Sarno TA, Gattaz WF, Forlenza OV. Protein levels of ADAM10, BACE1, and PSEN1 in platelets and leukocytes of Alzheimer's disease patients. Eur Arch Psychiatry Clin Neurosci. 2019;269:963-72.
61 Cooper C, Sommerlad A, Lyketsos CG, Livingston G. Modifiable predictors of dementia in mild cognitive impairment: a systematic review and meta-analysis. Am J Psychiatry. 2015;172:323-34.
62 Braak H, Braak E. Staging of Alzheimer's disease-related neurofibrillary changes. Neurobiol Aging. 1995;16:271-8; discussion 278-84.
63 Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992;256:184-5.
64 Roses AD. Apolipoprotein E affects the rate of Alzheimer disease expression: beta-amyloid burden is a secondary consequence dependent on APOE genotype and duration of disease. J Neuropathol Exp Neurol. 1994;53:429-37.
65 Querfurth HW, LaFerla FM. Alzheimer's disease. N Engl J Med. 2010;362:329-44.
66 Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, et al. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. Science. 2010;330:1774.
67 Knopman DS, Jack CR Jr, Wiste HJ, Weigand SD, Vemuri P, Lowe VJ, et al. Brain injury biomarkers are not dependent on β-amyloid in normal elderly. Ann Neurol. 2013;73:472-80.
68 Drachman DA. The amyloid hypothesis, time to move on: amyloid is the downstream result, not cause, of Alzheimer's disease. Alzheimers Dement. 2014;10:372-80.
69 Salloway S, Sperling R, Fox NC, Blennow K, Klunk W, Raskind M, et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. N Engl J Med. 2014;370:322-33.
70 Serrano-Pozo A, Qian J, Monsell SE, Blacker D, Gómez-Isla T, Betensky RA, et al. Mild to moderate Alzheimer dementia with insufficient neuropathological changes. Ann Neurol. 2014;75:597-601.
71 Monsell SE, Kukull WA, Roher AE, Maarouf CL, Serrano G, Beach TG, et al. Characterizing apolipoprotein E ε4 carriers and noncarriers with the clinical diagnosis of mild to moderate Alzheimer dementia and minimal β-amyloid peptide plaques. JAMA Neurol. 2015;72:1124-31.
72 Nelson PT, Dickson DW, Trojanowski JQ, Jack CR, Boyle PA, Arfanakis K, et al. Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. Brain. 2019; 142:1503-27.
73 Nelson PT, Schmitt FA, Lin Y, Abner EL, Jicha GA, Patel E, et al. Hippocampal sclerosis in advanced age: clinical and pathological features. Brain. 2011;134:1506-18.
74 Suemoto CK, Ferretti-Rebustini RE, Rodriguez RD, Leite RE, Soterio L, Brucki SM, et al. Neuropathological diagnoses and clinical correlates in older adults in Brazil: a cross-sectional study. PLoS Med. 2017;14:e1002267.
75 Forlenza OV, Loureiro JC, Pais MV, Stella F. Recent advances in the management of neuropsychiatric symptoms in dementia. Curr Opin Psychiatry. 2017;30:151-8.
76 Stella F, Laks J, Govone JS, de Medeiros K, Forlenza OV. Association of neuropsychiatric syndromes with global clinical deterioration in Alzheimer's disease patients. Int Psychogeriatr. 2016; 28:779-86.
77 Canevelli M, Blasimme A, Vanacore N, Bruno G, Cesari M. Mild behavioral impairment: ethical, methodological and clinical reflections. Neurosci Biobehav Rev. 2016;69:402-3.
78 de Oliveira AM, Radanovic M, Homem de Mello PC, Buchain PC, Dias Vizzotto A, Harder J, et al. An intervention to reduce neuropsychiatric symptoms and caregiver burden in dementia: preliminary results from a randomized trial of the tailored activity program-outpatient version. Int J Geriatr Psychiatry. 2019;34:1301-7.
79 Perry EK, Gibson PH, Blessed G, Perry RH, Tomlinson BE. Neurotransmitter enzyme abnormalities in senile dementia. Choline acetyltransferase and glutamic acid decarboxylase activities in necropsy brain tissue. J Neurol Sci. 1977;34:247-65.
80 Adler G, Mueller B, Articus K. The transdermal formulation of rivastigmine improves caregiver burden and treatment adherence of patients with Alzheimer's disease under daily practice conditions. Int J Clin Pract. 2014;68:465-70.
81 Sarno TA, Talib LL, Joaquim HP, Bram JM, Gattaz WF, Forlenza OV. Protein expression of BACE1 is downregulated by donepezil in Alzheimer's disease platelets. J Alzheimers Dis. 2017;55: 1445-51.
82 Aprahamian I, Stella F, Forlenza OV. New treatment strategies for Alzheimer's disease: is there a hope? Indian J Med Res. 2013; 138:449-60.
83 Forlenza OV, Diniz BS, Gattaz WF. Diagnosis and biomarkers of predementia in Alzheimer's disease. BMC Med. 2010;8:89.
84 Novak P, Schmidt R, Kontsekova E, Zilka N, Kovacech B, Skrabana R, et al. Safety and immunogenicity of the tau vaccine AADvac1 in patients with Alzheimer's disease: a randomised, double-blind, placebo-controlled, phase 1 trial. Lancet Neurol. 2017;16:123-34.
85 Timmers M, Streffer JR, Russu A, Tominaga Y, Shimizu H, Shiraishi A, et al. Pharmacodynamics of atabecestat (JNJ-54861911), an oral BACE1 inhibitor in patients with early Alzheimer's disease: randomized, double-blind, placebo-controlled study. Alzheimers Res Ther. 2018;10:85.
86 Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimers Res Ther. 2014;6:37.
87 Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. Nat Med. 2003;9:448-52.
88 Van Bulck M, Sierra-Magro A, Alarcon-Gil J, Perez-Castillo A, Morales-Garcia JA. Novel approaches for the treatment of Alzheimer's and Parkinson's disease. Int J Mol Sci. 2019;20(3). pii: E719. DOI: 10.3390/ijms20030719.
89 Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature. 1999;400:173-7.
90 Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, et al. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. Neurology. 2005;64:1553-62.
91 Penninkilampi R, Brothers HM, Eslick GD. Pharmacological agents targeting γ-secretase increase risk of cancer and cognitive decline in Alzheimer's disease patients: a systematic review and meta-analysis. J Alzheimers Dis. 2016;53:1395-404.
92 Winblad B, Andreasen N, Minthon L, Floesser A, Imbert G, Dumortier T, et al. Safety, tolerability, and antibody response of active Aβ immunotherapy with CAD106 in patients with Alzheimer's disease: randomised, double-blind, placebo-controlled, first-in-human study. Lancet Neurol. 2012;11:597-604.
93 Bouter Y, Lopez Noguerola JS, Tucholla P, Crespi GA, Parker MW, Wiltfang J, et al. Abeta targets of the biosimilar antibodies of Bapineuzumab, Crenezumab, Solanezumab in comparison to an antibody against N-truncated Abeta in sporadic Alzheimer disease cases and mouse models. Acta Neuropathol. 2015;130:713-29.
94 Sperling RA, Rentz DM, Johnson KA, Karlawish J, Donohue M, Salmon DP, et al. The A4 study: stopping AD before symptoms begin? Sci Transl Med. 2014;6:228fs13.
95 Bateman RJ, Benzinger TL, Berry S, Clifford DB, Duggan C, Fagan AM, et al. The DIAN-TU next generation Alzheimer's prevention trial: adaptive design and disease progression model. Alzheimers Dement. 2017;13:8-19.
96 Zhao J, Nussinov R, Ma B. Mechanisms of recognition of amyloid-β (Aβ) monomer, oligomer, and fibril by homologous antibodies. J Biol Chem. 2017;292:18325-43.
97 Bohrmann B, Baumann K, Benz J, Gerber F, Huber W, Knoflach F, et al. Gantenerumab: a novel human anti-Aβ antibody demonstrates sustained cerebral amyloid-β binding and elicits cell-mediated removal of human amyloid-β. J Alzheimers Dis. 2012;28:49-69.
98 Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature. 2016;537:50-6.
99 Budd Haeberlein S, O'Gorman J, Chiao P, Bussière T, von Rosenstiel P, Tian Y, et al. Clinical development of aducanumab, an anti-Aβ human monoclonal antibody being investigated for the treatment of early Alzheimer's disease. J Prev Alzheimers Dis. 2017;4:255-63.
100 Selkoe DJ. Alzheimer disease and aducanumab: adjusting our approach. Nat Rev Neurol. 2019;15:365-6.

101 Yang LB, Lindholm K, Yan R, Citron M, Xia W, Yang XL, et al. Elevated beta-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. Nat Med. 2003;9:3-4.
102 Egan MF, Kost J, Tariot PN, Aisen PS, Cummings JL, Vellas B, et al. Randomized trial of verubecestat for mild-to-moderate Alzheimer's disease. N Engl J Med. 2018;378:1691-703.
103 Braak H, Del Tredici K. Evolutional aspects of Alzheimer's disease pathogenesis. J Alzheimers Dis. 2013;33(Suppl 1):S155-61.
104 Ryan NS, Keihaninejad S, Shakespeare TJ, Lehmann M, Crutch SJ, Malone IB, et al. Magnetic resonance imaging evidence for presymptomatic change in thalamus and caudate in familial Alzheimer's disease. Brain. 2013;136:1399-414.
105 Sheline YI, Morris JC, Snyder AZ, Price JL, Yan Z, D'Angelo G, et al. APOE4 allele disrupts resting state fMRI connectivity in the absence of amyloid plaques or decreased CSF Aβ42. J Neurosci. 2010;30:17035-40.
106 Wirth M, Madison CM, Rabinovici GD, Oh H, Landau SM, Jagust WJ. Alzheimer's disease neurodegenerative biomarkers are associated with decreased cognitive function but not β-amyloid in cognitively normal older individuals. J Neurosci. 2013;33:5553-63.
107 Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 2010;9:119-28.
108 Edmonds EC, Delano-Wood L, Galasko DR, Salmon DP, Bondi MW; Alzheimer's Disease Neuroimaging Initiative. Subtle cognitive decline and biomarker staging in preclinical Alzheimer's disease. J Alzheimers Dis. 2015;47:231-42.
109 Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. J Neuropathol Exp Neurol. 2011;70:960-9.
110 Ossenkoppele R, Smith R, Ohlsson T, Strandberg O, Mattsson N, Insel PS, et al. Associations between tau, Aβ, and cortical thickness with cognition in Alzheimer disease. Neurology. 2019;92:e601-12.
111 Stella F, Radanovic M, Canineu PR, de Paula VJ, Forlenza OV. Anti-dementia medications: current prescriptions in clinical practice and new agents in progress. Ther Adv Drug Saf. 2015;6:151-65.
112 Grundman M. Editorial: Tau based therapeutics: alternative approaches in the war on Alzheimer's disease. J Prev Alzheimers Dis. 2019;6:151-2.
113 Herrmann A, Spires-Jones T. Clearing the way for tau immunotherapy in Alzheimer's disease. J Neurochem. 2015;132:1-4.
114 Qureshi IA, Tirucherai G, Ahlijanian MK, Kolaitis G, Bechtold C, Grundman M. A randomized, single ascending dose study of intravenous BIIB092 in healthy participants. Alzheimers Dement (N Y). 2018;4:746-55.
115 Rafii MS, Aisen PS. Alzheimer's disease clinical trials: moving toward successful prevention. CNS Drugs. 2019;33:99-106.
116 Cummings J, Blennow K, Johnson K, Keeley M, Bateman RJ, Molinuevo JL, et al. Anti-tau trials for Alzheimer's disease: a report from the EU/US/CTAD task force. J Prev Alzheimers Dis. 2019; 6:157-63.
117 Chen G, Rajkowska G, Du F, Seraji-Bozorgzad N, Manji HK. Enhancement of hippocampal neurogenesis by lithium. J Neurochem. 2000;75:1729-34.
118 Forlenza OV, De-Paula VJ, Diniz BS. Neuroprotective effects of lithium: implications for the treatment of Alzheimer's disease and related neurodegenerative disorders. ACS Chem Neurosci. 2014;5:443-50.
119 Dell'Osso L, Del Grande C, Gesi C, Carmassi C, Musetti L. A new look at an old drug: neuroprotective effects and therapeutic potential of lithium salts. Neuropsychiatr Dis Treat. 2016;12:1687-703.
120 Phiel CJ, Wilson CA, Lee VM, Klein PS. GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. Nature. 2003;423:435-9.
121 Nunes PV, Forlenza OV, Gattaz WF. Lithium and risk for Alzheimer's disease in elderly patients with bipolar disorder. Br J Psychiatry. 2007;190:359-60.
122 Kessing LV, Søndergård L, Forman JL, Andersen PK. Lithium treatment and risk of dementia. Arch Gen Psychiatry. 2008;65:1331-5.
123 Fajardo VA, Fajardo VA, LeBlanc PJ, MacPherson RE. Examining the relationship between trace lithium in drinking water and the rising rates of age adjusted Alzheimer's disease mortality in Texas. J Alzheimers Dis. 2018;61:425-34.
124 Forlenza OV, Diniz BS, Radanovic M, Santos FS, Talib LL, Gattaz WF. Disease modifying properties of long-term lithium treatment for amnestic mild cognitive impairment: a randomized controlled trial. Br J Psychiatry. 2011;198:351-6.
125 Forlenza OV, Radanovic M, Talib LL, Gattaz WF. Clinical and biological effects of long-term lithium treatment in older adults with amnestic mild cognitive impairment: randomised clinical trial. Br J Psychiatry. 2019 Apr 5;1-7. doi: 10.1192/bjp.2019.76. [Epub ahead of print]
126 Macdonald A, Briggs K, Poppe M, Higgins A, Velayudhan L, Lovestone S. A feasibility and tolerability study of lithium in Alzheimer's disease. Int J Geriatr Psychiatry. 2008;23:704-11.
127 Hampel H, Ewers M, Burger K, Annas P, Mortberg A, Bogstedt A, et al. Lithium trial in Alzheimer's disease: a randomized, single-blind, placebo- controlled, multicenter 10-week study. J Clin Psychiatry. 2009;70:922-31.
128 Nunes MA, Viel TA, Buck HS. Microdose lithium treatment stabilized cognitive impairment in patients with Alzheimer's disease. Curr Alzheimer Res. 2013;10:104-7.
129 Forlenza OV, Aprahamian I, de Paula VJ, Hajek T. Lithium, a therapy for AD: current evidence from clinical trials of neurodegenerative disorders. Curr Alzheimer Res. 2016;13:879-86.
130 Kook SY, Hong HS, Moon M, Ha CM, Chang S, Mook-Jung I. $A\beta_{1-42}$-RAGE interaction disrupts tight junctions of the blood-brain barrier via Ca^{2+}-calcineurin signaling. J Neurosci. 2012;32:8845-54.
131 Burstein AH, Grimes I, Galasko DR, Aisen PS, Sabbagh M, Mjalli AM. Effect of TTP488 in patients with mild to moderate Alzheimer's disease. BMC Neurol. 2014;14:12-12.
132 O'Connor E, Farrow M, Hatherly C. Randomized comparison of mobile and web-tools to provide dementia risk reduction education: use, engagement and participant satisfaction. JMIR Ment Health. 2014;1(1):e4.
133 Gitlin LN, Winter L, Dennis MP. Assistive devices caregivers use and find helpful to manage problem behaviors of dementia. Gerontechnology. 2010;9:408-14.
134 Trahan MA, Kuo J, Carlson MC, Gitlin LN. A systematic review of strategies to foster activity engagement in persons with dementia. Health Educ Behav. 2014;41(1 Suppl):70S-83S.
135 Tennstedt SL, Unverzagt FW. The ACTIVE study: study overview and major findings. J Aging Health. 2013;25(8 Suppl):3S-20S.
136 Ngandu T, Lehtisalo J, Solomon A, Levälahti E, Ahtiluoto S, Antikainen R, et al. A 2-year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. Lancet. 2015;385:2255-63.
137 Matthews FE, Stephan BC, Robinson L, Jagger C, Barnes LE, Arthur A, et al. A two decade dementia incidence comparison from the Cognitive Function and Ageing Studies I and II. Nat Commun. 2016;7:11398.
138 Satizabal CL, Beiser AS, Chouraki V, Chêne G, Dufouil C, Seshadri S. Incidence of dementia over three decades in the Framingham heart study. N Engl J Med. 2016;374:523-32.
139 Wu YT, Beiser AS, Breteler MM, Fratiglioni L, Helmer C, Hendrie HC, et al. The changing prevalence and incidence of dementia over time – current evidence. Nat Rev Neurol. 2017;13:327-39.
140 Lobo A, Launer LJ, Fratiglioni L, Andersen K, Di Carlo A, Breteler MM, et al. Prevalence of dementia and major subtypes in Europe: a collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. Neurology. 2000; 54(11 Suppl 5):S4-9.
141 Mayer F, Di Pucchio A, Lacorte E, Bacigalupo I, Marzolini F, Ferrante G, et al. An estimate of attributable cases of Alzheimer disease and vascular dementia due to modifiable risk factors: the impact of primary prevention in Europe and in Italy. Dement Geriatr Cogn Dis Extra. 2018;8:60-71.