Biomarkers: Our Path Towards a Cure for Alzheimer Disease

Rawan Tarawneh

Department of Neurology, The Ohio State University, Columbus, OH, USA.

Biomarker Insights
Volume 15: 1–15

DOI: 10.1177/1177271920976367

SAGE

ABSTRACT: Over the last decade, biomarkers have significantly improved our understanding of the pathophysiology of Alzheimer disease (AD) and provided valuable tools to examine different disease mechanisms and their progression over time. While several markers of amyloid, tau, neuronal, synaptic, and axonal injury, inflammation, and immune dysregulation in AD have been identified, there is a relative paucity of biomarkers which reflect other disease mechanisms such as oxidative stress, mitochondrial injury, vascular or endothelial injury, and calcium-mediated excitotoxicity. Importantly, there is an urgent need to standardize methods for biomarker assessments across different centers, and to identify dynamic biomarkers which can monitor disease progression over time and/or response to potential disease-modifying treatments. The updated research framework for AD, proposed by the National Institute of Aging- Alzheimer's Association (NIA-AA) Work Group, emphasizes the importance of incorporating biomarkers in AD research and defines AD as a biological construct consisting of amyloid, tau, and neurodegeneration which spans pre-symptomatic and symptomatic stages. As results of clinical trials of AD therapeutics have been disappointing, it has become increasingly clear that the success of future AD trials will require the incorporation of biomarkers in participant selection, prognostication, monitoring disease progression, and assessing response to treatments. We here review the current state of fluid AD biomarkers, and discuss the advantages and limitations of the updated NIA-AA research framework. Importantly, the integration of biomarker data with clinical, cognitive, and imaging domains through a systems biology approach will be essential to adequately capture the molecular, genetic, and pathological heterogeneity of AD and its spatiotemporal evolution over time.

KEYWORDS: Alzheimer disease, biomarker, synapse, neuron, inflammation

RECEIVED: September 30, 2020. **ACCEPTED:** October 13, 2020.

TYPE: Novel Biomarkers of Neurodegenerative Disorders: Updates and Challenges – Review

FUNDING: The author disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Institute of Health- National Institute of Aging under Award Number R21AG067755 (RT).

DECLARATION OF CONFLICTING INTERESTS: The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Rawan Tarawneh, Department of Neurology, The Ohio State University, MD, 395 W. 12th Avenue, Columbus, OH 43210, USA. Email: rawan.altarawneh@osumc.edu

Introduction

Alzheimer disease (AD) is a global health epidemic and a significant cause of morbidity and mortality among elderly populations.[1,2] Estimates from the Alzheimer's Association and the World Health Organization (WHO) suggest that there are currently about 5.8 million people in the US, and over 35 million people in the world, with AD.[2,3] In the absence of effective ways to cure or prevent this disease, and with the aging of populations, the number of individuals with AD is expected to triple in the next 30 years.[4] The Alzheimer's Association and WHO predict that at least 15 million people in the US, and over 115 million people in the world, will have AD by the year 2050.[2,3] Perhaps equally alarming are recent reports suggesting that a new diagnosis of AD is made every 65 seconds in the US.[2] Despite several large national and international efforts to find effective ways to prevent or slow down disease progression, to this day—over a century from when it was first described by Alois Alzheimer- AD remains a disease without a cure.[5]

Extracellular aggregates of amyloid-β (Aβ) peptides (in the form of amyloid plaques) and intracellular aggregates of hyperphosphorylated tau (in the form of neurofibrillary tangles) are the 2 main pathological hallmarks of AD.[6–8] Other pathological substrates such as inflammation,[9] immune dysregulation, vascular injury, oxidative stress,[10] mitochondrial dysfunction[11], and calcium-mediated excitotoxicity[12] also appear to play a role in AD pathogenesis. These different pathologies eventually culminate in neuronal injury, synaptic dysfunction, and neurodegeneration leading to cognitive, behavioral, and functional decline in affected individuals.[13] There has been increasing interest over the last few years in identifying novel imaging or molecular markers which can accurately capture the various pathological processes involved in AD.[14] This information will significantly improve our understanding of the interplay between various AD pathologies in different stages of the disease, and will be critical in identifying novel disease mechanisms and pathways which extend beyond the "plaque and tangle" model.[15]

We here review the main advances in the AD biomarker field over the last decade (Figure 1). We discuss current and future roles for fluid biomarkers in improving diagnostic accuracy, patient stratification, prognostic assessments, and monitoring potential response to disease-modifying therapies in clinical trials of AD. Importantly, we highlight the importance of biomarkers in improving our understanding of the biological construct of AD, and identifying novel therapeutic targets for AD pathologies which overlap with other disorders across the neurodegenerative spectrum.

Biomarkers of Pathological Substrates of AD

Biomarkers of core AD pathologies: Amyloid and Tau

CSF Aβ42, total tau, and tau phosphorylated at threonine 181 (p-tau181) levels reflect the 2 main pathologies in AD and are well-established AD biomarkers.[16,17] Lower CSF Aβ42 levels

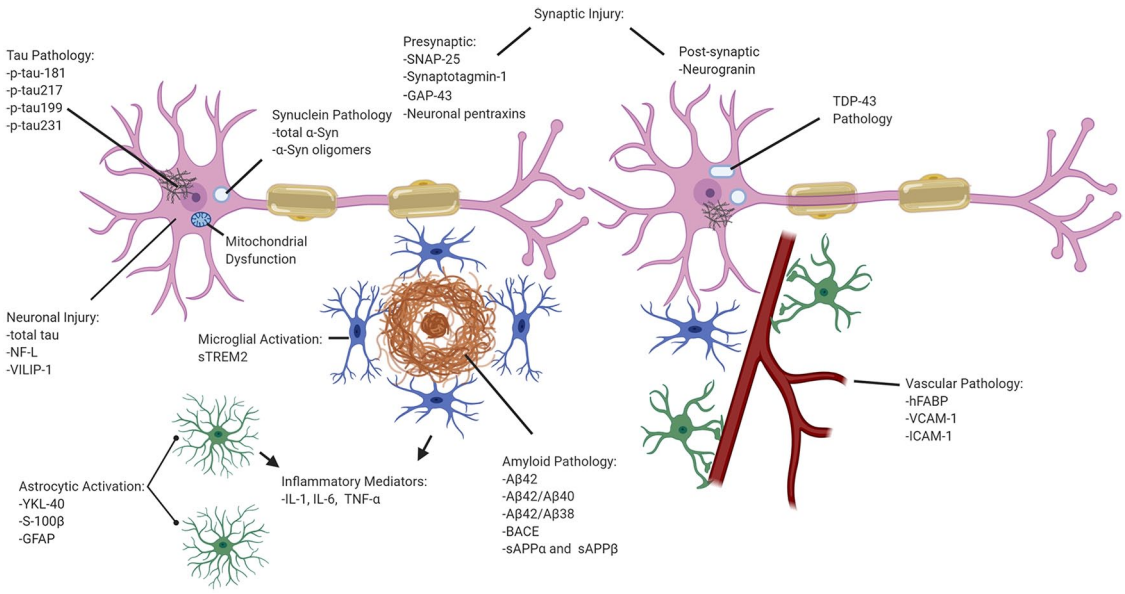

Figure 1. Biomarkers of different pathological substrates in Alzheimer disease (AD). This is a schematic diagram summarizing the most widely examined biomarkers that reflect different pathologies in AD. Abbreviations: Aβ, amyloid-peptide-β; BACE, β-secretase; GAP-43, growth-associated protein-43; GFAP, glial fibrillary acidic protein; hFABP, heart-type fatty acid binding protein; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; NF-L, neurofilament light chain; p-tau, hyperphosphorylated tau; sAPP, soluble amyloid CNS-derived exosomes precursor protein fragment; SNAP-25, synaptosomal-associated protein-25; sTREM2, soluble triggering receptor expressed on myeloid cells-2; α-Syn, α-synuclein; TDP-43, transactive response (TAR) DNA-binding protein-43; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; VILIP-1, visinin-like protein-1. This figure was created with BioRender.com.

are observed in AD compared to controls and result from amyloid aggregation in brain tissue via a sink mechanism,[18] while the aggregation of hyperphosphorylated tau in the form of neurofibrillary tangles and the accompanying neurodegeneration in AD brains are associated with increased levels of CSF p-tau181 and CSF tau.[17] Several clinicopathological studies have shown that the CSF biomarker phenotype of AD (ie, high CSF tau and/or p-tau181 and low CSF Aβ42) can be detected in the pre-symptomatic stages of disease and improves the diagnostic accuracy for AD from other causes of dementia in cognitively impaired individuals.[19] Moreover, the CSF tau/Aβ42 or CSF p-tau181/Aβ42 ratio is a strong predictor of the progression of AD pathology and future cognitive impairment in cognitively normal individuals over a 5-10 year follow-up period.[20,21]

While CSF levels of shorter Aβ peptides (ie, Aβ40 and Aβ38) have limited diagnostic utility in AD,[22] measurements of the CSF Aβ42/Aβ40 or Aβ42/Aβ38 ratios offer several advantages over absolute CSF Aβ42 levels. CSF Aβ42/Aβ40 and Aβ42/Aβ38 ratios are more closely associated with amyloid load on amyloid positron-emission tomography (PET) scans,[23] better differentiate AD from non-AD dementias,[23] and may be a better measure of target engagement in clinical trials of amyloid-based treatments than CSF Aβ42 alone. The use of the CSF Aβ42/Aβ40 ratio corrects for confounding effects arising from possible differences in CSF dynamics or rates of amyloid production among different individuals and reduces the influence of various pre-analytical factors on Aβ measurements.[24,25] Furthermore, in combination with CSF Aβ42, CSF Aβ40 offers a useful measure for target engagement of β-secretase (BACE1) inhibitors.[26] Reduced CSF Aβ42 and Aβ40 levels along with an increase in shorter fragments such as CSF Aβ37 and Aβ38 may be useful in tracking biological response to treatment with γ-secretase modulators.[27]

Although CSF p-tau181 has perhaps been the most widely examined form of hyperphosphorylated tau in AD, other p-tau species have also been investigated as potential AD biomarkers.[28] One comparative biomarker study examined the diagnostic accuracy of 3 CSF p-tau species (p-tau181, p-tau231, and p-tau199) in the same cohort.[28] In this study, CSF p-tau231 and p-tau199 demonstrated comparable specificity to CSF p-tau181 in differentiating AD from healthy controls. CSF p-tau231 and p-tau181, but not CSF p-tau199, provided high sensitivity and specificity in differentiating AD from non-AD dementias.[28] CSF p-tau231 improved the discrimination of AD from frontotemporal dementia (FTD), while CSF p-tau181 was useful in differentiating AD from Lewy body dementia (LBD).[28]

Importantly, more recent studies have shown that CSF p-tau217 levels are increased in AD and provide better diagnostic performance in differentiating AD from non-AD dementias than CSF p-tau181.[29] In the Swedish BioFINDER study, baseline and longitudinal measurements of CSF p-tau217 correlated with cortical tau deposition, measured by the PET tau tracer [^{18}F] flortaucipir, to a better extent than CSF p-tau181.[29] Furthermore, in this study, CSF p-tau217 showed stronger correlations with CSF and PET measures of cortical amyloid deposition compared to CSF p-tau181.[29]

There has been great interest in identifying peripheral disease-specific markers of AD.[30] Blood-based biomarkers are less invasive, costly, and time-consuming than CSF markers; and therefore, are likely to be more accessible as screening and stratification tools in clinical settings and research trials. Several studies across different cohorts have shown that p-tau181 levels can be accurately measured in the blood.[31–33] Plasma p-tau181 levels are increased early in AD, differentiate AD from cognitively normal controls and from other dementias, and correlate with cortical tau and amyloid deposition on PET imaging and with tau pathology at autopsy.[34] Furthermore, plasma p-tau181 levels offer useful prognostic markers in AD as they increase with disease progression over time and predict future progression to AD dementia in individuals with mild cognitive impairment (MCI).[34]

More recently, plasma p-tau217 has garnered significant attention as a promising blood-based biomarker for AD.[35] In a cross-sectional study in 3 well-characterized cohorts, plasma p-tau217 more accurately distinguished neuropathologically-confirmed AD from non-AD dementias compared to plasma p-tau181 or brain atrophy measures on magnetic resonance imaging (MRI), and to a comparable degree to CSF p-tau217, CSF p-tau181, and tau PET scans.[35] Furthermore, plasma p-tau217 levels were found to be significantly increased ~20 years prior to anticipated symptom onset in presenilin-1 (PSEN1) E280A mutation carriers compared to non-carriers and correlated with lower memory performance.[35]

The identification and validation of accurate and reliable blood-based markers of amyloid pathology has been more challenging.[36] Plasma or serum Aβ42 levels are 10 to 100-fold lower than CSF levels,[36] and Aβ epitopes may be masked by Aβ binding to plasma proteins.[37] These factors, and others (eg, variable peripheral sources of Aβ), have hindered the ability to obtain reliable and consistent peripheral measures of Aβ across different laboratories and study cohorts using conventional enzyme-linked immunoassays.[36]

More recent advances in peripheral Aβ measurements using immunoaffinity-based assays such as immunomagnetic reduction (IMR),[38] single- molecule arrays (SIMOA),[39] and the combination of immunoprecipitation and liquid-chromatography mass-spectrometry (IPMS)[40] offer promising tools for accurate quantification of peripheral Aβ levels in AD and will likely facilitate the standardization of blood-based Aβ markers across different centers. Novel assays, which utilize immunoprecipitation coupled with mass spectrometry or SIMOA, have allowed the measurement of plasma Aβ with high precision and demonstrate the ability of plasma Aβ40/Aβ42 levels to accurately predict amyloid-positive PET scans in cognitively normal or impaired individuals.[41,42] The SIMOA platform has also been implemented successfully for plasma p-tau181 measurements, and demonstrated the ability of plasma p-tau181 to accurately predict increased brain amyloid and tau on PET scans.[43]

Other studies suggest that CSF levels of the β-secretase (BACE1) are increased in MCI due to AD and AD dementia compared to controls, especially in individuals with the apolipoprotein E4 *(APOE4)* genotype;[44] however, results from other studies have been conflicting. No changes in CSF BACE1 levels were observed following treatment with BACE inhibitors, although there was a significant reduction in downstream CSF Aβ42 levels in treated patients.[45] Other studies suggest that higher CSF levels of the amyloid precursor protein (APP) metabolism products, sAPPα and sAPPβ, improve the differentiation of AD from other dementias.[46]

Biomarkers of neuronal and synaptic injury

Several biomarkers of neuronal and synaptic injury have been identified in the last decade. Of these, the most promising markers of neuronal injury are neurofilament-light chain (NF-L)[47–49] and visinin-like protein-1 (VILIP-1).[20,50,51] Among the most promising markers of synaptic injury in AD are the post-synaptic protein neurogranin (Ng),[52,53] and the pre-synaptic proteins synaptosome–associated protein-25 (SNAP-25)[54,55] and synaptotagmin-1 (Syt-1),[56] although several others have been examined.[57] The abundant expression and relative neuronal specificity of these markers allow them to reliably reflect neuronal and synaptic loss, as their CSF levels correlate with damage to neuronal and synaptic structures and the release of abundant neuronal or synaptic constituents into the extracellular compartment in the setting of neurodegeneration.

Neurofilament-light chain (NF-L) is a soluble and highly expressed component of neuronal axons.[58] Elevated CSF and plasma NF-L levels measure neuronal injury and appear to be promising markers of AD severity and progression.[47,49] CSF NF-L levels are increased in the pre-symptomatic and early symptomatic stages of AD and correlate with cognitive decline, progression of brain atrophy, and decreased survival.[47] Furthermore, the implementation of ultrasensitive biomarker assays using SIMOA has allowed the quantification of NF-L in blood samples.[49] Plasma or serum NF-L levels differentiate pre-symptomatic and early symptomatic AD from controls in studies of familial and sporadic AD,[49,59] and accurately predict rates of disease progression over time, offering a useful, although relatively nonspecific, peripheral measure of disease severity.[59,60]

VILIP-1 is an abundant neuronal calcium-sensor protein which is widely distributed in the human brain.[61] Studies across different cohorts have shown that CSF VILIP-1 is a promising marker of neuronal injury in AD[20,50,51,62] with relative specificity for AD.[20] CSF VILIP-1 levels are increased in MCI due to AD and AD dementia compared to controls and other dementias.[20] CSF VILIP-1 levels are closely associated with markers of tau pathology, can predict rates of whole brain and regional atrophy[50] and amyloid load[20] in symptomatic and pre-symptomatic AD, respectively. Importantly, CSF VILIP-1 or VILIP-1/Aβ42 is potentially a stronger predictor of cognitive decline and whole brain or regional atrophy in cognitively impaired individuals, and a stronger predictor of future

cognitive impairment in cognitively normal individuals, than CSF tau, p-tau181, or Aβ42 and tau/Aβ42 or p-tau181/Aβ42, respectively.[20,50,51] The addition of CSF VILIP-1 to CSF tau, p-tau181, and Aβ42 increases the predictive utility of these markers to detect preclinical AD pathology. Plasma VILIP-1 levels are also increased in AD compared to controls, although to a lesser extent than CSF VILIP-1 levels.[20]

Synaptic loss is an early and important feature in AD.[63] Cortical and hippocampal synaptic density is reduced by approximately 30% and 50% respectively, in even the earliest symptomatic stages as a result of both neuronal loss and reduced synaptic density per neuron.[64,65] While reduced expression levels of several synaptic proteins are observed in postmortem studies of AD brains;[66] CSF levels of several pre-synaptic and post-synaptic proteins are increased in AD, likely due to synaptic degeneration and the release of abundant synaptic constituents into the extracellular space.

Ng is a promising biomarker of synaptic injury which has been studied by many groups across different centers.[53,67-69] Ng is a calmodulin-binding neuronal protein which is abundantly expressed in the post-synaptic membrane and is involved in synaptic plasticity, long-term potentiation (LTP), and memory functions.[53] Previous studies across different cohorts and using different immunoassays have shown that CSF Ng is a useful diagnostic and prognostic biomarker in AD; baseline CSF Ng levels strongly correlate with rates of cognitive decline and brain atrophy in AD,[53,67,68] correlate with cortical amyloid deposition on amyloid PET scans in cognitively normal individuals, and can predict future cognitive impairment in cognitively normal individuals at least as well as CSF tau and Aβ42 levels.[53] Moreover, CSF Ng levels offer relative diagnostic specificity for AD as they differentiate AD from most other neurodegenerative disorders.[69] Plasma Ng levels have limited utility as markers in AD; however, recent studies examining Ng in neuronally-derived blood exosomes report lower levels, reflective of reduced brain expression, in AD compared to controls,[70,71] and in individuals with MCI who progressed to dementia compared to those with MCI who did not progress.[71]

Fewer studies have examined pre-synaptic proteins as CSF biomarkers in AD. SNAP-25 is involved in vesicle docking, neurotransmitter release, and neurite outgrowth.[72] Higher CSF levels of soluble SNAP-25 fragments are observed in AD compared to controls, and correlate with brain atrophy and future risk of cognitive decline.[54,55] Altered CSF SNAP-25 levels have also been reported in primary psychiatric and other neurodegenerative conditions.[72] Two splicing variants of SNAP-25, referred to as SNAP-25a and SNAP-25b, which differ in 9 amino acid residues, have been identified.[73] Whether CSF levels of the 2 isoforms have different diagnostic or prognostic utility in AD remains to be investigated. Growth-associated protein-43 (GAP-43, neuromodulin) has important roles in axonal branching and synaptic plasticity,[74] and facilitates vesicle recycling via its interactions with synaptophysin and SNAP-25 in the pre-synaptic compartment.[75] CSF GAP-43 levels are increased in early symptomatic AD compared to controls and most other neurodegenerative conditions, and correlate with amyloid and tau pathology in AD.[76,77]

Synaptotagmin-1 (Syt-1) is a pre-synaptic protein which is part of the calcium-dependent vesicle translocation machinery and acts as the calcium sensor protein for fast synchronous release.[78] In a recent study, CSF Syt-1 levels measured using an immunoprecipitation- mass spectrometry-based assay were increased in the earliest symptomatic stages of AD, including MCI, compared to controls.[56] Another comparative biomarker study utilizing the same method for CSF Syt-1 quantification demonstrated increased CSF Syt-1 levels in MCI due to AD and AD dementia compared to controls and other dementias, although the diagnostic accuracy of CSF Syt-1 in differentiating MCI due to AD or AD dementia from controls in this cohort was lower than that of CSF Ng and SNAP-25aa40.[79]

Neuronal pentraxins (NPTX, NP) are pre-synaptic glycoproteins which play an important role in the assembly of α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA)-mediated excitatory synapses.[80] NP1 is a pro-apoptotic protein which downregulates synaptic density and is detected within dystrophic neurites in human AD brains.[81,82] Aβ oligomers stimulate the pre-synaptic release of NP1 which contributes to synaptic and mitochondrial deficits observed in the presence of amyloid pathology.[82] Conversely, the knockdown of NP1 in animal models is associated with an increased number of cortical excitatory synapses, enhanced neuronal excitability, and facilitation of hippocampal LTP.[81] Plasma NP1 levels are increased in MCI compared to controls and further increases are observed in MCI patients who progress to AD dementia.[83] Other studies suggest that CSF NP2 (ie, NPTX2) levels are associated with resting-state functional connectivity in cognitively normal elderly,[84] and that CSF levels of the pentraxin receptor, NPTXR, are associated with increased dementia severity in early symptomatic AD.[85] Other potential synaptic markers have been proposed including synapsin, synaptophysin, syntaxin-1B, actin-associated protein Arc, neurofascin, members of the Rab family, SV2A, contactin-2, and neurexins.[86-88] Data regarding the utility of these proteins as CSF markers of synaptic injury is currently limited, and further research may be warranted.

Biomarkers of neuroinflammation and immune dysregulation

Emerging data from animal and clinical studies suggest a significant role for innate and adaptive immune dysregulation in AD pathogenesis, and highlight the notion that immune dysregulation is a central pathological substrate of AD which may directly contribute to neurodegeneration.[9,89] The "immune hypothesis of AD" has been supported by a rapidly growing body of genetic,[90,91] histopathological,[92] and mechanistic[90] evidence. Disturbances in innate immunity including cytokine signaling, immune cell proliferation and migration, and

microglial activation, are observed in animal models of AD.[93] Large-scale genetic analyses suggest that over half of the AD risk loci are significantly enriched or uniquely expressed in immune cells.[94] The activation of inflammatory cascades exacerbates amyloid and tau pathologies in animal models, and may serve as a link between early Aβ deposits and subsequent tangle pathology in pre-symptomatic AD.[95]

Microglia are resident immune cells within the CNS which are derived from peripheral macrophages and play important roles in immune surveillance in the brain. In AD, microglia are primarily activated by extracellular Aβ, and mediate Aβ phagocytosis through the release of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α).[96] A state of immune dysregulation occurs in AD, in which chronically activated microglia are less capable of clearing amyloid deposits and exacerbate synaptic and neuronal damage by triggering p53-mediated apoptosis of neural progenitor cells (NPCs) and disturbing synaptogenesis.[97] Upregulation of the pro-inflammatory cytokines IL-18 and IL-12p70, both produced by microglia, is associated with increased amyloid pathology in cell cultures and animal models.[98,99] Conversely, increased expression of the anti-inflammatory cytokine, IL-10, is associated with reduced amyloid burden due to regulation of microglial activity,[100] and increased neuronal survival through the astrocytic release of transforming growth factor – β (TGF-β).[101]

Central and peripheral levels of cytokines and other inflammatory mediators, and their associations with the pathological burden and cognitive outcomes in AD, have been examined with mixed results.[102,103] Brain and CSF levels of TNF-α, which is derived from neurons, microglia, and astrocytes, are increased in AD, and correlate with the risk of progression from MCI to AD dementia.[104,105] Higher CSF levels of TNF-α converting enzyme (TACE, ADAM17) and soluble TNF receptors have also been reported in AD.[106] Data regarding blood TNF-α levels have been conflicting.[107] Increased microglial expression of IL-1 in AD is associated with amyloid and tau pathologies, cholinergic dysfunction, and impairment of LTP.[108] IL-1 interacts with βAPP, α-macroglobulin, and *APOE*, and genetic variants of IL-1 have been shown to influence risk for AD.[109] Increased plasma IL-1β levels, likely of central origin, have been reported in AD dementia, but not MCI, compared to controls.[110] Aβ increases microglial and astrocytic expression of IL-6 in cell cultures and IL-6 is associated with diffuse plaques in human AD brains.[111] A recent meta-analysis of 175 studies of blood inflammatory markers found higher levels of IL-1β, IL-2, IL-6, IL-18, interferon-γ, homocysteine, TACE, soluble TNF receptors 1 and 2, α1-antichymotrypsin, high-sensitivity C reactive protein, C-X-C motif chemokine ligand-10 (CXCL-10, also known as interferon- γ-induced protein-10 [IP-10]), epidermal growth factor, vascular cell adhesion molecule-1 (VCAM-1), and lower levels of IL-1 receptor antagonist and leptin, in AD compared with controls, and inverse correlations of peripheral IL-6 levels with cognition.[112] Plasma levels of the inflammatory marker, IP-10 (CXCL-10), differentiated AD from controls in a spatiotemporal analysis of data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort.[113]

Triggering Receptor Expressed on Myeloid Cells-2 (TREM2) is a receptor of the innate immune system which supports protective microglial functions including phagocytosis and chemotaxis.[114] Perhaps some of the most compelling evidence supporting a role for innate immune dysregulation in AD pathogenesis is provided by recent studies showing that TREM2 variants are associated with a significantly increased risk for AD.[114,115] Deficiency or loss of TREM2 is associated with reduced number and phagocytic ability of plaque-associated macrophages, increased amyloid plaque burden, and worse pathological outcomes in AD mouse models.[9,116] Furthermore, TREM2 haplo-deficiency in mice is associated with axonal dystrophy and increased soluble and insoluble tau pathology.[117] Several studies have demonstrated the utility of soluble TREM2 (sTREM2) as a CSF surrogate of TREM2-mediated microglial activation in sporadic and familial AD.[118,119] In one study, CSF sTREM2 levels were increased approximately 5 years prior to anticipated symptom onset in familial AD and following biomarker evidence of amyloid and tau deposition.[120] Converging data from animal models and longitudinal clinical studies suggest that changes in CSF sTREM2 levels are detected in even the earliest preclinical stages of AD, and that the direction of such alterations may be differentially affected by the presence of amyloid or tau pathology and disease stage.[9]

YKL-40 (also known as chitinase-3-like-1 protein) is a glycoprotein which is expressed by astrocytes near amyloid plaques and is involved in inflammation and angiogenesis.[121] Elevated CSF YKL-40 levels have been reported in several studies of pre-symptomatic and symptomatic AD compared to controls, and are associated with markers of amyloid, tau, and synaptic injury, but not *APOE4*, in AD.[22,122,123] YKL-40 levels are associated with lower cortical thickness in individuals with the *APOE4* genotype[124] or those with low CSF Aβ42[125] and predict progression from MCI to AD dementia.[126] Other studies have shown that CSF YKL-40 levels can predict the presence of tau pathology in AD.[123] CSF YKL-40 has demonstrated utility as a marker of astrogliosis in AD; however, it may not be useful in differentiating AD from other dementias.[127,128] CSF YKL-40 levels are influenced by gender, ethnicity, and genetic polymorphisms of the CHI3L1 gene.[129]

Other astrocytic proteins which have been examined as potential markers in AD include S-100β and glial fibrillary acidic protein (GFAP). Elevated CSF S-100β levels in AD likely reflect increased S-100β expression in astrocytes, and possibly microglia, surrounding amyloid plaques,[130] but are not specific for AD.[131] In AD, S-100β appears to be involved in the inflammatory response, and may contribute to tau pathology and increased amyloid deposition through activation of β-secretase.[132] GFAP is an intermediate filament expressed by

astrocytes near amyloid plaques. In AD brains, there is increased expression of both GFAP isoforms, GFAPα and GFAPδ, in astrocytic long processes and a gradual increase in GFAP+[1] isoform expression in a subset of astrocytes which correlates with disease progression.[133] Elevated CSF GFAP levels have been reported in AD compared to controls, with higher levels being observed in FTD compared to AD or LBD.[134]

Markers of adaptive immunity have also been examined in AD. Findings include altered regulatory T lymphocyte profiles,[135] recruitment of macrophages/monocytes via CCL2 or CX3CL1 into the brain,[136] lower peripheral numbers of CCR2+ monocytes in AD compared to controls, and production of anti-Aβ antibodies by B lymphocytes.[137] A recent study using mass spectrometry suggests the presence of a potential immune signature of AD, characterized by increased peripheral numbers of CD8+ T effector memory CD45RA+ (TEMRA) cells, negative correlations of CD8+ TEMRA cells with cognition, and the presence of a clonally expanded CD8+ TEMRA cell population in the CSF of AD patients compared to controls.[138] Other inflammatory mediators involved in AD which may be suitable targets for novel biomarker discovery include eotaxins,[139] CX3CL1 (also known as fractalkine),[140] and nuclear factor κB (NFκB).[141]

Biomarkers of vascular pathology

Approximately 50% to 80% of aging and AD brains have variable degrees of concomitant vascular pathology, including atherosclerosis, small vessel disease, microvascular degeneration, blood-brain barrier dysfunction and cerebral amyloid angiopathy.[142,143] Cerebrovascular alterations, including disturbances in the blood-brain barrier, are common in pre-symptomatic AD,[144] and are observed in AD mouse models prior to amyloid deposition or cognitive deficits.[145] Findings from a recent study which examined the spatiotemporal changes in various fluid and imaging markers of AD in the ADNI cohort suggest that vascular dysfunction is an early and important feature of sporadic AD which precedes Aβ and tau pathologies, glucose hypometabolism, and brain atrophy.[113] There is also growing evidence that vascular disturbances exacerbate other pathologies including amyloid and tau aggregation, oxidative stress, inflammation, and may directly contribute to synaptic loss and neurodegeneration.[145] Vascular and amyloid pathologies have bidirectional relationships in AD; Aβ-mediated activation of perivascular macrophages results in the formation of oxygen free radicals, culminating in endothelial dysfunction.[146] Conversely, disruption of the blood-brain barrier, and the subsequent oxidative stress promotes Aβ aggregation through the activation of β- and γ- secretases[147] and impairs clearance of Aβ peptides from the brain.[148]

Only a few potential markers of vascular pathology have been examined in AD. Heart-type fatty acid-binding protein (hFABP), a protein associated with myocardial ischemia, has been proposed as a vascular marker in AD.[113] CSF hFABP levels are increased in AD and vascular dementia, and individuals with MCI who convert to AD dementia compared to controls or individuals with MCI who remain stable.[149] In combination with other markers, CSF hFABP may improve the discrimination of AD from other dementias.[150] CSF hFABP levels are associated with CSF Aβ42[151] and with longitudinal brain atrophy in individuals with amyloid deposition.[152]

CSF levels of the vascular markers, VCAM-1, intercellular adhesion molecule-1 (ICAM-1), IL-15, and fms-related receptor tyrosine kinase (Flt-1), are increased in pre-symptomatic and symptomatic AD compared to controls, and correlate with cortical thinning; CSF VCAM-1 and ICAM-1 levels also correlate with future cognitive decline.[153] Higher plasma levels of VCAM-1, ICAM-1, endothelin (ET-1), adrenomedullin (ADM), atrial natriuretic peptide (ANP), and sphingolipids are observed in early symptomatic AD.[154] Other markers such as von Willebrand factor (vWF), monokine induced by γ-interferon (MIG) (also known as the chemokine C-X-C motif ligand-9 [CXCL-9]) have been examined in vascular dementia and may warrant investigation as potential markers of vascular injury in AD.[155]

Biomarkers of oxidative stress and mitochondrial dysfunction

Oxidative stress and mitochondrial dysfunction play important roles in several neurodegenerative disorders, including AD.[156] Structural and functional alterations in mitochondria, including reduced mitochondrial numbers, loss of specific mitochondrial enzymes, and impaired mitochondrial fission and fusion, have been reported in AD.[11] These changes create a favorable environment for oxidative stress and bioenergetic failure in vulnerable neuronal populations. Importantly, there is a bidirectional relationship between amyloid or tau pathologies and mitochondrial dysfunction in AD.[156,157] Direct interactions between Aβ peptides, APP, or tau aggregates and the mitochondrial membranes or enzymatic complexes impair mitochondrial transport and promote the accumulation of toxic oxygen-free radicals.[157,158] Conversely, mitochondrial dysfunction and oxidative stress interfere with normal APP processing, increase the expression of β-secretase, enhance Aβ toxicity, and promote tau phosphorylation via activation of the glycogen synthase kinase 3-β (GSK-3β) in cell cultures and/or animal models of AD.[157,159]

Alterations in complex IV activity in platelets, abnormal levels of oxidative markers (eg, protein carbonyls, 3-nitro tyrosine, 3,4-dihydroxyphenylalanine, superoxide dismutase, nitric oxide synthase-2 (NOS2), heat shock protein-60 (HSP60), HSP72, and thioredoxin reductase in lymphocytes, and abnormal blood markers of lipid peroxidation (eg, protein-bound 4-hydroxy-2-nonenal [HNE]) have all been reported in AD.[160,161] There is a paucity of reliable biomarkers of oxidative stress and mitochondrial dysfunction in AD, and further research in this area is needed.

Biomarkers of TAR-DNA binding protein (TDP-43) pathology

TAR-DNA binding protein (TDP-43) is an RNA and DNA binding protein which is involved in the regulation of RNA transcription and splicing.[162] Cytoplasmic inclusions of hyperphosphorylated or ubiquitinated TDP-43 in the brain and/or spinal cord are characteristic pathological features of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD).[163] TDP-43 pathology is present in 20% to 50% of AD brains and up to 75% of those with severe dementia.[164,165] In AD, TDP-43 pathology is predominantly found in limbic structures such as the hippocampus and amygdala, and colocalizes with severe neuronal loss.[166] Several studies report associations between TDP-43 pathology and higher rates of brain atrophy and cognitive impairment in AD.[166] Furthermore, TDP-43 pathology contributes to inflammation, mitochondrial dysfunction, and neuronal/synaptic injury in AD, and interacts with Aβ and tau pathologies.[166,167] Recent studies suggest the presence of distinct molecular patterns of TDP-43 pathology in AD, including differences in phosphorylation patterns and the prevalence of truncated species, which influence the clinical phenotype and the prevalence of behavioral symptoms.[168]

Higher plasma TDP-43 levels in AD,[169] and higher serum levels of TDP-43 related variants in individuals with pre-MCI who later progressed to AD dementia,[170] have been reported. The measurement of CSF TDP-43 has been technically challenging.[30] However, as CSF TDP-43 appears to be mostly derived from the blood, measurements of TDP-43 in blood exosomes may serve as a useful surrogate for extracellular TDP-43 levels in the brain.

Biomarkers of synuclein pathology

Abnormal aggregation of the pre-synaptic protein α-synuclein (α-Syn) is a characteristic pathologic feature of Parkinson disease (PD), LBD, and multiple system atrophy (ie, synucleinopathies). Clinicopathological studies suggest that synuclein aggregation is a common comorbid pathology in AD.[171] Variable degrees of α-Syn pathology are observed in the neocortex, limbic system, and substantia nigra of most AD brains at autopsy, and correlate with extrapyramidal symptoms, visual hallucinations, and more rapid cognitive decline.[172,173] In AD brains, α-Syn pathology colocalizes with tau, and to a lesser extent, Aβ pathology.[174,175] Experimental evidence from animal studies supports the presence of significant interactions of α-Syn with tau and GSK3β, and α-Syn pathology appears to promote tau hyperphosphorylation.[171]

Several studies have shown lower CSF α-Syn in PD and LBD compared to controls and AD;[176,177] however, data regarding these as markers in AD have been inconsistent.[171] Consensus regarding CSF α-Syn profiles in neurodegenerative disorders has been limited by differences in assay techniques and the species of α-Syn measured by different laboratories. Higher CSF α-Syn levels in MCI, prodromal AD dementia, and AD dementia compared to controls or other synucleinopathies have been reported,[178,179] and shown to be associated with cognitive scores and an *APOE4*-dose-dependent risk of progression from MCI to AD dementia.[180,181] Conversely, no differences in CSF α-Syn between AD and controls were found in other cohorts.[182] In a recent meta-analysis, CSF α-Syn levels, when measured using conventional immunoassays, did not reliably differentiate individuals with AD from other synucleinopathies.[171]

CSF levels of oligomeric and phosphorylated forms of α-Syn, when measured by conventional assays, are not significantly altered in AD compared to controls.[179] However, recent biomarker assays which utilize techniques such as protein misfolding cyclic amplification (PMCA) and real time quaking-induced conversion (RT-QuIC) offer great promise in detecting oligomeric forms of α-Syn at low concentrations in the CSF or blood, and will provide valuable tools to reliably measure synuclein pathology across different neurodegenerative disorders.[183–185]

"Notable Mentions" in Recent Biomarker Research

MicroRNAs

MicroRNAs (miRNAs) are non-coding regulatory RNA molecules which are involved in post-transcriptional gene silencing and have recently gained significant attention as potential biomarkers in AD due to their stability, widespread expression, and easy detection in several tissues, including blood. Since miRNAs can reflect different pathogenic mechanisms, they offer additional tools to improve our understanding of AD pathology. A recent study suggests the presence of a unique 7-miRNA signature of AD which has a high accuracy in differentiating AD from controls and is enriched with target mRNAs involved in lipid metabolism.[186] Other miRNA signatures in AD have been proposed,[187] including a panel of 10 miRNAs which are deregulated in early pre-symptomatic AD, almost 20 years prior to symptom onset and are associated with the immune system, stress response, nerve growth factor signaling, Wnt signaling, and Rho GTPases.[188] As miRNAs in blood or brain tissue offer promising markers for AD, it will be important to standardize assays, storage time, and quantification methods in order to allow for validation of results across different centers.

Exosomes

Exosomes are small extracellular vesicles (30-150 nanometers [nm] in diameter) which are released from almost all cell types and carry a large variety of molecular cargoes, including DNA, RNA, lipid, and protein. Brain-derived exosomes originate from neurons, oligodendrocytes, microglia, astrocytes, and endothelium and serve important functions in cell signaling, neuron-glia interactions, and cell waste recycling.[189] Recently, exosomes have been found to be a valuable resource to examine

brain biomarkers as they can be easily extracted from various body fluids (eg, blood, CSF, and urine) and can provide reliable measures of brain expression levels of different proteins and miRNAs.[190]

Exciting results have been obtained using exosome studies in the last few years. Levels of tau, p-tau181, p-S396-tau, and Aβ42 from brain-derived exosomes isolated from the blood were shown to be increased in AD compared to controls up to 10 years prior to symptom onset.[191] Blood exosome levels of several brain-derived synaptic proteins, including synaptophysin, synaptopodin, synaptotagmin-2, GAP-43, NPTX2, neurexin-2, synapsin-1, and Ng are decreased in AD compared to controls, and lower blood exosome levels of GluA4-containing glutamate receptor and neuroligin-1 correlated with cognitive decline.[70,192] Other studies have shown that individuals with AD have lower levels of cellular survival proteins in brain-derived blood exosomes compared to controls.[189] A few limitations, such as costly and time-consuming sample preparation, may preclude the use of exosome analyses in high-throughput studies. However, exosome studies still offer great promise in biomarker discovery or validation, as they overcome technical challenges which have hindered the accurate quantification of certain proteins (eg, α-Syn and Ng) in the blood, and represent important tools for drug delivery in trials of AD therapeutics.

Biomarkers and the Complex Clinicopathological Spectrum of Alzheimer Disease

Extensive research efforts and billions of dollars have been invested in the AD drug pipeline over the last 20 years; yet, results of clinical trials of disease-modifying therapies have largely been disappointing.[193] In some cases, clinical trial failures can be attributed to flaws in trial design, methodological concerns, suboptimal inter-rater reliability, inadequate power to detect desired study outcomes, and challenges related to translating data from animal models into human therapeutics.[194,195] Measuring disease-modifying effects over short durations of participant follow-up can be challenging while longer trial durations have higher rates of drop-out. Nevertheless, with the increasing numbers of failed trials over the last decade, it has become increasingly clear that failure of the AD research community to find an effective cure for AD cannot be solely attributed to flaws in clinical trial design or study methods.[194] Indeed, such failures have identified several gaps in our current understanding of AD pathology from a molecular and pathophysiological standpoint.[195] Importantly, data from clinical trials, clinicopathological, and animal studies of AD all support the notion that AD is not a unidirectional or linear disease process, but rather a multifactorial disease which involves complex, and often bi-directional, interactions between different pathological substrates.[144,196,197] New evidence suggests the presence of a significant degree of genetic, molecular, and pathological heterogeneity in AD[198-200]; so that the relative contributions of different pathologies may differ among individuals or among different stages of the disease within the same individual.[200]

The current proposed model of AD, supported by a large body of evidence from clinicopathological and animal studies, suggests the presence of a long preclinical phase of disease which spans 10 to 15 years prior to the first signs of memory loss or cognitive impairment.[201] This long preclinical (ie, pre-symptomatic) phase is characterized by early amyloid followed by tau pathology which subsequently cause neuronal loss and synaptic dysfunction. The first signs of cognitive impairment appear after significant neuronal and synaptic dysfunction has occurred in vulnerable brain regions.[201] Therefore, the preclinical phase represents a critical time-window during which individuals are the most likely to respond to disease-modifying treatments that target early amyloid or tau pathologies prior to significant neuronal or synaptic loss.[202,203] Most clinical trials of AD therapeutics stratify patients based on their clinical presentation (ie, cognitively impaired due to suspected AD pathology vs cognitively normal) and include individuals in the early symptomatic stages (ie, MCI or mild AD dementia) in the AD arm. However, our current understanding of the AD model suggests that by the time individuals with AD become symptomatic it may already be "too late" to intervene.[204] In other words, early symptomatic AD is perhaps synonymous with an already advanced stage of AD pathology from a molecular and pathological standpoint.[204] Furthermore, observations from failed clinical trials suggest that many individuals with a clinical diagnosis of AD are later found to have alternative non-AD diagnoses, which strongly suggests that a clinical diagnosis of AD alone is insufficient for inclusion into clinical trials.[205]

The current model of AD provides another possible explanation for the failure of investigational AD therapeutics. Most disease-modifying AD therapies examined in clinical trials target different elements of the amyloid cascade, including both soluble and insoluble forms of the Aβ peptide.[206-208] Conversely, there has been a relative paucity of investigational treatments in the AD drug pipeline which are directed against other pathological substrates including tau, oxidative stress, or calcium-mediated excitotoxicity.[209,210] The amyloid hypothesis of AD, which suggests that amyloid pathology is an essential step in AD pathogenesis, is supported by evidence from clinicopathological studies of dominantly inherited and sporadic AD.[211,212] However, it has become widely accepted that, although amyloid pathology is an early and possibly critical process in disease pathogenesis, amyloid deposition alone is insufficient to drive the disease into the symptomatic stages.[14,213,214] In fact, recent emerging data from clinical and animal studies strongly converge on the notion that tau pathology on a background of amyloid deposition plays a more important role in driving the pathological, radiological, and clinical disease progression, particularly in the late pre-symptomatic and early symptomatic stages of the disease.[213] Therefore, amyloid deposition is considered a pre-requisite for AD, but tau,[213] and possibly other

"secondary" pathologies,[14] are also needed to drive the disease into the symptomatic stages.[202,213,215]

Over the last decade, there has been a great interest in the identification of fluid biomarkers which can reliably measure different AD pathologies, and accurately reflect the key molecular, pathway, or cellular abnormalities that occur in different stages of the disease.[202] Importantly, there is an urgent need to identify "dynamic" biomarkers which track with disease progression over time and whose levels may change in response to disease-modifying treatments.[216] From a clinical standpoint, the incorporation of biomarkers into clinical practice will allow early detection of disease pathology in the preclinical stages, improve diagnostic accuracy in the early symptomatic stages, and inform prognostic assessments.[202] While these remain promising future goals, several current limitations must be addressed before these objectives can be realized into clinical practice. These include the need for biomarker validation across different cohorts[217] including those with diverse ethnic/racial backgrounds, standardization of the methods used in biomarker measurements across different laboratories,[217-219] and a better understanding of the longitudinal changes in biomarker measures over time in relationship to disease severity, age, sex, population- based differences, and therapeutic response within and between individuals.[219-222] Importantly, early detection will likely not translate into improved outcomes until effective disease-modifying therapies are identified, which can alter disease pathology and whose benefits extend beyond the stabilization of clinical symptoms.

From a research standpoint, the incorporation of biomarkers into the design of clinical trials has improved patient stratification, as it has allowed the inclusion of individuals in the pre-symptomatic stages of the disease prior to significant neuronal loss, and those who are at the highest risk for cognitive decline.[223] Such an approach allows the enrichment of clinical trials with participants who are the most likely to respond to disease-modifying drugs at a time when such treatments are most likely to be effective, and thereby increase the power of studies to detect drug effects during the relatively short durations of follow-up.[202] This has recently been applied in several clinical trials of investigational anti-amyloid therapies in cognitively normal individuals with significant amyloid deposition on amyloid PET scans, and those with AD genetic risk factors or disease-causing mutations. Additionally, the use of preclinical fluid or imaging biomarkers allows for the exclusion of cognitively normal individuals who harbor preclinical AD pathology from the study control groups, and improves the diagnostic accuracy of AD in symptomatic individuals compared to clinical assessments alone.[205]

Synaptic and neuronal loss reflects the outcome of different pathological substrates,[13,224] and is more closely associated with clinical, cognitive, and radiological disease progression than amyloid, tau, inflammation, or gliosis in AD brains.[225,226] Tau and Aβ oligomers contribute to cognitive impairment through direct toxic effects on synaptic structures, independently of aggregated amyloid or tau.[227] Therefore, fluid markers which capture neuronal, synaptic, and axonal injury reflect the cumulative outcome of different pathological substrates in AD, and are likely to be better predictors of baseline and longitudinal clinical, cognitive, and structural imaging outcomes than CSF tau or Aβ42.[20,50,53] Elevated levels of these markers in the presence of abnormal CSF tau and Aβ42 levels, signify a high risk for imminent cognitive decline in cognitively normal individuals. Furthermore, combining CSF markers of neuronal or synaptic injury with markers of amyloid and tau pathology significantly improves the collective ability of these markers to predict future cognitive decline over short follow-up periods (ie, 2-3 years).[20,53] Therefore, the inclusion of cognitively normal or impaired individuals who have evidence of amyloid and/or tau pathology, and elevated levels of neuronal, synaptic, or axonal injury markers into clinical trials of symptomatic or disease-modifying treatments will improve participant stratification and enrich trials with individuals who are the most likely to meet clinical or cognitive outcomes over short follow-up periods and thereby, increase the chances of trial success (eg, when measuring outcomes such as clinical or radiological disease progression). On the other hand, the inclusion of cognitively normal individuals who have evidence of significant amyloid and/or tau pathology, but have not yet developed significant neuronal or synaptic loss, may help identify individuals who are more likely to benefit from disease-modifying treatments that alter early pathologies in secondary prevention trials that delay or prevent progression of tau pathology, neuronal loss, and prevent future cognitive decline.

Furthermore, markers of synaptic, neuronal, and axonal injury offer valuable tools to measure responses to therapeutic interventions which target different pathologies, and monitor their change over time, independently of changes to primary therapeutic targets such as CSF Aβ42 or tau.[20,47] Therefore, these markers may provide useful secondary or tertiary outcome measures in clinical trials, and complement information provided by clinical, functional, or imaging assessments. While several limitations remain to be addressed regarding the need to standardize assay techniques and reference values across laboratories and among different cohorts, a few clinical trials have successfully implemented fluid markers of neuronal/synaptic injury as exploratory outcome measures (eg, CSF Ng and NF-L in the AHEAD trial).

Importantly, the use of biomarkers in clinical and translational AD research will address major limitations in our current understanding of AD pathology, including gaps in our understanding of the temporal sequence of events and the complex interactions between different AD pathologies over time.[223] Such information will inform future trials of AD therapeutics by highlighting novel disease mechanisms or interactions between different pathologies and identifying potential therapeutic targets.[202,223] Theoretically, the use of standardized AD biomarkers may allow the identification of different signatures of AD pathology, or different molecular subtypes of AD

among different individuals, which will ultimately pave the way for future personalized therapies when disease-modifying treatments become available.[228]

Consistent with this, the updated research framework proposed by the National Institute on Aging—Alzheimer's Association (NIA-AA) Work Group supports the notion that AD should be conceptualized as a clinicopathological spectrum of AD pathology—which can be detected via preclinical biomarkers—prior to its progression to a clinical diagnosis of MCI due to AD or AD dementia.[229] Consequently, the research framework proposes the use of a classification system for AD which includes fluid or imaging biomarkers of amyloid pathology (A), tau pathology (T), and neurodegeneration (N) [referred to as the "ATN" classification] to describe subgroups of individuals with AD pathology based on the presence or absence of biomarker evidence for each of these 3 pathologies. Evidence of neurodegeneration ("N") in the ATN framework is provided by CSF total tau, brain atrophy on MRI, or hypometabolism on fluoro-deoxyglucose (FDG)-PET scans; however, it has been suggested that other fluid markers of neuronal/synaptic injury reviewed herein (eg, Ng, NF-L, or VILIP-1) may be incorporated into this system in the future. Importantly, the implementation of this framework in future studies of AD will shift the conceptualization of AD from a clinical diagnosis to that of a biological construct which encompasses both preclinical and clinical stages.[204] This framework is synergistic with the biology-driven approach to drug development and has close parallels with the new AD staging system proposed by the US Food and Drug Administration (FDA).[230] Importantly, this framework will support clinical trials by ensuring the presence of the target pathology in trials of AD therapies, excluding participants with AD-related pathologies from clinical trials of non-AD dementias, and allowing assessment of target engagement for neuroprotective, and amyloid- or tau-based therapeutics. This approach will pave the way for a more precise description of pathological disease subtypes in different individuals and subsequently, a more personalized approach to interventional drug trials.

Although the ATN framework represents an important first step in the incorporation of biomarkers into diagnostic and stratification schemes, the framework-in its current format-has several limitations. There is a significant degree of heterogeneity among individuals with the same clinical phenotype regarding the number, degree, and distribution of different pathologies in the brain, and their rate of progression over time. The dichotomous classification of individuals based on biomarker evidence of pathology (ie, positive vs negative as proposed by the ATN) does not capture the full spectrum of AD-related pathologies among individuals, or the extent by which these pathologies may change over time or with treatment. Therefore, the framework may be supplemented by the addition of stage-specific markers or a severity staging scheme for the ATN pathologies. Additionally, the ATN framework emphasizes the importance of amyloid, tau, and neurodegeneration to achieve a biological definition of AD; however, it does not include or exclude other co-pathologies (eg, synuclein, TDP-43, and vascular pathology) which may contribute to neurodegeneration. Therefore, it will be important to incorporate other biomarkers in the design and interpretation of trials in which these co-pathologies may influence study outcomes. The definition of amyloid and tau pathologies in the ATN framework is limited to CSF or PET markers of Aβ42 or tau, respectively. Biomarkers which capture other forms of amyloid and tau pathologies (eg, soluble Aβ or tau oligomers) which are known to contribute to cognitive impairment may be needed to supplement the ATN framework in certain settings.

Importantly, clinical outcomes or benefits (ie, the ability to predict or delay a clinical milestone) are needed for drug approval. As some aspects of the AD pathological construct (eg, the presence of amyloid pathology; A(+)TN) have limited impact on cognitive outcomes, clinical assessment and staging tools need to be integrated with the ATN in order to allow the selection of cohorts with homogenous clinical phenotypes which would facilitate trial planning, sample size determinations, and outcome assessments. Together, these factors limit the use of ATN alone as an outcome measure in clinical trials of AD therapeutics.

Recently, there has been great interest in the development and validation of other biomarker-based approaches to improve the characterization and stratification of the AD construct in individuals at risk for cognitive impairment. This is particularly important given the great degree of overlap of different pathologies in several neurodegenerative disorders, and the variability of clinical phenotypes associated with any particular pathology. These newer data-driven approaches emphasize the role of "Big Data- Smart Data" analytics, including bioinformatics, mechanism-based modeling, simulation approaches, and unsupervised clustering as valuable tools to discern the pathobiological construct of disease in asymptomatic or symptomatic individuals.[231-233] Data-driven methods which use continuous, rather than categorical, biomarker variables will help quantify contributions from different pathologies and their spatiotemporal evolution over time regardless of clinical phenotype. Approaches which utilize network biology and pathway databases to examine multidirectional interactions and nonlinear associations between different pathologies, are more likely to generate patient strata which fully capture the complexity and heterogeneity of the disease than empiric approaches which focus on single biomarker data.[234,235] The integration of biomarker data with other domains (eg, clinical, cognitive, and imaging) through a systems biology approach will be essential to adequately reflect the molecular, genetic, and pathological heterogeneity of AD and its spatiotemporal evolution over time.[234,235] Importantly, integrated systems biology and computational modeling methods will be important to generate novel predictive models of disease progression or response to mechanism-based treatments among individuals with different

clinical phenotypes. The importance of biomarker-based approaches to AD and other neurodegenerative disorders relies in their ability to identify common pathological substrates across different clinical phenotypes of neurodegenerative disease. Such an approach will identify individuals who may benefit from investigational treatments which target a specific pathology or pathway regardless of the clinical phenotype (eg, anti-amyloid treatments which target amyloid pathology in individuals with the LBD phenotype). Overall, advanced data analytics that extend beyond empiric approaches will pave the way for novel drug discovery, target validation, and personalized approaches to disease treatment.

Conclusion and Future Directions

In conclusion, novel biomarker discovery is at the frontier of AD research and the search for an AD cure. Biomarkers offer valuable tools to measure and track different disease mechanisms, cellular alterations, and disturbances in pathways "in vivo", and will pave the way for the discovery of novel drug targets. Over the last decade, biomarkers have significantly improved our understanding of the complex pathophysiology of AD which has culminated in a modified research definition of the disease. However, further research is needed to identify biomarkers that reflect mitochondrial dysfunction, vascular disease, and calcium-mediated excitotoxicity in AD, and to standardize biomarker assessments across different laboratories. Personalized biomarker-based medicine paradigms are the future of drug discovery and will be an important leap towards finding a cure for individuals with AD and other disorders across the neurodegenerative spectrum.

ORCID iD

Rawan Tarawneh https://orcid.org/0000-0002-7328-9568

REFERENCES

1. Tarawneh R, Holtzman DM. The clinical problem of symptomatic Alzheimer disease and mild cognitive impairment. *Cold Spring Harb Perspect Med*. 2012;2(5):a006148.
2. The Alzheimer's Association. The Alzheimer's Association Report: Facts and Figures. 2019.
3. The World Health Organization and Alzheimer's Disease International. Dementia: A Public Health Priority. 2012.
4. Hebert LE, Weuve J, Scherr PA, Evans DA. Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. *Neurology*. 2013;80(19):1778-1783.
5. Hippius H, Neundorfer G. The discovery of Alzheimer's disease. *Dialogues Clin Neurosci*. 2003;5(1): 101-108.
6. Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. *Ann Neurol*. 1999;45(3):358-368.
7. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. 1991;82(4):239-259.
8. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med*. 2011;1(1): a006189.
9. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimers Dement*. 2018;4:575-590.
10. Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. *Free Radic Biol Med*. 1997;23(1):134-47.
11. Swerdlow RH. Mitochondria and mitochondrial cascades in Alzheimer's disease. *J Alzheimers Dis*. 2018;62(3):1403-1416.
12. Wang R, Reddy PH. Role of glutamate and NMDA receptors in Alzheimer's disease. *J Alzheimers Dis*. 2017;57(4):1041-1048.
13. Price JL, Ko AI, Wade MJ, Tsou SK, McKeel DW, Morris JC. Neuron number in the entorhinal cortex and CA1 in preclinical Alzheimer disease. *Arch Neurol*. 2001;58(9):1395-1402.
14. Fessel J. Amyloid is essential but insufficient for Alzheimer causation: addition of subcellular cofactors is required for dementia. *Int J Geriatr Psychiatry*. 2018;33(1):e14-e21.
15. Zetterberg H, Schott JM. Biomarkers for Alzheimer's disease beyond amyloid and tau. *Nat Med*. 2019;25(2):201-203.
16. Blennow K. Cerebrospinal fluid protein biomarkers for Alzheimer's disease. *NeuroRx*. 2004;1(2):213-225.
17. Andreasen N, Minthon L, Davidsson P, et al. Evaluation of CSF-tau and CSF-abeta42 as diagnostic markers for Alzheimer disease in clinical practice. *Arch Neurol*. 2001;58(3):373-379.
18. Fagan AM, Mintun MA, Mach RH. Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. *Ann Neurol*. 2006;59(3): 512-519.
19. Ferreira D, Perestelo-Pérez L, Westman E, Wahlund L-O, Sarría A, Serrano-Aguilar P. Meta-review of CSF core biomarkers in Alzheimer's disease: The state-of-the-art after the new revised diagnostic criteria. *Front Aging Neurosci*. 2014;6:47.
20. Tarawneh R, Angelo GD', Macy E. Visinin-like protein-1: diagnostic and prognostic biomarker in Alzheimer disease. *Ann Neurol*. 2011;70(2):274-285.
21. Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. *Arch Neurol*. 2007;64(3):343-349.
22. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol*. 2016;15(7):673-684.
23. Janelidze S, Zetterberg H, Mattsson N, et al. CSF Aβ42/Aβ40 and Aβ42/Aβ38 ratios: better diagnostic markers of Alzheimer disease. *Ann Clin Transl Neurol*. 2016;3(3):154-165.
24. Wiltfang J, Esselmann H, Bibl M. Amyloid β peptide ratio 42/40 but not Aβ42 correlates with phospho-Tau in patients with low- and high-CSF Aβ40 load. *J Neurochem*. 2007;101(4):1053-1059.
25. Gervaise-Henry, Watfa G, Albuisson E, et al. Cerebrospinal fluid Aβ 42/Aβ 40 as a means to limiting tube- and storage-dependent pre-analytical variability in clinical setting. *J Alzheimers Dis*. 2017;57:437-445.
26. Kennedy ME, Stamford AW, Chen X, et al. The BACE1 inhibitor verubecestat (MK-8931) reduces CNS β-amyloid in animal models and in Alzheimer's disease patients. *Sci Transl Med*. 2016;8(363):363ra150.
27. Olsson F, Schmidt S, Althoff V, et al. Characterization of intermediate steps in amyloid beta (Aβ) production under near-native conditions. *J Biol Chem*. 2014;289(3):1540-1550.
28. Hampel H, Buerger K, Zinkowski R, et al. Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: a comparative cerebrospinal fluid study. *Arch Gen Psychiatry*. 2004;61(1):95-102.
29. Janelidze S, Stomrud E, Smith R, et al. Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. *Nat Commun*. 2020;11(1):1683.
30. Molinuevo JL, Ayton S, Batrla R. Current state of Alzheimer's fluid biomarkers. *Acta Neuropathologica*. 2018;136(6):821-853.
31. Lantero Rodriguez J, Karikari TK, Suarez-Calvet M, et al. Plasma p-tau181 accurately predicts Alzheimer's disease pathology at least 8 years prior to post-mortem and improves the clinical characterisation of cognitive decline. *Acta Neuropathologica*. 2020;140(3):267-278.
32. Tatebe H, Kasai T, Ohmichi T, et al. Quantification of plasma phosphorylated tau to use as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and down syndrome. *Mol Neurodegener*. 2017;12(1):63.
33. Yang C-C, Chiu M-J, Chen T-F, Chang H-L, Liu B-H, Yang S-Y. Assay of plasma phosphorylated tau protein (Threonine 181) and total tau protein in early-stage Alzheimer's disease. *J Alzheimers Dis*. 2018;61:1323-1332.
34. Janelidze S, Mattsson N, Palmqvist S, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med*. 2020;26(3):379-386.
35. Palmqvist S, Janelidze S, Quiroz YT, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA*. 2020;324(8):772-781.
36. Blennow K, Zetterberg H. Understanding biomarkers of neurodegeneration: Ultrasensitive detection techniques pave the way for mechanistic understanding. *Nat Med*. 2015;21(3):217-219.
37. Kuo Y-M, Emmerling MR, Lampert HC, et al. High levels of circulating Aβ42 are sequestered by plasma proteins in Alzheimer's disease. *Biochem Biophys Res Commun*. 1999;257(3):787-791.
38. Yang C.-C, Yang S-Y, Chieh J-J, et al., Biofunctionalized magnetic nanoparticles for specifically detecting biomarkers of alzheimer's disease in vitro. *ACS Chem Neurosci*. 2011;2(9):500-505.

39. Zetterberg H, Mörtberg E, Song L, et al. Hypoxia due to cardiac arrest induces a time-dependent increase in serum amyloid beta levels in humans. *PLoS One*. 2011;6(12):e28263.
40. Schindler SE, Bollinger JG, Ovod V. High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology*. 2019;93(17):e1647-e1659.
41. Nakamura A, Kaneko N, Villemagne VL. High performance plasma amyloid-β biomarkers for Alzheimer's disease. *Nature*. 2018;554(7691):249-254.
42. Vergallo A, Mégret L, Lista S, et al., Plasma amyloid β 40/42 ratio predicts cerebral amyloidosis in cognitively normal individuals at risk for Alzheimer's disease. *Alzheimers Dement*. 2019;15(6):764-775.
43. Mielke MM, Hagen CE, Xu J, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimer's Dement*. 2018;14(8):989-997.
44. Ewers M, Zhong Z, Bürger K, et al. Increased CSF-BACE 1 activity is associated with ApoE-epsilon 4 genotype in subjects with mild cognitive impairment and Alzheimer's disease. *Brain*. 2008;131(Pt 5):1252-1258.
45. Timmers M, Barão S, Broeck BV, et al. Bace1 dynamics upon inhibition with a bace inhibitor and correlation to downstream Alzheimer's disease markers in elderly healthy participants. *J Alzheimers Dis*. 2017;56:1437-1449.
46. Lewczuk P, Kamrowski-Kruck H, Peters O, et al. Soluble amyloid precursor proteins in the cerebrospinal fluid as novel potential biomarkers of Alzheimer's disease: a multicenter study. *Mol Psychiatry*. 2010;15(2):138-145.
47. Zetterberg H, Skillbäck T, Mattsson N, et al., Association of cerebrospinal fluid neurofilament light concentration with alzheimer disease progression. *JAMA Neurol*. 2016;73(1):60-67.
48. Zetterberg H. Neurofilament light: a dynamic cross-disease fluid biomarker for neurodegeneration. *Neuron*. 2016;91(1):1-3.
49. 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(5):557-566.
50. Tarawneh R, Head D, Allison S, et al. Cerebrospinal fluid markers of neurodegeneration and rates of brain atrophy in early Alzheimer disease. *JAMA Neurol*. 2015;72(6):656-65.
51. Tarawneh R, Lee J-M, Ladenson JH, Morris JC, Holtzman DM. CSF VILIP-1 predicts rates of cognitive decline in early Alzheimer disease. *Neurology*. 2012;78(10):709-719.
52. Mattsson N, Insel PS, Palmqvist S, et al. Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer's disease. *EMBO Mol Med*. 2016;8(10):1184-1196.
53. Tarawneh R, D'Angelo G, Crimmins D, et al. Diagnostic and prognostic utility of the synaptic marker neurogranin in Alzheimer disease. *JAMA Neurol*. 2016;73(5):561-571.
54. Zhang H, Therriault J, Kang MS, et al. Cerebrospinal fluid synaptosomal-associated protein 25 is a key player in synaptic degeneration in mild cognitive impairment and Alzheimer's disease. *Alzheimers Res Ther*. 2018;10(1):80.
55. Brinkmalm A, Brinkmalm G, Honer WG, et al. SNAP-25 is a promising novel cerebrospinal fluid biomarker for synapse degeneration in Alzheimer's disease. *Mol Neurodegener*. 2014;9:53.
56. Öhrfelt A, Brinkmalm A, Dumurgier J, et al. The pre-synaptic vesicle protein synaptotagmin is a novel biomarker for Alzheimer's disease. *Alzheimer's Res Ther*. 2016;8(1):41.
57. Camporesi E, Nilsson J, Brinkmalm A. Fluid biomarkers for synaptic dysfunction and loss. *Biomark Insights*. 2020;15:1177271920950319.
58. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol*. 2018;14(10):577-589.
59. Preische O, Schultz SA, Apel A, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nat Med*. 2019;25(2):277-283.
60. Cantó E, Barro C, Zhao C, et al., Association between serum neurofilament light chain levels and long-term disease course among patients with multiple sclerosis followed up for 12 years. *JAMA Neurol*. 2019;76(11):1359-1366.
61. Braunewell KH, Riederer P, Spilker C, Gundelfinger ED, Bogerts B, Bernstein HG. Bbnormal localization of two neuronal calcium sensor proteins, visinin-like proteins (VILIPS)-1 and -3, in neocortical brain areas of alzheimer disease patients. *Dement Geriatric Cogn Disord*. 2001;12(2):110-116.
62. Luo X, Hou L, Shi H, et al. CSF levels of the neuronal injury biomarker visinin-like protein-1 in Alzheimer's disease and dementia with Lewy bodies. *J Neurochem*. 2013;127(5):681-690.
63. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann Neurol*. 1990;27(5):457-464.
64. Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol*. 1991;30(4):572-580.
65. Scheff SW, Price DA, Schmitt FA, Mufson EJ. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiol Aging*. 2006;27(10):1372-1384.
66. Reddy PH, Mani G, Park BS, et al. Differential loss of synaptic proteins in Alzheimer's disease: Implications for synaptic dysfunction. *J Alzheimers Dis*. 2005;7(2):103-117.
67. Mattsson N, Insel PS, Palmqvist S, et al. Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer's disease. *EMBO Mol Med*. 2016;8(10):1184-1196.
68. Thorsell A, Bjerke M, Gobom J. Neurogranin in cerebrospinal fluid as a marker of synaptic degeneration in Alzheimer's disease. *Brain Res*. 2010;1362:13-22.
69. Wellington H, Paterson RW, Portelius E, et al. Increased CSF neurogranin concentration is specific to Alzheimer disease. *Neurology*. 2016;86(9):829-835.
70. Goetzl EJ, Kapogiannis D, Schwartz JB, et al. Decreased synaptic proteins in neuronal exosomes of frontotemporal dementia and Alzheimer's disease. *FASEB J*. 2016;30(12):4141-4148.
71. Winston CN, Goetzl EJ, Akers JC, et al., Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. *Alzheimers Dement*. 2016;3(1):63-72.
72. Antonucci F, Corradini I, Fossati G, Tomasoni R, Menna E, Matteoli M. SNAP-25, a known presynaptic protein with emerging postsynaptic functions. *Front Synaptic Neurosci*. 2016;8:7.
73. Bark IC, Wilson MC. Human cDNA clones encoding two different isoforms of the nerve terminal protein SNAP-25. *Gene*. 1994;139(2):291-292.
74. John BD. Molecular mechanisms, biological actions, and neuropharmacology of the growth-associated protein GAP-43. *Curr Neuropharmacol*. 2006;4(4):293-304.
75. Holahan MR. A shift from a pivotal to supporting role for the growth-associated protein (GAP-43) in the coordination of axonal structural and functional plasticity. *Front Cell Neurosci*. 2017;11:266.
76. Sjögren M, Davidsson P, Gottfries J, et al. The cerebrospinal fluid levels of tau, growth-associated protein-43 and soluble amyloid precursor protein correlate in Alzheimer's disease, reflecting a common pathophysiological process. *Dement Geriatr Cogn Disord*. 2001;12(4):257-264.
77. Sandelius Å, Portelius E, Källén Å, et al. Elevated CSF GAP-43 is Alzheimer's disease specific and associated with tau and amyloid pathology. *Alzheimers Dement*. 2019;15(1):55-64.
78. Chang S, Trimbuch T, Rosenmund C. Synaptotagmin-1 drives synchronous Ca2+-triggered fusion by C2B-domain-mediated synaptic-vesicle-membrane attachment. *Nat Neurosci*. 2018;21(1):33-40.
79. Tible M, Sandelius Å, Höglund K, et al. Dissection of synaptic pathways through the CSF biomarkers for predicting Alzheimer disease. *Neurology*. 2020;95(8):e953-e961.
80. Xu D, Hopf C, Reddy R, et al. Narp and NP1 form heterocomplexes that function in developmental and activity-dependent synaptic plasticity. *Neuron*. 2003;39(3):513-528.
81. Figueiro-Silva J, Gruart A, Bernard Clayton K, et al. Neuronal pentraxin 1 negatively regulates excitatory synapse density and synaptic plasticity. *J Neurosci*. 2015;35(14):5504-5521.
82. Abad MA, Enguita M, DeGregorio-Rocasolano N, Ferrer I, Trullas R. Neuronal pentraxin 1 contributes to the neuronal damage evoked by amyloid-β and is overexpressed in dystrophic neurites in Alzheimer's brain. *J Neurosci*. 2006;26(49):12735-12747.
83. Ma QL, Teng E, Zuo X, et al. Neuronal pentraxin 1: a synaptic-derived plasma biomarker in Alzheimer's disease. *Neurobiol Dis*. 2018;114:120-128.
84. Soldan A, Moghekar A, Walker KA, et al. Resting-state functional connectivity is associated with cerebrospinal fluid levels of the synaptic protein NPTX2 in non-demented older adults. *Front Aging Neurosci*. 2019;11:132.
85. Begcevic I, Tsolaki M, Brinc D, et al. Neuronal pentraxin receptor-1 is a new cerebrospinal fluid biomarker of Alzheimer's disease progression. *F1000Res*. 2018;7:1012.
86. Poirel O, Mella S, Videau C, et al. Moderate decline in select synaptic markers in the prefrontal cortex (BA9) of patients with Alzheimer's disease at various cognitive stages. *Sci Rep*. 2018;8(1):938.
87. Wesenhagen KEJ, Teunissen CE, Visser PJ, Tijms BM, et al. Cerebrospinal fluid proteomics and biological heterogeneity in Alzheimer's disease: A literature review. *Crit Rev Clin Lab Sci*. 2020;57(2):86-98.
88. Nikolaienko O, Patil S, Eriksen MS, Bramham CR, et al. Arc protein: a flexible hub for synaptic plasticity and cognition. *Semin Cell Dev Biol*. 2018;77:33-42.
89. Hopperton KE, Mohammad D, Trépanier MO, Giuliano V, Bazinet RP. Markers of microglia in post-mortem brain samples from patients with Alzheimer's disease: a systematic review. *Mol Psychiatry*. 2018;23(2):177-198.
90. Liu C, Chyr J, Zhao W, et al. Genome-wide association and mechanistic studies indicate that immune response contributes to Alzheimer's disease development. *Front Genet*. 2018;9:410.
91. Kunkle BW, Grenier-Boley B, Sims R, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. *Nat Genet*. 2019;51(3):414-430.
92. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. *Mol Neurodegener*. 2019;14(1):32.
93. Heneka MT, Golenbock DT, Latz E. Innate immunity in Alzheimer's disease. *Nat Immunol*. 2015;16(3):229-236.

94. Jansen IE, Savage JE, Watanabe K, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat Genet*. 2019;51(3):404-413.
95. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimers Dement*. 2018;4:575-590.
96. Solito E, Sastre M. Microglia function in Alzheimer's disease. *Front Pharmacol*. 2012;3:14-14.
97. Guadagno J, Swan P, Shaikh R, Cregan SP. Microglia-derived IL-1beta triggers p53-mediated cell cycle arrest and apoptosis in neural precursor cells. *Cell Death Dis*. 2015;6:e1779.
98. Sutinen EM, Pirttilä T, Anderson G, Salminen A, Ojala JO. Pro-inflammatory interleukin-18 increases Alzheimer's disease-associated amyloid-beta production in human neuron-like cells. *J Neuroinflammation*. 2012;9:199.
99. Vom Berg J, Prokop S, Miller KR, et al. Inhibition of IL-12/IL-23 signaling reduces Alzheimer's disease-like pathology and cognitive decline. *Nat Med*. 2012;18(12):1812-1819.
100. Martinez B, Peplow PV. Amelioration of Alzheimer's disease pathology and cognitive deficits by immunomodulatory agents in animal models of Alzheimer's disease. *Neural Regen Res*. 2019;14(7):1158-1176.
101. Lobo-Silva D, Carriche GM, Castro AG, Roque S, Saraiva M. Balancing the immune response in the brain: IL-10 and its regulation. *J Neuroinflammation*. 2016;13(1):297.
102. McCaulley ME, Grush KA. Alzheimer's disease: Exploring the role of inflammation and implications for treatment. *Int J Alzheimers Dis*. 2015:2015;515248.
103. Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, Herrmann N. A meta-analysis of cytokines in Alzheimer's disease. *Biol Psychiatry*. 2010;68(10): 930-941.
104. Fillit H, Ding WH, Buee L. Elevated circulating tumor necrosis factor levels in Alzheimer's disease. *Neurosci Lett*. 1991;129(2):318-320.
105. Tarkowski E, Andreasen N, Tarkowski A, Blennow K. Intrathecal inflammation precedes development of Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2003;74(9):1200-1205.
106. Jiang H, Hampel H, Prvulovic D, et al. Elevated CSF levels of TACE activity and soluble TNF receptors in subjects with mild cognitive impairment and patients with Alzheimer's disease. *Molecular Neurodegener*. 2011;6(1):69.
107. Brosseron F, Krauthausen M, Kummer M, Heneka MT. Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: a comparative overview. *Mol Neurobiol*. 2014;50(2):534-544.
108. Park J-C, Han S-H, Mook-Jung I. Peripheral inflammatory biomarkers in Alzheimer's disease: a brief review. *BMB Reports*. 2020;53(1):10-19.
109. Mrak RE, Griffin WS. Interleukin-1 and the immunogenetics of Alzheimer disease. *J Neuropathol Exp Neurol*. 2000;59(6):471-476.
110. Italiani P, Puxeddu I, Napoletano S, et al. Circulating levels of IL-1 family cytokines and receptors in Alzheimer's disease: new markers of disease progression? *J Neuroinflammation*. 2018;15(1):342.
111. Lee KS, Chung JH, Choi TK, Suh SY, Oh BH, Hong CH. Peripheral cytokines and chemokines in Alzheimer's disease. *Dement Geriatr Cogn Disord*. 2009; 28(4):281-287.
112. Lai KSP, Liu CS, Rau A, et al. Peripheral inflammatory markers in Alzheimer's disease: a systematic review and meta-analysis of 175 studies. *J Neurol Neurosurg Psychiatry*. 2017;88(10):876-882.
113. Iturria-Medina Y, Sotero RC, Toussaint PJ, Mateos-Pérez JM, Evans AC, Alzheimer's Disease Neuroimaging Initiative. Early role of vascular dysregulation on late-onset alzheimer's disease based on multifactorial data-driven analysis. *Nat Commun*. 2016;7(1):11934.
114. Jay TR, von Saucken VE, Landreth GE. TREM2 in neurodegenerative diseases. *Molecular Neurodegener*. 2017;12(1):56.
115. Raha AA, Henderson JW, Stott SRW, et al. Neuroprotective effect of TREM-2 in aging and Alzheimer's disease model. *J Alzheimers Dis*. 2017;55(1):199-217.
116. Jiang T, Zhang Y-D, Gao Q, et al. TREM1 facilitates microglial phagocytosis of amyloid beta. *Acta Neuropathol*. 2016;132(5):667-683.
117. Yuan P, Condello C, Keene CD, et al. TREM2 haplodeficiency in mice and humans impairs the microglia barrier function leading to decreased amyloid compaction and severe axonal dystrophy. *Neuron*. 2016;90(4):724-739.
118. Suarez-Calvet M, Morenas-Rodríguez E, Kleinberger G, et al. Early increase of CSF sTREM2 in Alzheimer's disease is associated with tau related-neurodegeneration but not with amyloid-beta pathology. *Mol Neurodegener*. 2019;14(1):1.
119. Heslegrave A, Heywood W, Paterson R, et al. Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease. *Molecular Neurodegener*. 2016;11:3.
120. Suárez-Calvet M, Araque Caballero MÁ, Kleinberger G, et al. Early changes in CSF sTREM2 in dominantly inherited Alzheimer's disease occur after amyloid deposition and neuronal injury. *Sci Transl Med*. 2016;8(369):369ra178.
121. Libreros S, Iragavarapu-Charyulu V. YKL-40/CHI3L1 drives inflammation on the road of tumor progression. *J Leukoc Biol*. 2015;98(6):931-936.
122. Baldacci F, Toschi N, Lista S, et al. Two-level diagnostic classification using cerebrospinal fluid YKL-40 in Alzheimer's disease. *Alzheimers Dement*. 2017;13(9):993-1003.
123. Baldacci F, Lista S, Cavedo E, Bonuccelli U, Hampel H. Diagnostic function of the neuroinflammatory biomarker YKL-40 in Alzheimer's disease and other neurodegenerative diseases. *Expert Rev Proteomics*. 2017;14(4):285-299.
124. Gispert JD, Monté GC, Suárez-Calvet M, et al. The APOE ε4 genotype modulates CSF YKL-40 levels and their structural brain correlates in the continuum of Alzheimer's disease but not those of sTREM2. *Alzheimer's Dement*. 2016;6:50-59.
125. Alcolea D, Vilaplana E, Pegueroles J. Relationship between cortical thickness and cerebrospinal fluid YKL-40 in predementia stages of Alzheimer's disease. *Neurobiol Aging*. 2015;36(6):2018-2023.
126. Kester MI, Teunissen CE, Sutphen C, et al. Cerebrospinal fluid VILIP-1 and YKL-40, candidate biomarkers to diagnose, predict and monitor Alzheimer's disease in a memory clinic cohort. *Alzheimers Res Ther*. 2015;7(1):59.
127. Mattsson N, Tabatabaei S, Johansson P, et al. Cerebrospinal fluid microglial markers in Alzheimer's disease: elevated chitotriosidase activity but lack of diagnostic utility. *Neuromolecular Med*. 2011;13(2):151-159.
128. Craig-Schapiro, Perrin RJ, Roe CM, et al. YKL-40: a novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biol Psychiatry*. 2010;68(10): 903-912.
129. Llorens F, Thüne K, Tahir W, et al. YKL-40 in the brain and cerebrospinal fluid of neurodegenerative dementias. *Mol Neurodegener*. 2017;12(1):83.
130. Cristóvão JS, Gomes CM. S100 Proteins in Alzheimer's disease. *Front Neurosci*. 2019;13:463.
131. Steiner J, Bogerts B, Schroeter ML, Bernstein H-G. S100B protein in neurodegenerative disorders. *Clin Chem Lab Med*. 2011;49:409-424.
132. Cristóvão JS, Gomes CM. S100 proteins in Alzheimer's disease. *Front Neurosci*. 2019;13:463.
133. Kamphuis W, Middeldorp J, Kooijman L, et al. Glial fibrillary acidic protein isoform expression in plaque related astrogliosis in Alzheimer's disease. *Neurobiol Aging*. 2014;35(3):492-510.
134. Ishiki A, Kamada M, Kawamura Y, et al. Glial fibrillar acidic protein in the cerebrospinal fluid of Alzheimer's disease, dementia with Lewy bodies, and frontotemporal lobar degeneration. *J Neurochem*. 2016;136(2):258-261.
135. Ciccocioppo F, Lanuti P, Pierdomenico L, et al. The characterization of regulatory t-cell profiles in Alzheimer's disease and multiple sclerosis. *Sci Rep*. 2019;9(1):8788.
136. Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: their role in infection and inflammation. *J Leukoc Biol*. 2007;81(3):584-592.
137. Rezai-Zadeh K, Gate D, Szekely CA, Town T. Can peripheral leukocytes be used as Alzheimer's disease biomarkers? *Expert Rev Neurother*. 2009;9(11):1623-1633.
138. Gate D, Saligrama N, Leventhal O, et al. Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer's disease. *Nature*. 2020;577(7790):399-404.
139. Mroczko B. The Assessment of eotaxin-1 (CCL-11) concentrations in the cerebrospinal fluid of patients with mild cognitive impairment and Alzheimer's disease. *Alzheimers Dement*. 2018;14(7):P776.
140. Zujovic V, Benavides J, Vigé X, Carter C, Taupin V. Fractalkine modulates TNF-alpha secretion and neurotoxicity induced by microglial activation. *Glia*. 2000;29(4):305-315.
141. Hayden MS, West AP, Ghosh S. NF-kappaB and the immune response. *Oncogene*. 2006;25(51):6758-6780.
142. Petrovitch H, Webster Ross G, Steinhorn SC, et al. AD lesions and infarcts in demented and non-demented Japanese-American men. *Ann Neurol*. 2005;57(1):98-103.
143. Kalaria RN, Ballard C. Overlap between pathology of Alzheimer disease and vascular dementia. *Alzheimer Dis Assoc Disord*. 1999;13 Suppl 3:S115-S1123.
144. Iturria-Medina Y, Carbonell FM, Sotero RC. Multifactorial causal model of brain (dis)organization and therapeutic intervention: Application to Alzheimer's disease. *Neuroimage*. 2017;152:60-77.
145. Cortes-Canteli M, Iadecola C. Alzheimer's disease and vascular aging. *J Am Acad Cardiol*. 2020;75(8):942-951.
146. Cheignon C, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C, Collin F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biol*. 2018;14:450-464.
147. Salminen A, Kauppinen A, Kaarniranta K. Hypoxia/ischemia activate processing of Amyloid Precursor Protein: impact of vascular dysfunction in the pathogenesis of Alzheimer's disease. *J Neurochem*. 2017;140(4):536-549.
148. Chakraborty A, de Wit NM, van der Flier WM, de Vries HE. The blood brain barrier in Alzheimer's disease. *Vascul Pharmacol*. 2017;89:12-18.
149. Olsson B, Hertze J, Ohlsson M, et al. Cerebrospinal fluid levels of heart fatty acid binding protein are elevated prodromally in Alzheimer's disease and vascular dementia. *J Alzheimers Dis*. 2013;34(3):673-679.
150. Chiasserini D, Biscetti L, Eusebi P, et al. Differential role of CSF fatty acid binding protein 3, α-synuclein, and Alzheimer's disease core biomarkers in Lewy body disorders and Alzheimer's dementia. *Alzheimers Res Ther*. 2017;9(1):52.
151. Leung YY, Toledo JB, Nefedov A, et al. Identifying amyloid pathology–related cerebrospinal fluid biomarkers for Alzheimer's disease in a multicohort study. *Alzheimers Dement*. 2015;1(3):339-348.

152. Desikan RS, Thompson WK, Holland D, et al. Heart fatty acid binding protein and Aβ-associated Alzheimer's neurodegeneration. *Mol Neurodegener*. 2013;8(1):39.
153. Janelidze S, Mattsson N, Stomrud E, et al. CSF biomarkers of neuroinflammation and cerebrovascular dysfunction in early Alzheimer disease. *Neurology*. 2018;91(9):e867-e877.
154. Ewers M, Mielke MM, Hampel H. Blood-based biomarkers of microvascular pathology in Alzheimer's disease. *Exp Gerontol*. 2010;45(1):75-79.
155. Wallin A, Kapaki E, Boban M, et al. Biochemical markers in vascular cognitive impairment associated with subcortical small vessel disease - A consensus report. *BMC Neurology*. 2017;17(1):102.
156. Hawking ZL. Alzheimer's disease: the role of mitochondrial dysfunction and potential new therapies. *Biosci Horizons*. 2016;9:hzw014.
157. Cheng Y, Bai F. The association of tau with mitochondrial dysfunction in Alzheimer's disease. *Front Neurosci*. 2018;12:163.
158. Devi L, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK. Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *J Neurosci*. 2006;26(35):9057-9068.
159. Perez Ortiz JM, Swerdlow RH. Mitochondrial dysfunction in Alzheimer's disease: Role in pathogenesis and novel therapeutic opportunities. *Br J Pharmacol*. 2019;176(18):3489-3507.
160. Skoumalová A, Hort J. Blood markers of oxidative stress in Alzheimer's disease. *J Cell Mol Med*. 2012;16(10):2291-2300.
161. Valla J, Schneider L, Niedzielko T. Impaired platelet mitochondrial activity in Alzheimer's disease and mild cognitive impairment. *Mitochondrion*. 2006;6(6):323-330.
162. Prasad A, Bharathi V, Sivalingam V, Girdhar A, Patel BK. Molecular mechanisms of TDP-43 misfolding and pathology in amyotrophic lateral sclerosis. *Front Mol Neurosci*. 2019;12:25.
163. Arai T, Hasegawa M, Akiyama H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun*. 2006;351(3):602-611.
164. Amador-Ortiz C, Lin W-L, Ahmed Z, et al. TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann Neurol*. 2007;61(5):435-445.
165. Uryu K, Nakashima-Yasuda H, Forman MS. Concomitant TAR-DNA-binding protein 43 pathology is present in Alzheimer disease and corticobasal degeneration but not in other tauopathies. *J Neuropathol Exp Neurol*. 2008;67(6):555-564.
166. Huang W, Zhou Y, Tu L, et al. TDP-43: from Alzheimer's disease to limbic-predominant age-related TDP-43 encephalopathy. *Front Mol Neurosci*. 2020;13:26.
167. Wilson AC, Dugger BN, Dickson DW, Wang D-S. TDP-43 in aging and Alzheimer's disease - a review. *Int J Clin Exp Pathol*. 2011;4(2):147-155.
168. Tomé SO, Vandenberghe R, Ospitalieri S, et al. Distinct molecular patterns of TDP-43 pathology in Alzheimer's disease: relationship with clinical phenotypes. *Acta Neuropathologica Commun*. 2020;8(1):61.
169. Foulds P, McAuley E, Gibbons L, et al. TDP-43 protein in plasma may index TDP-43 brain pathology in Alzheimer's disease and frontotemporal lobar degeneration. *Acta Neuropathol*. 2008;116(2):141-146.
170. Williams SM, Schulz P, Rosenberry TL, Caselli RJ, Sierks MR. Blood-based oligomeric and other protein variant biomarkers to facilitate pre-symptomatic diagnosis and staging of Alzheimer's disease. *J Alzheimers Dis*. 2017;58:23-35.
171. Twohig D, Nielsen HM. α-synuclein in the pathophysiology of Alzheimer's disease. *Mol Neurodegener*. 2019;14(1):23.
172. Iseki E. Dementia with Lewy bodies: reclassification of pathological subtypes and boundary with Parkinson's disease or Alzheimer's disease. *Neuropathol*. 2004;24(1):72-78.
173. Olichney JM, Galasko D, Salmon DP, et al. Cognitive decline is faster in Lewy body variant than in Alzheimer's disease. *Neurology*. 1998;51(2):351-357.
174. Ishizawa T, Mattila P, Davies P, Wang D, Dickson DW. Colocalization of tau and alpha-synuclein epitopes in Lewy bodies. *J Neuropathol Exp Neurol*. 2003;62(4):389-397.
175. Clinton LK, Blurton-Jones M, Myczek K, Trojanowski JQ, LaFerla FM. Synergistic Interactions between Abeta, tau, and alpha-synuclein: acceleration of neuropathology and cognitive decline. *J Neurosci*. 2010;30(21):7281-7289.
176. Wennström M, Surova Y, Hall S, et al. Low CSF levels of both α-synuclein and the α-synuclein cleaving enzyme neurosin in patients with synucleinopathy. *PLoS One*. 2013;8(1):e53250.
177. van Steenoven I, Majbour NK, Vaikath NN, et al. α-Synuclein species as potential cerebrospinal fluid biomarkers for dementia with lewy bodies. *Movement Disord*. 2018;33(11):1724-1733.
178. Korff A, Liu C, Ginghina C, Shi M, Zhang J, Alzheimer's Disease Neuroimaging Initiative. α-synuclein in cerebrospinal fluid of alzheimer's disease and mild cognitive impairment. *J Alzheimers Dis*. 2013;36:679-688.
179. Majbour NK, Chiasserini D, Vaikath NN, et al. Increased levels of CSF total but not oligomeric or phosphorylated forms of alpha-synuclein in patients diagnosed with probable Alzheimer's disease. *Sci Rep*. 2017;7:40263.
180. Vergallo A. Association of cerebrospinal fluid α-synuclein with total and phospho-tau181 protein concentrations and brain amyloid load in cognitively normal subjective memory complainers stratified by Alzheimer's disease biomarkers. *Alzheimers Dement*. 2018;14(12):1623-1631.
181. Twohig D, Rodriguez-Vieitez E, Sando SB, et al. The relevance of cerebrospinal fluid α-synuclein levels to sporadic and familial Alzheimer's disease. *Acta Neuropathol Commun*. 2018;6(1):130.
182. Berge G, Sando SB, Albrektsen G, et al. Alpha-synuclein measured in cerebrospinal fluid from patients with Alzheimer's disease, mild cognitive impairment, or healthy controls: a two year follow-up study. *BMC Neurol*. 2016;16(1):180.
183. Shahnawaz M, Tokuda T, Waragai M, et al. Development of a biochemical diagnosis of parkinson disease by detection of α-synuclein misfolded aggregates in cerebrospinal fluid. *JAMA Neurol*. 2017;74(2):163-172.
184. Fairfoul G, McGuire LI, Pal S, et al. Alpha-synuclein RT-QuIC in the CSF of patients with alpha-synucleinopathies. *Ann Clin Transl Neurol*. 2016;3(10):812-818.
185. Candelise N, Schmitz M, Da Silva Correia SM, et al. Applications of the real-time quaking-induced conversion assay in diagnosis, prion strain-typing, drug pre-screening and other amyloidopathies. *Expert Rev Mol Diagn*. 2017;17(10):897-904.
186. Kumar P, Dezso Z, MacKenzie C, et al. Circulating miRNA biomarkers for Alzheimer's disease. *PLoS One*. 2013;8(7):e69807.
187. Guo R, Fan G, Zhang J, et al. A 9-microRNA signature in serum serves as a noninvasive biomarker in early diagnosis of Alzheimer's disease. *J Alzheimers Dis*. 2017;60(4):1365-1377.
188. Swarbrick S, Wragg N, Ghosh S, Stolzing A. Systematic review of miRNA as biomarkers in Alzheimer's disease. *Mol Neurobiol*. 2019;56(9):6156-6167.
189. Goetzl EJ, Boxer A, Schwartz JB, et al. Low neural exosomal levels of cellular survival factors in Alzheimer's disease. *Ann Clin Transl Neurol*. 2015;2(7):769-773.
190. Coleman BM, Hill AF. Extracellular vesicles–Their role in the packaging and spread of misfolded proteins associated with neurodegenerative diseases. *Semin Cell Dev Biol*. 2015;40:89-96.
191. Fiandaca MS, Kapogiannis D, Mapstone M, et al. Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study. *Alzheimers Dement*. 2015;11(6):600-7.e1.
192. Goetzl EJ, Abner EL, Jicha GA, Kapogiannis D, Schwartz JB. Declining levels of functionally specialized synaptic proteins in plasma neuronal exosomes with progression of Alzheimer's disease. *FASEB J*. 2018;32(2):888-893.
193. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimers Res Ther*. 2014;6(4):37.
194. Mehta D, Jackson R, Paul G, Shi J, Sabbagh M. Why do trials for Alzheimer's disease drugs keep failing? A discontinued drug perspective for 2010-2015. *Expert Opin Investig Drugs*. 2017;26(6):735-739.
195. Aisen PS, Cummings J, Jack CR Jr. On the path to 2025: understanding the Alzheimer's disease continuum. *Alzheimers Res Ther*. 2017;9(1):60.
196. Iqbal K, Grundke-Iqbal I. Alzheimer's disease, a multifactorial disorder seeking multitherapies. *Alzheimers Dement*. 2010;6(5):420-424.
197. Carreiras MC, Mendes E, Jesus Perry M, Francisco AP, Marco-Contelles J. The multifactorial nature of Alzheimer's disease for developing potential therapeutics. *Curr Top Med Chem*. 2013;13(15):1745-1770.
198. Ferreira D, Wahlund LO, Westman E. The heterogeneity within Alzheimer's disease. *Aging*. 2018;10(11):3058-3060.
199. Devi G, Scheltens P. Heterogeneity of Alzheimer's disease: consequence for drug trials? *Alzheimers Res Ther*. 2018;10(1):122.
200. Au R, Piers RJ, Lancashire L. Back to the future: Alzheimer's disease heterogeneity revisited. *Alzheimers Dement*. 2015;1(3):368-370.
201. Jack CR Jr, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol*. 2013;12(2):207-216.
202. Tarawneh R, Holtzman DM. Biomarkers in translational research of Alzheimer's disease. *Neuropharmacology*. 2010;59(4-5):310-322.
203. Tarawneh R, Holtzman DM. Critical issues for successful immunotherapy in Alzheimer's disease: development of biomarkers and methods for early detection and intervention. *CNS Neurol Disord Drug Targets*. 2009;8(2):144-159.
204. Sperling RA, Karlawish J, Johnson KA. Preclinical Alzheimer disease-the challenges ahead. *Nat Rev Neurol*. 2013;9(1):54-58.
205. Cummings J. Lessons learned from Alzheimer disease: clinical trials with negative outcomes. *Clin Transl Sci*. 2018;11(2):147-152.
206. Honig LS, Vellas B, Woodward M, et al. Trial of solanezumab for mild dementia due to Alzheimer's disease. *N Engl J Med*. 2018;378(4):321-330.
207. Salloway S, Sperling R, Brashear HR. Phase 3 trials of solanezumab and bapineuzumab for Alzheimer's disease. *N Engl J Med*. 2014;370(15):1460.
208. Salloway S, Sperling R, Fox NC, et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N Engl J Med*. 2014;370(4):322-333.
209. Cummings J, Morstorf T, Lee G. Alzheimer's drug-development pipeline: 2016. *Alzheimers Dement*. 2016;2(4):222-232.
210. Cummings J, Lee G, Ritter A, Zhong K. Alzheimer's disease drug development pipeline: 2018. *Alzheimers Dement*. 2018;4:195-214.

211. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*. 2002;297(5580):353-356.
212. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med*. 2016;8(6):595-608.
213. Kametani F, Hasegawa M. Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. *Front Neurosci*. 2018;12:25.
214. Morris GP, Clark IA, Vissel B. Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer's disease. *Acta Neuropathol Commun*. 2014;2:135.
215. Murray ME, Lowe VJ, Graff-Radford NR, et al. Clinicopathologic and 11C-Pittsburgh compound B implications of Thal amyloid phase across the Alzheimer's disease spectrum. *Brain*. 2015;138(Pt 5):1370-1381.
216. Lawrence E, Vegvari C, Ower A, et al. A systematic review of longitudinal studies which measure Alzheimer's disease biomarkers. *J Alzheimers Dis*. 2017;59(4): 1359-1379.
217. Humpel C. Identifying and validating biomarkers for Alzheimer's disease. *Trends Biotechnol*. 2011;29(1):26-32.
218. Molinuevo JL, Blennow K, Dubois B, et al. The clinical use of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: a consensus paper from the Alzheimer's Biomarkers Standardization Initiative. *Alzheimers Dement*. 2014;10(6):808-817.
219. Vanderstichele H, Bibl M, Engelborghs S, et al. Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: a consensus paper from the Alzheimer's Biomarkers Standardization Initiative. *Alzheimers Dement*. 2012;8(1):65-73.
220. Li G, Shofer JB, Petrie EC, et al. Cerebrospinal fluid biomarkers for Alzheimer's and vascular disease vary by age, gender, and APOE genotype in cognitively normal adults. *Alzheimers Res Ther*. 2017;9(1):48.
221. Koran MEI, Wagener M, Hohman TJ, Alzheimer's Neuroimaging Initiative. Sex differences in the association between AD biomarkers and cognitive decline. *Brain Imaging Behav*. 2017;11(1):205-213.
222. Ferreira D, Rivero-Santana A, Perestelo-Pérez L, et al. Improving CSF biomarkers' performance for predicting progression from mild cognitive impairment to alzheimer's disease by considering different confounding factors: a meta-analysis. *Front Aging Neurosci*. 2014;6:287.
223. Mattsson N, Carrillo MC, Dean RA, et al. Revolutionizing Alzheimer's disease and clinical trials through biomarkers. *Alzheimers Dement*. 2015;1(4): 412-419.
224. Selkoe DJ. Alzheimer's disease is a synaptic failure. *Science*. 2002;298(5594): 789-791.
225. DeKosky ST, Scheff SW, Styren SD. Structural correlates of cognition in dementia: quantification and assessment of synapse change. *Neurodegeneration*. 1996;5(4):417-421.
226. Masliah E, Mallory M, Hansen L, DeTeresa R, Alford M, Terry R. Synaptic and neuritic alterations during the progression of Alzheimer's disease. *Neurosci Lett*. 1994;174(1):67-72.
227. Spires-Jones TL, Hyman BT. The intersection of amyloid beta and tau at synapses in Alzheimer's disease. *Neuron*. 2014;82(4):756-771.
228. Teng E, Yamasaki TR, Tran M, Hsiao JJ, Sultzer DL, Mendez MF. Cerebrospinal fluid biomarkers in clinical subtypes of early-onset alzheimer's disease. *Dement Geriatr Cogn Disord*. 2014;37(5-6):307-314.
229. Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14(4): 535-562.
230. U.S. Food and Drug Administration. Early Alzheimer's disease: developing drugs for treatment; draft guidance for industry. Federal Register. 2018 (7060-1).
231. Toschi N, Lista S, Baldacci F. Biomarker-guided clustering of Alzheimer's disease clinical syndromes. *Neurobiol Aging*. 2019;83:42-53.
232. Geerts H, Dacks PA, Devanarayan V, et al. Big data to smart data in Alzheimer's disease: The brain health modeling initiative to foster actionable knowledge. *Alzheimers Dement*. 2016;12(9): 1014-1021.
233. Haas M, Stephenson D, Romero K. Big data to smart data in Alzheimer's disease: Real-world examples of advanced modeling and simulation. *Alzheimers Dement*. 2016;12(9):1022-1030.
234. Hampel H, Toschi N, Babiloni C, et al. Revolution of Alzheimer precision neurology. Passageway of systems biology and neurophysiology. *J Alzheimers Dis*. 2018;64(s1):S47-S105.
235. Hampel H, Vergallo A, Aguilar LF, et al. Precision pharmacology for Alzheimer's disease. *Pharmacol Res*. 2018;130:331-365.