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Biomarkers in Alzheimer's disease drug development

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Abstract Developing new therapies for Alzheimer's disease (AD) is critically important to avoid the impending public health disaster imposed by this common disorder. Means must be found to prevent, delay the onset, or slow the progression of AD. These goals will be achieved by identifying disease-modifying therapies and testing them in clinical trials. Biomarkers play an increasingly important role in AD drug development. In preclinical testing, they assist in decisions to develop an agent. Biomarkers in phase I provide insights into toxic responses and drug metabolism and in Phase II proof-of-concept trials they facilitate go/no-go decisions and dose finding. Biomarkers can play a role in identifying presymptomatic patients or specific patient subgroups. They can provide evidence of target engagement before clinical changes can be expected. Brain imaging can serve as a primary outcome in Phase II trials and as a key secondary outcome in Phase III trials. Magnetic resonance imaging is currently best positioned for use in large multicenter clinical trials. Cerebrospinal fluid (CSF) measures of amyloid beta protein (Aβ), tau protein, and hyperphosphorylated tau (p-tau) protein are sensitive and specific to the diagnosis of AD and may serve as inclusion criteria and possibly as outcomes in clinical trials targeting relevant pathways. Plasma measures of Aβ are of limited diagnostic value but may provide important information as a measure of treatment response. A wide variety of measures of detectable products of cellular processes are being developed as possible biomarkers accessible in the cerebrospinal fluid and plasma or serum. Surrogate markers that can function as outcomes in pivotal trials and reliably predict clinical outcomes are needed to facilitate primary prevention trials of asymptomatic persons where clinical measures may be of limited value. Fit-for-purpose biomarkers are increasingly available to guide AD drug development decisions.

Keywords: Alzheimer's disease; Drug development; Biomarkers; Amyloid imaging; FDG PET; FDDNP; MRI; Proteomics; Clinical Dementia Rating; ADAS-cog; MMSE

1. Introduction

Alzheimer's disease (AD) is a progressive brain disorder that becomes increasingly common with aging. As the global population ages, AD is rapidly becoming an urgent public health challenge; it now affects 35 million individuals worldwide and is projected to affect 115 million by 2050 if effective therapeutics are not developed [1]. Current therapies for AD provide symptomatic relief either by improving symptoms above baseline or by delaying decline [2,3]. Increasing insight into the molecular mechanisms of AD has provided multiple potential targets for disease-modifying therapeutic intervention. The study of the molecular neurobiology of beta-amyloid (Aβ) protein, tau protein, and hyperphosphorylated tau (p-tau), and intracellular signaling pathways, mitochondrial dysfunction, synaptic abnormalities, and cell death have identified multiple exploitable steps potentially vulnerable to pharmacologic manipulation [2,4]. Agents with putative disease-modifying properties have been identified and have entered preclinical and clinical testing [2,4]. Over 80 compounds—most of them being assessed for disease-modification—are currently in clinical trials for the treatment of AD [5].

Proof of efficacy of AD treatment depends on demonstrating benefits on the clinical measures in patients. However, biomarkers may provide faster, more convenient answers to some questions and are playing increasingly diverse roles in drug development (Fig. 1). Biomarkers may provide new insights into the neurobiology of AD and generate new and

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novel therapeutic targets. Target validation biomarkers demonstrate the interaction between the candidate agent and the immediate or proximal target [6]. Disease-related biomarkers can assist in patient selection, sample stratification, course prediction, and defining disease severity. Some biomarkers may identify patients uniquely responsive to a specific therapy and have ''theranostic'' utility. Pharmacodynamic biomarkers play a role in demonstrating target engagement, dose selection, and treatment response. Pharmacokinetic biomarkers measure aspects of drug absorption, distribution, metabolism, and excretion. Toxicity biomarkers detect adverse effects and may allow the prediction of adverse events. Approval of generics and development of new formulations requires bioequivalence measures that are another form of biomarker [7]. Biomarkers may assist in decision-making in early clinical development [6]. Biomarkers may inform corporate decisions regarding go or no-go decisions, prioritization of compounds within a company's portfolio, or potential expanded or new indications. Biomarkers may be helpful in accelerating early attrition of flawed compounds, decrease cycle time, and decrease development costs [8–10]. Valid biomarkers have commercial value when developed as a product.

In this review, definitions of biomarkers are presented, the diverse uses of biomarkers relevant to drug development in AD are summarized, and the potential role of biomarkers to assess efficacy of AD therapeutics is described. Individual biomarkers are discussed and their implementation in different phases of drug development is reviewed. The purpose of the review is to describe recent advances in biomarkers relevant to the AD drug development process and describe how these new measures can best be integrated into development programs.

2. Definitions and terminology

A biomarker (biological marker) is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [11]. When used in clinical trials, a biomarker may be defined as a laboratory measure that reflects the activity of the disease process [9].

A surrogate marker (or surrogate endpoint) is a biomarker intended to substitute for a clinical endpoint (defined as a characteristic or variable that reflects how a patient feels, functions, or how long a patient survives). It is expected to predict clinical benefit, harm, or lack of benefit or harm [11]. The features of a surrogate marker from a regulatory perspective are provided in Table 1. There are no validated surrogate markers in AD, that is, no biomarker has been shown to reliably predict clinical outcome in AD clinical trials [12].

Biomarkers need not meet criteria for surrogacy to be useful in drug development. The role of biomarkers in assessing the efficacy of drug development in AD is to demonstrate proof-of-principle (POP) or proof of pharmacology and target engagement with the expectation—which is unproven

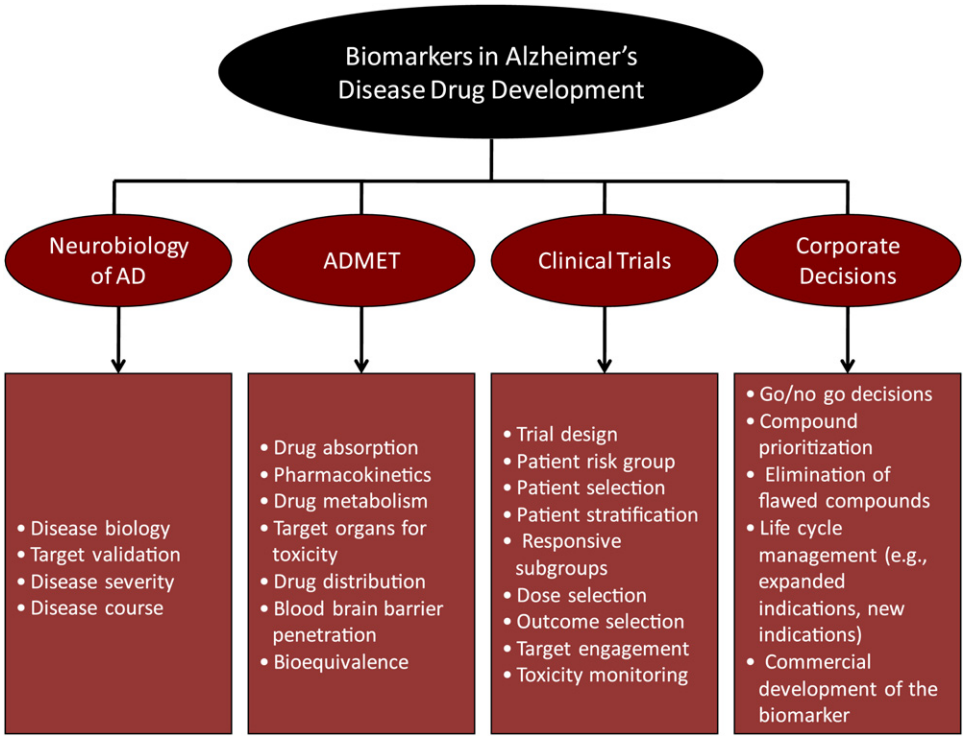

Fig. 1. The role of biomarkers in drug development (AD, Alzheimer's disease; ADMET, absorption, distribution, metabolism, excretion, toxicity).

Table 1
Regulatory features of a valid surrogate biomarker (reproduced with permission from Ref. 9)

It is an objectively measured indicator of the AD pathogenic processes
It measures a feature central to AD-related cell death
The biological relationship between the biomarker and the disease process is known
It has been shown to be valid and reliable under clinical trial conditions
The relationship between the biomarker and the clinical outcome is ''strong'' (e.g., predicts a clinically meaningful benefit)
The effect on the biomarker is responsible for the clinical benefit (as supported by a correlation between the two in clinical trials and a linkage between the two in preclinical studies)
All of the actions of the compound being assessed are known and the clinical benefit is attributable to the biological effect on the disease measured by the biomarker
The relationship between the biomarker and the clinical benefit has been observed across several drugs and across multiple classes of drugs
The risk of causing harm to the patients being treated on the basis of a biomarker does not outweigh its potential benefit

as yet—that the pharmacologic effect will translate into clinical benefit. Biomarkers help de-risk Phase III development programs lacking Phase II clinical proof-of-concept data [13]. As more data are accumulated linking biomarker changes to clinical outcomes, the value of biomarkers in drug development will increase [13].

The U.S. Food and Drug Administration has articulated a mechanism for qualifying a biomarker whereby a potential biomarker can be integrated into a clinical development program and the results presented as part of a New Drug Application (NDA) [14]. Figure 2 summarizes the steps of the qualification process. Biomarkers may be designated as exploratory, probable valid, and known valid depending on the data linking the biomarker to the disease process. A qualified valid biomarker can play an important role in providing data acceptable to regulatory agencies in support of indication claims and labeling.

Disease biomarkers may be relevant to state, trait, and rate [15]. Trait markers represent risk factors and do not change with the presence of the disease. Trait biomarkers indicating an increased risk of AD include the apolipoprotein E ε4 (*APOE* ε4) allele [16,17], APOJ [18,19], CR1 [19], PICALM [18], SORL1 [20], and TOMM40 [21]. State markers indicate the presence of the disease process and include medial temporal atrophy (MTA) on magnetic resonance imaging (MRI), amyloid imaging, and cerebrospinal fluid (CSF) Aβ and tau measures. Rate biomarkers track disease progression; progressive atrophy detected by MRI and hypometabolism observed on fluorodeoxyglucose (FDG) positron emission tomography (PET) are rate biomarkers that correlate with disease severity.

3. Biomarkers and the molecular neurobiology of AD

Figure 3 shows a model of AD molecular biology based on the emerging understanding of the cascade of events involved in this complex disorder [22]. Nearly all current AD drug development targets some aspect of this cascade. The cascade begins with the cleavage of the amyloid precursor protein (APP) sequentially by β- and γ-secretase (Fig. 4). The 42 amino acid Aβ fragment self-assembles into oligomers; neurotoxicity is thought to reside primarily in the oligomeric species of Aβ. Aβ42 also has direct pharmacologic effects on synaptic function, impairing memory and long-term potentiation in animal models [23]. Neurofibrillary tangle formation, oxidation, excitotoxicity, inflammation, synaptic compromise, demyelination, mitochondrial dysfunction, and neurodegeneration follow the interaction with oligomeric Aβ [22]. Aβ may also fibrillize to form insoluble aggregates that compose the neuritic plaques characteristic of AD. Neuritic plaques are surrounded by a halo of astrocytes, microglial cells, and dystrophic neuritis, suggesting that a gradient of toxic Aβ species surrounds the plaque and is in equilibrium with fibrillar Aβ [24].

The extent to which biomarkers reflect brain pathology is critical to their consideration. Hippocampal volume on MRI has been shown to correlate with cell loss [25] and with Braak stage of neurofibrillary tangle formation [26]. Loss of gray matter as shown by MRI voxel-based morphometry (VBM) correlates with the Braak stage [27]. A Structural Abnormality Index score that rates the degree to which a magnetic resonance (MR) image corresponds to a typical library of AD images also correlated strongly with the Braak stage [28]. The ligand used in Pittsburgh Compound B (PIB) PET labels fibrillar amyloid at postmortem [29,30]. Hypometabolism on FDG-PET correlated with reduced synaptophysin levels (a measure of synaptic integrity) at postmortem in studies of nonhuman primates [31]. Reduced CSF Aβ42 correlates well with Aβ neuropathology at autopsy in human beings [32,33], and elevated CSF tau levels correlate with neurofibrillary tangles [34]. Biomarker-pathology relationships can be difficult to establish as biomarkers are often collected early in the disease process, long before the patient succumbs to the illness and well before studies of brain pathology. The pathology data support a model of AD in which Aβ is deposited early in the course of AD before cognitive decline. In the second phase of the illness, neurofibrillary tangles develop, neurons die, brain atrophy occurs, and cognition declines [35]. An ordered sequence of changes in the biomarker reflects this process.

Biomarkers are collected from a variety of biological compartments (e.g., imaging of brain, CSF Aβ, and tau levels from lumbar CSF) and each compartment provides a different window on the pathological processes of AD (Fig. 5). Imaging biomarkers provide insight into the topographic distribution of pathologic changes, whereas fluid biomarkers do not. Fluid biomarkers may appear in the extra-central nervous system (CNS) compartments by diffusion or facilitated transport and are subject to metabolism and excretion; the status of these mechanisms will also affect the relationship of the biomarker to the brain disease.

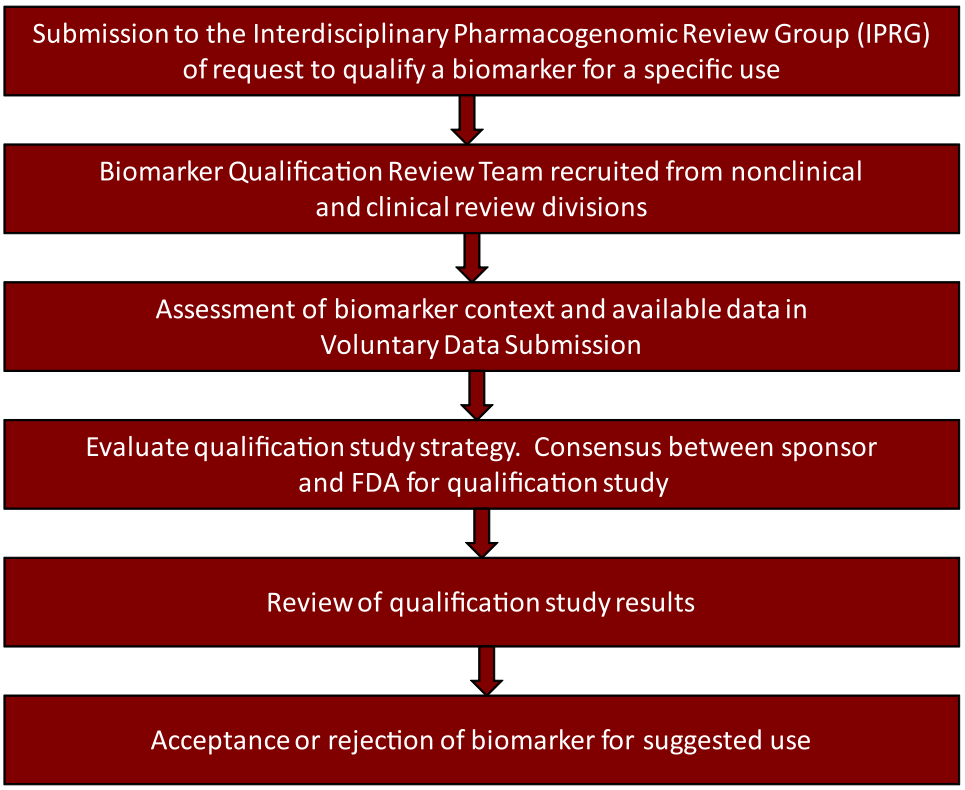

Fig. 2. Biomarker qualification process of the U.S. Food and Drug Administration.

4. Aβ physiology in healthy persons and in AD

4.1 Cerebrospinal fluid Aβ

Interpreting biomarkers in AD depends on a thorough understanding of Aβ physiology in healthy individuals and how this is altered in AD. The production rate of CSF is 20–30 mL/hr; fractional production of Aβ is 7.6% per hour, and clearance is 8.3% per hour in young normal individuals, indicating that in persons without AD, approximately 8% of total CSF Aβ is produced and removed each hour [36]. This is consistent with turning over the Aβ content of the CSF approximately twice daily (once every 13 hours).

In healthy individuals, Aβ is cleared from the brain by breakdown within the CNS, transported from the brain to the blood across the blood–brain barrier, and transported from the brain to the CSF. CSF Aβ is transported to the venous blood through the arachnoid granulations [24]. Aβ derived from APP may be degraded by a variety of enzymes (neprilysin, insulin degrading enzyme, plasminogen activator inhibitor, and others). Aβ is actively transported into the brain from the blood through the receptor for advanced glycation end products (RAGE) and is actively transported out of the brain by low-density lipoprotein receptor-related protein (LRP) [22,37,38]. LRP-1 facilitates the transport of Aβ40, whereas LRP-2 and Apo J (also known as clusterin) mediate transport of Aβ42 across the blood–brain barrier [22]. P-glycoprotein mechanisms are also involved in amyloid transport from the brain to the blood [39,40]. A small amount of Aβ (10% of that leaving the brain) crosses the brain-CSF barrier into the CSF by bulk flow [24].

Dysfunction of transport mechanisms that normally remove Aβ from the brain may contribute to the pathology of

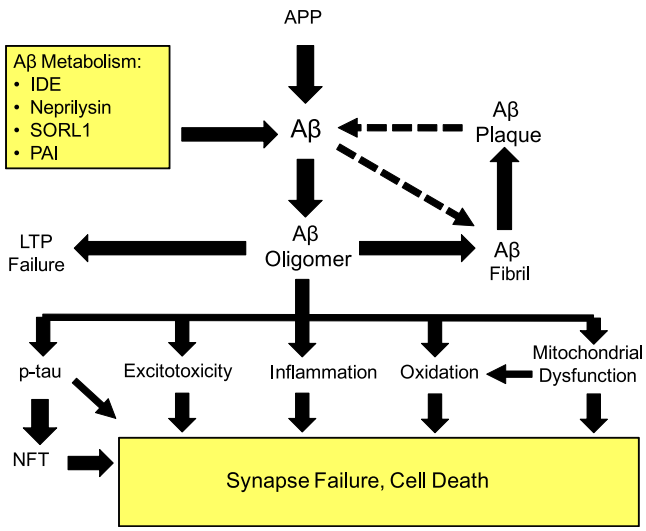

Fig. 3. Amyloid-beta (Aβ) metabolism and related neurotoxic cascades (Aβ, amyloid beta protein; APP, amyloid precursor protein; IDE, insulin degrading enzyme; SORL1, sortrilin 1; PAI, plasminogen activator inhibitor; LTP, long term potentiation; NFT, neurofibrillary tangle; p-tau, phosphorylated tau protein).

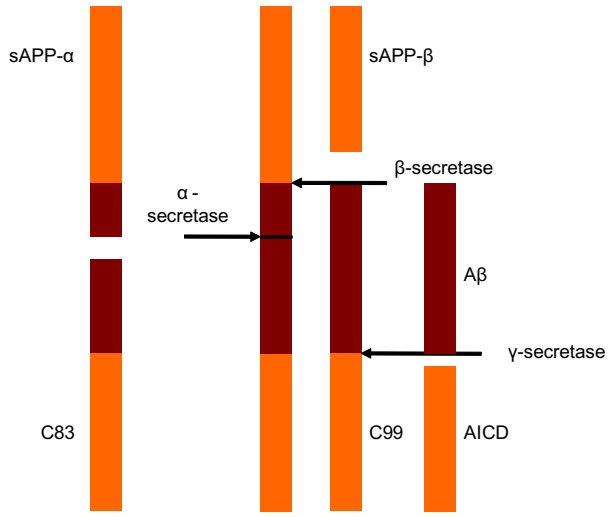

Fig. 4. Amyloid precursor protein (APP) processing to form amyloid-beta (Aβ) protein. On the left, the non-amyloidogenic pathway is initiated by α-secretase cleavage of APP within the Aβ fragment producing soluble APP (sAPP) alpha and a C83 fragment; on the right β-secretase cleaves APP to produce the N-terminal of C99, then gamma-secretase cleaves C99 to create the C-terminus of Aβ plus the AICD fragment (AICD, amyloid intracellular domain).

AD [39,41]. RAGE is overexpressed in AD suggesting that increased Aβ transport into the brain may contribute to the amyloid pathology [42]. The altered transport mechanisms into and out of the brain in AD, as well as increased Aβ deposition in the brain, may help to account for the observation wherein plasma and CSF Aβ (Aβ40 and Aβ42) levels are correlated in healthy control subjects but not in those with AD [43].

In healthy individuals, Aβ levels are 50 times higher in CSF than in plasma and most of the CSF Aβ is of neuronal origin [36], although small amounts of non-neuronal Aβ may enter CSF from the blood (Fig. 6). Aβ levels vary from 1.5- to 4-fold when measured serially over 36-hour periods [44]. This indicates the importance of standardizing times of CSF collection in clinical trials where Aβ levels are an outcome parameter.

Monomeric Aβ42 rapidly and dynamically aggregates into oligomers. Of the total soluble Aβ42 in the brain, approximately 75% is oligomeric [30]. The amount of soluble Aβ42 in the brain is approximately 50 times greater than the level of soluble Aβ42 measurable in the CSF of AD patients [45]. Moreover, the amount of insoluble fibrillar Aβ present in plaques in the brain is approximately 100-fold greater than the amount of soluble Aβ in the brain [30]. Plaques may serve as a reservoir of Aβ that can be solubilized. Thus, sources of soluble Aβ may include newly synthesized Aβ, Aβ derived from the huge amyloid mass present in insoluble plaques, and Aβ derived from peripheral sources and transported into the brain by RAGE [24,30].

4.2 Plasma Aβ

Most plasma Aβ is of neuronal origin, but some also originate from the muscles, liver, kidneys, and lungs [46,47] (Fig. 6). Some studies have found elevated muscle Aβ in AD compared with healthy control subjects, and this source may contribute to the Aβ in the plasma of AD patients [48]. Changes in peripheral Aβ after the administration of anti-Aβ compounds, such as secretase inhibitors, may reflect in part an effect on these peripheral sources. Free Aβ redistributes to tissues within about 1 minute and has a terminal elimination half-life of approximately 10 minutes in the blood [49]. Approximately 90% of Aβ is catabolized in the liver and the remaining 10% is degraded in peripheral tissues or excreted in the urine [49]. Most peripheral Aβ—approximately 95%—is bound to serum proteins [50,51] (5% is

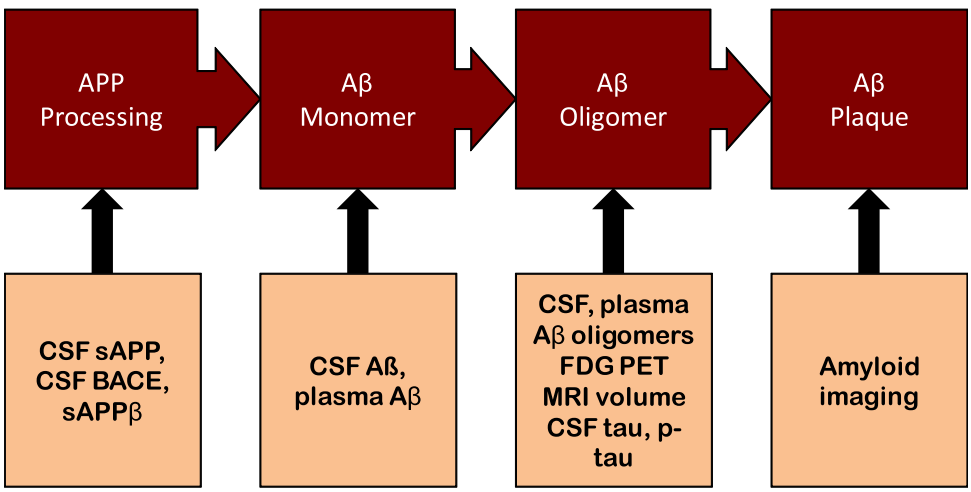

Fig. 5. Biomarkrs for each step in the amyloid cascade. APP processing can be assessed as sAPP α, in CSF; BACE protein and activity are measured in CSF and sAPPβ is a measureable product of BACE cleavage of APP; Aβ monomers can be measured in CSF and plasma; a few studies suggest that oligomers can be measured in plasma and CSF; oligmers are toxic and affect synaptic function as measured by FDG PET, neurodegeneration and atrophy as measured by MRI and death of neruons with release of tau and p-tau in CSF; amyloid imaging measures fibrillar Aβ deposited as neuritic plaques. (FDG PET, fluorodeoxyglucose positron emission tomography; BACE, Aβ-site APP cleaving enzyme)

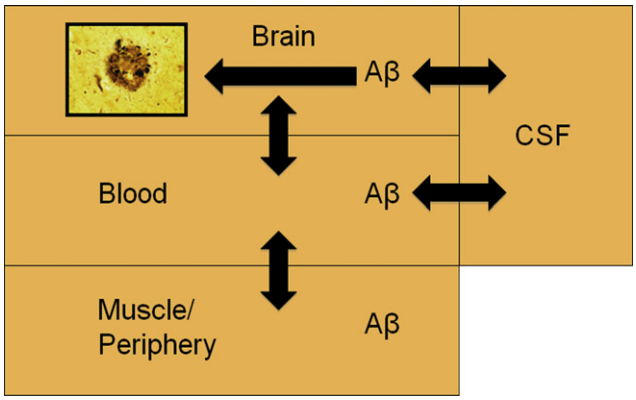

Fig. 6. Biological compartments with amyloid-beta (Aβ) protein (CSF, cerebrospinal fluid).

bound to lipoproteins and 90% to albumin). The half-life of Aβ bound to albumin in serum is 2–3 weeks [49]. There are at least 300 times more amyloid binding proteins in plasma than in CSF [22]. Binding of Aβ in plasma may prevent oligomerization and fibrillization, thereby preventing both peripheral toxicity and peripheral deposition observed in the brain [22,50,51].

Mean plasma Aβ (Aβ40, Aβ42) does not discriminate between healthy control subjects and those with AD [52,53]. Most studies have failed to show that Aβ42 levels distinguish between patients with mild cognitive impairment (MCI) who progress to AD dementia and those who do not[54]. Aβ40 and Aβ42 levels increase with age and those with increased Aβ40 levels are at increased risk for AD [55]. Most studies suggest that those with low Aβ42 levels have a greater risk of developing MCI and dementia [55–59]. Mutations causing AD increase the level of Aβ42 in plasma [60]. Aβ42 declines toward normal levels as AD begins suggesting that—in parallel with CSF Aβ42—there is a reduction peripherally as the Aβ is deposited in the brain [61]. Oligomeric Aβ has been difficult to measure; recent studies show

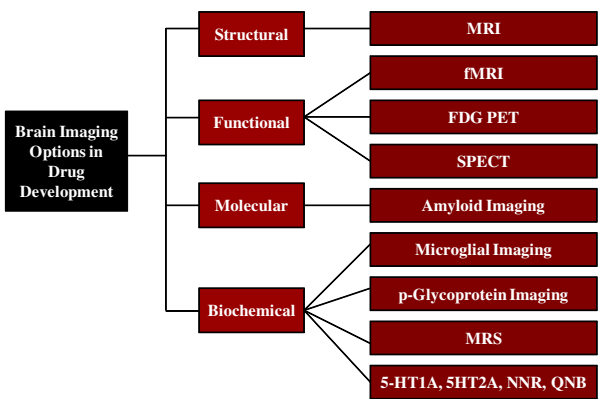

Fig. 7. Role of brain imaging in Alzheimer's disease (AD) drug development. (FDG PET, fluorodeoxyglucose positron emission tomography, MRI, magnetic resonance imaging, MRS, magnetic resonance spectroscopy, QNB, 123 I-iodo-quinuclidinyl-benzilate; NNR, neuronal nicotinic receptor binding; HT, hydroxytrypamine).

decreasing oligomeric as well as monomeric plasma Aβ in patients with AD [62].

In healthy elderly persons, plasma Aβ (Aβ40 and Aβ42) levels are correlated; this predictable relationship is lost in MCI and in AD, where these two measures are not related consistently [43,63].

5. Specific biomarkers potentially useful in AD drug development

5.1 Cerebrospinal fluid

5.1.1. Levels of Aβ, tau, and p-tau

The approximately 50% reduction in CSF Aβ42 levels in AD is attributed to trapping of the peptide in Aβ plaques in the brain [64], although one-third of the variance in amyloid imaging is not predicted by CSF Aβ levels [65]. Aβ42 levels are reduced and total tau and p-tau levels are increased in AD [66–68]; Aβ40 levels remain unchanged or may be moderately increased [64]. CSF Aβ levels remain unchanged after onset of the dementia phase of the illness, whereas tau levels may show a slight increase [69–71]. Reduced Aβ42 and elevated total tau and p-tau levels do not correlate with the Mini-Mental Status Examination (MMSE) score in AD but they do predict a more rapid decline [72]. The stability of Aβ levels in the CSF implies that a dynamic equilibrium is reached between amyloid production and its removal and degradation. This stability may reflect the following altered CSF dynamics in AD patients as compared with healthy persons: CSF production is reduced and increasing cerebral atrophy leads to increased CSF volumes [73,74]; levels of brain-derived proteins are higher in ventricular CSF as compared with lumbar CSF (1.5:1 for tau) [73]; Aβ measures in CSF are based on detection of the monomeric Aβ species; and oligomerization of the Aβ contributes to the reduced levels of monomeric Aβ in the CSF of persons with AD [75]. Low CSF levels of Aβ42 and elevated levels of total tau or p-tau predict progression from MCI to AD dementia with 83% sensitivity, 72% specificity, 62% positive predictive value, and 88% negative predictive value [67]. Aβ-like peptides generated by β- and γ-secretase are elevated in CSF of AD and MCI patients [76].

Total tau levels are thought to reflect neuronal degeneration and are elevated in several neurological disorders. Phosphorylation of tau reflects the specific pathophysiology of AD leading to neurofibrillary tangles. In most studies, p-tau measures have greater sensitivity and specificity for the diagnosis of AD than total tau measures [77]. It is thought that these intracellular proteins are released from dying cells and migrate from the interstitial fluid of the brain to the CSF.

Relationships between CSF biomarkers and brain imaging have been studied. CSF Aβ42 (but not total tau or p-tau) correlated with whole-brain atrophy in cognitively normal persons whereas CSF tau and p-tau (not Aβ42) correlated with brain atrophy in mild AD [78]. Baseline CSF p-tau levels

correlated with hippocampal atrophy at baseline and with rates of hippocampal atrophy in patients with AD [79]. These observations suggest that tau abnormalities and MRI changes reflect neurodegeneration, whereas altered Aβ levels precede neurodegenerative changes [35].

CSF measures of Aβ42, total tau, and p-tau have limited relationships to clinical outcomes. They do not correlate with Clinical Dementia Rating-Sum of Boxes (CDR-sb) or MMSE scores in cross-sectional studies of healthy elderly patients with amnestic MCI or mild AD [80]. Log (total tau/Aβ42) was predictive of progression from amnestic MCI to AD type dementia [81]. In patients with very mild AD, the rate of dementia progression is predicted by lower baseline Aβ42 and higher tau or p-tau levels [82]. Patients with higher levels (total tau >800 ng/L) performed worse on MMSE and Alzheimer's Disease Assessment Scale—cognitive portion (ADAS-cog) [83] assessments.

A few studies showed that candidate AD therapies were effective on CSF parameters. Levels of Aβ42 declined after treatment with PBT2 [84], MK-0752 [85], talsaclidine [86], AF102B [87], tramiprosate [88], and intravenous immunoglobulin [46]. Treatment with the antibody LY2062430 (solazumab) increased serum Aβ and CSF Aβ [89]. Both simvastatin [90] and AN-1792 [91] lowered CSF tau levels in clinical trials.

5.1.2. Aβ production

It is possible to measure CSF Aβ production and clearance by using a stable isotope labeling kinetic (SILK) technique [36]. Production and clearance is 8% per hour in young healthy control subjects. LY-450139, a γ-secretase inhibitor has been shown to decrease Aβ production in AD [92]; however, there was no change in CSF Aβ levels after 3 months of treatment with this agent [93].

5.1.3. Other CSF measures

Many other proteins have been measured in the CSF in an effort to develop better biomarkers (Table 2). Most of these have been reported in small cohorts of patients with limited technical validation of the methods and require verification in larger populations with confirmatory findings from other laboratories. These are exploratory markers and integration into trials awaits further study. Choosing a biomarker closely linked to the mechanism of action of candidate therapies provides direct insight into the potential for altering the disease process. Biomarkers specific to the mechanisms of the agent may be combined with measures of AD progression to assess target engagement and effect of the disease in AD drug development programs. CSF isoprostane levels—a measure of oxidative injury—are among the most promising emerging disease-related measures [103,104].

5.2 Neuroimaging

Several brain imaging techniques have become available to characterize the structure, function, composition, and molecular pathology of the brain in AD (Fig. 7).

5.2.1. Magnetic resonance imaging

MR brain imaging can be divided into structural techniques including volumetric MRI, diffusion-weighted MRI, diffusion tensor imaging (DTI), and magnetization transfer ratio (MTR), or functional techniques such as perfusion MRI, blood oxygenation level-dependent functional MRI (fMRI), and arterial spin labeling (ASL) [128]. Structural MR studies can use manual region of interest analyses or computer-based approaches such as tissue segmentation, global boundary shift integral measures, VBM, or tensor-based morphometry (TBM) [128]. MRI morphometric measures are the most promising biomarkers to detect drug efficacy in AD clinical trials. MRI can also have a critical role in detecting evidence of drug-related toxicity in clinical trials including vasogenic edema and microhemorrhages [128]. Table 3 summarizes the uses of MRI relevant to AD drug development.

Volumetric MRI is the method of choice for clinical trials of disease-modifying agents. MR imaging devices are widely available, the measures have high test-retest reliability, and correlations between MR measures of atrophy and neuronal loss have been established [27,73,128]. Methods applicable to multisite studies have been established and shown to work in large-scale studies such as the Alzheimer's Disease Neuroimaging Initiative (ADNI) [132–134]. Atrophy is not specific to AD, occurring in normal aging and other dementias; and MRI does not give intervention-specific information relevant to specific mechanistic aspects of drugs. Drug-placebo differences in atrophy progression in trials of disease-modifying therapeutic agents would provide important but nonspecific support for a neuroprotective drug effect.

MTA identifies patients with underlying AD-type pathology; in patients with MCI, MTA predicts progression to AD-type dementia. Using a rating scale ranging from 0 (no atrophy) to 4 (severe atrophy), DeCarli et al [135] found that of those with scores of ≤1, 29.1% progressed to dementia over a 3-year period (10% per year) compared with 45.7% of those with scores >1, 60% of those with scores of ≥2, and 75% of those with scores >2. MTA has been reported to correlate with scores on the ADAS-cog, paragraph recall, and the digit symbol substitution test in patients with MCI.

Hippocampal atrophy also identifies patients with AD dementia, and atrophy in this region progresses more rapidly in AD dementia (4.66% annualized atrophy) compared with healthy elderly control subjects (1.41% annualized atrophy) [136]. High-dimensional modeling using elastic registration methods has shown that it is possible to perform subfield mapping of the hippocampus. Greater atrophy of the CA1 region of the hippocampus correlated with delayed recall performance and predicted which MCI patients would progress to AD dementia [137] (Fig. 8).

The rate of ventricular enlargement is 1.3 cm^3/year in healthy elders, 2.5 cm^3/year in persons with amnestic MCI, and 7.7 cm^3/year in those with AD [138]. Whole-brain atrophy has been reported as on the order of 0.5% in healthy

Table 2
CSF measures potentially applicable to clinical trials of Alzheimer's disease

Measure	Biology
β-secretase (BACE-1)	Increased activity in CSF of MCI but not of AD; activity may be decreased in AD; BACE-1 activity was weakly associated with BACE protein levels; CSF Aβ levels correlated with BACE activity [94]
Aβ42	Decreased in AD and in MCI who progress to AD dementia [68]
Aβ oligomers ([ADDLs])	Increased in AD [95]
Tau, p-tau	Increased in AD and in MCI who progress to AD dementia [68]
APPs-α	Soluble APP α is decreased in AD; correlated with MMSE scores [96,97]
APPs-β	APPs-β is a product of APP cleavage by BACE-1; levels are normal in AD; this measure could be important as a measure of BACE-1 inhibition [97]
APLIβ	Fragments generated by β- and γ- secretase; elevated in MCI and AD [76]
SorLA/LR11	Decreased in mild to moderate AD and in postmortem samples of CSF from patients with advanced AD [98]
Neprilysin	Aβ-degrading enzyme; decreased in MCI that progressed to AD dementia and in very mild AD; increased in concert with tau levels during disease progression [99]
VLP-1	Elevated in patients with AD as compared with the healthy elderly population; elevated in AD with the ε4/ε4 genotype; correlated with p-tau; correlated with MMSE score [100]
12(S)-HETE acid and 15(S)-HETE	Products of 12/15 lipoxygenase enzyme; elevated in AD and in MCI; correlated with tau and lipid peroxidation [101]
SAP	Glycoprotein that binds Aβ and initiates the complement cascade; lower levels in CSF of MCI patients who progressed to AD dementia [102]
F2-isoprostanes	Elevated in patients with AD as compared with hospitalized persons without neurologic disease and patients with ALS [103–105]; progressive increase in patient with MCI developing AD dementia [106]
8-OHdG	Increased in CSF in AD; marker of oxidative stress [107]
GFAP	Cytoskeletal protein expressed primarily by astrocytes [108]
Neuroserpins	Increased in patients with AD as compared with controls and dementia with Lewy bodies [109]
ACT	ACT is produced in AD brain by reactive astrocytes in response to high levels of the pro-inflammatory cytokine interkeukin-1 secreted by reactive microglia; increased in AD as compared with healthy controls [109; correlated with cognition [110]; not correlated with serum levels of ACT [111]
CRP	Inflammatory marker; increased in AD and MCI [112]
TGF-β1	Cytokine indicative of an inflammatory response; elevated in CSF in AD (not serum) [113]
PDS and TTR	PDS/TTR is increased in AD and MCI, not in healthy controls or nonAD disease-control subjects; distinguished AD with 100% sensitivity and 93% specificity [114]
Dkk-3	Dkk-3 is a secreted glycoprotein; increased in patients with AD as compared with controls; not increased in MCI or depression [115]
Soluble IL-1 sIL-1RII	sIL-1RII is decreased in mild to moderate AD; normal in patients with MCI who progress to AD dementia and in severe AD [116]
HO-1	Reduced in AD as compared with healthy elderly control subjects [117]
RBP	Decreased in patients with AD and MCI as compared with healthy elderly control subjects; levels were lower in AD than MCI and correlated with disease severity. Not all studies have found diminished levels [118]
Kallikrein-6	A protein highly expressed in the cells of the choroid plexus; increased in AD [119]
Haptoglobin precursor allele 1	Decreased in patients with AD and MCI as compared with healthy elderly control subjects; levels were lower in AD than MCI and correlated with disease severity [120]
NPR	Elevated in AD not PD [121]
Glutamine synthetase	Increased in AD and vascular dementia [122]
Proteomic 23 protein panel	Distinguished AD from nonAD neurological comparison group and healthy controls with 94% sensitivity and 94% specificity [123]
Proteomic 17 protein panel	Distinguished MCI that progressed to AD dementia from MCI that did not; upregulated proteins included C3a anaphylatoxin des-Arg, C4a anaphylatoxin des-Arg, ubiquitin, and phosphorylated C-terminal fragment of osteopontin, a proinflammatory cytokine [124]
Proteomic 15 protein panel	Panel distinguished patients with AD from healthy control subjects; seven of the 15 differentiated AD from frontotemporal dementia [125]
NCAM-120, α-dystroglycan	Elevated in AD and in PD [121]
N^E(gamma-glutamyl)lysine	Increased in AD and vascular dementia; levels above 120 mM/L were 77% sensitive and 72% specific for the diagnosis of AD [126]
Nerve growth factor (NGF)	Elevated in AD when examined in patients with p-tau/Aβ42 ratios >10 [127]

Abbreviations: BACE, beta-amyloid-site APP cleaving enzyme; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; ADDLs, amyloid-β-derived diffusible ligands; APLIβ, Aβ-like peptides; SorLA/LR11, sortilin-related receptor; VLP-1, visinin-like protein; HETE, hydroxyeicosatetraenoic; SAP, serum amyloid protein; ALS, amyotrophic lateral sclerosis; OHdG, hydroxy-2'-deoxyguanosine; GFAP, glial fibrillary acid protein; ACT, alpha-1-antichymotrypsin; CRP, C-reactive protein; TGF-β1, transforming growth factor-β1; PDS, protein complex of prostaglandin-D-synthase; TTR, transthyretin; Dkk-3, dickkppf homolog 3; IL-1, interleukin-1; sIL-1RII, soluble interleukin-1 receptor subtype II; HO-1, heme oxygenase-1; RBP, retinol binding protein; NPR, neuronal pentraxin receptor; NCAM, neural cell adhesion molecule-1; NGF, nerve growth factor.

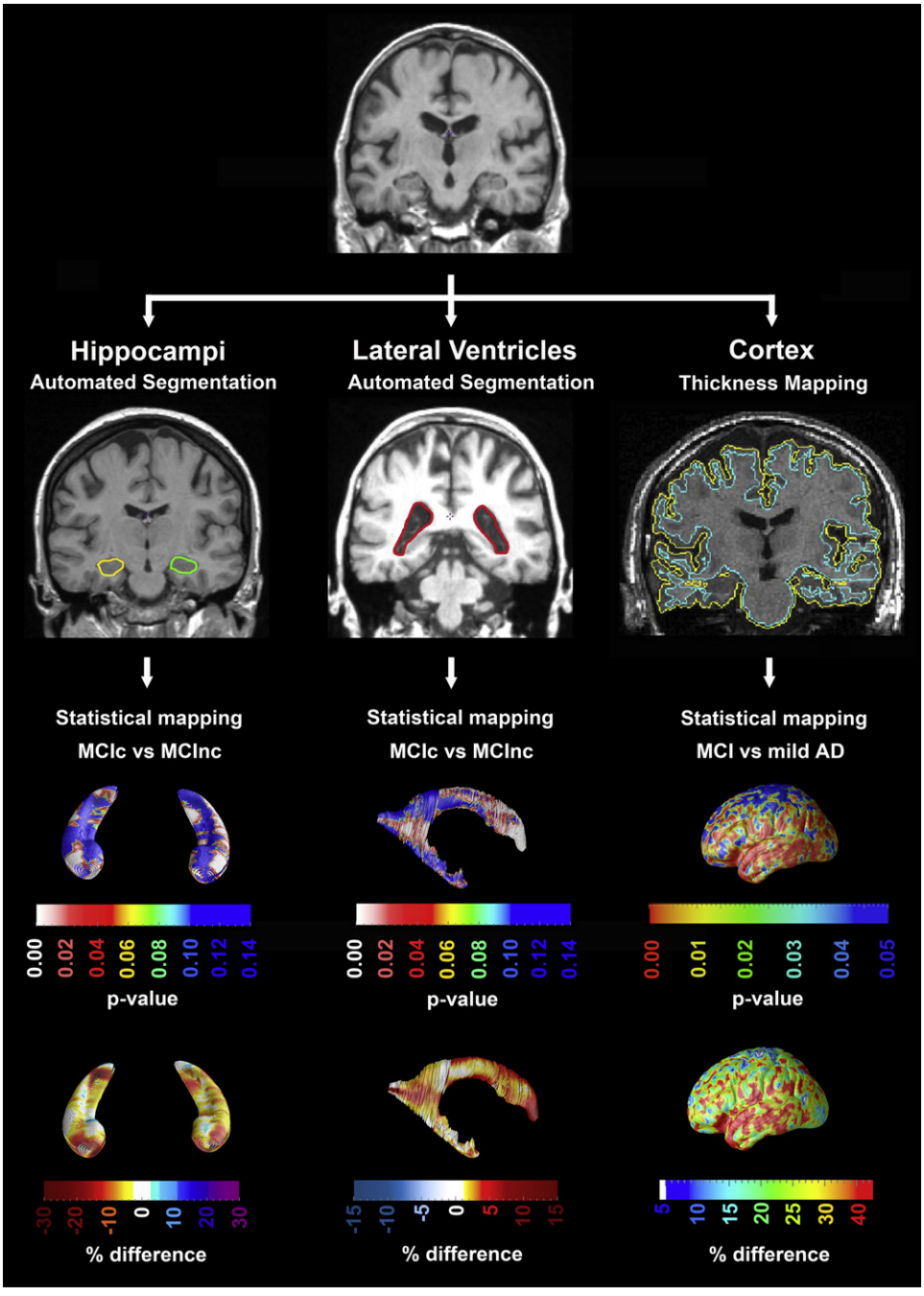

Fig. 8. MRI with 3D structural reconstruction to show hippocampal atrophy (left column), ventricular enlargement (center column), and cortical thickness (right column) (images courtesy of Liana Apostolova, MD and Kristy Hwang, UCLA) (AD, Alzheimer's disease; MCIc, mild cognitive impairment that converted to AD; MCInc, MCI did not convert to AD).

control subjects and 1.4%–2.4% per annum in mild–moderate AD [132]. In patients included in a clinical trial and manifesting mild-to-moderate AD, rates for whole-brain atrophy and ventricular enlargement were correlated with changes in MMSE, ADAS-cog, Disability Assessment for Dementia, and Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change [139]. Clinical Dementia Rating (CDR) was correlated with whole-brain atrophy but not with ventricular enlargement. Jack et al [138] found significant relationships between ventricular enlargement, and MMSE and CDR scores. The relationship between whole-brain atrophy or ventricular enlargement and ADAS-cog scores has been confirmed by other investigators and extended to patients with MCI who had progressed to AD within 12 months [140]. Autopsy studies showed that the rate of brain atrophy as measured by MRI (whole brain volume and ventricular volume) is related to cognitive measures and to neurofibrillary tangle burden as determined by Braak stage; however it is not related to Aβ neuritic plaque burden or to the total of neuritic and diffuse plaque burden [141].

Table 3
MRI techniques potentially applicable to clinical trials of Alzheimer's disease

MRI technique	Potential application in clinical trial
MRI hippocampal volumetric measures	Hippocampal volume atrophies less in the treatment group as compared with the placebo group
Hippocampal subfield (radial atrophy) mapping	MCI patients with greater atrophy of CA1 atrophy are more likely to progress to AD dementia; this could be used as an outcome measure if correlated with clinical measures or to identify patients for study entry
MRI whole brain volumetric measures	Whole brain volumes decreases less (there is greater preservation of volume) in the treatment group as compared with the placebo group
Ventricular volume measures	Ventricular volume increases more in the placebo group than in the treatment group
FLAIR sequence	Detects white matter images including vasogenic edema and encephalitis [129]
GRE images	Detects microbleeds occurring spontaneously in AD and occurring as a complication of immunotherapy [130]
SWI	SWI maximizes sensitivity to magnetic susceptibility effects and is more sensitive than GRE in detecting cerebral microhemorrhages [131]
MRI cortical thickness mapping	The cortex undergoes less thinning in the treatment group as compared with the placebo group
DWI and DTM	Greater decline in fractional anisotropy as a measure of axonal integrity in the posterior corpus callosum, cingulate white matter, and other fiber tracts in the placebo group as compared with the treatment group
TBM	Greater atrophy in placebo group as compared with the control groups
fMRI	Activation of the brain by asking subjects to perform tasks when they are under the scanner reveals differences between patients and controls and could demonstrate drug-placebo differences
MRS	Proton MRS may show differences in NAA as a marker of cell viability in treatment and placebo groups

Abbreviations: MRI, magnetic resonance imaging; MCI, mild cognitive impairment; AD, Alzheimer's disease; FLAIR, fluid-attenated inversion recovery; GRE, gradient echo; SWI, susceptibility-weighted imaging; DWI, diffusion-weighted imaging; DTM, diffusion tensor mapping; TBM, tensor-based morphometry; MRS, magnetic resonance spectroscopy; NAA, *N*-acetyl aspartate.

AD patients with younger age at onset, who exhibited generalized rather than focal hippocampal atrophy, and who were not carriers of the *APOE* ε4 genotype progressed more rapidly on measures of whole-brain atrophy [142] suggesting that such clinical factors could be used to construct populations with more rapid atrophy and to optimize the ability to distinguish drug-placebo differences with this biomarker.

The medial forebrain region can be measured on MRI and has been shown to be atrophic in AD. Atrophy was measurable up to 4.5 years before the onset of symptoms [143].

AD is associated with progressive cortical thinning reflecting the loss of brain substance. VBM can be used to assess the gray matter volume and surface-based methods provide measures of cortical thickness. Cortical thinning distinguished between AD and healthy control subjects with 83% sensitivity and 93% specificity [144]. Cortical thinning occurs in a regionally-specific manner with the greatest atrophy in the rostral medial temporal cortex (14% thinning), inferior temporal regions (11%), inferior parietal cortex (9.6%), and superior frontal cortical areas (7.8%) in mild AD compared with healthy elderly control subjects [145]. Regional thinning was inversely correlated with the CDR-sb. This topographical pattern of thinning was detected in MCI and in the normal elderly persons with positive PIB scans, indicating that the regional pattern of atrophy may be a biosignature of AD.

TBM uses computational techniques to make 3-dimensional profiles of brain atrophy across different groups or over time [146]. Its approaches have been applied to the ADNI sample of AD, MCI, and normal elderly individuals. TBM can be automated and applied to large scale clinical trials. In MCI, temporal TBM measures correlated with MMSE and CDR scores and showed a rapid progression of atrophy in patients with MCI who had progressed to dementia as compared with those who had not progressed to dementia. In AD, temporal lobe TBM correlated with CSF p-tau and p-tau/Aβ_{42} ratio [146]. Temporal lobe atrophy in AD correlated with decreased logical memory on neuropsychological testing but not with changes in MMSE, CDR, and immediate or delayed logical memory.

Diffusion-weighted imaging (DWI) and DTI measure white matter of the brain with the potential to show drug-placebo differences in clinical trials. DTI measures fractional anisotropy (FA) and mean diffusivity based on diffusion gradients to molecular motion produced by diffusion barriers in the brain. The resulting FA image is a marker of fiber loss in cerebral fiber tracts. Differences between AD and MCI and their respective controls have been established [147]. The method has not been widely integrated into trials [133,148].

MTR measures the ratio of efficiency of magnetization exchange between protons in the tissue compared with the surrounding water. It is more sensitive than conventional MRI as a measure of changes in the white matter. MTR measures are less robust than those demonstrated with DTI [149].

Decisions regarding the specific structural MRI measures to be used in a trial will be guided by the hypotheses of the trial, the population being studied, and the anticipated effects of the candidate agent. For example, hippocampal measures may be optimal for identifying patients with predementia AD, whereas ventricular enlargement or whole-brain atrophy may be superior to track disease progression and correlate with clinical outcomes [138]. Recent subregional analyses using TBM-like approaches showed that smaller sample sizes may be required to document changes in entorhinal cortex and inferior and medial temporal structures than more global measures; relationships to clinical trial measures have not yet been demonstrated [150].

Blood oxygenation level-dependent fMRI measures the changes in regional deoxyhemoglobin concentrations during

task performance [15]. In patients with AD, memory tasks are associated with reduced hippocampal and parahippocampal activation compared with healthy control subjects. Using a novel-versus-repeated stimulus as an fMRI activation task, Diamond et al [151] showed correlations between ADAS-cog total scores and ADAS-cog verbal memory scores and left superior temporal and left prefrontal cortical activation. Reports of the results of activation studies in MCI are less consistent [15]. Preliminary comparative studies showed a greater sensitivity and classification accuracy of medial temporal entorhinal cortex atrophy compared with fMRI for distinguishing AD from MCI, and MCI from healthy elderly subjects [152]. fMRI requires substantial patient cooperation and investigator expertise and may be best considered for small POP studies.

ASL is an MR technique that is sensitive to cerebral blood flow and reveals patterns in the changes of blood flow similar to those seen with single photon emission computed tomography (SPECT) in AD with diminished flow in parietal, frontal, and posterior cingulate cortex [153]. Patients with MCI have similar but less marked and less extensive perfusion deficits with ASL measures [153].

Magnetic resonance spectroscopy (MRS) allows investigation of the chemical constituents of the brain. Proton MRS (1H MRS) measures proton-containing metabolites in the brain. *N*-acetyl aspartate (NAA) is a marker for neuronal integrity; myoinositol is a glial metabolite that increases with CNS inflammation; and choline is a measure of membrane phospholipids and membrane turnover [15,154]. In one longitudinal study, NAA declined 12.36% in AD patients compared with a 0.94% decline in normal elderly individuals and the changes correlated with global clinical measures [15,155]. NAA/Creatine (Cr) ratio is decreased in AD [156] and a low NAA/Cr ratio at baseline predicts progression of MCI to AD dementia [157]. Standardization techniques have been developed to allow its use in multicenter trails [156].

When used to measure concentrations of labeled agents in the brain, MRS can assess compounds present in micromolar (10^{-6}) quantities, whereas PET and SPECT can measure ligands of receptor occupancy of ligands in nanomolar (10^{-9}) to picomolar (10^{-12}) concentrations [158] making these approaches substantially more sensitive in this setting.

5.2.2. Amyloid imaging

N-methyl [^{11}C]-2-(4'-methylaminophenyl)-6-hydroxybenzothiazole or PIB [159] labels insoluble amyloid and as such provides a reliable means of identifying patients with fibrillar neurotic Aβ plaques. PIB imaging reveals an amyloid burden in 20%–30% of the normal elderly individuals, 60% of patients with MCI, and 95%–100% of those with clinically diagnosed AD [160–163]. PIB binds to fibrillar amyloid and labels mature neuritic plaques most intensely; diffuse plaques are relatively unlabeled; and the amorphous plaques of the cerebellum are not detected at all [29,30]. Vascular amyloid is also labeled. The stoichiometric relationship of PIB and Aβ is known, with >500 PIB binding sites per 1000 Aβ molecules [164]. The pattern of PIB binding in some autosomal dominant cases of AD differs from the pattern observed in sporadic AD cases with greater binding in the striatum of familial cases [165]. Soluble amyloid including monomers and oligomers are not labeled by PIB. Neurofibrillary tangles are not labeled with PIB although occasional ''ghost'' tangles in the neuropil have been found to be PIB positive in postmortem samples [29]. Amyloid is not specific to AD and a positive PIB signal is not equivalent to a diagnosis of AD; patients with dementia with Lewy body dementia, Down syndrome, and amyloid angiopathy also have evidences of amyloidosis on PIB imaging [166].

Test-retest reliability of PIB over short periods of time is in the range of 3%–7% [159]; a 10%–20% decrease in insoluble Aβ load should be detectable with 90% power with PIB in the course of clinical trials [24,30,167].

There was only a slight increase in the PIB signal when patients with AD were followed longitudinally for up to 2 years [138,159,168]. There are several possible explanations for the stable PIB signal and the following multiple factors may contribute to its stability: amyloid plaque formation may plateau in the early phases of the AD process; plaque may develop and disappear dynamically without changing the total amyloid signal; plaques may grow in size without presenting more binding sites for the PIB ligand; inflammation in the region of the plaques may increase and alter local PIB binding; and progressive atrophy of cortex in the course of AD may blunt the PIB signal since the cortical ribbon will become smaller over time [159,167].

Patients with MCI who have positive PIB scans are highly likely to progress to dementia of the AD-type, whereas those with negative scans are not [169,170], suggesting that PIB identifies MCI patients who do not yet meet criteria for dementia in the early phases of AD. Half of the PIB positive MCI patients progressed to AD-type dementia within 1 year and 80% progressed to dementia within 3 years [171]. MCI and AD patients with *APOE* ε4 genotype have higher PIB uptake levels and progress to dementia more rapidly than those without the ε4 allele [160,162,171,172]. PIB signal is related to ε4 gene dose [173].

When PIB is present at baseline in cognitively normal persons, it strongly predicted MRI atrophy in a 2-year follow-up period [168,174]. This suggests that PIB imaging indicative of an amyloid burden in cognitively normal individuals establishes the presence of early AD. PIB imaging and MRI atrophy are not correlated in most regions of the brain. In AD, frontal lobes have high PIB retention and little atrophy, whereas anteromedial temporal regions have little PIB retention and significant cortical thinning [134]. Correlations between atrophy and PIB binding have been reported in hippocampal and amygdalar regions [175] and some investigators also found correlations between whole-brain atrophy and whole-brain and regional PIB retention [176].

PIB correlates with episodic memory impairment in healthy elderly individuals and in persons with MCI but

not with memory loss in AD [161,163,170,174]. In AD, CDR-sb scores explain 10%–20% of the variance in PIB uptake, suggesting a limited relationship between PIB signal and global dementia severity [177]. Tolboom et al [178] found a relationship between delayed recall and PIB in patients with AD.

PIB correlates inversely with CSF Aβ42 when all diagnostic groups are combined (healthy elderly, MCI, AD) [170,179], and inverse correlation between PIB signal and CSF Aβ42 has been found specifically in the AD population. No correlation was found with CSF Aβ40, p-tau, plasma Aβ40, or plasma Aβ42 [179]. Some studies found correlations between PIB and total tau [170], whereas others did not [179]. In one study of persons with MCI, 87% had abnormal PIB imaging of which only 54% had low CSF Aβ [180]. In cross-sectional studies, CSF Aβ42 levels account for approximately two-thirds of the variance of the PIB signal [65].

PIB does not bind to Aβ plaques in transgenic (tg) mouse models of AD (PS1/APP tg mouse) [164]. However, when PIB is produced with high specific radioactivity, it effectively labels amyloid plaques in tg mice (APP23), suggesting that PIB might be useful as a translational tool that can help to bridge the gap between preclinical and clinical studies [181].

^{11}C-PIB has a short half-life and the availability of an 18-flourine (^{18}F) Aβ tracer would facilitate wider availability of Aβ imaging. Preliminary studies of ^{18}F-flutemetamol, a PIB derivative radiolabeled with ^{18}F, have been reported [182]. Florpiramine-F18 (18F-AV-45) is a fluorine-based ligand developed for amyloid imaging that has the ability to discriminate AD cases from the normal control subjects and had good test-retest reliability (Fig. 9) [183,184]. Waragai et al [185] reported the clinico-imaging correlations of 2-(2-[2-demethylaminothiazol-5-yl]ethenyl)-6-(2-[Fluoro]ethoxy)benzoxazole (BF-227). They found that like other amyloid ligands, there was greater binding in MCI patients who progressed to dementia and little correlation with cognition. A stilbene derivative that labels Aβ has also been developed as an Aβ ligand for imaging; trans-4-(*N*-methyl-amino-4'-(2-(2-(2 [^{18}F]fluoro-ethoxy)-ethoxy)-ethoxy)-stilbene has been shown to identify clinically diagnosed AD patients with 100% sensitivity and 90% specificity [186]. Amyloid binding ligands applicable to SPECT imaging are being developed [184]. SPECT is more widely available than PET, and SPECT ligands could be implemented for diagnostic purposes.

Another ligand being studied in AD is 2-(1-[6-[2-F-18]fluoroethyl)(methyl)amino]-2-naphthyl]ethylidene)malononitrile (FDDNP) [187]. This ligand labels both tau and Aβ. Binding of FDDNP distinguished MCI from controls and AD from MCI; area under the curve analyses suggested a better discrimination among groups by FDDNP than FDG-PET regional hypometabolism or MTA on MRI. Higher binding of FDDNP was observed in MCI patients who progressed to dementia of the AD-type compared with those who did not [187]. FDDNP provides a high signal in the medial temporal regions whereas PIB does not, indicating that this regional signal is derived primarily from the presence of neurofibrillary tangles [188]. There is a more robust positive correlation between FDDNP binding and CSF tau compared with the association between FDDNP and CSF Aβ [189]. Tolboom et al [178] reported a significant association between FDDNP-binding and immediate recall.

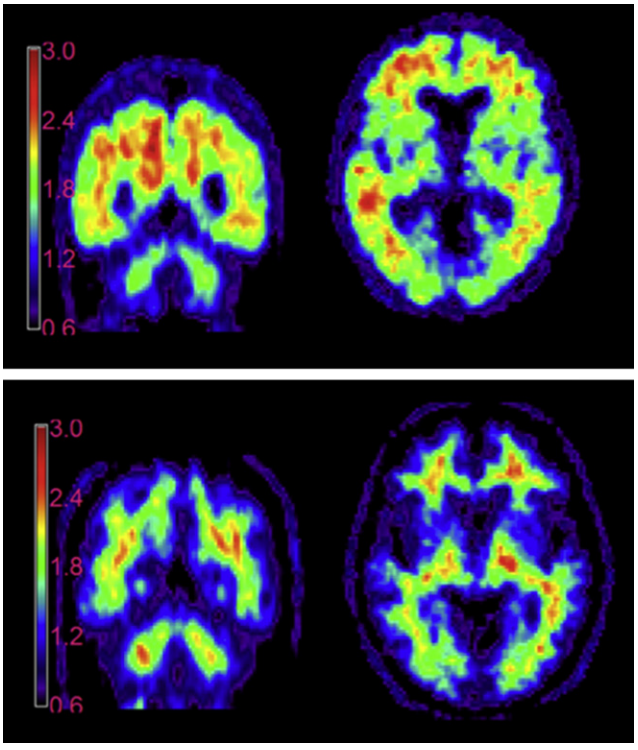

Fig. 9. Representative [18F]AV-45 PET images. Average of two consecutive 5 minute PET brain images (obtained 50–60 minutes after injection) from a 77-year-old female, a mild AD patient with an MMSE of 24 (top) and an 82-year-old male, a cognitively healthy control with an MMSE of 30 (bottom), after an injection with 10 mCi [18F]AV-45. Experimental conditions and imaging and computational parameters were identical for the two subjects. Counts are shown as ratio to the average of the grey matter in cerebellum for each subject (standard uptake value regional [SUVR]) (figure courtesy of M Pontecorvo, Avid Pharmaceuticals).

Appropriate roles for amyloid imaging in drug development include patient selection for anti-amyloid agents (this might include persons with normal cognition and positive scans, MCI with positive amyloid imaging, or patients with a clinical diagnosis of AD and positive amyloid scans), monitoring agents that increase Aβ clearance such as immunotherapy, or prevention of Aβ deposition in those known to be at risk for AD such as persons carrying a presenilin or APP mutation. FDDNP imaging may be useful in Aβ and tau-related therapies.

5.2.3. Fluorodeoxyglucose positron emission tomgraphy

FDG-PET demonstrates cerebral cortical metabolism and the signal is derived mostly from synaptic activity, with 95% of FDG localizing to the synapses and 5% to cell bodies [190,191]. FDG-PET demonstrates reduced metabolism in

the posterior cingulate cortex, precuneus, parietotemporal cortex, and frontal cortex of patients with AD that worsens over time [192–195] (Fig. 10). In one longitudinal study (1.5–2.5 years) of patients with mild-to-moderate AD, regional FDG metabolic rate declined by 20%. FDG-PET correlated with the MMSE score at baseline and at follow-up [196]. FDG-PET correlates with ADAS-cog and with activities of daily living (ADL) measures both cross-sectionally and longitudinally, and low glucose metabolism at baseline predicts a decline in ADAS-cog and ADL scores [191].

Cerebral glucose metabolism correlates with CSF Aβ levels—higher CSF Aβ correlated with better cerebral metabolism [197,198]. CSF total tau or p-tau typically shows correlations with diminished regional metabolism [197,199,200], although not all investigations have supported this association [198].

Most studies have identified lower whole brain and regional (parietal, temporal, cingulate) metabolism in patients with MCI who progressed to AD-type dementia [195,201–204]; however, a few investigators did not find any difference in FDG regional metabolism between MCI patients who did and those who did not progress to AD-type dementia [170].

Lower cortical metabolism correlates with higher PIB signals in temporal and parietal areas; the high PIB signal in frontal regions without a corresponding decrease in FDG-PET signal suggests that amyloid burden is not responsible for the neuronal dysfunction [196]. FDG and PIB signals have high diagnostic agreement for AD but only moderate agreement in patients with MCI [205]. FDG-PET is useful in distinguishing frontotemporal dementia from AD and can assist in excluding non-AD dementia patients from clinical trials [206].

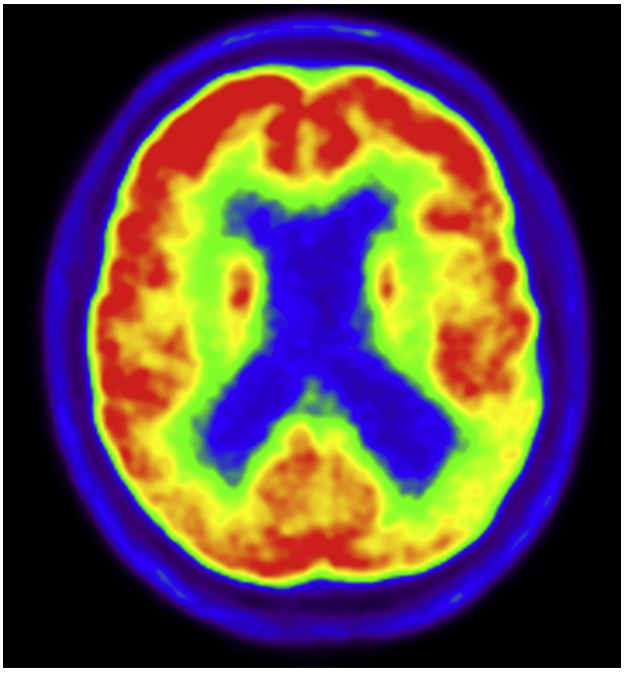

Fig. 10. Fluorodeoxyglucose (FDG) positron emission tomography (PET); transaxial image at the level of the ventricular bodies showing reduced metabolism (yellow) in the posterior parietal and parietal-occipital junction region of both hemispheres compared with the normal metabolic activity (red) of the frontal lobes.

5.2.4. Other imaging modalities

SPECT studies of cerebral perfusion provide data similar to FDG-PET, with evidence of reduced activity in parietotemporal cortex in AD. Statistical approaches can identify hypoperfusion in the posterior cingulate and precuneus in very early AD or predementia MCI [207].

4-[F-18]fluoro-N-/-N-(2-pyridinyl)benzamine or is a selective serotonin 1A (5-HT_{1A}) molecular imaging probe that has been shown to preferentially label the pyramidal cells of the hippocampus and limbic system. The ligand shows a decreased signal in MCI that is further diminished in AD and correlates with clinical assessments, decreased FDG-PET signal intensity, and increased FDDNP signal [208,209]. As a measure of neuronal viability, this approach could be used to assess neuroprotective effects of disease-modifying compounds.

5-HT_{2A} receptors can be visualized with PET using ^{123}I-5-I-R91150, a selective 2A receptor antagonist. This signal is also diminished in AD compared with healthy elderly control subjects [210]. [18-F]Deuteroaltanserin PET is an alternate method of visualizing 5-HT_{2A} receptors and has shown diminished binding in anterior cingulate regions in AD [211].

Activated microglia express peripheral benzodiazepine binding sites that can be detected with (R)-PK11195 C-11 PET. Normal aging is associated with an increased signal in the thalamus; patients with AD showed evidence of activation in entorhinal, temporoparietal, and cingulate cortices [212]. Regional microglial activation correlates with regional amyloid deposition on PIB imaging and is inversely correlated with MMSE scores [213]. Such imaging could have a role in POP studies assessing the effects of drug treatment on microglial activation.

^{123}I-idodo-quinuclidinyl-benzilate demonstrates reduced M1/M4 cholinergic receptor binding in AD [214]. In trials, such imaging could show reduced binding with displacement from receptors with cholinergic therapies or a drug-placebo difference in loss of cholinergic cells with successful neuroprotective interventions. C-11-N-methy-4-piperidyl-acetate measures acetylcholinesterase activity and reflects the integrity of the presynaptic cholinergic system [215]. ^{123}I-51A-85380 reveals reduced alpha-4-β-2 nicotinic receptor binding in AD [216] and can be used to study the integrity of the nicotinic cholinergic system.

Verapamil is a specific ligand for the permeability (p)-glycoprotein transporter that actively removes substances from the brain, including Aβ, and transports them across the blood–brain barrier into the circulatory system [217,218]. C11-verapamil provides a means of imaging this system. The p-glycoprotein activity is reduced in normal aging and further declines in AD [217,219]. C11- verapamil PET can

function as a biomarker for agents affecting this transport mechanism.

5.3. Blood, plasma, and serum

5.3.1. Aβ and APP

Plasma Aβ is derived from peripheral as well as central sources [47]. As noted, there is substantial overlap between levels in patients with AD and healthy control subjects [53]. Although of limited diagnostic significance, plasma Aβ could track a treatment response and decreases have been documented after treatment with a γ-secretase inhibitor [220].

Plasma Aβ is bound to immunoglobulin M and immunoglobulin G antibodies [51], and the increases in plasma Aβ observed in the course of immunotherapy may partly be ascribed to the binding of Aβ to the antibody; Aβ then has the prolonged half-life of the antibody [46].

In AD, there may be a shift of plasma APP isoforms from the larger (130 kDa) form to the smaller (106–110 kDa) form. Using a ratio of 0.57, Borroni et al [221] showed the test to have a sensitivity of 88.2% and a specificity of 89.4%. The reduction correlated with disease severity as assessed by cognitive and global assessments.

5.3.2. Other blood measures

Oxidative damage is considered a major pathway of neuronal injury in AD and markers of lipid peroxidation may provide a peripheral means of detecting oxidative injury and assessing the effect of agents that decrease oxidation either as a primary mechanism of action or as a consequence of ameliorating neuronal injury. 8,12-iso-iFP2a-VI is an isoprostane that appears to be sensitive to lipid peroxidation in patients with AD [222]. It has been found to be elevated in CSF of patients with AD and to correlate with memory impairments and CSF tau levels [223].

Many proteins have been measured in blood, serum, plasma, or platelets in an attempt to find a peripheral marker for AD (Table 4). Most of the observations are unconfirmed across laboratories and no consensus has emerged on a single measure or combination of measures that have reproducible sensitivity and specificity. Proteomic profiles for healthy aging, predementia, MCI, and AD are being pursued and progress is anticipated [234,235]. Proteomic profiles may change after treatment and provide a means of assessing the success of intervention [260]. Assessing the effect of a candidate agent in a drug development program involves choosing biomarkers specifically linked to the mechanism of the agent and combining it with a measure of its effect on AD.

5.4. Electrophysiology

If the agents used to treat AD produce changes in the electrophysiologic rhythms at the resting state, then pharmacoelectroencephalography (pharmaco-EEG) can detect whether the agent has entered the brain as well as document the time-course for entering and exiting the brain. This information is relatively limited in terms of specificity but can be used to ensure blood–brain barrier penetration in humans. Successful treatment with a disease-modifying agent might be marked by less deterioration in EEG patterns than observed in a placebo group; the high degree of variability of EEG patterns and fluctuations with arousal states reduces the attractiveness of EEG as an outcome measure.

There have been relatively few studies of electrophysiologic biomarkers relevant to AD drug development. Using spectral analysis of EEG patterns, Ueda et al [261] demonstrate power variations in MCI and AD. Patients with MCI and abnormal N400 and N600 word repetition effects were much more likely to progress to AD-type dementia within 3 years than those with normal responses (88% vs. 20%) [262]. Induced θ activity captured while subjects performed an n-back test (press a lever when the letter shown is identical to the last one seen) was significantly less in patients with MCI who progressed to AD dementia than in those who did not [263]. This indicates that attentional networks are compromised in very early AD and that this abnormality can be detected by EEG. Tests such as these might be used to identify patients at high risk to progress to AD dementia. The ability to apply such measures in multisite trials requires demonstration.

Evoked responses to odd-ball novel tone stimuli distinguish healthy controls from AD with reasonable sensitivity and specificity [264]. N200 latencies are prolonged in patients with MCI who progress to AD dementia compared with those who do not [265]. Somatosensory-evoked responses were found to be larger in with MCI patients than in normal control subjects or patients with AD [266]. The utility of such techniques to detect drug-placebo differences in multisite studies is unknown.

5.4. Other biomarkers

Little research as been done on biomarkers present in the urine or saliva of patients with AD. Neural thread protein has been reported in urine in AD [267]. Salivary proteomics is making progress as a useful approach to biomarkers in cancer and may have applications in AD [268].

5.5. Biomarker combinations

Different biomarkers assess different biological processes in different anatomical compartments and provide complementary information (Fig. 5). In addition, biomarkers report on the processes that differ in trajectory and sequence across the temporal course of AD and have a different role in different phases of the disease [269]. Combinations of biomarkers will provide a more comprehensive assessment of the response of AD to therapy than a single biomarker. Using combinations also increases the likelihood that a change corresponding to clinical

response will be found because biomarkers will have different sensitivities and different predictive abilities. Figure 11 provides an example of how intervention early in the AD pathogenesis cascade could be reflected in sequential events assessed by biomarkers.

The combination of Aβ and tau/p-tau measures has been extensively studied and the combination of these two measures has greater sensitivity and specificity for diagnosis of AD than either measure alone [270–272]. Measures of CSF tau, Aβ, and brain atrophy contribute independently to predicting clinical progression over time [80,81]. deLeon et al [73] found that combined use of MRI and CSF measures incrementally improved the early diagnosis of AD. Adding pathological levels of Aβ42 and total tau and p-tau amplify the ability of regional parietal blood flow to detect which patients with MCI will progress to AD. Walhovd et al [273] studied patients with MCI and healthy control subjects using FDG-PET, MRI, and DTI. Morphometry showed higher diagnostic accuracy but was improved when buttressed with data from other imaging modalities; each modality contributed to variance in memory performance. Data from the AddNeuroMed Consortium showed that the combination of neuropsychological and regional volumes best discriminated AD from MCI as well as MCI from healthy control subjects [274].

The combination of a target engagement biomarker with a disease-related biomarker has proved to be particularly valuable in drug development [275] and may be a model in the use of biomarker combinations in AD drug development. The optimal combination of biomarkers will be determined by the mechanism of action of the agent, the question to be resolved (e.g., dose, profile of effects, regulatory application), and the plausibility of applying the measures in the proposed study (e.g., small number of specialized sites in POP studies vs. many sites in large pivotal studies).

6. Integration of biomarkers into AD drug development plan

Biomarkers may play a role in disease-modifying drug development programs to demonstrate a pharmacologic effect in preclinical studies, and to support POP in phase II or phase III pivotal trials. Biomarkers are often used in phase II to support clinical measures or as an alternative on which to base the decision to proceed to phase III. Phase III studies require demonstration of clinical efficacy and biomarkers can support an NDA but will not reduce sample size on study duration.

The only clinical trial designs that support a claim for disease modification without concomitant biomarker data are the staggered start and randomized withdrawal approaches. Given the uncertainties associated with implementing these designs, most disease-modification development programs depend on biomarkers. The data will be integrated into the NDA to support a claim for disease-modification related labeling. If biomarkers are in the package insert they can be discussed as part of the marketing of the compound and will help inform prescribers, patients, caregivers, and payers of the value of the compound. Biomarkers included in labeling are not necessarily surrogate markers that could substitute for clinical markers, but they must be validated—measured by an analytical test system with well established test characteristics—and qualified—supported in an established scientific framework or body of evidence that elucidates the physiologic, toxicologic, pharmacologic, or clinical significance of the results (Table 1, Fig. 2).

Biomarkers will play an important role in phase III clinical trials of agents for which a disease modification-related claim is to be sought. To support a disease-modifying claim, the trial must show a drug-placebo difference on primary clinical outcomes (cognitive measure and global or functional measure) and on a biomarker; the clinical outcome and the biomarker must be correlated to support the claim that the two sets of measures reflect an effect on a common neurobiological pathway. Drug-placebo differences on clinical outcomes in conjunction with significantly correlated drug-placebo differences on a biomarker and supported by preclinical studies establishing the mechanism of action and excluding other potential effects of the candidate agent would comprise a comprehensive and integrated data platform for an NDA.

The requirement for a correlation between clinical outcomes and biomarker outcomes in clinical trials of disease-modifying agents helps inform which biomarkers are most likely to perform well as trial outcomes. Amyloid brain levels as revealed by amyloid imaging and CSF Aβ42 and tau/p-tau levels have only weak correlations with clinical measures [80,163,177]. Drug-induced changes in these measures may be less likely to correlate with changes in cognition as compared with biomarkers that correlate well with measures of clinical change such as volumetric MRI, FDG-PET, FDDNP, and possibly some serum measures [139,178,187,191]. However, a drug-induced change in a biomarker could be correlated with a drug-induced change in clinical outcomes even if these measures are not correlated in the untreated state.

6.1 Biomarkers in preclinical development

Biomarker development should begin as soon as the decision is made to progress a candidate compound into preclinical development. Use of biomarkers in animal models will provide insight into the mechanism of action of the compound and the magnitude of its effect in that specific model. Details of collection, storage, and measurement can be prepared in this setting. Of multiple biomarkers assessed in the preclinical phase, one or a few can be prioritized for use in human studies. These biomarkers can be assessed in phase I or II and a decision can be made as to their potential value in phase III. Biomarkers developed in conjunction with one program may have applications in the assessment of follow-on compounds or in programs addressing related pathways. Evolving databases of biomarkers may help

Table 4
Blood, platelet, plasma, and serum measures potentially applicable to clinical trials of AD.

Measure	Biology
APP ratio	AD patients have an APP ratio shift toward lower weight immunoreactive bands [221,224]; lower APP ratio predicts cognitive decline [225] and is correlated with cognitive status in AD [226]; sensitivity was 86%–88% and specificity 88%–89% in distinguishing patients with AD and healthy control subjects [227,228]
Plasma Aβ40 and Aβ42	Plasma measures do not differentiate normal from AD or MCI; levels decrease with secretase inhibition and may be an indicator of target engagement
Serum isoprostanes	Elevated in AD; correlate with memory impairment [223]
Platelet β-secretase activity	Increased activity in AD as compared with controls [229]
Leptin	Decreased incidence of AD with higher leptin levels [230]
Aβ phagocytosis by macrophages	Macrophages from patients with AD have impaired phagocytosis of Aβ [231]
ALZAS protein antibodies	Increased in MCI and mildly elevated in autopsy-based studies in patients with AD as compared with healthy controls [232]
TNF-α	Elevated levels in AD are associated with increased rate of progression; indicative of chronic inflammation[233]
Proteomic 5-protein biosignature	Distinguished patients with AD from healthy elderly subjects [234]; subset of proteins from Ray and colleagues [235]
Proteomic 18-protein biosignature	Proteomic analysis revealed 18 proteins involved in the immune response, hematopoiesis and apoptosis that distinguished patients with AD from control subjects and identified MCI that progressed to AD [235]
ECE-2	Degrades Aβ; upregulated in AD [236]
Hydroxyoctadecadienoic acid	Measure of oxidative stress; increased in patients with AD as compared with normal controls; increased with increasing dementia severity [237]
Oxidized purines in nuclear DNA of peripheral lymphocytes	Measure of oxidative damage; differentiated patients with AD from healthy control subjects [238]
Melanotransferrin	Protein involved in brain iron transport; increased serum levels in AD [239].
8-OHdG	Marker of oxidative damage to DNA in peripheral lymphocytes; significantly elevated in patients with AD as compared with healthy controls [107,240]
Protein carbonyls	Indicator of nitrosative stress; elevated in plasma and blood cells of AD and in plasma in MCI [107]
3-NT and dityrosine	Indicator of nitrosative stress; elevated in plasma and blood cells of AD [107]
Plasma glutathione and its metabolite cysteinglycine	Antioxidant proteins reduced in patients with MCI and AD as compared with healthy control subjects [241]
GGT	Modulates cellular uptake of glutathione; levels elevated in patients with AD as compared with control subjects [242]
Telomere shortening of chromosomes from peripheral blood mononuclear cells	Telomere length on T-cells correlated with cognitive measures; inversely correlated with serum level of the proinflammatory cytokine TNF-α [243]
Inflammatory proteins (α-1-antichymotrypsin, IL-6, C-reactive protein)	Higher levels in elderly subjects associated with an increased risk for AD [244]; cytokines are subject to many influences and correlation with disease progression has been limited [245]
CRP	Low plasma levels of high-sensitivity CRP are associated with more rapid functional decline in patients with established AD [246]
IL-6	Increased in AD [247]; inflammatory cytokine
IL-6 receptor	Reduced in AD and in other neurological disease; correlated with MMSE [248,249]
Plasma αTNF-α	Increased in AD [247]
Plasma IFN-α	IFN-α is increased in patients with MCI as compared with those with AD and healthy control subjects [247]
Platelet COX 2	Platelet levels raised in patients with AD and MCI as compared with healthy control subjects [247]
ACT	ACT is produced in AD brain by reactive astrocytes in response to high levels of the pro-inflammatory cytokine interkeukin-1 secreted by reactive microglia; increased in AD-CSF; correlated with cognition [110]
Serum cystatin C	Lower levels in cognitively normal men are associated with an increased risk of developing AD [250]
Plasma soluble CD40 levels	Elevated in patients with MCI who progress to AD dementia as compared with healthy control subjects and patients with MCI who do not progress to AD dementia [70]. Increased in AD and inversely correlated with MMSE scores [251]. CD40 interacts with the CD40 ligand to elicit activation of immune cell and to up-regulate costimulatory molecules and cytokines
MCP-1	Chemokine indicator of inflammation; elevated in MCI and early AD, not severe AD; progressively decreased in patients with MCI who progressed to AD dementia [252]
Homocysteine	Increased in patients with AD as compared with healthy control subjects [241,253]
EPC-CFUs	Capacity to regenerative vascular endothelium; reduced in AD [254]
RAGE	Plasma levels of soluble RAGE reduced in AD as compared with vascular dementia or healthy elderly control subjects [255]. Reduced levels have not been confirmed by all studies [241]
PlsEtn	PlsEtn is decreased in AD with limited to no overlap with normal controls; assessed in five independent populations [256]. Correlated with severity of dementia. Decreased synthesis and increased degradation in AD may contribute to the diminished serum levels
TDP-43	Increased in the plasma of 22% of patients with AD and 46% of patients with FTD [257]. Could be used to assess impact of agents with a specific effect on this subpopulation of patients with AD

(*Continued*)

Table 4
Blood, platelet, plasma, and serum measures potentially applicable to clinical trials of AD. (*Continued*)

Measure	Biology
Serum trans-activator regulatory (TAR) neuronal pentraxin receptor (NPR	NPR is elevated in AD as compared with healthy control subjects; elevated in MCI but was lesser when compared with AD [121]
24S-hydroxy-cholesterol	Index of brain cholesterol metabolism; normal in AD; could function as a measure of treatment elect with agent affecting cholesterol metabolism [258]
VCAM-1; ICAM-1	Candidates for markers of microvascular pathology in AD; increased plasma levels in AD [259]

Abbreviations: APP, amyloid precursor protein; AD, Alzheimer's disease; MCI, mild cognitive impairment; ALZAS, plasma Alzheimer associated; TNF, tumor necrosis factor; ECE-2, endothelin-converting enzyme-2; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; 3-NT, 3-nitrotyrosine; GGT, gamma glutamyltrasferase; IL-6, interleukin-6; CRP, plasma C-reactive protein; TNF-α, plasma tumor necrosis factor-α; IFN, interferon; COX 2, cylooxygenase-2; ACT, alpha-1-antichymotrypsin; MCP-1, monocyte chemotactic protein-1; EPC-CFUs, endothelial progenitor cells colony forming unit; RAGE, receptor for advanced glycation end products; PlsEtn, serum ethanolamine plasmalogen; TDP-43, plasma trans-activator regulatory DNA binding protein-43; NPR, serum trans-activator regulatory neuronal pentraxin receptor; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intracellular adhesion molecule-1.

companies prioritize compounds as relationships between biomarker changes and clinical effects are established [276].

In AD drug development programs, tg mouse models are commonly used to assess the effect of anti-Aβ therapies. The mice typically recapitulate part of the AD neurobiology, such as amyloid deposition, but often lack other components of the disease observed in human beings (e.g., tangle formation, cell loss). Typically, tg species are created with human mutations and more closely resemble autosomal dominant familial AD than sporadic late-onset AD. Among the many tg species available, the tg2576 is the most widely used. This mouse carries an APP mutation and over-produces Aβ42 [277]. The APP-ArcSwe tg mouse has 2 APP mutations and deposits insoluble Aβ that more closely resembles the type of Aβ observed in human AD [278]. A triple tg mouse bearing APP, presenilin, and tau mutations recapitulates most of the pathological features of human AD [279]. Testing in at least two AD models and in models most closely resembling the human disease may increase the predictive ability of preclinical testing for outcomes in human trials.

Imaging modalities used in or being considered for human AD trials have been assessed in tg mouse models in AD: MRI studies of brain atrophy have been accomplished in APP-PS1 mice [280]; MRI shows DTI abnormalities in

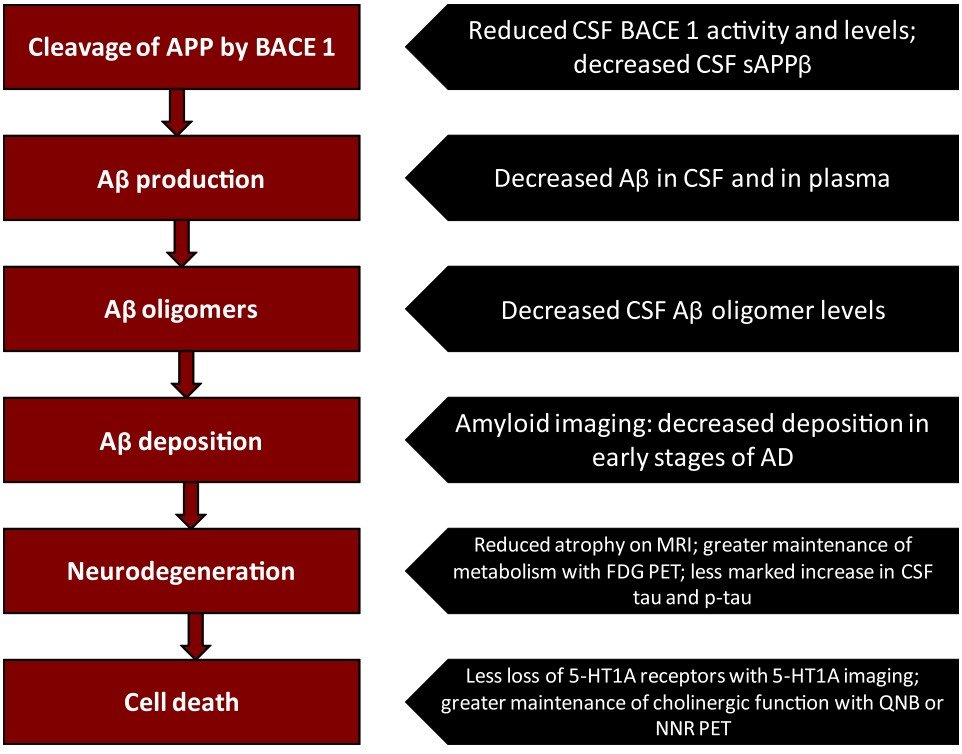

Fig. 11. Example of biomarkers that might be integrated into a clinical development plan to assess the effect of a BACE-1 inhibitor; the potential sequential events as measured by biomarkers are shown. Measures of BACE-1 activity are a direct pharmacologic measure of enzyme inhibition; additional measures provide insight into the effects of enzyme inhibition on amyloid production and deposition, and neurodegeneration. The black boxes state the anticipated benefit compared with placebo.

PDAPP tg mouse model [281]; PIB has demonstrated amyloid pathology in tg mouse models [181]; and microglial imaging in vivo with PK11195 has been shown to correlate with histopathologic evidence of activated microglia in the APP/PS1 tg mouse model [282]. CSF Aβ and tau have been studied in tg mouse models of AD and in non-human primates [283,284]. The differences among animal models and between models of AD and human AD must be considered when evaluating the results of treatment studies [285,286].

6.2. Phase 0

Phase 0 trials conducted under an exploratory Investigational New Drug application refer to pilot studies of new drugs administered in limited doses to small number of patients without therapeutic intent [287,288]. Preclinical pharmacology and toxicology requirements are less extensive than for traditional Investigational New Drug approaches. Phase 0 studies quantify a specific drug effect and allow rational decisions concerning further drug development. Of the biomarkers available in AD drug development, the SILK technique [24,36,92] and ligand-based imaging might be applicable in this setting.

6.3. Biomarkers in phase I trials

Figure 12 provides an overview of how biomarkers might be integrated into a comprehensive drug development program; details of a development plan will differ for any specific agent.

Phase I trials usually involve normal control subjects although immunotherapy studies typically involve patients with AD from onset; development programs for other agents often include patients with AD in phase Ib trials. The major role for biomarkers in phase I first-in-human trials is the detection of toxic adverse events. Biomarkers of liver and cardiac injury are especially important (e.g., liver function tests and electrocardiography) [289]. CSF Aβ can be measured in normal subjects and used, for example, in dose-escalation studies. In this setting, the CSF findings are not subject to the influences of aberrant metabolism or deposition [290,291]. CSF Aβ, tau, and p-tau, however, are altered in AD and drug effects seen in normal subjects might not be recapitulated in patients with AD [292]. SILK studies can also be initiated in phase I in normal control subjects to assess Aβ production and clearance [24,36,92].

6.4 Biomarkers in phase II trials

Biomarkers are critically important in phase II of AD drug development programs. They can serve as the primary outcome measures of POP trials that will help in making informed decisions as to whether to advance a compound to phase III [13,293]; alternatively, they can serve as key secondary outcomes in longer (12–18 month) trials where clinical efficacy is the primary outcome [294]. Safety

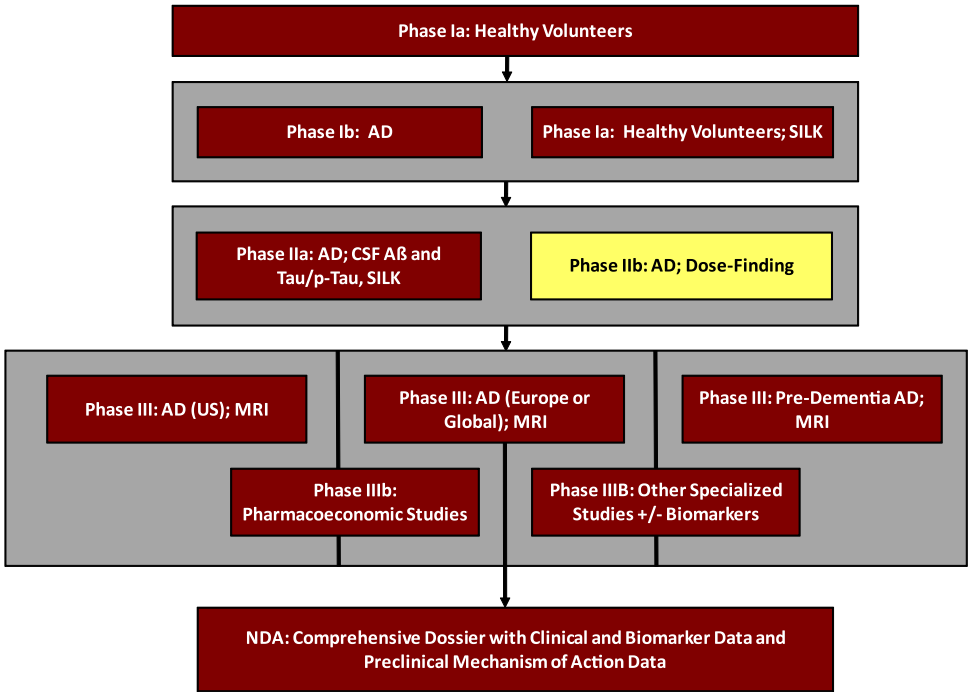

Fig. 12. Example of a comprehensive drug development program with integrated biomarkers to develop a comprehensive integrated NDA supported by biomarker and clinical data. The aim is to include biomarker information in the labeling to facilitate the discussion of these observations with the end-user community after marketing approval. (AD, Alzheimer's disease; SILK, stable isotope-labeled kinetics; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; NDA, new drug application; p-tau, phospho-tau. SILK is useful primarily in the development of β- and γ-secretase inhibitors or agents that increase the clearance of Aβ.

information in the intended treatment population is also monitored with biomarkers in phase II.

In trials involving patients with MCI, biomarkers can select those patients with underlying AD as the cause of the cognitive impairment. MCI is a heterogeneous category comprised of some patients in the earliest phases of AD, some in early stages of non-AD dementia, some who will remain with mild impairment for long periods of time, and some who will recover to normal cognition [295,296]. Drugs targeted on specific aspects of AD should be tested in trials of patients in whom the AD substrate is present. MCI patients who have the AD CSF profile of diminished Aβ and elevated tau and p-tau are highly likely to progress to AD-type dementia within the next few years [297]. This optimizes the opportunity to observe a drug-placebo difference with an effective agent. Similarly, patients with amyloid imaging showing an amyloid burden in the brain [170], FDG-PET demonstrating hypometabolism in the parietal lobes or temporal lobes (Fig. 10) [202], or MRI with hippocampal atrophy [154] or cortical thinning [27] are at high risk for progression to AD dementia. Using the ADNI sample, McEvoy et al [144] demonstrated that MCI patients with an AD pattern of atrophy (mesial and lateral termporal, cingulate, orbitofrontal cortex) progressed to AD dementia at a rate of 29% per year compared with 8% annually for those without the AD pattern. Using these biological measures to assure the presence of AD as the cause of the MCI will optimize the ability to demonstrate POP with an anti-AD agent.

Biomarkers that can serve as outcomes and provide information on target engagement and biological effects include the SILK technique; CSF Aβ, tau, and p-tau; and possibly isoprostanes. Longer term trials could use MRI measures of atrophy or amyloid imaging. Multiple doses are included in phase II trials to provide information on which dose or doses should be advanced to phase III; this decision can be supported by biomarker observations. Among the current key unknowns are the time frames for affecting specific measures (e.g., if Aβ production is reduced, when might a change in tau or p-tau ensue) and the relationship of the biomarker to the clinical outcome (e.g., what degree of secretase inhibition might translate into a measurable clinical benefit).

Enrichment strategies using biomarkers can be considered in phase II trials. Kinkingnehun et al [298] showed that AD patients with more extensive cortical atrophy as shown by VBM on entry into the study had more rapid decline in cognition than those with less atrophy. Patients were matched for dementia severity at baseline. Stone et al [299] found that AD patients who were *APOE* ε4 carriers progressed more rapidly in trials than those lacking the ε4 allele. More rapid rate of decline in the placebo groups enhances the opportunity to observe a drug-placebo difference of an efficacious agent within the time frame of the trial.

Scheltens and Barkhof [132] championed the use of MRI in disease-modifying trials to exclude patients with nondegenerative causes of dementia, identify patients with patterns of atrophy suggestive of non-AD degenerative dementias, exclude those with significant vascular comorbidity from AD trials, and establish a prespecified degree of MTA to support the diagnosis of AD. Standardization of acquisition, continuous quality assurance, and centralized image assessment are keys to successful implementation of MR measures into clinical trials. Studies with the ADNI sample provide an example of semi-automated quantitative techniques applicable to larger scale clinical trials [144]. Similarly, the AddNeuroMed Consortium combined a harmonized acquisition protocol, quality control, centralized analysis hub and automated image analysis to demonstrate the feasibility of MRI in multicenter trials [300].

It is in phase II that a biomarker might currently be substituted for a clinical outcome. Changes observed in untreated patients have been used to model sample sizes needed to show drug-placebo differences. On the basis of a sample from a clinical trial of mild-to-moderate AD patients, Jack et al [301] estimated that a 1-year trial would need 21 patients per arm to demonstrate a 50% reduction in hippocampal atrophy and 54 patients per arm to show a 50% difference in temporal horn enlargement (90% power). This compares with 241 patients to show a similar difference on the MMSE and 320 for the ADAS-cog. Scheltens and Barkhof [132] estimated that 102 patients per arm would be required to show a 25% treatment effect with 90% power using hippocampal atrophy measures. Using whole-brain atrophy rates, Fox et al [302] estimated that a sample size of 207 per arm would be needed to show a 20% reduction in atrophy, with 90% power assuming a 10% dropout rate and a 10% rate of scan pair unusability. Thirty-three per arm would be required to show a 50% reduction in atrophy rate—comparable with the calculations of Jack et al [301]. On the basis of the observations from the placebo arm of the AN1792 trial, Ridha et al [139] calculated that for changes of 50% magnitude, the number of subjects per arm would be 305 if MMSE was the outcome, 220 for the ADAS-cog, 199 for CDR, 61 for whole-brain atrophy, and 59 for ventricular enlargement. Based on ADNI samples and using ventricular enlargement to predict sample size, the number of patients required to show a 20% drug-placebo difference in AD, AD who were ε4 carriers, AD who did not have an ε4 allele, and MCI patients was 342, 468, 257, and 1180, respectively, corresponding numbers for the ADAS-cog were 1607, 2100, 1370, and >20,000 [303].

Using TBM, Hua et al [146] found that only 48 AD patients or 88 MCI patients per treatment group would be required in a clinical trial to demonstrate a 25% reduction in atrophy rate with 80% power. Chou et al [304] correlated baseline ventricular morphology with subsequent change over one year in MMSE, global CDR, and CDR-SB in patients with MCI. They showed that 120 subjects would be needed to correlate ventricular enlargement with MMSE, CDR, and CDR-SB.

CSF Aβ measures or amyloid imaging would be appropriate biomarkers for agents affecting Aβ metabolism or

deposition. Not all patients will agree to have a lumbar puncture, and amyloid imaging may not be sufficiently widely available for use in large global trials. Collecting these data on a subset of patients may be a viable alternative; control of biases that might render the participants nonrepresentative of the entire trial population must be addressed. Thal et al [305] calculated sample sizes needed to show a 25% drug-placebo difference in CSF Aβ or tau levels assuming an alpha of .05 and 80% power. They concluded that in each arm of the study, a sample size of 40 and 36 would be required for changes in total tau and in CSF Aβ42 in patients with AD, respectively. Studies have shown substantial intersite variability in CSF measures and centralized measures or standardized intersite reliability measures are required for trials [67,306].

FDG-PET can be considered for phase II trials. Techniques to reduce interscanner variability are being developed for application in trials [307]. The number of AD patients per arm in a 12-month study designed to show a 20% treatment effect is calculated to be 62 (all brain regions combined), 28–36 for a 30% treatment effect, 16 for a 40% effect, and 10 for a 50% effect [305,308]. When individual regions are used rather than whole brain measures, the number of patients required to show a drug-placebo difference increases (101 patients per arm to show a 33% treatment effect in typical regions). The numbers required are much smaller than the number of patients needed to show a drug-placebo difference on clinical measures.

6.5 Biomarkers in phase III trials

MRI is the technology most readily applicable to large phase III multisite clinical trials. Coordination of data collection protocols, site-networking requirements, user-authentication protocols, data archiving, and computational requirements enable acquisition of large-scale purpose-driven datasets appropriate for regulatory quality clinical trials [309]. ADNI, European ADNI, and AddNeuroMed protocols provide models for accomplishing these ends.

All these projections are based on extrapolations from placebo-control groups of past trials or natural history observations and remain to be verified in prospective trials with efficacious agents. It is also uncertain how a reduction in one parameter (e.g., 30% reduction in Aβ production) will translate into a downstream measure such as cerebral atrophy.

Enrichment strategies based on biomarker features can be considered in phase III trials. For example, MTA and MRI would enrich either MCI or clinically diagnosed AD population for the presence of the AD process. Patient selection criteria will be incorporated into the labeling of the agent and will establish the treatment population to which the approved treatment can be applied. Payers may use these criteria for payment decisions.

Biomarkers in phase III may be used to support the NDA with its specific labeling and indications claim.

6.6 Biomarkers in primary prevention trials

Primary prevention trials involve persons who are without cognitive impairment at the time of randomization. To capture enough events to allow demonstration of a drug-placebo difference, these trials must be large (possibly several thousand patients) and sufficiently long to have enough patients reach an endpoint to allow a well-powered conclusion about treatment efficacy. Potential outcomes of primary prevention trials include progression to MCI, progression to AD-type dementia, cognitive decline without reaching a specific diagnostic state, or impact on a biomarker causally linked to AD.

The use of biomarkers in the latter setting will require development of surrogate endpoints that can substitute for a clinical outcome. The process of establishing surrogate status is complex and requires biological plausibility based on epidemiologic evidence, animal models, mechanism-of-action information, success in clinical trials including several agents and several classes of agents, and a favorable risk-benefit ratio including the public health effects of false negative and false positive outcomes on the measure and the adverse effects of drugs used on the basis of the surrogate [9,310,311]. Table 1 lists the elements expected in developing a surrogate marker and Fig. 13 summarizes the process [276,312].

When no treatment is available, the disease has disastrous consequences (as is the case of AD), and a biomarker is reasonably likely on the basis of epidemiologic, therapeutic, and pathophysiologic evidence to predict clinical outcome, then a drug can be approved based on an unvalidated biomarker [9]. Drugs approved with unvalidated surrogates must provide meaningful therapeutic benefit, be superior to existing products, and be subject to postmarketing studies to establish the predictive relationship of the biomarker to the clinical outcome.

There are several populations that might participate in primary prevention trials. All persons over a specified age could be included or additional factors may be required to increase the likelihood of some participants manifesting cognitive impairment in the course of the trial. The Alzheimer's Disease Anti-inflammatory Prevention Trial, for example, required a minimum age of 70 and a positive family history of dementia for participation [313]. A sample size of 2,625 was estimated to provide 80% power to establish a 30% reduction in the incidence of AD.

Reiman et al [314] estimated the sample size of each arm of a clinical trial in which a cognitively normal population of individuals who were >50 years was enriched with the *APOE* ε4 genotype and FDG-PET was the outcome measure in a 2-year trial. Using metabolism of the posterior cingulate region as the outcome, 130, 58, 33, and 22 patients would be required for a 20%, 30%, 40%, and 50% reduction in disease progression, respectively. Using combined brain regions 39, 19, 12, and 8 patients per arm would be required for these drug-placebo difference rates. Such sample sizes are easily achievable.

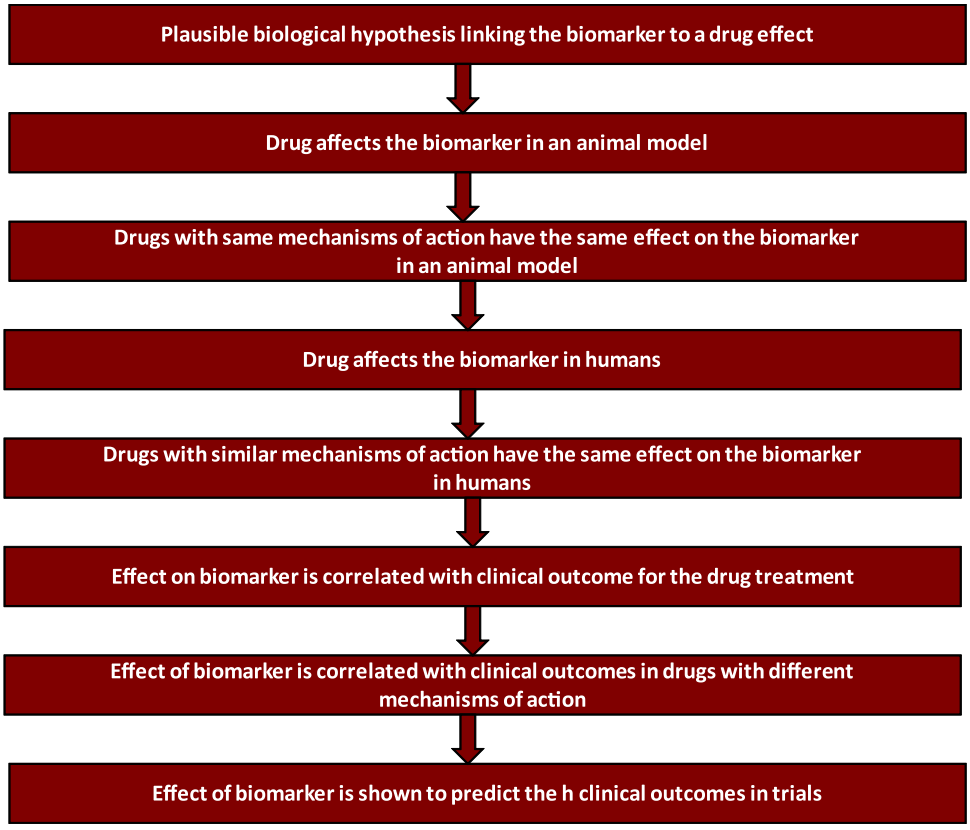

Fig. 13. Process by which a biomarker is shown to be a surrogate marker.

Biomarkers can serve to identify patients at elevated risk for AD. Fig. 14 shows the continuum of biomarker change in AD and indicates how biomarkers might be selected to construct such a trial population. Loss of hippocampal volume may be detectable as long as 6 years before the onset of AD dementia [154,315]. This suggests that hippocampal atrophy could be used to identify a population of normal elderly patients with the earliest changes of AD. Burgmans et al [316] reported that cognitively normal older patients who progressed to dementia over a 6-year observation period had significantly smaller prefrontal volumes on MRI than those who did not progress. deLeon et al [73] and Scheinin et al [168] demonstrated that reduced FDG metabolism in entorhinal cortex predicted a decline to MCI (sensitivity 83% and specificity 85%). When brain amyloid (positive amyoid imaging) is present at baseline in cognitively normal persons, it strongly predicted MRI atrophy in a 2-year follow-up period. Amyloid imaging could play an important role in identifying persons with the earliest form of AD in those who are cognitively normal but who would progress to MCI and to AD.

Patients carrying genetic mutations known to cause AD comprise an obvious population for prevention trials since progression to AD is certain and the age of symptom appearance is relatively stereotyped within families. Ringman et al [317] used FA measures derived from DTI to assess asymptomatic carriers and matched noncarriers. Carriers had significantly decreased mean whole brain white matter FA, as well as FA of the columns of the fornix, the area of the perforant pathway bilaterally, and the left orbitofrontal lobe compared with noncarriers. Similarly carriers were shown to have higher serum levels of Aβ42 [60] and altered cortical evoked potentials [318]. The drug-placebo difference in FA, serum Aβ, or evoked potentials might serve as outcome measures in trials in this population.

7. Discussion

Many biomarkers are available for integration into drug development programs, new tests are rapidly emerging, and numerous measures with claims to biomarker status can be anticipated. Choosing among biomarkers for a drug development program requires a fit-for-purpose determination of the biomarker including whether good laboratory practice standards have been developed for measuring the biomarker; sensitivity and specificity of the biomarker for the specific application in the trial have been determined, preclinical performance is already known, likelihood of correlating with the clinical measures has been established, concordance with the mechanism of action of the treatment being assessed has been determined, regulatory expectations are known, product labeling connotations are anticipated, and plausibility in the

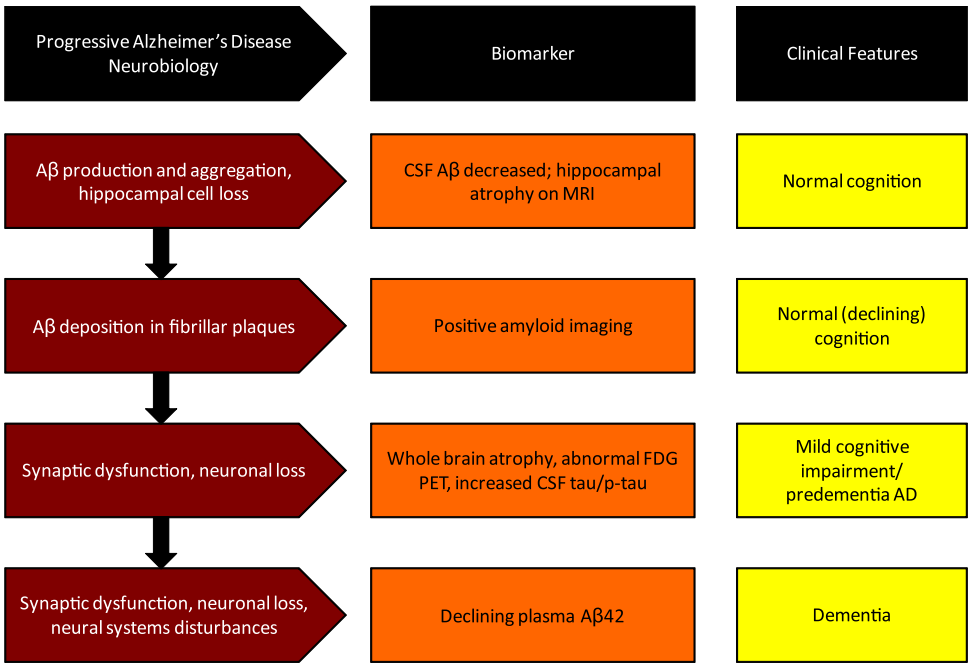

Fig. 14. Putative sequence of events in AD; the neurobiology changes over time as reflected in the biomarker profile and the clinical state of the patient (Aβ, amyloid beta protein; AD, Alzheimer's disease; CSF, cerebrospinal fluid; FDG-PET, fluorodeoxyglucose positron emission tomograph; MRI, magnetic resonance imaging; p-tau, phospho-tau).

type of trial planned is established [319]. For protein measures (e.g., Aβ, tau), pre-analytic considerations include availability of a reference standard, array platform, calibration, and sample collection and handling; method validation includes assay range-finding, accuracy and precision, sensitivity, specificity, and stability [320].

Grading of biomarkers has been proposed based on the level of information available on a biomarker from early animal observation through study of predictability, accuracy, and reproducibility in human beings [8,321]. Less information will be available for exploratory biomarkers used early in drug development compared with the extensive data required for biomarkers being considered for phase III trials [275].

Much of current drug development focuses on affecting Aβ including decreasing production, increasing degradation, reducing transport across the blood–brain barrier from the serum or increasing transport to the serum, inhibiting aggregation, or removing fibrillar and nonfibrllar forms of amyloid. Biomarkers are being developed to measure the outcomes of interventions on these processes (Fig. 5). Toxic species of amyloid may have the following two detrimental effects important in AD: (1) in animal models, soluble Aβ has been shown to have a direct inhibitory effect on synaptic function and long-term potentiation, and (2) soluble oligomers are neurotoxic and are thought to play a pivotal role in the initiating a complex cascade of events leading to cell death [22]. Anti-Aβ therapies might be anticipated on this basis to have acute symptomatic effects, long-term effects on disease course, or both. Most biomarkers in development measure aspects of the amyloid process or cell degeneration. Amyloid is an intermediate target in the process of cell death and anti-Aβ therapies will be successful to the extent that interrupting Aβ production and deposition leads to a decrease in synaptic dysfunction and cell loss. Combinations of biomarkers of the downstream effects of therapy on cell survival (e.g., MRI atrophy, serotonin or cholinergic cell function, and CSF measures reflecting cell death) in conjunction with biomarkers showing the effects on Aβ (e.g., CSF Aβ levels, Aβ production, Aβ clearance) may provide more insight into drug effects than measures focusing exclusively on Aβ.

There is increasing evidence that Aβ is not the immediate cause of neuronal death in AD. Fibrillar amyloid is present in the cognitively normal elderly individuals at autopsy; fibrillar amyloid is found in 20%–30% of cognitively normal elderly individuals studied with amyloid imaging; amyloid plaques have weak relationships with severity of cognitive decline in AD; amyloid imaging and CSF Aβ remain stable in AD despite worsening cognition; amyloid imaging is not correlated with changes in FDG-PET (a measure of synaptic function); amyloid imaging in persons with focal presentations of AD show nonfocal cortical Aβ deposition; and Aβ pathology in tg animal models of AD is not associated with neurofibrillary tangle formation or cell loss [22]. In contrast, autopsies of persons in different stages of AD show increasingly severe pathological changes including Aβ deposits; there is a constantly expanding ''front'' of AD pathology in the course of AD; there is a relatively rapid (two times per day) turnover of Aβ in the CSF suggesting that production is on-going over

the course of AD; and total Aβ levels (including soluble and fibrillar forms) correlate with severity of cognitive deficits [162,322,323]. Possible explanations for these discrepant bodies of information include the role of cognitive reserve in limiting symptoms early in the course of Aβ pathology; saturation of the PIB signal to limit its sensitivity to change; length of exposure to Aβ may be an important determinant of toxicity; or a second process such as tau hyperphosphoryation and aggregation or oxidative brain injury is primarily responsible for the symptomatic phase of AD [324]. Biomarkers will play a critical role in characterizing the pathological process of AD, prioritizing these alternative explanations, and guiding drug development for the most promising therapeutic targets.

Most biomarker studies currently approach AD as a single phenotype occurring along a spectrum of severity. This may belie the complexity of the illness and the corresponding heterogeneity of some biomarker changes [325,326]. Biomarkers may reflect the specific neurobiology of progression of disease (e.g., higher levels of oxidation products with greater cellular involvement); genetic and molecular changes of specific phenotypes (e.g., those with and without psychosis) [327]; or the presence of comorbid conditions (e.g., changes in white matter seen on neuroimaging [328]. Progress in understanding the neurobiology of specific phenotypes of AD and the corresponding biomarker abnormalities may reduce variability in biomarker measures, allow identification of specific subgroups, and facilitate treatment targeting unique neurobiological pathways, disease stages, or clinical phenomena.

Biomarkers in drug development are intended to increase the safety, assist in drug candidate and dose choice, reduce cycle-time and development costs, assist in decision-making, and support the NDA [329]. Progress is being made in developing biomarkers relevant to AD drug development especially those that characterize disease progression. These biomarkers can play a role in patient selection for trial participation and disease progress monitoring. Target engagement biomarkers are needed and advances have been made in measures of beta-amyloid-site APP cleaving enzyme activity as well as Aβ production and clearance in CSF. The combination of a target engagement and disease progression biomarker has assisted drug development in non-AD areas and may be optimal to advances anti-AD therapies. No biomarker has established predictive validity for clinical changes in AD, and establishing these relationships is critical to advancing AD drug development. When predictive associations are known, drug development can be accelerated and prevention trials considered.

Biomarkers are part of the solution to the challenge of improving success rates in AD drug development. They must be accompanied by excellent strategies in drug discovery, medicinal chemistry and lead optimization, dose selection and clinical trial conduct. Biomarkers comprise a critical part of the repertoire of drug development activities required to develop new therapeutics for AD [330].

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