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Review

Developing predictive CSF biomarkers—A challenge critical to success in Alzheimer's disease and neuropsychiatric translational medicine

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ABSTRACT

The need to develop effective treatments for Alzheimer's disease has been confounded by repeated clinical failures where promising new chemical entities that have been extensively characterized in preclinical models of Alzheimer's disease have failed to show efficacy in the human disease state. This has been attributed to: the selection of drug targets that have yet to be shown as causal to the disease as distinct from being the result of the disease process, a lack of congruence in the animal models of Alzheimer's disease, wild-type and transgenic, to the human disease, and the enrollment of patients in proof of concept clinical trials who are at too advanced a stage of the disease to respond to any therapeutic. The development of validated biomarkers that can be used for disease diagnosis and progression is anticipated to improve patient enrollment in clinical trials, to develop new animal models and to identify new disease targets for drug discovery. The present review assesses the status of current efforts in developing CSF biomarkers for Alzheimer's disease and briefly discusses the status of CSF biomarker efforts in schizophrenia, depression, Parkinson's disease and multiple sclerosis.

Contents

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1. Introduction

The use of biomarkers for both the diagnosis of human disease states and their progression can be traced back to the 25th century B.C. when the Chinese Emperor, Huang Di also known as Huang Ti, authored the *Huang-di nei-jing*, the first recorded Chinese medical text [1,2]. A widely used quote ascribed to this work states that "the superior physician helps before the early budding of disease.... To administer medicines to diseases which have already developed is comparable to the behavior of those persons who begin to dig a well after they have become thirsty"[2], an avocation that remains as important in medicine today as it did nearly 5000 years ago.

The modern discipline of clinical chemistry was established early in the 19th century [4] on the basis that "data obtained from a body fluid sample could be used to infer information about the status of the living organism from which it came". Virtually anything that can be measured in a biological system, *in vivo* or *ex vivo*, has the potential to function as a biomarker of health and disease and includes simple and complex chemicals as well as bacteria and viruses. Glucose, urea, hemoglobin, cholesterol, lipids, etc., present in blood, urine, cerebrospinal fluid (CSF), feces, sweat, lung exhalations, etc., as well as brain volume, heart rate, tumor mass, body temperature, lung tidal volume, urine output, body mass index, cognitive function, gait, etc., represent phenotypic biomarkers are all potentially useful biomarkers as are imaging techniques [5] that include ultrasound, thermography, MRI and PET scanning that can be used in a non-invasive manner to assess the "status of the living organism".

In 2001, the Biomarkers Definitions Working Group [6] derived a consensus definition of a biomarker 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". In addition to the examples above, this definition can also be extended to include the measurement of plasma levels of any new chemical entity (NCE) being evaluated as a potential therapeutic agent, the plasma concentration of an NCE over time being a biomarker for the normal biological processes of compound absorption, distribution, and elimination [ADME; 7]. In the drug discovery process, biomarkers can be used to assess the pharmacokinetic/pharmacodynamic (PK–PD) relationship of an NCE [8–10], to assess its access to its designated target in the body and its target residence time [11] and to determine the efficacy, selectivity and safety of an NCE.

Many biomarkers have gained general acceptance and are widely used to diagnose disease occurrence and severity (e.g., blood pressure and heart disease, blood glucose and diabetes) and to determine PK–PD parameters and safety in both preclinical and early phases of clinical trials for NCEs in diverse therapeutic areas. Biomarkers for NCE/drug efficacy tend to be disease specific and, in some instances, are specific to the therapeutic target. Thus serum cholesterol, an indication of metabolic dysfunction, can be used as a surrogate marker for cholesterol deposition, atherosclerotic plaque formation and coronary heart disease. Drug-induced (statins, CETP-inhibitors) decrease in plasma cholesterol is a biomarker for inhibition of HMG-CoA reductase activity and, indirectly, cardiovascular health [12,13]. The established relationship between cholesterol and coronary artery disease led to the approval of simvastatin for the lowering of cholesterol, a surrogate endpoint [14] with subsequent demonstration of efficacy for the treatment of morbidity and mortality associated with cardiovascular disease, the clinical endpoint [15].

2. Biomarkers

The availability of reliable, predictive biomarkers has become a key, if not the major factor, in translational medicine initiatives in drug discovery [16–19] with an absolute emphasis on their reliablity and predictivity. *Reliability* describes the ability to measure a given biomarker in an accurate, consistent manner with minimal variability in multiple patient samples. *Predictivity* is that the biomarker, once reliably measured, can be used to provide information on the presence and progression of a disease state and the effect of therapeutic interventions, including drugs, on disease status and outcome. Another factor in defining a disease related biomarker is its uniqueness for that disease. Measuring C-reactive protein (CRP), an acute-phase protein, was considered at one point to be superior to serum cholesterol in diagnosing heart disease risk, reflecting its increased levels following arterial damage [20]. However an assessment of individuals with CRP variants found that those with high CRP levels showed no increase in cardiovascular disease risk as compared to those with a normal or low CRP [21]. Thus CRP measurement can add value to other biomarkers (including phenotypic ones) used for cardiovascular disease state assessment, even though it is a non-specific biomarker of inflammation [22].

In drug discovery and development, the ability to identify/qualify/validate predictive biomarkers can decrease the risk in the drug approval process and facilitate clinical trial design [18,19,23]. The formation of a Biomarker Consortium [24,25], a partnership between the US National Institutes of Health (NIH), the FDA, the CMS (Centers for Medicare and Medicaid Services), the pharmaceutical and biotechnology industries, academic medical research institutions and non-profit disease research and patient advocacy groups underlines the importance of biomarkers for the future of biomedical research.

The identification of robust, predictive biomarkers for CNS translational medicine and drug discovery, despite considerable investment, remains a major challenge.

A biomarker is accepted as being potentially useful when it is closely linked causally or by correlation to a relevant biological outcome. It then becomes useful in the diagnosis and treatment of disease when it is a diagnostic or staging tool, a prognostic indicator of the natural course of the disease, or a predictor or indicator of a clinical response to an intervention [6]. When used for disease diagnosis, the variability in the measurement of an analyte, gene, protein, chemical or phenotype, can blur the line between data obtained from a 'normal', control individual and that originating from a patient with the targeted disease (Fig. 1). An ideal biomarker would therefore be binary, absent in a healthy individual, only present in the disease state and increasing with the severity of the latter. The challenge(s) in identifying such analytes is considerable and requires time-consuming and costly measurements in large patient cohorts, ideally following patients from disease diagnosis to postmortem confirmation of their disease state and its severity, and including not only healthy controls and

[2] While this is a widely cited quotation, recent translations of the *Neijing Suwen* [1] and the *Huang Di Nei Jing Su Wen* [2] fail to provide this precise version. Weil [3] cites a somewhat longer version of the quote from p.105 of [2].

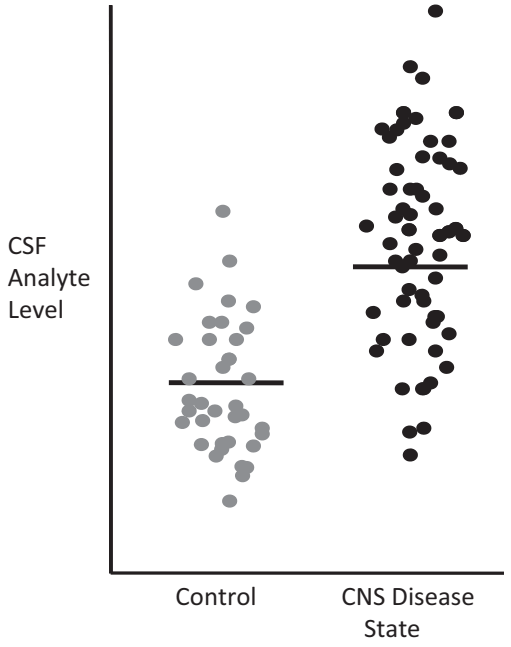

Fig. 1. Cartoon of diagnostic data points. The cartoon shows a stylized amalgamation of typical data sets from the biomarker literature. An analyte is measured, in this instance in the CSF, from a cohort of age and gender matched controls (control) and a disease cohort. The means of the data sets are shown using the horizontal bars and clearly delineated analyte differences between the two groups. However, there is considerable overlap due to data scatter such that a "normal" individual, based on the use of this biomarker approach, could be diagnosed as have the disease in question. Conversely, an individual with the disease could be diagnosed as normal highlighting the need for a battery of biomarkers rather than one in isolation.

patients with the disease being targeted but also well defined patient groups with other diseases as negative controls. As noted, biomarkers predictive of the efficacy of an NCE based on preclinical disease models have become an absolute imperative in the transition of the NCE from animals to humans. At their most basic, the plasma levels of an NCE showing efficacy in a putative animal models can be used to target similar levels in humans. Dose proportionality is however subject to both the vagaries of human ADME characteristics versus those in a rat or mouse or even a non-human primate and also the ability of humans to communicate subjective changes in response to NCE administration, an ability lacking in other species. Thus, irrespective of how many times the question is asked, there is no species that is uniformly predictive of human metabolism and NCE responses. The ability to use efficacious plasma levels of an NCE in preclinical models to predict human dose ranging can give needed assurance to proceed to early clinical phases for the disease. The availability of *bona fide* disease-related biomarkers can reduce the risk of failure, or identify early problems with NCEs and terminate them prior to costly Phase II clinical trials.

2.1. Biomarker validation

Biomarker development for use in early phases of NCE testing has been particularly useful in PK–PD modeling where attrition rates due to undesirable PK and bioavailability profiles were dramatically reduced between 1991 and 2000 [26]. Biomarkers for efficacy and toxicology/safety are anticipated to reduce the current NCE attrition rate that has been in evidence for more than a decade [27].

Validation of biomarkers, is a complex and time consuming process that has usually been agreed to "by debate, consensus and the passage of time" [28]. Ideally, a biomarker should be a surrogate marker of disease status making it useful for both diagnosis and for assessing drug effects. A validated surrogate biomarker is one where there is evidence that a drug-induced effect on the surrogate while an "unvalidated" surrogate biomarker is one that is "reasonably likely" to predict clinical benefit [23], the latter type of is however unlikely to receive widespread support anymore than a drug that that is "reasonably likely" to affect disease progression would be anticipated to receive regulatory approval. Thus the path forward in determining the sensitivity and selectivity of a biomarker is determined by its "beta" testing in the clinical setting, Biomarker validation is most advanced in the area of safety and toxicity where a number of assays are already in widespread use within the pharmaceutical industry in the context of the International Conference on Harmonization (ICH) guidelines. One example is inhibition of the hERG (human *ether-a-go-go*-related gene) K^+ channel (I_{Kr}, Kv11.1) that can result in QTc prolongation with the potential for *torsades de pointes*, ventricular fibrillation, and sudden death which is the subject of the ICH S7B guidance [29] that requires NCEs be studied indirectly (e.g., binding assay, ion flux assay) or directly (e.g., hERG/I_{Kr} current study) in discovery and development phases as a basis for decision-making in NCE selection [30–32]. A retrospective study [33] comparing hERG block potency with drug-induced QTc prolongation assessed via thorough QT (TQT) clinical studies of some 40 known drugs in a diversity of therapeutic indications found that higher preclinical safety margins provided less confidence in predicting prolongation in the clinic. In addition to suggesting that additional preclinical assays and adaptive strategies were required to reliably mitigate QTc prolongation risk, this study also highlighted a need for caution in data interpretation and prediction even when established 'gold standard' biomarkers were used. This is of concern when such assays are used to decide the fate of new NCE series. It has also been suggested that using QTc prolongation assessment as an absolute binary, rather than relative (e.g., therapeutic index-based) filter, is itself a major contributor to NCE attrition [34]. Screening *in vitro* for cytochrome P450 (CYP) enzyme inhibition and induction to predict potential drug–drug interactions is another widely used early discovery biomarker screen [35–37]. In humans, NCEs are metabolized by a limited number of P450 enzymes, primarily CYP3A4, such that these are prioritized in human liver microsomal studies [36,37]. CYP inhibition can increase NCE concentrations leading to unexpected toxicities while CYP induction can reduce drug plasma concentrations below efficacious levels or increase metabolite-mediated toxicities. Ideally, NCEs that are advanced into development will have minimal effects on both the hERG channel and CYPs as part of the lead optimization process. Early screening for these liabilities will reduce later stage failures due to unacceptable safety profiles, although, as noted, the biomarkers used are not wholly predictive either positively or negatively and cannot replace later phase safety studies.

Pathway analysis [38] represents a useful technique for predicting and interpreting preclinical and clinical toxicities associated with the inhibition of a discrete molecular target. Similarly, 'omic'-based approaches including proteomics [39], metabolomics [40,41] and transcriptomics [35] can be used to evaluate changes in RNA and protein expression in response to NCEs and their metabolites and may be useful in assessing issues of inhibition of specific molecular targets as they relate to side effects and toxicity [7]. Toxicogenomics [42], using gene microarrays [43] and high throughput screening, the latter a key element of the Tox21 initiative [44], has the potential to identify NCE and molecular target-based toxicity. However, despite considerable promise as tool for compound selection and advancement has still to achieve a prominent role in decision making [45] as the pharmaceutical industry remains apprehensive about how the data will be used by regulatory agencies in real NCE submissions

since 'omic' methods have not been thoroughly validated and since the observed changes are not yet validated biomarkers of toxicity [46]. 'Omic' approaches still hold promise however, in the discovery phase for compound selection and for the early identification of possible safety biomarkers that can then be pursued using more established methods.

Biomarkers for efficacy additionally offer the hope of producing new drug therapies more efficiently, primarily by reducing the length of clinical trials and possibly by potentially reducing the numbers of human subjects required to observe a significant effect of drug treatment [23]. The possibility exists that clinical trials can be shortened if an effect on a surrogate biomarker can be observed relatively quickly as compared to more traditional survival outcomes as the measure of therapeutic efficacy, e.g., an effect of antihypertensive medications on reduction of blood pressure rather than on a reduction in mortality due to stroke or overt cardiovascular disease as a consequence of hypertension. The value of a validated biomarker is thus self-evident both from a cost and risk reduction perspective. However, the process of validation is not something to be undertaken lightly, especially in the field of CNS disease states and disorders where the target organ, the brain, is more inaccessible than other organs to invasive interrogation due to the blood–brain-barrier. In addition, the causality for the majority of CNS disease states is unknown.

The most challenging aspect of developing useful biomarkers for disease progression is that such biomarkers are ultimately confirmed only when they are found useful for predicting clinical efficacy of a NCE. Currently used therapeutics in both psychiatric and neurological diseases provide only symptomatic effects rather than truly modifying core pathophysiology of the disease. Thus, current biomarker development for disease modification proceeds without the presence of gold standard treatments to validate novel biomarker endpoints. Nonetheless, the ability to develop predictive biomarkers for psychiatric disorders, e,g., schizophrenia, depression, autism, bipolar disorders, etc., and for neurological diseases including stroke, Alzheimer's disease (AD), Parkinson's disease (PD) and other neurodegenerative disorders has the potential to revolutionize the diagnosis of these disorders leading to better treatments and better outcomes. For instance, the ability to rapidly diagnose a stroke and its severity using a validated biomarker as contrasted to the present exclusionary diagnosis would markedly decrease the morbidity and mortality associated with this disorder.

The present Commentary focuses on the current state of the search for CNS biomarkers for disease diagnosis, progression and the assessment of NCE efficacy, with a special focus on putative CSF biomarkers for three CNS disease states, two psychiatric – schizophrenia and depression, and one neurodegenerative- AD.

3. CNS biomarkers

The search for predictive biomarkers for CNS disorders began in earnest with the advent of neuropsychiatric genetics [47,48] and proteomics [49]. Prior to this, the technology available had limited studies to gross assessments of CNS function, e.g., measuring changes in urinary analytes, a paradigm that by both definition and practicality was inversely comparable to the butterfly effect in chaos theory. The accuracy and relevance of a urinary analyte to what was occurring within the brain, given that the analyte would need to traverse the blood–brain barrier, enter the circulation, pass through several thousand feet of blood vessels to reach the kidney and then be filtered through the glomeruli, stored in the bladder at body temperature along with other body waste for unknown time periods is questionable. Further potential delays in urine analysis following micturition into a container that may or may not contain acid to prevent bacterial growth and may or may not be stored in a refrigerator can seriously weaken the value of the biomarker analyte with the potential to markedly distort data outcomes especially when sources other than the brain had the potential to produce the analyte. Thus a "pink spot" in urinary chromatograms in the 1960s, identified as dimethoxyphenylethylamine, initially associated with schizophrenia [50] was subsequently questioned when it was also found in the urine of normal individuals and patients with Parkinson's disease [51]. The limitations of urinary biomarkers for CNS disease diagnosis were further highlighted by reports of an increase of truncated nerve growth factor receptor in the urine of mildly demented AD patients [52] that were also found [53] in the urine of individuals with congestive heart failure.

While direct access to brain tissue or fluids is the most appropriate way to obtain samples that reflect real time activities in the brain, this is only possible during invasive surgical procedures. An example of the value of this approach involved a study in epileptics that unequivocally demonstrated the role of adenosine as an endogenous anticonvulsant [54]. In this study, depth electrodes modified to include a microdialysis probe were implanted in the hippocampi of patients with intractable complex partial epilepsy and samples collected bilaterally before, during and after a single, spontaneous-onset seizure. Seizures that originated in one hippocampus and propagated to the contralateral hippocampus were associated with 6–31-fold increases in extracellular adenosine levels with a greater increase in the epileptogenic hippocampus that reflected adenosine concentrations of approximately 65 μM. Since adenosine *in vitro*, at concentrations of 40–50 μM can depress epileptiform activity, the levels found in the brain during seizures have the potential to suppress seizures *in vivo*.

In the absence of being able to circumvent the blood–brain barrier by direct access to brain tissue and fluids, cerebrospinal fluid (CSF) is the nearest approximation as many CNS drugs can diffuse freely between the interstitial fluid of the brain and CSF [55] as can analytes, increasing the likelihood that drug concentrations and analyte changes in the brain can be indirectly measured in the CSF [56–58]. Obtaining CSF while less invasive than surgery still remains an invasive procedure, requiring a lumbar puncture that can lead to post-lumbar puncture headache (PLPH) characterized by headache, back pain, dizziness, nausea and vomiting [59] that can be reduced in incidence by using smaller gauge needles [60] and can be treated with opioids. Spinal taps to obtain CSF for biomarker assessment are rapidly becoming a routine procedure, especially in the area of AD with analytes being measured by ELISA and mass spectroscopy-based methodologies [61].

The global CNS biomarker market was estimated to be $1.3 billion in 2009 and anticipated to increase to more than $3.0 billion by 2015 [62].

The clinical endpoints for psychiatric and neurodegenerative disorders, in addition to morbidity and mortality, involve tests of motor, cognitive, or memory function using clinical rating scales to measure impairment and can seldom be substantiated by clinical laboratory or neuroimaging findings. Although these functional rating scales are to a major extent surrogate endpoints, they are generally accepted as clinical endpoints in clinical research and efficacy trials and have generally been validated prior to or as a part of efficacy trials [63]. One example of a CNS surrogate efficacy biomarker is the decrease in the frequency of new gadolinium-enhanced lesions observed during magnetic resonance imaging (MRI) scans in trials of immunologically-based treatments for multiple sclerosis. These gadolinium-enhanced lesions were quantified together with several clinical endpoint measures [64]. Currently, pharmaceutical companies use onset of new gadolinium-enhanced lesions as a primary efficacy measure during Phase II proof-of-concept trials as an indication that an immunological treatment will result in clinically significant modulation of

multiple sclerosis symptoms over a 1–2 year period in Phase III studies.

It is unlikely that any of the many biomarkers under evaluation for psychiatric and neurodegenerative diseases will advance to the level of validation to be accepted as surrogate endpoints and supplant traditional clinical endpoints in the near future. A valid biomarker, as already noted, would be one that is unequivocally associated with disease causality. Thus the product(s) of each disease-associated candidate gene and its associated pathways would be a likely biomarker candidate. In schizophrenia, GWAS (genome wide association studies) have identified more than 30 disease-associated genes that include neuregulin, reelin, *DTNBP*, *RGS4*, *DISC1*, *CMYA5* and the α7 NNR [65] the putative role of which in disease causality remains open to subjective interpretation in the context of the existing dopamine/glutamate (DA/G) hyper/hypofunction hypotheses [66] or remain obscure and have yet to be useful in producing novel, next generation CNS drugs [67]. The most recent gene association for schizophrenia, *CMYA5* (cardiomyopathy associated 5 gene or *myospryn*) was identified in 20 independent samples involving more than 33,000 participants [68]. Myospryn can bind to dysbindin [69], the protein product of the *DTNBP1* (dystrobrevin binding protein 1) gene that has been identified as a major schizophrenia susceptibility factor [70]. The function of myospryn in schizophrenia is presently unclear although its reported association with left ventricular hypertrophy [71], make it, despite the GWAS-derived association a somewhat questionable biomarker for schizophrenia.

A major hurdle in biomarker development involves the accuracy of disease diagnosis, a classical catch-22 situation where validated biomarkers have the potential to markedly improve patient diagnosis and where more accurate patient diagnosis can enhance the value of identified biomarkers. Differences in disease conceptualization between basic researchers and clinicians in the drug discovery process represent a major confound to this process. As an example, for the preclinical researcher, schizophrenia as a disease can be defined in terms of D2/5-HT_{2A} receptor blockade and modulation of NMDA receptor functionality. For the practicing psychiatrist, the same disease is viewed in terms of positive, negative and cognitive states resulting in significant functional impairment as defined in DSM-IV (*Diagnostic and Statistical Manual of Mental Disorder-IV*) [72] the canon for psychiatric disease diagnosis and for the psychiatric phenotypes accompanying neurodegenerative disorders. DSM is however, based on symptomatic phenomenology rather than objective causality and has thus been viewed as "arbitrary or hazy" [73] in the context of the molecular target-based research environment that almost uniformly reflects current approaches to drug discovery in the CNS area [74]. One glaring example [67] of the imprecision inherent in the DSM-IV TR framework relates to the diagnosis of depression. This involves 10 distinct criteria, five of which necessary need to be present simultaneously for a diagnosis of depression. With 10 criteria, two patients can have 5 each, leading to a diagnosis of depression with none of these in common. Another example is the diagnostic dilemma experienced by clinicians when faced with the first psychotic break by a patient that with time may better match current DSM-IV criteria for schizophrenia or bipolar disorder. Significant overlap exists for genetic vulnerability of these two disorders questioning whether they should be considered together, or remain separate. However, superior treatment response to lithium and a developing differential treatment response pattern for ketamine and i.v. scopolamine in bipolar patients as opposed to patients appearing with a treatment course better approximating the Kraepelinian viewpoint does argue for the current nosology.

As a result of this current diagnostic uncertainty, the NIMH has undertaken a new research-based initiative to classify psychiatric disorders in the context of newly designed Research Domain Criteria (RDoC). The latter of which are based on a neural circuitry approach and involve five distinct domains: negative emotionality, positive emotionality, cognitive processes, social processes and arousal/regulatory symptoms [75]. Using this framework, disease phenotypes would be more concretely linked, or dissected, with a basis in molecular causality that more accurately reflects the preclinical framework of the drug discovery process. However, the challenge for the RDoC initiative and for CNS drug discovery will be to create a transparent link between the research and the clinic. One way in which this can occur is at the level of the biomarkers that are validated by being directly associated with disease causality and will be reinforced with efforts to improve preclinical animal models so that they more appropriately reflect the disease than the putative mechanism of the disease [67,76,77].

4. Biomarkers for Alzheimer's disease

4.1. Alzheimer's disease

Alzheimer's disease (AD), also known as senile dementia of the Alzheimer type (SDAT), is the most common form of dementia, reflected as a decline in cognitive function with associated behavioral disturbances that include depression, anxiety, suspiciousness, agitation, anger, delusions and/or hallucinations. With an unknown casualty and currently incurable, this neurodegenerative disorder currently affects some 35 million individuals worldwide [78] with estimates varying between 115 million individuals being affected by 2050 [79] or 1 in 4 of the population [80]. A new diagnostic framework for AD has recently been proposed [81] that considers the disease as a clinical and symptomatic entity involving both predementia and dementia phases, such that an absence of dementia does not preclude an AD diagnosis.

The only treatments approved for the treatment of AD are palliative and include the cholinesterase inhibitors, donepezil, galantamine and rivastigmine, that address the cholinergic deficit hypothesis of AD, and the NMDA receptor antagonist, memantine. These agents are generally considered to have marginal efficacy [82,83].

Numerous potential causes of/risk factors for AD have been postulated [78,84–86] and include: aging, brain inflammation produced by infection, trauma and/or cerebrovascular disease; oxidative stress; altered ATP production/mitochondrial dysfunction; anesthesia from surgery; a host response [87]; environmental pollutants (e.g., automobile exhaust); aluminum cookware; stress; diet including diabetes [88,89]; overuse of antibiotics; chemotherapeutic use; the absence of robust intellectual stimuli in present day society and dysfunctional neurogenesis [90]. Most recently, AD has been suggested to be due to a dysfunctional synaptic mTOR pathway [91], a proteopathy [92] and increased oxysterol levels [93].

4.2. Genetic associations with Alzheimer's disease

Some 120 gene loci associated with AD have been identified using GWAS [94,95]. These include four genes "unequivocally associated with AD" [96] – *APP* (amyloid precursor protein), *APOE* (apolipoprotein E), *PSEN1* (presenilin 1) and *PSEN2* (presenilin 1). Of these, *APP*, *PSEN1* and *PSEN2* are associated with an autosomal dominant, early-onset familial form of AD {EOAD} that accounts for 2% of all cases of AD [96]. *APOE* is considered a risk gene for late-onset familial AD and for the more common sporadic form of the disease, late onset AD [LOAD] that is responsible for approximately 80% of AD cases. Other candidate genes identified by GWAS include *GAB2* (GRBS2-associated binding protein 2), *CHRNB2* (neuronal nicotinic acetylcholine receptor β2 subunit), *CH25H* (cholesterol

25-hydroxyase), *PGBD1* (piggyBac transposable element derived 1), *LMNA* (protein laminin A/C), *CST3* (cystatin C), *PCK1* (phosphoenolpyruvate carboxykinase 1), *ACE* (angiotensin converting enzyme), *MAPT* (microtubule associated protein-τ) and SORL1 (neuronal apolipoprotein E receptor). Irrespective of the contribution of environmental factors and genetic risk factors to AD onset, age remains a major risk factor, although AD is not thought to be part of the normal aging process.

4.3. Alzheimer's disease hallmarks

Histopathological studies of brain from AD patients, the earliest dating back to 1906 with Alzheimer's original identification of the disease, have identified two key hallmarks associated with AD – *amyloid plaques* and *neurofibrillary tangles* (NFTs) [78,84,97]. NFTs are comprised of hyperphosphorylated tau, a microtubule associated protein. Whether these core disease hallmarks, also known as tombstones, are: causative to; the result of; or the products of an endogenous protective response to [87,98]; the disease remains unknown with no definitive data currently available.

Nonetheless, the majority of biomedical research and drug discovery efforts in AD conducted over the past 15 years has focused on identifying compounds or biologicals to reduce the amyloid load in the brain [84,86] and, to a lesser extent, to reduce tau hyperphosphorylation and associated NFT formation [99–101]. Despite considerable efforts and a number of clinical candidates, little progress has been made in clinically validating either of these two events as key to AD causality or in discovering viable new therapeutics for the treatment of AD [85], instead, there have been several notable Phase III failures including inhibitors of γ-secretase, one of the key enzymes involved in amyloid production, tarenflurbil [102] and semagacestat [103] and of the putative mitochondrial pore modulator, latrepirdine (dimebon) [104] while biologicals including vaccines [105] and antibodies [106] have shown mixed results in attenuating the progress of AD with significant safety issues.

The outcomes of these various trials have been interpreted in terms of; (i) an insufficient understanding of the mechanism(s) of action of the NCEs tested; (ii) intrinsic shortcomings in the drug-like properties of the clinical candidates tested [103,104] or; the inclusion of AD patients in clinical trials who were too advanced in their disease progression to benefit from any type of therapeutic intervention. Since these results have "challenged the primacy of Aβ in AD pathophysiology" [103], they have provided a body of data that amyloid may not be causative in AD pathophysiology [85,87,107]. This has major implications in developing appropriate biomarkers for the disease, especially since the latter are critical in diagnosing AD at a sufficiently early stage that putative therapeutic interventions may be effective, especially as regards to patient enrollment in clinical trials. The following sections reflect the status of published AD CSF Biomarker research up until the last quarter of 2010. Given the intense efforts in the area, it is unlikely to be up-to-date by the time it is in print.

4.4. Biomarker approaches to Alzheimer's disease diagnosis and assessment of disease progression

The search for predictive biomarkers for AD is a high priority in neurodegenerative disease research and is underlined by the lack of progress in identifying new treatments for the disease. Part of the NIH Biomarker Consortium is the ADNI (Alzheimer's Disease Neuroimaging Initiative) that is funding multicenter initiatives to identify biomarkers for AD [108]

Biomarker development for AD – to both diagnose the disease and to assess its progression – currently encompasses six different, but inter-related approaches: (i) behavioral assessment of the patient including measurements of cognitive status [81,109] that include the ADAS-Cog (Alzheimer's Disease Assessment Scale-cognitive [110]) and MMSE (Mini-Mental State Examination [111]) scales; (ii) assessment of changes in brain volume [112]; (iii) alterations in brain metabolism [113,114]; (iv) measurement of biomarker load within the brain [114–117]; (v) CSF biomarker profiles [116–120] and (vi) post mortem confirmation of AD histopathology. The predictive utility of these various biomarker approaches, individually or collectively, has yet to be established despite numerous publications that claim to have discovered a "gold standard" of AD diagnosis in a patient cohort. Indeed, it has been noted [121] that given the many factors involved in establishing steady-state levels of Aβ in the CSF – production, aggregation, clearance and bidirectional transport across the blood–brain barrier – it is "difficult to predict what different amyloid-targeting treatments may do to CSF Aβ levels". Conversely, this would also complicate interpretation of what demonstrated changes in CSF Aβ may relate to in the brain, especially as predictive power of CSF Aβ markers is "suboptimal" and of "limited diagnostic usefulness" [121].

Comparison and/or integration of the results derived from these different biomarker approaches in some instances are confirmatory of initial findings and, in others, contradictory. Additionally, there are cases of diagnosed AD in which postmortem assessment failed to confirm the presence of plaques and tangles while individuals diagnosed as lacking AD were found to have these 'tombstones". In a cohort of 43 adults aged 65–88 who did not have clinical AD, PET scanning identified 9 individuals with β-amyloid deposits in brain using PET imaging who were cognitively equivalent to 29 individuals lacking amyloid deposits and 5 participants with "intermediate" evidence of amyloid deposits [121]. The finding that individuals with "significant amyloid burden" are cognitively normal suggests either a high level of cognitive reserve or that amyloid deposits are insufficient to cause AD. These findings echo post-mortem studies in which brains of individuals with normal cognition were found to have significant amyloid deposits [122].

4.4.1. CSF amyloid as a biomarker of AD

Amyloid, the product of APP (amyloid precursor protein) exists in a variety of isoforms of 36–43 amino acids in length. APP is a substrate for the aspartyl proteases, α-, β- and γ-secretase, the latter of which leads to C-terminal fragments of APP that include Aβ peptide, the most common forms of which are $A\beta_{40}$ and $A\beta_{42}$ (also known as $A\beta_{1-40}$ and $A\beta_{1-42}$) [78,84] $A\beta_{40}$ is the most common form with the more toxic $A\beta_{42}$ form representing 10% of Aβ. Other fibrillar and soluble forms of Aβ have been associated with neurotoxicity in animal models and include $A\beta_{25-35}$, and $A\beta_{31-35}$ [123], Aβ -derived diffusible ligands (ADDLs [124]), Aβ dimers [125] and Aβ oligomers [126,127]. Other Aβ peptides found in CSF include $A\beta_{1-14}$, $A\beta_{1-15}$ and $A\beta_{1-16}$ that reflect a novel APP- processing pathway [128] and 20 other truncated Aβ peptides [129]. Despite the complexity of the Aβ isozymes potentially present the CSF, the focus on CSF amyloid biomarkers has been the measurement of $A\beta_{42}$ [130] that undergoes a decrease in CSF in patients diagnosed as converting from MCI to AD [131], an assumption being that this reflects increased accumulation of $A\beta_{42}$ in brain forming plaques [132] and thus reflects brain Aβ load [120,133]. Analysis of amino-truncated $A\beta_{42}$ peptides, as compared with $A\beta_{42}$ alone, improved the ability to differentiate stable MCI patients versus those progressing to AD [134]. The pattern of carboxy-truncated Aβ ($A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-39}$) versus $A\beta_{40}$ and $A\beta_{42}$ has been suggested as useful in both diagnosing AD and differentiating it from other neurodegenerative diseases including PD, dementia with diffuse Lewy bodies and Creutz-

feldt-Jakob disease [135,136] although specificity remains an open question [137,138]. In addition, it has been noted that due to interpatient heterogeneity, a CSF $A\beta_{42}$ biomarker may not improve the efficiency of running clinical trials in AD [139]. In this study, clinical trial simulation using data from the ADNI showed that a cohort of 148 patients with amnestic MCI who had low levels of CSF $A\beta_{42}$ scored significantly worse in ADAS-Cog and other AD behavioral tests than 51 patients with high CSF $A\beta_{42}$ underscoring concerns that brain amyloid deposits as measured indirectly by CSF $A\beta_{42}$ levels do not necessarily lead to AD [122].

4.4.2. CSF tau phosphorylation as a biomarker of AD

The second CSF core biomarker for AD is tau protein [140] that has been the subject of biomarker research for the past 15 years. Tau protein exists in six isoforms of 352–441 amino acids in length that are subject to a variety of posttranslational modifications [141] and, presumably, function. Of the 79 serine and threonine phosphorylation sites on the longest isoform of tau, 4R/2N, approximately 40 have been verified [142] of which 25 have been identified as sites of "abnormal phosphorylation" [99]. The phosphorylation state of tau is the net result of a balance of kinase and phosphatase activity. Much of the activity in tau-based drug discovery has been focused on selective finding inhibitors of "tau kinase", a combination of the activity of two serine/theronine kinases that can phosphorylate tau – glycogen synthase kinase 3 (GSK3; tau protein kinase I), cyclin-dependent kinase 5 (CDK5; tau protein kinase II) and a third kinase, extracellular signal-regulated kinase 2 (ERK2), from the possible 518 member kinase family, as a possible therapeutic approach to treating AD [99–101]. Other kinases that are possible targets to prevent tau hyperphosphorylation are casein kinase 1 [141], AMP-activated protein kinase (AMPK [143]) and DYRK1A and AKAP-13 [144].

From a biomarker perspective, total tau (t-tau), a generic measure of cortical axon damage associated with AD, multiple sclerosis [140,145], stroke and Creuzfeldt-Jacob disease, and phosphorylated tau (p-tau) are increased by three fold in the CSF of confirmed AD patients [131]. Of the 40 or so phosphorylation sites on tau, six – $pThr^{181}$ (phosphothreonine-181), $pSer^{199}$, $pSer^{202}/pThr^{205}$ (AT8 site), $pSer^{214}/pSer^{212}$ (AT100 site), $pThr^{231}/pSer^{235}$ (TG3 site) and $pSer^{396}/pSer^{396}$ (PHF1 site) – have been associated with tau hyperphosphorylation and to screen NCEs for potential "tau kinase" inhibitory activity. While $pSer^{199}$ and $pThr^{231}$ ($p\text{-}tau_{231}$) have been evaluated as CSF biomarkers [147,148], $pThr^{181}$ (also designated as $p\text{-}tau_{181}$ or $P\text{-}Tau_{181P}$) is the most widely used CSF biomarker to assess tau hyperphosphorylation [149,150] having similar diagnostic accuracy to $p\text{-}tau_{231}$ [151]. Like $A\beta_{42}$, the diagnostic value of both t-tau and $p\text{-}tau_{181}$ has been questioned in terms of their specificity as AD biomarkers [153].

4.4.3. Combination CSF amyloid and tau phosphorylation biomarkers—a signature for AD?

Given the current limitations of the predictive value of $A\beta_{42}$, t-tau and $p\text{-}tau_{181}$ as AD biomarkers alone, these have been used together to develop a "CSF AD signature", again, with mixed results [131,153–157]. While some studies indicate that the combination $A\beta_{42}$, t-tau and $p\text{-}tau_{181}$ biomarker signature in CSF has high predictivity in identifying cases of prodromal AD in MCI patents [131,132,152], there is considerable intersite variability that can confound biomarker accuracy [154]. Measurements of CSF Aβ42, and p-tau181 levels as an "AD signature" – reduced CSF Aβ42 and increased CSF p-tau181 concentrations – were used independently of a clinical diagnosis to stratify patient groups [157]. This AD signature was found in 90%, 72%, and 36% of patients with AD, mild cognitive impairment (MCI), and cognitively normal groups respectively [157]. The cognitively normal group with an AD signature were enriched in apolipoprotein E4 alleles. Validation of these findings in two further data sets showed that 64/68 (94% sensitivity) of autopsy-confirmed AD patients were classified with an AD signature while 57 MCI patients followed for 5 years had a sensitivity of 100% in progressing to AD based on their biomarker signature. The presence of a CSF AD signature in cognitively normal subjects was interpreted by the authors as an indication of AD pathology being present and detectable far earlier than previously envisioned in disease progression. While this study has generated considerable interest based on a null hypothesis approach where patient CSF biomarkers were measured prior to conventional diagnosis, another study [158] only showed changes in CSF t-tau in the control group.

4.4.4. Other CSF biomarkers for AD

As the AD signature approach based on the amyloid and tau causality hypothesis of AD continues to evolve, other CSF biomarkers are also being assessed. These include CSF cytokines [159,160] – specifically TGFβ increases in AD CSF [159] – CSF proteomic profiles [161], clusterin [162] and IgG antibodies from the adaptive immune system [163]. The latter is a field of intense efforts, despite the challenges in analyzing proteome profiles, and involves the study of differences in the CSF proteome inn AD, MCI and control subject groups [161,164–168]. One study [167] reported changes in a variety of CSF proteins including α-2-macroglobulin, α1-antichymotrypsin, α1-antitrypsin, complement and heat shock proteins, cathepsin D, enolase and creatine. The ADNI is also generating CSF proteomic profiles as part of its "*Use of Targeted Multiplex Proteomic Strategies to Identify Plasma-Based Biomarkers in Alzheimer's Disease*" [108].

4.5. Complementing CSF biomarkers for early AD diagnosis

As already discussed, achieving adequate sensitivity and selectivity for predicting which MCI patients will progress to AD within a 1–2 year period may remain quite challenging relying only on CSF biomarkers. Another approach, reviewed elsewhere in this special issue, suggests that connectivity changes in the default mode network measured during resting state of MRI studies may improve the sensitivity and selectivity in predicting earlier AD progression [169]. Thus, use of a more extensive biomarker profile, rather than any single measure may help ensure that MCI progression studies become a more viable strategy for discovering disease-modifying therapeutics.

4.6. Ethical considerations of CSF biomarkers for AD

While a validated CSF biomarker(s) for AD is essential in the clinical trial setting for appropriate patient enrollment, there has been considerable debate [170–172] as to the practical value of an AD diagnosis, especially in the prodromal AD state, to both a patient and his/her caregivers when there remains no effective treatment, palliative or disease-modifying. To inform a patient that they will progress from MCI to AD and that there is no drug available has to many been the cause of significant ethical issues. That patients with normal cognitive function can have a CSF AD signature [157], may not represent an enhanced ability to diagnose earlier stage AD but rather a false positive. Such concerns no doubt contributed to the background to the January, 2011 FDA advisory panel review of the amyloid imaging agent, florbetapir [117] that while very positive [173] recommended by a 13–3 vote that this agent is not ready for approval "at the present time" [174]. In the context of individuals having evidence of amyloid deposits in the absence of AD, it was noted that physicians "will have to use their clinical judgment, taking into account a patient's symptoms, in

deciding what the scan results mean" [173], an event that will occur in the absence of any effective treatment.

5. Biomarkers for other CNS disease states

Despite the caveats related to the reliability, predictability, selectivity and sensitivity of CSF biomarkers for AD, the latter area is far more advanced than other CNS disease areas, especially psychiatric. The following section provides a brief overview of the current status of CSF biomarker research in the areas of schizhophrenia, depression, PD and multiple sclerosis.

5.1. Schizophrenia

While the focus on the potential use of CSF biomarkers [175,176] for schizophrenia disease progression is a major advance on the urine "pink spots" of the mid 20th century [50,51], it is far less advanced than in AD. As already noted, the causal factors for schizophrenia remain unknown with a large number of genetic associations and environmental considerations [65], any, all of which, or none, could be potential biomarkers. Genetic vulnerabilities of very low effect size for schizophrenia interact with environmental factors as early as the second trimester *in utero* to program a range of developmental abnormalities that are finally reflected in the first schizophrenic break occurring often occurring in the early 20 s in males and by the early 30 s in females [177]. While current preclinical screening batteries appear to predict the clinical efficacy of novel treatments for positive symptoms of schizophrenia, therapeutics to optimally treat negative symptoms and cognitive dysfunction may need to begin prior to the first psychotic break. Even after the initial psychotic break, compliance to therapeutic treatments is challenging. If premorbid treatment is necessary for schizophrenic negative symptoms and cognitive impairment, then the challenge of ensuring treatment compliance in patients with premorbid increases in suspiciousness and denial is even greater than current treatment with atypical antipsychotics. Especially when considering safety aspects of drug treatment.

N-glycosylation of proteins in the CSF and serum of schizophrenic patients appears to hold promise as a CSF biomarker endpoint [178] but requires further replication and characterization. Clearly, an integrated, more in-depth understanding of normal brain development, altered developmental trajectories in schizophrenia, and moving beyond the current DSM-IV diagnoses will be necessary to bring CSF biomarkers for schizophrenia into a useful context.

5.2. Depression

The potential use of CSF biomarkers is even more challenging for major depressive disorders (MDD) than for schizophrenia. For example, clinical differentiation between bipolar depression and MDD is not possible until an episode of mania or hypomania define patients with manic-depressive illness versus MDD. Beyond that, most clinicians recognize that there is probably considerable heterogeneity among patients with MDD according to DSM-IVR criteria. Depression occurring first in late life (>60 years of age) with white hyperintensities on MRI is probably different with respect to etiology, and perhaps pathophysiology, than depression first occurring earlier in life. Beyond this, there is no agreement as to whether melancholic features (characterized by neurovegetative symptoms like decreased sleep, increased appetite or weight change) or psychotic features define separate groups or are reflections of disease severity. While there are currently hypotheses about the etiology/pathophysiology of MDD such as inflammatory changes, CSF biomarkers for depression will need to be defined and refined with respect to identifiable neuroimaging, genetic, and therapeutic findings for distinct subgroups of depressed patients. An increase in CSF $A\beta_{42}$ in elderly women which contrasts with the lower levels occurring in AD, together with altered CSF/serum albumin ratios that may be indicative of altered vascular events, has been associated with depression [179]. Whether this a potential diagnostic for depression [180,181] or a CSF event related to the early stages of AD remains to be determined.

5.3. Parkinson's disease

Parkinson's disease (PD) is another neurological disease where the primary pathophysiology is known with much greater precision than for psychiatric diseases such as schizophrenia and mood disorders. PD is characterized in the initial stages by a progressive loss of monoamine containing neurons from the brainstem that lead to prominent motor features reflecting the degeneration of dopamine containing neurons. A number of therapeutic approaches are currently being investigated for the dystonias that appear associated with prolonged direct or indirect agonism of dopamine receptors. Earlier diagnostic biomarkers and disease progression biomarkers are needed to most efficiently and rapidly advance disease-modifying treatments for this insidious, progressive neurodegenerative disease [182]. In April 2010, the Michael J. Fox Foundation launched a Parkinson's Progression Markers Initiative to identify neuroimaging, genetic, and CSF or blood biomarkers that may be used in clinical trials to demonstrate changes in disease progression [183]. This initiative is similar in intent to the ADNI effort in AD [108] in that funding from PD foundations, pharmaceutical and biotechnology companies will be used to supplement resources from academic centers and government agencies. Given that misfolded proteins represent a common feature of almost all neurodegenerative disease states, it is not surprising that efforts to standardize biomarker acquisition and assessment for the proteome are a priority.

Research to date has identified some 70–80 proteins that are different in PD patients compared to healthy controls [184,185]. Among these are apolipoprotein H/β-2-glycoprotein 1 (Apo-H), ceruloplasmin, and chromogranin B (secretogranin I) that are relatively unique to PD, although they may only possess sensitivity in the range of 56–67%. Alterations in CSF α-synuclein levels have been observed in relation to PD [186], While CSF complement proteins, cytokines and various antibodies to infection have been investigated as PD biomarkers, of these, SOD-1, the mitochondrial anti-oxidant DJ-1, osteopontin, BDNF, IL-1β, TNF-α, TIMP-1, and IL-6 as well as α-synuclein are biomarker candidates that have still to be validated [185].

Like efforts in the AD field, a panel of eight CSF proteins (BDNF, IL-8, VDBP, β2-microglobulin, apoAII, apoE, plus tau and $A\beta_{42}$) appears to classify PD patients with 95% sensitivity and 95% specificity [184]. Unfortunately, the overlap between dysregulated proteins in brain tissue compared to CSF of PD patients is very low. It remains to be seen whether this feature is primarily due to the inherent insensitivity in detecting proteins that are expressed in the CSF at concentrations as much as a thousand-fold less than in the blood or may reflect differing compartmentalization of the brain interstitial fluid and CSF.

5.4. Multiple sclerosis

Multiple sclerosis (MS), which most often presents with multiple demyelinating episodes separated in time and space throughout the CNS, is another neurodegenerative disorder where CSF biomarkers will play an important role. A variety of CSF proteins are under evaluation as potential biomarkers for MS and reflect various aspects of disease status and progression and

include markers related to inflammation and immune dysfunction, demyelination, oxidative stress, remyelination/repair, glial activation/dysfunction and neuroaxonal damage [187]. Clinically, the presence of oligoclonal bands in the CSF can complement MRI neuroimaging and the clinical correlations in patients presenting with a clinically isolated syndrome before definitive diagnosis of MS is made [188]. CSF proteomics are also being investigated as a means for differentiate between patients with the more common relapsing remitting (RR) form or the primary progressive (PP) form of MS, the latter which appears to have an attenuated immunological response. While most of the same proteins are altered in the CSF of patients with both clinical forms compared to healthy controls, there are a handful of proteins that may differentiate these two forms of MS that also lack clear differences with respect to genetic, immunological and neuropathological profiles [189]. These proteomic CSF and CNS tissue studies are likely to be critical as this field is currently attempting to better define the grey matter pathology and other factors that appear to track the progressive impairment of everyday function with greater fidelity than immunologically-mediated gadolinium-enhanced lesions [64].

6. Future directions

Much of the attrition in advancing NCEs from their identification as preclinical research leads to successful Phase II proof of concept trials in the CNS area is predicated on predictive animal models and informed and robust patient diagnosis with validated biomarkers being a necessary part of the translational model. While the limitations of animal models, wild type and transgenic, and their congruence – or lack thereof – with the human disease state have been the subject of considerable concern in the CNS area [67,76,77,190].

These concerns have been further compounded by issues related to the relationship of disease diagnoses based on DSM-IV criteria [67,73,75]. Thus models of AD, PD, schizophrenia, depression, etc., in which NCEs perform equivalently or superior to present standards of care – where these exist – have not accurately predicted the human clinical response. In addition to limitations on the animal models – which in many instances are little more than pharmacodynamic models with soft phenotypic readouts [191] – much of the preclinical characterization effort for NCEs is based on a target-based approach using "normal" cloned human receptors that may not reflect these entities, or their associated signaling pathways, in diseased tissues. In addition to diagnostic criteria and assumptions of disease causality at the molecular level, the latter of which is often multifactorial, subjectivity in patient responses to NCE administration – the placebo response – can further confound the translational interface in CNS drug research.

Against this backdrop, validated biomarkers, phenotypic, imaging and analyte based, can provide an additional level of confidence in advancing drug-like NCEs to the clinic with an enhanced expectation of predicting efficacy. Validated biomarkers also have the potential to develop new animal models that are congruent with the human disease state being targeted and also to stratify patient cohorts for inclusion in early clinical trials, especially those intended for proof of concept for an NCE. Given the imprecision associated with CNS disease diagnosis, the ambiguity in clinical trial outcomes and the caveats associated with the biomarkers discussed, the reader may ask which of these is the highest priority in terms of being addressed to which the answer is that each is contingent on the others. Successful clinical trials will provide validation of the disease target at which the drug discovery effort was targeted while more precision in disease diagnostics and the biomarkers associated with the disease will facilitate clinical trials. Inevitably, improving the CNS drug discovery paradigm is less amenable to inflexible binary dictates, e.g., naïve one gene, one disease-type approaches, but more to informed iterations on tangible progress that is made in any one area. In this context, the probable approval of florbetapir [171–174] is predicated on the dictate that amyloid is causative in the etiology of AD. If it is not, the ability to accurately diagnosis the disease with high selectivity and specificity – scatter grams like Fig. 1 notwithstanding – will certainly aid in providing a better means to identify *bona fide* new targets.

However, in the context of hypotheses about the deceleration of drug development and how much biomarkers can promise should bring other mitigating factors into context. Namely, have questionable assumptions been made about the most attractive CNS drug targets over the last few years? As an example, in psychiatry the NCEs discovered by serendipity [74,192] in the 1950s and 1960s possessed a rich repertoire of pharmacological properties [193] with many of these drugs being "magic shotguns" rather than selective agents. While the therapeutic effects of tricyclic antidepressants (TCAs) generally have been attributed to blockade of norepinephrine (NET) and/or serotonin (SERT) transporters, these drugs can also potently block 5-HT_{2A}, 5-HT_{2C}, α1 adrenergic, histamine H_1, and muscarinic receptors [194]. Blockade of these receptors can also contribute to the antidepressant effects of TCAs in the clinic. Selective serotonin reuptake inhibitors (SSRIs) have been an advance over TCAs with respect to safety and tolerability. However, several head-to-head comparisons between the TCA clomipramine and either paroxetine or citalopram by the Danish University Antidepressant Group in the late 1980s have suggested that SSRIs may be less efficacious than TCAs [195,196] with subsequent independent confirmation of this conclusion [197,198]. However, meta-analyses of efficacy comparisons between TCAs and SSRIs can be compromised by design flaws in many of the pharmaceutical trials that include the use of suboptimal dose treatment with TCAs. More recent work has suggested that selective NET inhibitors (SNRIs; selective norepinephrine reuptake inhibitors) may be less efficacious and less safe in treating depression than SSRIs [199]. Activation of 5-HT and adrenergic receptors by SSRIs and SNRIs may also contribute to the antidepressant efficacy of the TCAs, SSRIs, SNRIs, etc. Thus, is it any surprise that current drug discovery focused on developing new antidepressants based on interactions with single monoaminergic receptors has failed to result in novel therapeutics?

In addition to the challenges of optimal target selection and validation, the challenges faced by the pharmaceutical industry in discovering new medications in therapeutic areas with generic competition demand new biomarker approaches, especially for targets related to disease modification in both neurological and psychiatric disease states.

As noted, biomarker development is most advanced for AD with respect to both CSF and neuroimaging endpoints as a consequence of investment in the ADNI collaboration [108]. However, as biomarkers are identified and their specificity evaluated between different disease states, it is clear that a CSF biomarker on its own will be insufficient to diagnose a disease, its progression and the effects of an NCE on the latter. Thus it is unlikely that CNS disease states will lend themselves to "one stop" biomarkers like serum cholesterol and lipids [14,15]. Also, as noted, total tau levels in CSF can be a measure of several abnormalities in the brain [140,146] while Aβ42, a designated core CSF biomarker for AD is also altered as a result of depression [180,181]. An additional caveat is the degree of scatter in CSF analyte data points between designated controls and disease cohorts. This is illustrated in the cartoon in Fig. 1 abstracted from several published data sets on CSF biomarkers where the biomarker cannot definitively differentiate between control and diseased subjects and may thus have the liability of detecting false positives and negatives in the absence of

additional diagnostic criteria. This highlights the need for a biomarker battery rather than one in isolation.

Nonetheless, biomarker consortia [24,25] similar to the ADNI [108] and the Michael J. Fox initiative [183] involving academic funding agencies, academia, regulatory agencies and the pharmaceutical industry for CNS indications where disease modifying therapeutics are unavailable to provide an ultimate "gold standard" validation of new treatment modalities. These will be necessary to create the necessary fundamental clinical discoveries in psychiatric disorders including schizophrenia, major depression, bipolar disorders, autism, etc., and in neurological diseases like PD and MS. Development of biomarkers to supplement clinical endpoints will be complementary to pharmacodynamic biomarkers including imaging [169] and will provide increasing confidence that the CNS target has been engaged in the clinic setting, enhancing translational success [200], the relevance of CNS biomedical research in the 21st century [201] and avoiding the current trend to diminish efforts in CNS drug discovery in pharma [202].

Declaration of interest

DGF is an employee of EnVivo Pharmaceuticals who are actively researching therapeutic approaches to Alzheimer's disease. GJM is a clinician at Abbott Laboratories working in Clinical Development for Psychiatric and Neurological disorders. MW is a pharma industry consultant with a primary background in CNS drug discovery. This review was conceived, written and edited by DGF, GJM and MW.

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