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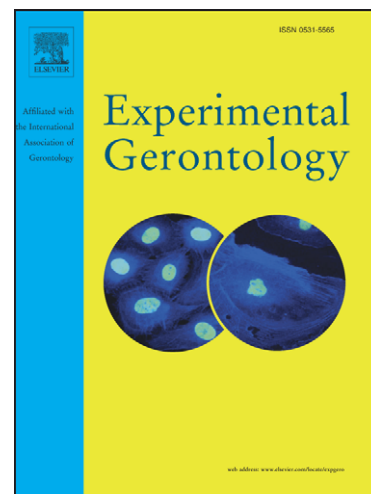
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Invited Full Review for Experimental Gerontology

Total and Phosphorylated Tau Protein as Biological Markers of Alzheimer's Disease

Harald Hampel^{*1,2,3}, Kaj Blennow⁴, Leslie M. Shaw⁵, Yvonne C. Hoessler²,

Henrik Zetterberg⁴, and John Q. Trojanowski⁵

¹ Discipline of Psychiatry, School of Medicine & Trinity College Institute of Neuroscience (TCIN), Laboratory of Neuroimaging & Biomarker Research, Trinity College, University of Dublin, The Adelaide and Meath Hospital Incorporating The National Children's Hospital (AMiNCH), Tallaght, Dublin, Ireland

² Alzheimer Memorial Center, Department of Psychiatry, Ludwig-Maximilian University, Munich, Germany

³ Department of Psychiatry, University of Frankfurt, Frankfurt/Main, Germany

⁴ Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at University of Gothenburg, Mölndal, Sweden

⁵ Center for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, Institute on Aging, the University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

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***Corresponding author:**

Harald Hampel, M.A., M.D., M.Sc.
Discipline of Psychiatry
School of Medicine & Trinity College Institute of Neuroscience (TCIN)
Trinity College, University of Dublin
Trinity Center for Health Sciences
The Adelaide and Meath Hospital Incorporating The National Children's Hospital (AMiNCH, Tallaght, Dublin 24, Ireland
Email: harald.hampel@med.uni-muenchen.de; harald.hampel@tcd.ie
Phone (admin): +353 1-896-3706
Fax (admin): +353-1-896-1313

Abstract

Advances in our understanding of tau-mediated neurodegeneration in Alzheimer's disease (AD) are moving this disease pathway to center stage for the development of biomarkers and disease modifying drug discovery efforts. Immunoassays were developed detecting total (t-tau) and tau phosphorylated at specific epitopes (*p-tauX*) in cerebrospinal fluid (CSF), methods to analyse tau in blood are at the experimental beginning. Clinical research consistently demonstrated CSF t- and p-tau increased in AD compared to controls. Measuring these tau species proved informative for classifying AD from relevant differential diagnoses. Tau phosphorylated at threonine 231 (*p-tau231*) differentiated between AD and frontotemporal dementia, tau phosphorylated at serine 181 (*p-tau181*) enhanced classification between AD and dementia with Lewy bodies. T- and p-tau are considered "core" AD biomarkers that have been successfully validated by controlled large-scale multi-center studies. Tau biomarkers are implemented in clinical trials to reflect biological activity, mechanisms of action of compounds, support enrichment of target populations, provide endpoints for proof-of-concept and confirmatory trials on disease modification. World-wide quality control initiatives are underway to set required methodological and protocol standards. Discussions with regulatory authorities gain momentum defining the role of tau biomarkers for trial designs and how they may be further qualified for surrogate marker status.

Keywords:

Alzheimer's disease, MCI, neurodegeneration, biomarker, prediction, diagnosis, blood, CSF, tau, p-tau.

Running Title: Total Tau and P-Tau as Biomarkers in AD

Background

The “Biomarker Working Group of the National Institute on Aging” (Frank et al., 2003) made first critical suggestions regarding implementation of Alzheimer’s disease (AD) biomarker candidates for clinical trials and recommended a specified panel of “core”, feasible” biomarker candidates for validation within the worldwide Alzheimer’s Disease Neuroimaging Initiative (ADNI). Therefore, in this review and update on tau-related biomarkers we focus on established, pre-selected and validated (by international consensus processes) “core feasible” biomarkers, which have been evaluated in several studies by independent international research groups (with the greatest available level of evidence), and give a practical guide to their implementation in clinical routine and their potential role in clinical trials. Biomarkers may have a key role in AD trials in the baseline evaluation of patients eligible for the trial and as diagnostic markers to enrich the patient sample with pure AD cases (Blennow and Hampel, 2003; Carrillo et al., 2009; Hampel et al., 2008). First international multi-center reliability studies using core, feasible biomarker candidates from *cerebrospinal fluid (CSF)* have been successfully concluded (Buerger et al., 2009; Shaw et al., 2009). Among other current uses of tau-related core feasible biomarkers in such trials is for patient stratification and enrichment (Hampel and Broich, 2009).

AD is a progressive neurodegenerative disorder that causes dementia in approximately 10% of individuals older than 65 years (Blennow et al., 2006). One of its typical brain lesions is neurofibrillary tangles (NFTs) that consist of hyperphosphorylated forms of the microtubule associated protein tau that is assembled into paired helical filaments or PHFs (Grundke-Iqbal et al., 1986; Kosik et al., 1986; Lee et al., 1991; Nukina and Ihara, 1986; Wood et al., 1986). Tau expression is high in non-myelinated cortical axons, especially in the regions of the brain that are involved in memory consolidation such as the limbic cortex including the hippocampus (Trojanowski et al., 1989). Hyperphosphorylation of tau causes the protein to

detach from the microtubules, thereby destabilizing microtubules and compromising axonal transport (Bramblett et al., 1993; Ishihara et al., 1999). While tau phosphorylation promotes axonal and synaptic plasticity in the developing brain (Lovestone and Reynolds, 1997), it is pathological in the adult brain and specifically related to a group of disorders referred to as tauopathies, which includes AD and some forms of frontotemporal dementia (FTD) (Ballatore et al., 2007).

Tau proteins may be considered promising candidate biomarkers for Alzheimer-type axonal degeneration and NFT formation. However, molecular characterization of (CSF) tau presents an analytical challenge for several reasons. One is the high heterogeneity of the protein: in the adult human brain there are six different tau isoforms produced from a single gene by alternative mRNA splicing (Figure 1). This heterogeneity is compounded by extensive post-translational modifications, including phosphorylation, glycosylation and oxidation of the protein (Hernandez and Avila, 2007). Of a potential 79 serine and threonine phosphorylation sites in the longest isoform, 39 different sites have been verified (Hanger et al., 2007). It is not clear if tau phosphorylation plays a *critical* role in regulating the propensity of the protein to aggregate, nor is it known if the hyperphosphorylation of tau (triggered by increased rate of phosphorylation and/or decreased rate of dephosphorylation) and tangle formation are a cause or a consequence of AD (Ballatore et al., 2007). However, most studies, both in animal models (Gotz et al., 2001; Lewis et al., 2001) and those in longitudinally followed elderly humans (Gustafson et al., 2007; Stomrud et al., 2007), suggest that tau pathology may be downstream of the amyloidogenic cascade in AD, but it is clear that tau pathology alone causes neurodegeneration as exemplified by familial and sporadic tauopathies (Ballatore et al., 2007).

Another technical challenge is the low concentration of tau in CSF, ranging from approximately 300 ng/L in healthy individuals to 900 ng/L in AD patients (Blennow and

Hampel, 2003). Considering that this quantity is distributed over many different modified forms and six splice variants, the amount available for analysis of each molecular species falls close to the detection limit of most assays. Nevertheless, tau was recently immunoprecipitated from human CSF and characterized by mass spectrometry (Portelius et al., 2008), showing that CSF tau indeed is amenable to detailed molecular characterization. Pilot data using immunoprecipitation and western blot recently revealed a tau isoform pattern specific to progressive supranuclear palsy (Borroni et al., 2009; Borroni et al., 2008), suggesting that certain tau isoforms may reflect disease-specific neurodegenerative processes. These studies, however, warrant replication.

CSF *t-tau* and *p-tau* assays – method development and diagnostic performance

Enzyme-linked immunosorbent assay (ELISA) has been used extensively to determine CSF tau concentrations in clinical samples. Initial studies utilized antibodies insensitive to the modification status of the protein, thereby measuring the total tau (*t-tau*) protein concentration (Figure 1). The first study in which *t-tau* could be successfully analyzed in CSF was published in 1995 and showed that the *t-tau* concentration was significantly elevated in AD patients compared with controls as well as in patients with other neurodegenerative disorders (Arai et al., 1995; Blennow et al., 1995). Of the more than 50 studies conducted on AD patients and controls to date, almost all have shown an increase in *t-tau* in AD patients by approximately 300% with a sensitivity and specificity of 80-90% (Blennow and Hampel, 2003; Blennow and Zetterberg, 2009; Hampel et al., 2008; Shaw et al., 2009) (Table 1).

As described by Buerger et al. (Buerger nee Buch et al., 1999) t-tau has a statistically significantly greater discriminative power in the young (<70y/o), compared to the old-old (>70y/o) study population of healthy age-matched control subjects, AD cases and comparisons with major-depression. Therefore, the effect of age on t-tau concentrations and

proposed age-related cut-offs (Buerger nee Buch et al., 1999) should be taken into account, particularly in studies investigating t-tau as a single diagnostic marker, however, this does not seem to contribute additional diagnostic value in current multi-center validation trials using the combined set of the three core feasible CSF biomarkers.

When comparing the CSF *t-tau* concentration of AD patients with that of other neurodegenerative diseases, such as FTD or vascular dementia (VaD), the specificity drops to approximately 50-60%, rendering *t-tau* of limited use as a diagnostic marker for distinguishing AD from other dementing illnesses (Blennow and Hampel, 2003; Shaw et al., 2007). In fact, *t-tau* seems to be a general marker of damage to cortical axons, a view substantiated by results from studies of stroke, brain trauma and Creutzfeldt-Jakob disease (Blennow et al., 2005; Hesse et al., 2001; Ost et al., 2006; Otto et al., 2002; Riemenschneider et al., 2003; Zetterberg et al., 2006).

By using antibodies recognizing specific phosphorylated motifs in the tau amino acid sequence (*p-tau*), some *p-tau* isoforms (mainly *p-tau181*, *-199* and *-231*) were found that appeared to be more characteristic to AD. *P-tau231* and *p-tau181* can be used to distinguish AD from control groups and even from FTD, *dementia with Lewy bodies (DLB)*, VaD and major depression (Bian et al., 2008; Buerger et al., 2003; Grossman et al., 2005; Hampel et al., 2004a; Hampel and Teipel, 2004; Vanmechelen et al., 2000). *For review of t- and p-tau as biomarkers for FTD see Hampel and Teipel (2004).* While other *p-tau* species also have been measured such as *p-tau199*, *p-tau199+202*, as well as *p-tau396+404*, most studies have focused on *p-tau231* and *p-tau181* (for review see Blennow and Hampel, 2003).

CSF *p-tau* levels correlate with cognitive decline in patients with mild cognitive impairment (MCI) (Buerger et al., 2002a) and with neocortical NFT-pathology in AD (Buerger et al., 2006). Furthermore, both *t-tau* and *p-tau* predict rate of cognitive decline in different stages of AD (Blom et al., 2009; Buerger et al., 2005; Samgard et al., 2009) and concentration of *p-*

tau231 declined longitudinally from mild to moderate AD (Hempel et al., 2001) and correlated significantly at baseline with rate of hippocampal atrophy in mild to moderate AD, acting as an indicator of structural disease progression (Hempel et al., 2005). In a recent European multi-center-study CSF *p-tau* reliably predicted AD in subjects with MCI with high accuracy (80%) as a single biomarker in a relatively short but clinically relevant observation interval of 1.5 years (Ewers et al., 2007). Finally, numerous studies have shown that a combination of the three core feasible biomarker candidates *t-tau*, *p-tau* and the 42 amino acid form of *amyloid beta* ($A\beta_{42}$) can be used with optimized accuracy to detect incipient AD in subjects with MCI with positive and negative predictive values of >80% (Hansson et al., 2006; Herukka et al., 2005; Mattsson et al., 2009; Zetterberg et al., 2003) (Table 2), and addition of *Apolipoprotein E* (*APOE*) genotype adds further to these measures as shown in a recent study from ADNI (Shaw et al., 2009). *These results imply that CSF t-tau and p-tau are useful both as diagnostic markers for AD and as markers of disease intensity. Using the monoclonal antibodies (MAbs) AT180 and AT270 which recognize p-tau181+231, respectively, on human tau (Goedert et al., 1994), an ELISA method was developed to measure p-tau181+231 (Blennow et al., 1995). A marked increase was found in AD compared with controls and patients with other neurodegenerative disorders (Blennow et al., 1995).*

As reviewed in Brunden et al. (Brunden et al., 2009), tau is normally phosphorylated at multiple serine and threonine residues, and tau hyperphosphorylation reduces microtubule binding and may enhance aggregation. Therefore, it is possible that changes in protein kinases and/or phosphatases could enhance tau phosphorylation. A number of kinases and phosphatases have been implicated as contributing to tau hyperphosphorylation including the kinases glycogen syntase kinase 3 (GSK-3), cyclin-dependent kinase 5 (CDK-5) and microtubule-affinity regulating kinase (MARK) while protein phosphatase 2A (PP-2A)

appears to be the most plausible phosphatase involved in this abnormal posttranslational modification of tau (Matsuo et al., 1994). However, additional post-translational modifications may also contribute to tau pathology and dysfunction. For example, tau undergoes a specific type of serine/threonine O-glycosylation and these modifications can reduce the extent of tau phosphorylation. Thus, a decrease in tau O-glycosylation could result in increased hyperphosphorylation. Tau can also be tyrosine phosphorylated, sumoylated and nitrated, but it is not fully understood what effects these modifications have on tau.

T-tau and p-tau protein in clinical trials

The implementation of biochemical markers in trials on potential disease modifying compounds is still in its infancy. Phosphorylation of tau is currently hypothesized to be a downstream product of A β toxicity, and an increase in *t-* and/or *p-tau* levels in CSF is thought to reflect neuronal cell death with release of tau-related proteins into the extracellular CSF compartment. The temporal relationship between impacting A β production or aggregation and tau phosphorylation and neuronal cell death has not been defined, and the expected effect on tau of anti-A β therapies is uncertain. Tau-related therapies might inhibit new paired helical filament and tangle formation or the molecular sequence of a specific tau *hyperphosphorylation pattern in AD* without impacting existing levels of these measurable proteins in CSF. A small number of patients included in the phase IIa AN1792 active vaccination trial who had lumbar punctures and tau measures evidenced a mean significant decrease in CSF *t-tau* concentrations (Gilman et al., 2005) in vaccinated AD cases versus placebo-treated controls, however, the anti-A β passive immunization trial AAB-001 showed no statistically significant effect on CSF tau concentrations (Grundman and Black, 2008). In a recent placebo-controlled randomized single-blinded multicenter study lithium (Hampel et al., 2009b) was tested for the treatment of AD. As the primary outcome variables in vivo CSF and

plasma biomarkers were used (*t-tau*, *p-tau*, A β 42, GSK-3). Over the observation period of 10 weeks, lithium-treated patients did not show a significant change in CSF or plasma markers or neuropsychological performance when compared to placebo treated patients at two timepoints. This disappointing clinical and biomarker outcome of this proof-of-principle trial led to the final termination of another planned large *National Institutes of Health (NIH) Alzheimer's Disease Cooperative Study (ADCS)* lithium trial program in the US, therefore sacrificing a potentially effective treatment approach in the light of conceptual design problems regarding the implementation and value of biomarkers as outcomes in a particular trial. In conclusion, it may have been premature to pull lithium from trial because of a lack of change in tau biomarkers since lithium targets GSK-3 which also modulates A β . In order to reduce interpretation caveats at late stages of phase 2 and 3 trials, biomarker candidates for the various roles and functions in trials should be best co-developed with drugs as an integrated part of the trial program and best driven through preclinical to clinical stages.

Currently, the relationship between the diminished CSF A β or *t- and p-tau concentrations* and clinical outcome in clinical trials is still unknown. *The degree of pathological changes in the levels of CSF A β , t- and p-tau* has not been credibly linked to a specific degree of improvement on clinical outcomes such as the *Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog)*, the *Mini-Mental State Examination (MMSE)*, or the *Clinical Dementia Rating Scale (CDR)*. Reducing the levels of A β , *t- and p-tau* would indicate that a biological target has been hit. This might be sufficient in some cases to support advancing clinical trials (go- no go decision to proceed to next phase) of potentially disease modifying compounds. In pivotal trials they might play a supporting role to clinical outcomes. Although decreasing A β , *t- and p-tau* is the primary objective of anti-amyloid agents such as secretase inhibitors, changing CSF measures should be viewed as an intermediary goal; avoiding disease progression and irreversible neuronal death is the main objective of treatment (Table

3). The quantitative linkage between turnover, release, expression and concentration of core CSF measures and cell death is not yet established in humans, however, measures of *t-* and *p-tau* might most likely indirectly reflect neuronal cell death. More than in trials on potential disease modifiers, future prevention trials might depend entirely on biomarker outcomes. Therefore, validating core biomarkers, such as *CSF t- and p-tau* for use in primary prevention trials is clearly another key research priority.

To date, based on accumulated evidence from the rapidly progressing international controlled multi-site biomarker validation field (Ewers et al., 2007; Hansson et al., 2006; Mattsson et al., 2009; Vemuri et al., 2009a, b; Visser et al., 2009), tau-related biomarker information can be suggested for support recruitment and inclusion criteria, regarding enhanced diagnostic accuracy between AD and age-matched controls, for predicting AD in subjects with MCI, predicting time of cognitive decline and/or conversion to AD, enrichment of target populations for proof-of-concept trials with reduced sample variance, lower sample size and trial cost, may serve as a additional safety measures, and in the continued absence of a validated surrogate marker, may substantiate the claim for disease modification as secondary outcomes in addition to the demonstration of efficacy in the required clinical co-primary outcomes cognition and function.

To this end, further multi-site and assay quality control issues (see ongoing international QC-program supported by the Alzheimer Association) need to be solved and in close collaboration with regulators qualification and validation of *CSF tau* biomarkers is urgently required for effective use in trials (for conceptual review see Cummings, 2009; Frank et al., 2003; Hampel and Broich, 2009; Hampel et al., 2009a) (for detailed review and discussion of other studies with preliminary findings that assessed tau biomarkers in AD treatment trials where there was either a treatment effect that tested for the possible value of biomarkers as

indices of (a) neurodegeneration and possible reversal see Gilman et al., 2005 or where there was a (b) specific biochemical effect see Lannfelt et al., 2008).

Tau proteins in peripheral blood?

There are very little data on tau levels in peripheral bodily fluids so much more research is needed to confirm and extend these studies as it is not entirely clear how increased brain levels of tau in AD might be reflected in elevated levels of tau in blood. However, the continuous production of CSF requires that it exit the subarachnoid space surrounding the brain and presumably, as CSF is drained through the subarachnoid granulations into the venous circulation, products released from the brain into the CSF could be conveyed to blood when CSF enters the venous circulation. However, there might be peripheral sources of tau that could account for tau being present in the blood such as when minor peripheral nerve injuries occur that release tau from injured nerves into soft tissues so it can be absorbed by the local vasculature. Thus, while the initial reports on the detection of tau in blood are promising, further research is needed in order to exploit these findings for the development of a validated assay to measure tau in blood.

The CSF is in direct contact with the brain interstitial fluid and thus probably provides a more accurate measure than peripheral blood of tau metabolism. However, despite the low frequency of post-lumbar puncture headache (Blennow et al., 1993; Peskind et al., 2005), spinal tap is commonly regarded as an invasive and time-consuming procedure that is difficult to implement in large studies. Although certain positron emission tomography techniques seem to visualize NFTs directly in living patients (Small et al., 2006), the procedure is very expensive and has limited availability. Hence, determination of tau proteins in peripheral blood would be of great value, especially in large clinical studies. In spite of considerable efforts, however, reliable methods to determine tau protein levels in serum or plasma in

patients with neurodegenerative diseases are still lacking. One pilot study reported detectable tau immune reactivity in plasma without any clear relation to dementia diagnoses (Ingelson et al., 1999). Another study reported transient increases in serum tau protein levels measured by conventional ELISA in a subset of stroke patients (Bitsch et al., 2002). These data are promising but highlights the need for improved assays with lower detection limit and higher specificity in order to make them useful in the evaluation of patients with neurodegenerative diseases, in which the release of tau proteins to the peripheral circulation most likely is much lower than in acute stroke. For comprehensive review of blood-based biomarker candidates in AD see Schneider et al., 2009; and Ewers et al., 2009.

Conclusions

Based on the foregoing, it is clear that measures of *t-tau* and *p-tau* in CSF are informative for the diagnosis of AD and the distinction of AD from other neurodegenerative disorders including FTD, but it is equally evident that no single biomarker in CSF or neuroimaging modality will be sufficient for the early reliable diagnosis and prediction of AD. Indeed, biomarkers have a number of critical applications beyond diagnosis of the disease state including identifying those at greatest risk for AD among cognitively normal individuals and those with MCI as well as for use as indicators of disease progression and response to disease modifying therapies. Thus, even at this advanced stage in the quest for “ideal” core AD biomarkers, the use of algorithms that combine measures of CSF *t-tau*, *p-tau*, A β 42, the APOE genotype as well as validated and suitable neuroimaging modalities (such as MRI-based hippocampal or mediotemporal lobe indices) (Chou et al., 2009; Schuff et al., 2009; Vemuri et al., 2009a, b) appear to be the most informative for the applications discussed here. Indeed, as disease modifying therapies that target A β 42 and tau move dynamically forward in ongoing clinical trials, it will be increasingly relevant to have at hand these and other

biomarkers that will accelerate the pace of finding interventions to prevent, arrest or reverse AD (Brunden et al., 2009; Hampel et al., 2009c). For comprehensive reviews on biomarkers in AD reflecting both microvascular alternations and amyloidogenic cascade related molecular mechanisms in AD see Hampel et al. (2009c) and Ewers et al. (2009).

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Legends

Figure 1. Six different tau isoforms produced from a single gene by alternative mRNA splicing are presented. The blue, green, and yellow boxes correspond to exon 2, 3, and 10, respectively. The longest isoform is composed of 441 amino acids. In the most commonly used commercial assay for *t-tau* (INNOTEST® hTau Ag), AT120 is used as capture antibody and HT7 and BT2 as detection antibodies. This assay will detect all different tau isoforms irrespectively of differential splicing and phosphorylation status.

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Table 1. Completed p-tau and t-tau studies of AD

Design	Reference	Study Title	Study Population	Bioassay
Multicenter	(Arai et al., 1995)	Tau in cerebrospinal fluid: a potential diagnostic marker in Alzheimer's disease	AD (n=70), non-AD neurological diseases (n=96, HC (n=19)	Sandwich ELISA
Monocenter	(Arai et al., 1997)	Effect of genetic risk factors and disease progression on the cerebrospinal fluid tau levels in Alzheimer's disease	Probable AD (n=62)	Sandwich ELISA
Monocenter	(Shoji et al., 1998)	Combination assay of CSF tau, A beta 1-40 and A beta 1-42(43) as a biochemical marker of Alzheimer's disease	AD (n=55), Non-AD dementias (n=23), OND (n=45), HC (n=34)	ELISA (A/S Nunc, Kamstrup, Denmark)
Multicenter	(Andreasen et al., 1999a)	Sensitivity, specificity, and stability of CSF-tau in AD in a community-based patient sample	probable AD (n=274), Possible AD (n=133), Depression (n=28), HC (n=65)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Buerger nee Buch et al., 1999)	Cerebrospinal fluid tau protein shows a better discrimination in young old (<70 years) than in old old patients with Alzheimer's disease compared with controls	AD (n=38), Major depression (n=19), HC (n=28)	ELISA
Multicenter	(Hulstaert et al., 1999)	Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF	AD (n=150), Non-AD dementias (n=79), OND (n=84), HC (n=100)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Multicenter	(Kohnken et al., 2000)	Detection of tau phosphorylated at threonine 231 in cerebrospinal fluid of Alzheimer's disease patients	AD (n=27), Non-AD (n=31)	Sandwich ELISA

Monocenter	(Sjogren et al., 2000)	CSF levels of tau, beta-amyloid(1-42) and GAP-43 in frontotemporal dementia, other types of dementia and normal aging	AD (n=60), FTD (n=17), subcortical white-matter dementia (n=24), Parkinson's disease (n=23), Dysthymia (n=19), HC (n=32)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Multicenter	(Andreasen et al., 2001)	Evaluation of CSF-tau and CSF-Abeta42 as diagnostic markers for Alzheimer disease in clinical practice	Probable AD (n=105), Possible AD (n=58), VaD (n=23), MCI (n=20), DLB (n=9), OND (n=3), Other psychiatric disorders (n=5), HC (n=18)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Multicenter	(Itoh et al., 2001)	Large-scale, multicenter study of cerebrospinal fluid tau protein phosphorylated at serine 199 for the antemortem diagnosis of Alzheimer's disease	AD (n=236), Non-AD-demented and nondemented diseases (n=239), HC (n=95)	Sandwich ELISA (HT-7; Innogenetics and Anti-PS199; Mitsubishi Chemical Corporation)
Multicenter	(Parnetti et al., 2001)	CSF phosphorylated tau is a possible marker for discriminating Alzheimer's disease from dementia with Lewy bodies. Phospho-Tau International Study Group	AD (n=80), DLB (n=43), HC (n=40)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Sjogren et al., 2001a)	The cerebrospinal fluid levels of tau, growth-associated protein-43 and soluble amyloid precursor protein correlate in Alzheimer's disease, reflecting a common pathophysiological process	AD (n=47), FTD (n=14), VaD (n=17), HC (n=12)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Sjogren et al., 2001b)	Both total and phosphorylated tau are increased in Alzheimer's disease	Probable AD (n=41), Possible AD (n=19), FTD (n=18), SAE (n=17), Parkinson's Disease (n=15), HC (n=17)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)

Multicenter	(Buerger et al., 2002b)	Differential diagnosis of Alzheimer disease with cerebrospinal fluid levels of tau protein phosphorylated at threonine 231	Probable AD (n=82), FTD (n=26), LBD (n=17), VaD (n=20), OND (n=26), HC (n=21)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Nagga et al., 2002)	Cerebrospinal fluid phospho-tau, total tau and beta-amyloid(1-42) in the differentiation between Alzheimer's disease and vascular dementia	Possible AD (n=23), Probable AD (n=50), AD with relevant cerebrovascular disease (n=14), Possible VaD (n=39), Probable VaD (n=36), Cognitively impaired (n=13), HC (n=27)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Buerger et al., 2003)	Differentiation of geriatric major depression from Alzheimer's disease with CSF tau protein phosphorylated at threonine 231	Depression (n=34), Probable AD (n=64), Possible AD (n=17), HC (n=21)	ELISA (Molecular Geriatrics Corporation, Vernon Hills, Ill.)
Multicenter	(Gomez-Tortosa et al., 2003)	Cerebrospinal fluid markers in dementia with lewy bodies compared with Alzheimer disease	Probable AD without parkinsonism (n=33), DLB (n=25), HC (n=46)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Maddalena et al., 2003)	Biochemical diagnosis of Alzheimer disease by measuring the cerebrospinal fluid ratio of phosphorylated tau protein to beta-amyloid peptide42	Probable AD (n=51), Non-AD dementias (n=30), OND (n=19) HC (n=31)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Schonknecht et al., 2003)	Cerebrospinal fluid tau levels in Alzheimer's disease are elevated when compared with vascular dementia but do not correlate with measures of cerebral atrophy	AD (n=88), VaD (n=23), Major Depression (n=25), HC (n=17)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Sunderland et al., 2003)	Decreased beta-amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease	AD (n=131), HC (n=72)	Sandwich ELISA

Multicenter	(Hampel et al., 2004a)	Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: a comparative cerebrospinal fluid study	Probable AD (n=108), FTD (n=24), DLB (n=22), VaD (n=7), HC (n=45)	ELISA
Monocenter	(Lewczuk et al., 2004)	Neurochemical diagnosis of Alzheimer's dementia by CSF Abeta42, Abeta42/Abeta40 ratio and total tau	AD (n=22), Non-AD dementias (n=11), HC (n=35)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Schoonenboom et al., 2004)	Amyloid beta(1-42) and phosphorylated tau in CSF as markers for early-onset Alzheimer disease	EAD (n=47), FTD (n=28), HC (n=21)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Jia et al., 2005)	Cerebrospinal fluid tau, Abeta1-42 and inflammatory cytokines in patients with Alzheimer's disease and vascular dementia	Probable AD (n=39), Probable VaD (n=38), HC (n=35)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Ibach et al., 2006)	Cerebrospinal fluid tau and beta-amyloid in Alzheimer patients, disease controls and an age-matched random sample	AD (n=76), Non-AD dementias (n=48), Mental disorders (n=26), HC (n=39)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Monocenter	(Kapaki et al., 2007)	Cerebrospinal fluid tau, phospho-tau 181 and beta-amyloid1-42 in idiopathic normal pressure hydrocephalus: a discrimination from Alzheimer's disease	Probable AD (n=67), iNPH (N=18), HC (n=72)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Multicenter	(Blennow et al., 2007)	Longitudinal stability of CSF biomarkers in Alzheimer's disease	AD (n=59)	Sandwich ELISA
Monocenter	(Boban et al., 2008)	Cerebrospinal fluid markers in differential diagnosis of Alzheimer's disease and vascular dementia	Probable AD (n=12), VaD (n=9), HC (n=12)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Multicenter	(Haense et al., 2008)	CSF total and phosphorylated tau protein, regional glucose metabolism and dementia severity in Alzheimer's disease	AD (n=38)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
Multicenter	(Shaw et al., 2009)	Cerebrospinal fluid biomarker signature in Alzheimer's Disease Neuroimaging Initiative Subjects	Probable AD (n=100), MCI (n=196), HC (114); Autopsied AD (n=56), HC (n=52)	Multiplex xMAP Luminex (INNO-BIA AlzBio3; Innogenetics, Gent, Belgium)

Monocenter	(Welge et al., 2009)	Combined CSF tau, p-tau181 and amyloid-beta 38/40/42 for diagnosing Alzheimer's disease	AD (n=44), Depressive cognitive complainers (n=25), Non-AD dementias (n=87)	Sandwich ELISA (Innotest; Innogenetics, Gent, Belgium)
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Abbreviations: AD = Alzheimer's disease; MCI = mild cognitive impairment; HC = healthy controls; OND = other neurological diseases; FTD = frontotemporal dementia; VaD = vascular dementia; DLB = dementia with Lewy bodies; SAE = subcortical arteriosclerotic encephalopathy; iNPH = idiopathic normal pressure hydrocephalus; EAD = early-onset Alzheimer disease

Table 2. Diagnostic performance of CSF tau and amyloid related biomarkers in the MCI stage of Alzheimer's disease

Reference	Year	Design	Numbers included	AD-associated change	Diagnostic performance
(Andreasen et al., 1999b)	1999	Mono-center longitudinal MCI-control study	16 MCI-AD patients and 15 age-matched controls	Low CSF A β 42, high CSF t-tau	Sensitivity 88%, specificity 80%
(Riemenschneider et al., 2002)	2002	Mono-center longitudinal MCI study	28 MCI patients, 10 of whom developed AD	Low CSF A β 42, high CSF t-tau	Sensitivity 90%, specificity 90%
(Zetterberg et al., 2003)	2003	Mono-center longitudinal MCI study	53 MCI patients, 22 of whom developed AD	Low CSF A β 42, high CSF t-tau, high CSF p-tau181	Sensitivity 68%, specificity 97%, PPV 94%, NPV 81%
(Hampel et al., 2004b)	2004	Mono-center longitudinal MCI-AD-control study	52 MCI patients, 93 AD patients and 10 controls	Low CSF A β 42, high CSF t-tau	Sensitivity 59-83%, specificity 90-100%
(Herukka et al., 2005)	2005	Mono-center longitudinal MCI-control study	78 MCI patients, 23 of whom developed AD, 46 controls	Low CSF A β 42, high CSF t-tau, high CSF p-tau181	Sensitivity 91%, specificity 56%
(Hansson et al., 2006)	2006	Mono-center longitudinal MCI study	137 MCI patients, 57 of whom developed AD	Low CSF A β 42, high CSF t-tau, high CSF p-tau181	Sensitivity 95%, specificity 83%, PPV 81%, NPV 96%
(Herukka et al., 2007)	2007	Mono-center longitudinal MCI study	79 MCI patients, 33 of whom developed AD, 60 controls	Low CSF A β 42, high CSF t-tau, high CSF p-tau181	Low levels of CSF A β 42 predicted progression to AD
(Hansson et al., 2007)	2007	Mono-center longitudinal MCI study	137 MCI patients, 57 of whom developed AD	Low A β 42/A β 40 ratio	Sensitivity 87%, specificity 78%
(Bouwman et al., 2007)	2007	Mono-center longitudinal MCI study	59 MCI patients, 30 of whom developed AD	Low CSF A β 42, high CSF t-tau	Patients with abnormal values at baseline had higher risk of developing AD. Sensitivity and specificity missing.
(Brys et al., 2007)	2007	Mono-center longitudinal MCI-control study	65 MCI patients, 22 of whom developed AD, 21 controls	Low CSF A β 42, low A β 42/A β 40 ratio, high CSF t-tau, high CSF p-tau231	Sensitivity 68-86%, specificity 60-91%

(Mattsson et al., 2009)	2009	Multi-center longitudinal MCI study	529 AD patients, 304 controls and 751 MCI patients, 271 of whom developed AD	Low CSF A β 42, high CSF t-tau, high CSF p-tau181	Sensitivity 83%, specificity 72%, PPV 62%, NPV 88%
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Abbreviations: AD = Alzheimer's disease; MCI = mild cognitive impairment; PPV = positive predictive value; NPV = negative predictive value.

Table 3. Potential disease modifying agents in development targeting p-tau and t-tau

Sponsor	Study	Follow-up	Primary outcomes	Assessment	Other outcomes
Lundbeck (Phase IV)	Memantine and Changes of Biological Markers and Brain PET Imaging in Alzheimer's Disease	24 weeks	Biological markers of CSF, 18[F]-FDG-PET of brain, cognitive function	Measurement of biological markers, PET-Imaging, cognitive and functional measures	Behaviour and activities of daily living, short term memory
North Shore Long Island Jewish Health System, Forest Laboratories (Phase IV)	Magnetic Resonance Spectroscopy Study of Memantine in Alzheimer's Disease	48 weeks	Relative levels of myo-inositol (mI), N-acetylaspartate (NAA), total creatine (Cr), and choline (Cho) by single voxel 1H MRS	MMSE, ADAS-cog, ADCS-ADL, NPI, Trails A and B, Animal Naming and FAS verbal fluency, logical memory, visual reproduction, digit span, clock-drawing, presidents, Rey-Osterreith figure copying and recall, judgment of line orientation, semantic distance	CSF beta-amyloid, tau, and phosphorylated tau levels
Bristol-Myers Squibb (Phase II)	A Multicenter, Double Blind, Placebo-Controlled, Safety and Tolerability Study of BMS-708163 in Patients With Prodromal Alzheimer's Disease	52 weeks	Adverse events	Measurement of biological markers, MRI, cognitive and functional measures	To assess the predictive value and longitudinal behavior of CSF biomarkers (A β 40, A β 42, total Tau, phosphorylated Tau) and volumetric MRI, Assess drug effects on progression to dementia, Global clinical impression as assessed with the Clinical Dementia Rating-Sum of Boxes (CDR-SB), an instrument designed to measure the severity of cognitive symptoms in daily life

Hamilton Health Sciences, The Physicians' Services Incorporated Foundation	Effects of Doxycycline and Rifampicin on Biomarkers of Alzheimer's Disease in the Cerebrospinal Fluid		Clinical Dementia Rating scale, Standardized Alzheimer's disease Assessment Scale - cognitive subscale	Biochemical markers of A β (1-40) and A β (1-42), P-tau and T-tau, matrix metalloproteinases (MMP-2, MMP-9), pro-inflammatory cytokines (IL-1beta, TNF-alpha), and anti-inflammatory cytokines (IL-4 and IL-10)	
National Institute on Aging (NIA) (Phase II)	Predictors of Cognitive Decline in Normal Aging		The relationship between changes in brain volume, CSF levels, and memory performance	Neuropsychological data, magnetic resonance imaging (MRI), and cerebrospinal fluid (CSF)	
Eli Lilly and Company (Phase III)	Effects of LY450139, on the Progression of Alzheimer's Disease as Compared With Placebo (IDENTITY-2)	21 months	Safety and tolerability; Rate of cognitive and functional decline in AD over time	Brain-scanning techniques (FDG-PET, vMRI, AV-45-PET); Biochemical measures; ADAS-Cog, CDR, MMSE; NPI; Qol-AD; RUD-Lite; EQ-5D Proxy	Determine levels of peptides in blood and spinal fluid that might relate to Alzheimer's disease; Evaluate changes in thinking and memory; Evaluate changes in daily living activities; Determine levels of study drug in blood and CSF
Eli Lilly and Company (Phase III)	Effect of LY450139 on the Long Term Progression of Alzheimer's Disease	21 months	Rate of cognitive and functional decline in Alzheimer's disease over time	Brain-scanning techniques (FDG-PET, vMRI, AV-45-PET); Biochemical measures; ADAS-Cog, CDR, MMSE; NPI; Qol-AD; RUD-Lite; EQ-5D Proxy	Effect of long term treatment, Energy usage (metabolism) seen on FDG-PET, Brain size (volume) seen with AD on vMRI, Amount of brain amyloid plaque seen in AD on AV-45-PET, Tau, Safety, Quality of life
National Institute on Aging (NIA)	Amyloid Plaque and Tangle Imaging in Aging and Dementia	2 years		Clinical and neuropsychological assessments, structural MRI and/or PET scans	

Pfizer (Phase II)	Multiple IV Dose Study Of PF-04360365 In Patients With Mild To Moderate Alzheimer's Disease	24 months	Safety and tolerability	Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog); Disability Assessment for Dementia (DAD); plasma/CSF Abeta; CSF tau and phosphotau; CSF protein, RBCs, WBCs and glucose; Immunogenicity (anti-drug antibodies)	Pharmacokinetics
Octapharma (Phase II)	Study of Octagam 10% on the Treatment of Mild to Moderate Alzheimer's Patients	24 weeks	Change in amyloid beta peptide concentration of the blood plasma from immediately prior to the last IVIG infusion calculated over 2 or 4 weeks	Measurement of biological markers, MRI and PET-Imaging, cognitive and functional measures	Changes from baseline in MRI, PET scan, neuropsychometric testing results, autoantibody concentrations in the blood and in CSF, CSF tau and pTau concentrations
Grifols Biologicals Inc. (Phase II)	Efficacy and Safety of Plasma Exchange With 5% Albutein in Beta-Amyloid Peptide Clearance in Cerebrospinal Fluid	1 year	To determine whether plasma exchange with 5% human albumin is able to modify the concentration of beta-amyloid peptide in cerebrospinal fluid (CSF) in the treatment group of patients with Alzheimer's disease	Measurement of biological markers	To assess the variations in other parameters (tau protein, beta-secretase, gamma-secretase, nicastrin, etc.), To determine whether plasma exchange with 5% human albumin is able to modify the plasma concentration of beta-amyloid

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Figure 1

