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Committee for Medicinal Products for Human Use (CHMP)

Qualification opinion of Alzheimer's disease novel methodologies/biomarkers for the use of CSF AB 1-42 and t-tau signature and/or PET-amyloid imaging (positive/ negative) as a biomarkers for enrichment, for use in regulatory clinical trials – in mild and moderate of Alzheimer's

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Comments should be provided using this template. The completed comments form should be sent to Qualification@ema.europa.eu

Keywords	*Qualification opinion, PET/CSF Biomarker, Alzheimer's disease*

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Background information as submitted by the applicant

In follow-up to the positive Qualification Opinion on the use of cerebrospinal fluid (CSF) biomarkers in predementia AD adopted on 14-Apr- 2011 (EMA/CHMP/SAWP/102001/2011), BMS is requesting an additional qualification advice and ultimately, a qualification opinion, on an additional biomarker [amyloid positron emission tomography (PET) imaging)] for patient selection in both predementia and mild to moderately severe AD clinical studies, and to expand the positive Qualification Opinion on CSF biomarkers in predementia AD for application in clinical studies of amyloid-targeted therapies in mild to moderately severe AD.

RATIONALE

AD is a serious neurodegenerative disease that begins with memory loss and progresses to severe impairment of activities of daily living, leading to death approximately 8 years on average from time of diagnosis of dementia (Brookmeyer 2002). The cause of AD is currently unknown but pathologic, genetic, and nonclinical evidence suggests that amyloid beta (Aβ) peptides and specifically, the highly amyloidogenic isoform Aβ42 (with 42 residues), are involved in the pathogenesis of AD (Artavanis-Tsakonas 1999).

Currently, clinical diagnosis of AD is probabilistic. That is, it is estimated that approximately 15% to 20% (Rinne & Någren, 2010) of patients currently enrolled in clinical trials evaluating treatments for mild to moderate AD do not have the underlying pathology, and the actual number in the clinical setting is up to 25% (Klatka 1996, Pearl 1997, Rasmusson 1996, Schneider 2010). A definitive diagnosis of AD for a demented patient requires a histopathological evaluation of the number and localization of neuritic plaques and neurofibrillary tangles upon autopsy (Consensus 1997). The most recent publication of the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association [NINCDS-ADRDA] criteria (McKhann 2011) includes the category of 'pathophysiologically proved AD dementia' that is consistent with the previous consensus. Plaques primarily consist of Aβ that are formed by a sequential proteolytic cleavage of the amyloid precursor protein (APP) first by APP-cleaving enzyme (BACE) to generate the NH-terminal domain and then by gamma (γ)-secretase to form the COOH terminal domain. Increase in the toxic species of Aβ is considered to be an early event in the disease course. Patients with mild cognitive impairment, who do not meet the criteria for dementia of AD, can already show abnormal (low) levels of Aβ in the cerebrospinal fluid (CSF) (Fagan 2007, Hansson 2006). Aβ40 is the most abundant form of Aβ synthesized (80% to 90%), while Aβ42 is most tightly linked with AD pathogenesis. In particular, mutations that lead to rare, familial forms of AD implicate Aβ42 aggregates as the primary toxic species (Wolfe 2004); current evidence suggests that oligomeric, protofibrillar and intracellular Aβ42 are essential for initiation and progression of AD (Caughey 2003, Cleary 2005, Wilson 2003). Based on the amyloid hypothesis, inhibitors of the enzymes that form Aβ42, in particular BACE and γ-Secretase, have the potential to function as disease-modifying therapeutics for AD.

Current approved treatments are for patients who have been clinically diagnosed with mild to severe Alzheimer's dementia, and provide only modest and transient benefits. Thus, there is great interest in studying AD earlier in the disease process, and investigating whether the use of potentially disease-

modifying agents can alter the long-term course of the illness and prevent the neurodegenerative cascade associated with the disease.

Pathologic evidence obtained at post-mortem of patients with dementia of the Alzheimer's type shows several characteristic neuropathologies, including extracellular plaques, intracellular tangles, and neurodegeneration (Consensus 1997, Grundman 2004, Walsh 2004). Plaques consist primarily of amyloidogenic Aβ peptides that are formed by a stepwise proteolytic cleavage of APP, ending with cleavage by the γ-secretase complex. Aβ40 is the most abundant form of Aβ synthesized (80% to 90%), while Aβ42 is most tightly linked with AD pathogenesis. Although the most prominent form of Aβ in an AD brain is fibrillar Aβ42 accumulated in plaques, current evidence suggests that soluble Aβ, likely oligomeric Aβ42, contributes to cognitive deficits (Caughey 2003, Cleary 2005). Genetic evidence shows that mutations in the APP and components of the γ-secretase complex (the presenilin [PS]-1 and PS-2 genes) lead to rare, familial forms of AD that implicate Aβ42 aggregates as the primary toxic species (Selkoe 2001).

Nonclinical models show that APP over expression leads to plaques and cognitive deficits due to Aβ overproduction in mice (Kobayashi 2005). Studies in both transgenic and wild type animal models demonstrate that γ-secretase inhibitors can reduce brain Aβ levels (Barten 2005, Best 2005, Lanz 2006). The amount of Aβ-reduction needed for clinical benefit in AD is presently unknown. Modest decreases (15% to 30%) in Aβ synthesis by γ-secretase inhibition reversed cognitive deficits and prevented synaptic deficits in transgenic mice models (Comery 2005).

The collective evidence suggests that reducing total Aβ synthesis by inhibiting the γ-secretase complex, therefore reducing Aβ42 levels, might have the potential to intervene in the disease process of AD and thus slow down or delay the progression of the disease.

In addition to amyloid plaque deposition, the formation of neurofibrillary tangles is a central defining feature of AD pathology (Consensus 1997, Grundman 2004, Walsh 2004). Neurofibrillary tangles are intraneuronal aggregates composed of hyperphosphorylated tau protein. Tau is a microtubule-associated protein found primarily in axons. In AD, tau hyperphosphorylation has been hypothesized to elicit tau dissociation from microtubules leading to structural axonal instability and the formation of paired helical filaments, the major component of neurofibrillary tangles (Meraz-Rios 2010). Although the science around soluble tau remains incomplete, soluble forms of tau are detectable in CSF and increased levels of both tau and phosphorylated tau (p-tau) occur in AD. Interestingly, injury to neurons resulting from stroke, head injury, Creutzfedlt-Jakob (CJD) disease and other types of infectious or neurodegenerative insult will also produce increases in CSF tau (Bahl 2009, Hesse 2001, Zemlan 1999). Thus, elevated tau is not specific to AD. The lack of specificity of total tau (t-tau) is offset by the fact that within the heterogeneous class of dementia, elevations in phosphorylated tau is relatively unique to dementia of the AD type (Le Bastard 2010). Natural history studies have shown that during AD disease progression, increased brain amyloid burden (as evidenced by amyloid PET imaging or low CSF Aβ42 levels) can take place well before clinical symptoms (Aisen 2010). The appearance of elevated CSF tau, on the other hand, is often associated with clinical symptoms and dementia (Aisen 2010). As with p-tau, the combinatorial use of increased CSF tau and low CSF Aβ42

improves specificity for AD and is also useful in identifying cognitively impaired subjects at imminent risk of progression to dementia (Blennow 2010). The coincident pathological appearance of both tau aggregates and amyloid pathology in AD has lead to multiple hypotheses that mechanistically link the two pathologies. One prevailing hypothesis poses amyloid pathology as the major driver of tau hyperphosphorylation, yet another poses that tau dendritic signaling mediates amyloid pathology and a third argues for synergistic concordance of the contributing pathologies (Ittner 2011). If amyloid and tau are indeed mechanistically linked, then it is plausible that an amyloid-modulating therapy could impact tau pathology. What remains clear is that 1) amyloid plaque and neurofibrillary tangle pathology remains a defining feature of AD, and 2) in patients at risk of progressing to AD, a pathological signature for CSF Aβ42 and tau can be detected. Recent evidence is emerging showing that in patients with a CSF AD pathological signature, increased brain amyloid burden is highly concurrent (Fagan 2006, Jagust 2010) suggesting both CSF and amyloid PET imaging are useful biomarker tools for AD clinical trials.

Questions and evidence to support proposed biomarkers

Background

Recently, the EMA published (16-May-2011) a positive qualification opinion enabling the use of low CSF Aβ42 and high CSF tau as enrichment tools for clinical studies of amyloid targeted therapies in predementia AD (EMA/CHMP/SAWP/102001/2011). One objective of this follow-up QP is to seek a broadened use of CSF biomarkers as tools to enrich clinical trials in AD dementia patients (mild to moderately severe) with neuropathology most likely to benefit from treatment with amyloid modulating therapies. The other objective is to support qualification of PET-amyloid imaging as a second biomarker to be used as an enrichment tool in clinical studies of amyloid targeted therapies in patients with predementia AD and in patients with mild to moderately severe AD.

Methods

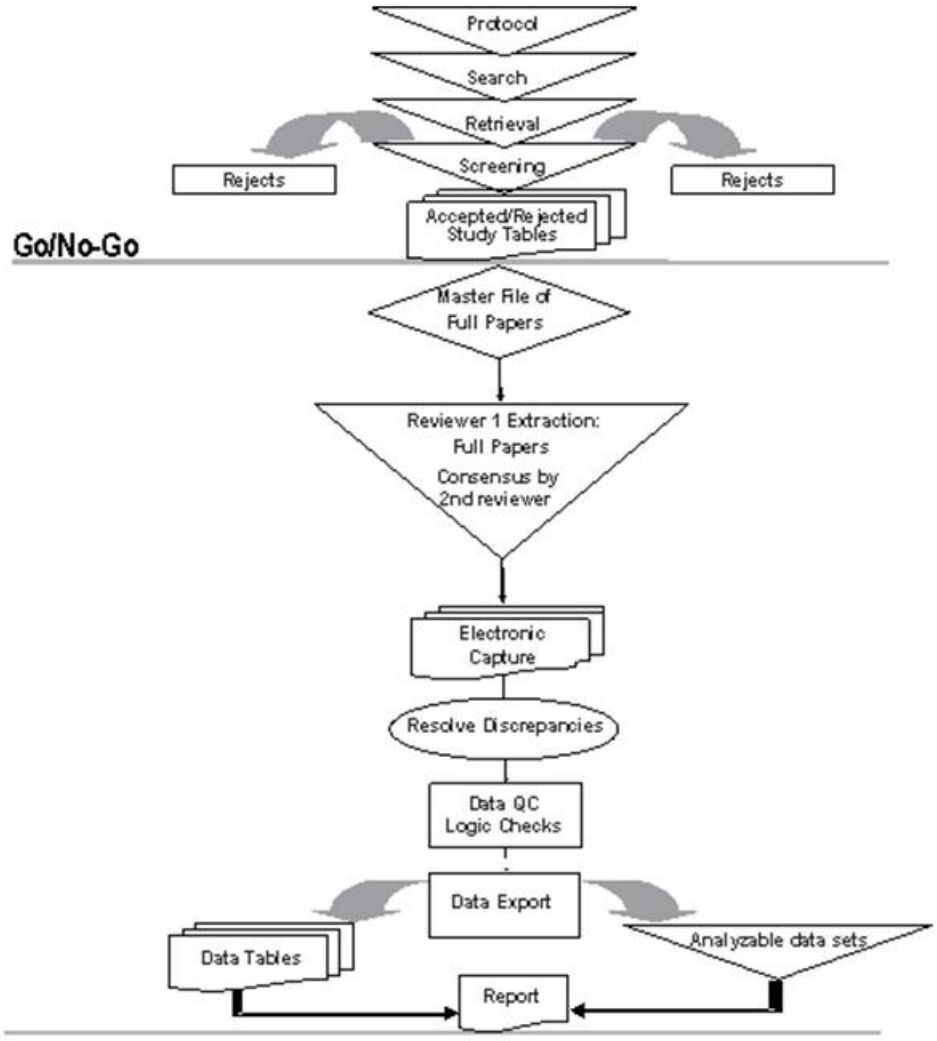
The prior systematic literature review BMS submitted in support of the positive qualification opinion for the use of CSF Aβ42 and total tau in predementia AD was expanded to include articles that examined the levels of CSF biomarkers in autopsy confirmed AD and non AD dementia subjects. In addition, historical meta-analyses describing low CSF Aβ42 and elevated CSF tau in AD subjects were summarized and updated through April 2011.

A new systematic literature search on all amyloid PET imaging radiotracers (e.g. amyloid imaging and binding of the Florbetapir AV-45 fluorinated PET ligand, Pittsburgh Compound B: PiB, Bayer Florbetaben ligand) was undertaken as a means to estimate potential positive/negative predictive value, hazard/odds ratios, or sensitivity/specificity of amyloid PET to identify AD neuropathology at both the predementia stage, as well as in patients with mild to moderately severe dementia. Further, as CSF biomarkers have been qualified for clinical trial enrichment in predementia AD, data demonstrating correlation between amyloid PET and CSF biomarkers is provided adding strong supportive evidence that amyloid PET can also be used as an equally valid measure for identifying AD

neuropathology for clinical trial enrichment. The review also sought to establish the correlation of performance of amyloid PET with CSF biomarkers on AD diagnosis in the mild, moderate, and severe stages of dementia. Finally, as this QP seeks to broaden the scope of qualification to include both predementia and dementia stages of AD, the predictive value of amyloid PET imaging in AD dementia was examined by correlating amyloid PET imaging results with autopsy results, currently the only means for a definitive AD diagnosis.

Procedures for this systematic review followed established methods used in the evolving science of systematic review research (Cook et. al, 1997; Alderson et al., 2003). A flow diagram outlining the systematic review process is included below in Figure 1.

Figure 1: Flow Diagram of Systematic Review



Sources of literature and published data that have used amyloid-related biomarker criteria included randomized clinical trials, non-interventional observational studies, open-label trials, retrospective database studies, and consortia and investigator driven work such as the Alzheimer's Disease

Neuroimaging (ADNI), Development of Screening Guidelines and Diagnostic Criteria for Predementia Alzheimer's Disease (DESCRIPA), and the VU Medical Center cohort (VUMC).

The source of data was limited to studies published in English in the last 10 years (since 2001). The comprehensive literature search included all articles published between January 2001 and March 15, 2011, and included both electronic and manual components. The electronic search was performed in MEDLINE (via PubMed) and EMBASE.

In addition to searching MEDLINE and EMBASE, a manual search of the reference lists of all accepted studies, as well as the reference lists of recent reviews and meta-analyses, supplemented the above electronic searches to ensure optimal literature identification and retrieval.

Study eligibility was determined by 2 reviewers, who used abstracts of publications and full papers when necessary. Two levels of study screening were performed. Level I screening was performed on abstracts downloaded from the literature searches noted above. At Level I screening, any study with a definite exclusion criterion was rejected.

Heterogeneity was explored once the final list of included studies was prepared. The comparability of patient populations, length of treatment, and baseline patient characteristics was assessed.

Results of systematic review for amyloid PET imaging

The literature search through MEDLINE, EMBASE, and manual bibliography checks yielded 1322 citations, not including duplicate citations from the various sources. Of these, 1196 titles and abstracts were rejected during abstract (Level I) screening. Corresponding full papers of the remaining 126 citations were retrieved for further, in-depth review and underwent Level II screening. Of the full papers retrieved, 109 were rejected at Level II screening or during data extraction, leaving a total of 17 relevant studies for this review. The reasons for rejection were: no CSF, autopsy-only data, or no progression to AD (k=41); no PET data (k=19); FDG-PET-only data (k=16); abstract, review, case report, meta-analysis, etc. (k=17); not MCI or AD subjects (k=11); and fewer than 10 subjects in the study (k=5). The final dataset of accepted studies consisted of 17 studies.

Cohort 1: 6 Studies

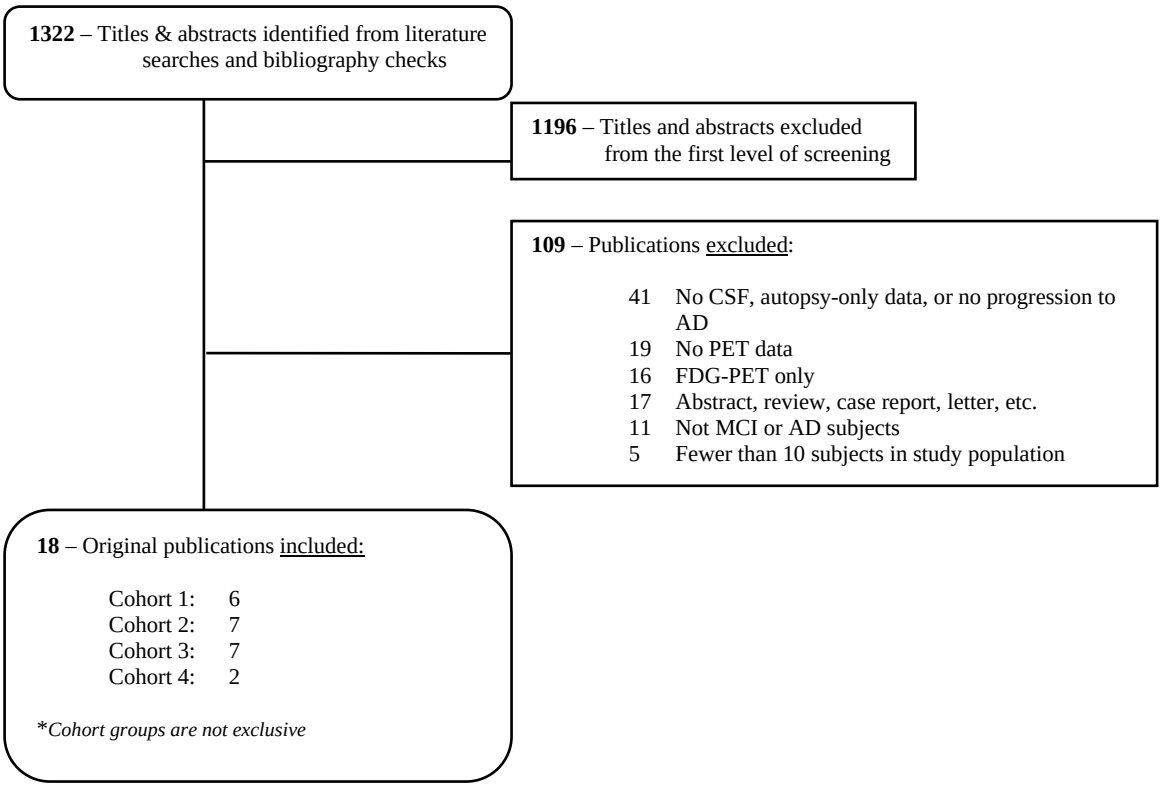
Cohort 2: 7 Studies

Cohort 3: 7 Studies

Cohort 4: 2 Studies

Several studies reported information on subjects in more than one cohort, and therefore data were extracted in their respective tables. A summary of the report extraction flow is included in Figure 2 below.

Figure 2: Summary of Study Evaluation and Inclusion



Question 1

Mild to moderate AD

CSF Biomarkers: In clinical studies of amyloid targeted therapies in mild to moderate dementia of the Alzheimer's type, are there sufficient data to support the use of CSF Aβ42 and total-tau concentrations as biomarkers for enrichment, by excluding patients who are unlikely to have underlying AD pathology?

Applicant's position

While the clinical diagnosis of AD is believed to be adequate for determining treatment initiation with currently available symptomatic therapies, significant rates (approximately 25% on average) of misdiagnosis have been revealed upon autopsy-based confirmation of the disease (Klatka 1996, Pearl 1997, Rasmusson 1996, Chui et al., 2003, Schneider 2010). A key step in the diagnostic process is to exclude other causes of dementia, relying typically on clinical assessment and MRI. However, clinical and MRI assessments cannot identify underlying neurofibrillary or amyloid pathology known to be hallmarks of AD. Inclusion of AD patients that do not exhibit evidence of AD pathology can confound results for studies of targeted amyloid modulating therapies. Furthermore, patients with underlying AD pathology (i.e., amyloid plaques or an abnormal CSF signature) are most likely to benefit from treatment with drugs targeting the pathophysiology of AD and would therefore have a more favorable benefit/risk profile. Noting limitations concerning the accuracy of clinical diagnosis for AD, the CHMP Guideline on Medicinal Products for the Treatment of Alzheimer's Disease and other Dementias

(CPMP/EWP/553/95 Rev. 1, dated 24-Jul- 2008) commented how improved sensitivity and specificity of diagnosis within clinical trials can benefit from emerging technical methods, specifically PET and lumbar punctures to assess CSF profile. To date, pivotal trials in populations with mild-to-moderate dementia have not made use of this guidance. With the advent of additional supportive data in the literature, the sponsor agrees that use of amyloid PET or CSF biomarkers, cited by the CHMP Guideline, can improve identification of the population of mild-to-moderately severe AD that truly suffer from an underlying AD pathology.

Use of such methods is also supported by current draft recommendations updating diagnostic criteria for dementia of the Alzheimer's type, from three independent working groups: National Institutes of Health and Alzheimer's Association Working Group (NINCDS-AA); American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-V) Neurocognitive Working Group; and International Working Group for New Research Criteria for the Diagnosis of AD. While these criteria have only recently been published and therefore are not fully adopted, a role for biomarker demonstration of underlying AD pathology is included in all of them, ranging from core diagnostic criteria (International Working Group: Dubois 2010) to enhancing confidence in a diagnosis of probable AD dementia for predominantly research purposes (NINCDS-AA: McKhann 2011) to use in atypical presentations without prominent memory impairment (DSM-V: Jeste 2010).

Recently the CHMP issued a positive opinion to qualify the use of a CSF biomarker signature (based on low Aβ42 and high t-tau) to identify patients with MCI who are at greater risk to evolve into AD dementia. The scientific basis for this opinion is that a CSF signature indicates an underlying pathology of AD. Hence, it would be expected that a CSF biomarker signature would also be useful for increasing confidence that patients with clinically diagnosed dementia of the Alzheimer's type truly have an underlying AD pathology. Support for the use of CSF biomarkers in enhancing the accuracy of a diagnosis of AD-dementia is supported by (1) CSF biomarker data in autopsy confirmed subjects and (2) data comparing CSF biomarker levels in AD dementia to controls or to non-AD dementia.

(1) Autopsy confirmed supportive data

There is a significant percentage of patients diagnosed with AD who actually have a non-AD dementia. Studies examining the accuracy of clinical diagnosis using either NINCDS-ADRDA or DSM III clinical criteria versus autopsy confirmation report diagnostic sensitivity and specificity ranging from 53-90% and 56-80%, respectively (Chui et al., 2003, Table 1). In general, sensitivity is relatively high and specificity is low suggesting that a diagnosis of AD is rarely missed using standard criteria. In current BMS protocols in mild-to-moderate AD, inclusion is based on meeting criteria for clinical diagnosis of probable AD based on NINCDS-ADRDA and DSM-IV-TR criteria. Given the low specificity of current clinical diagnosis, it is highly likely a significant percentage of enrolled dementia patients would have non-AD dementia. In order to enrich for dementia of the Alzheimer's type, evidence of AD pathology determined by CSF biomarkers will be utilized as an exclusion criterion.

Table 1: Accuracy of Clinical Diagnosis vs Autopsy Confirmations

Criteria	No. of studies	PPV: mean (sd)	NPV: mean (sd)	Sensitivity: mean (sd)	Specificity: mean (sd)	LR+: mean (sd)
NINCDS/ADRDA (probable or possible AD)	10	0.84 (0.11)	0.66 (0.16)	0.90 (0.07)	0.56 (0.22)	2.9 (2.4)
NINCDS/ADRDA (probable AD)	5	0.91 (0.08)	0.45 (0.12)	0.65 (0.15)	0.76 (0.20)	4.4 (5.0)*
DSM-III (dementia of the Alzheimer type)	3	0.86 (0.09)	0.58 (0.16)	0.53 (0.21)	0.88 (0.07)	4.8 (1.0)

AD, Alzheimer's disease; NPV, negative predictive value; LR+, positive likelihood ratio; PPV, positive predictive value; sd, standard deviation.
*Only able to calculate LR for four of the five studies.

Source: Chui et al., 2003

Use of CSF biomarkers can improve the odds that only clinically-diagnosed AD patients with evidence of AD pathology are treated with amyloid modulating treatments. In the context of diagnostic terms, these probabilities are often expressed as positive and negative predictive values and positive and negative likelihood ratios.

Positive predictive values (PPV) provide information on the probability that a subject may in fact have the disease when the test is positive. PPV is a measure of how frequently a positive test result is correct. Negative predictive values (NPV) provide information on the probability that a subject does not have the disease when the test is negative; NPV is a measure of how frequently a negative test is correct.

Alternatively, likelihood ratios, when used in the context of diagnostic assessments, provides an estimate of how much a test result will change the odds of having (or not having) the disease. The positive likelihood ratio is the ratio of the probability that a patient has the disease if he has tested positive, divided by the probability that a patient has the disease if he has tested negative. The negative likelihood ratio is the probability that a patient does not have the disease if he tests positive, divided by the probability that a patient does not have the disease if he tests negative. Thus, likelihood ratios offer an estimate of how much more likely a patient is to have the disease given a positive test (positive likelihood ratio), or a negative test (negative likelihood ratio). Positive likelihood ratios significantly greater than 1 indicate that the test is predictive of disease; negative likelihood ratios significantly less than 1 indicate that the test is predictive of absence of the disease. In either case, if the likelihood ratio result is close to 1 then that test is of limited use in diagnosing the presence or absence of disease. A general rule of thumb is that positive likelihood ratios between 2 and 5 provide modest incremental improvements to the ultimate diagnosis while likelihood ratios of greater than 5 provide a significant improvement.

In an effort to establish the validity of using CSF Aβ42 and t-tau biomarkers to enrich for AD neuropathology in clinically diagnosed AD dementia, a literature review was conducted examining CSF levels in autopsy confirmed AD dementia cases. Autopsy literature reports were placed into two

categories: 1) those that reported sensitivity and specificity values based upon CSF biomarkers (See Table 1) and 2) those that reported correlations with CSF biomarkers to neuropathological amyloid plaque and neurofibrillary tangle criteria. Studies reporting sensitivity and specificity results were compared based upon positive likelihood ratios that could be calculated from sensitivity and specificity information. In general, CSF biomarkers yielding positive likelihood ratios ranging from 2-5 were judged as providing modest, yet significant, improvement over existing tests whereas CSF biomarkers with positive likelihood ratios greater than 5 were perceived as providing significant improvement over current standards. There were a total of 14 studies reviewed with 11 studies reporting sensitivity and specificity results based on CSF biomarker data. (Clark 2003, Grossman 2005, Engelborghs 2008, Bian 2008, Koopman 2009, Roher 2009, Shaw 2009, Tapiola 2009, de Meyer 2010, Brunnstrom 2010, de Jager 2010). Table 2 summarizes autopsy studies with reported sensitivity and specificity values.

Table 2: Summary of CSF Aβ42 and Total tau in Autopsy-confirmed Subjects

Study, Year Center Collection time	N Autopsy Confirmed	Assay	Findings in Autopsy-confirmed AD	Comparison Group	Negative / Positive Likelihood Ratio	Negative/Positive Predictive Value	Sensitivity/ Specificity
Clark et al., 2003 Univ Penn Ante mortem LP Controls non-autopsy	74 AD 13 OD	Tau Innotest Aβ - Suzuki	↑ Tau in AD, prion, ALS, ganglioma ↓Aβ42 in AD, DLB, prion	Tau ADvC	0.18 / 5.3	NR / 87%	84% / 85%
				Tau ADvOD	0.4 / 2.32	NR / 80%	72% / 69%
				Aβ42 ADvC	NR	NR	NR
				Aβ42 ADvOD	NR	NR	NR
				Tau & Aβ42	NR	NR	NR
Grossman et al., 2005 Univ Penn Ante mortem LP ROC included non-autopsy	11 FTD 17 AD	Tau Innotest Aβ - Suzuki	↑ Tau in AD ↓Aβ42 in AD	Tau ADvC	NR	NR	NR
				Tau ADvFTD	0.32 / 4.2	NR / 94.7%	74% / 82/4%
				Aβ42 ADvC	NR	NR	NR
				Aβ42 ADvFTD	0.89 / 1.07	NR / 79.4%	37% / 58.8%
				Tau & Aβ42 ADvOD	NR	NR	NR
Engelborghs et al., 2008 Inst Born-Bunge Ante mortem LP	73 AD 27 OD	Tau and Aβ42 Innotest	↑ Tau AD, CJD ↓ Aβ42 AD	Tau ADvC	NR	NR	NR
				Tau ADvFTD	NR	NR	NR
				Aβ42 ADvC	NR	NR	NR
				Aβ42 ADvFTD	NR	NR	RN
				Tau&Aβ42 ADvOD	0 / 7.7	NR	100 % / 87 %

Table 2: Summary of CSF Aβ42 and Total tau in Autopsy-confirmed Subjects

Study, Year Center Collection time	N Autopsy Confirmed	Assay	Findings in Autopsy-confirmed AD	Comparison Group	Negative / Positive Likelihood Ratio	Negative/Positive Predictive Value	Sensitivity/ Specificity
Bian et al., 2008 Univ Penn & Erasmus Ante mortem LP Control CSF nonautopsy	19 AD 30 FTD	Tau Innotest Aβ42 - Suzuki	↑ Tau in AD ↓Aβ42 in AD	Tau ADvC	NR	NR	NR
				Tau ADvFTD	0.35 / 6.64	NR / 81.25 %	68.4 % / 89.7%
				Aβ42 ADvC	NR	NR	NR
				Aβ42 ADvFTD	NR	NR	NR
				Tau&Aβ42 ADvFTD	0.22 / 23.2	NR / 93.75%	78.9 % / 96.6 %
Koopman et al., 2009 Inst Born-Bunge Ante mortem LP	95 AD 50 OD	Tau and Aβ42 Innotest	↑ Tau in AD ↓Aβ42 in AD	Tau ADvC	NR	NR	NR
				Tau ADvOD	0.53 / 1.9	NR	65% / 66%
				Aβ42 ADvC	NR	NR	NR
				Aβ42 ADvOD	0.53 / 2.0	NR	74% / 62 %
				Tau&Aβ42 ADvOD	NR	NR	NR
Roher et al., 2009 Sun Health Res Inst Postmortem Ventricle	47 AD 43 Con 17 OD	Tau and Aβ42 Innotest	No change Tau ↓ Aβ42 in AD	Tau ADvC	NR	NR	NR
				Tau ADvOD	NR	NR	NR
				Aβ42 ADvC	0.34 / 2.0	ND	79% / 61%
				Aβ42 ADvOD	0.49 / 4.1	ND	58 % / 86%
				Tau&Aβ42 ADvOD	NR	NR	NR

Table 2: Summary of CSF Aβ42 and Total tau in Autopsy-confirmed Subjects

Study, Year Center Collection time	N Autopsy Confirmed	Assay	Findings in Autopsy-confirmed AD	Comparison Group	Negative / Positive Likelihood Ratio	Negative/Positive Predictive Value	Sensitivity/ Specificity
Shaw et al., 2009 Univ Penn Ante mortem LP	56 AD	Tau and Aβ42 Alzbio3	↑ Tau AD ↓ Aβ42 AD	Tau ADvC	0.3 / 9.0	95.2% / 81.8%	69.6 %/ 92.3%
				Tau ADvOD	NR	NR	NR
				Aβ42 ADvC	0.05 / 4.2	73.8% / 90.7%	96.4 % / 76.9%
				Aβ42 ADvOD	NR	NR	NR
				Tau/Aβ42 ADvC	0.17 / 5.6	84.6 % / 85.7 %	85.7% / 84.6 %
Tapiola et al., 2009 Univ Kuopio Finland Ante mortem LP	79 AD 29 OD 15 OND	Tau and Aβ42 Innotest	↑ Tau with↑ Braak; ↓ Aβ42 with ↑ neuritic plaque #	Tau ADvC	ND	ND	ND
				Tau ADvBraak	0.32 / 3.3	NR	75.5% / 76.9%
				Aβ42 ADvC	ND	ND	ND
				Aβ42 ADvNP	0.24 / 4.5	ND	80% / 82.1%
				Tau/Aβ42 ADvBraak	0.23 / 10.3	ND	79.1 % / 92.3%
				Tau/AB42 ADvNP	0.18 / 7.9	ND	84.2 % / 89.3%
De Meyer et al., 2010 Inst Born-Bunge Ante mortem	73 AD	Tau and Aβ42 Innotest	↑ Tau AD ↓ Aβ42 AD	Tau ADvC	NR	NR	NR
				Tau ADvOD	ND	ND	ND
				Aβ42 ADvC	0.15 / 2.5	NR	91% / 62%
				Aβ42 ADvOD	ND	ND	ND

Table 2: Summary of CSF Aβ42 and Total tau in Autopsy-confirmed Subjects

Study, Year Center Collection time	N Autopsy Confirmed	Assay	Findings in Autopsy-confirmed AD	Comparison Group	Negative / Positive Likelihood Ratio	Negative/Positive Predictive Value	Sensitivity/ Specificity
				Tau/Aβ42 ADvC	NR	NR	NR
Brunnstrom et al., 2010 Univ Lund Ante mortem	8 AD 35 OD	Tau and Aβ42 Innotest	↑ Tau AD ↓ Aβ42 AD	Tau ADvC	ND	ND	ND
				Tau ADvOD	1.09 / 0.95	18% / 80%	63% / 34%
				Aβ42 ADvC	ND	ND	ND
				Aβ42 ADvOD	0.69 / 1.18	22 % / 86 %	75 %/ 36 %
				Tau & Aβ42 ADvOD	NR	NR	NR
de Jager et al., 2010 OPTIMA, UK Ante mortem LP	177 AD 63 OD 3 C	Tau and Aβ42 Innotest	↑ Tau AD ↓ Aβ42 AD	Tau ADvC	ND	ND	ND
				Tau ADvOD	0.24 / 2.32	77%/83%	92% / 61%
				Aβ42 ADvC	ND	ND	ND
				Aβ42 ADvOD	0.24 / 5.3	44%/97%	80% /85%
				Tau/Aβ42 ADvC	ND	ND	ND

C- Control subjects; OD - Other Dementia, OND - Other neurological disease; ND - no data; NR - Not reported; NP - Neuritic Plaques; FTD - Frontotemporal dementia

*estimated from table reports of raw data

**Total numbers from based upon latest numbers from individual institutions.

Further details on these studies are provided below:

- In a paper by Clark et al. (2003), ante-mortem CSF Aβ42 and total tau levels were obtained from 74 AD and 33 other dementia and neurological subjects including dementia with Lewy Body (DLB, N= 3), frontotemporal dementia (FTD, N=10) and prion diseases (e.g. Creutzfeldt-Jakob disease-CJD, N=8). In addition, individual samples from amyotrophic lateral sclerosis, progressive supranuclear palsy, Parkinson's disease, Huntington's disease, progressive multifocal leukoencephalopathy, ganglioma and multiple sclerosis were examined (N=8). CSF was also obtained from 73 control subjects, 4 of whom went on to autopsy confirmation. When comparing AD to other dementias, CSF t-tau was the most robust measure. Inclusion of CSF Aβ42 did not improve AUC or diagnostic sensitivity and specificity, thus numbers were not reported. Although there was overlap in individual subjects between AD CSF Aβ42 and t-tau levels, average levels of CSF Aβ42 were higher in AD and average levels of t-Tau were lower in DLB and FTD compared to AD suggesting some utility of the CSF markers in differentiating AD from other forms of dementia.

- In a study by Grossman et al. (2005) focusing on the CSF profile in 17 autopsy confirmed FTD cases compared to autopsy confirmed AD cases, CSF t-tau again was able to differentiate AD from FTD with CSF Aβ42 being less informative. Cases from non-autopsy confirmed CSF samples were combined with autopsy confirmed data to derive a sensitivity for t-tau of74% and specificity 82.4% using AD vs FTD comparisons. CSF Aβ42 did not differentiate AD from FTD in this study and sensitivity for CSF Aβ42 was 37% with specificity at 58.8%. Earlier studies from the University of Pennsylvania reported Aβ values using a research use only assay (RUO) based on antibodies described by Suzuki et al. (1994). Consistent with previous studies from the UPenn group (Clark et al., 2003), Tau proved most informative in differentiating AD from other dementias, such as FTD.

- In an updated study by Engelborghs (2008), ante-mortem CSF samples were obtained from 100 autopsied subjects with a clinical diagnosis of dementia (majority with AD pathology confirmed at autopsy) and 100 (non-autopsy) controls. The 100 autopsy confirmed cases were classified as AD or non AD. CSF Aβ42 levels differentiated AD from controls, but did not significantly differ between AD and non-AD. However, combining CSF t-tau with CSF Aβ42 significantly improved the ability to distinguish AD-dementia from non-AD and controls with high sensitivity (100%) and specificity (87%) similar to findings reported by Clark et al. (2003) and Grossman et al. (2005).

- In a study focusing on FTD and AD from UPenn and additions from the Erasmus cohort, Bian et al. (2008) reported on the utility of CSF biomarkers in differentiating AD from FTD specifically in autopsy confirmed samples. A subset of FTD subjects were familial genetic cases. CSF t-tau levels were significantly higher and CSF Aβ42 levels were significantly lower in AD vs FTD. CSF t-tau showed reasonable sensitivity of 68.4% and specificity of 90%. The use of t-tau/Aβ42 ratio showed a sensitivity of 79% and specificity of 97%. CSF Aβ42 sensitivity and specificity were not reported by the authors. CSF t-tau appeared to be most important in driving differentiation between AD and FTD.

- In what appears to be an updated report (Engelborghs et al., 2007) of CSF analysis from autopsy confirmed subjects from the Institute of Born-Bunge in Antwerp Belgium, Koopman et al. (2009) report performance of CSF Aβ42 and t-tau in differentiating AD from other dementias including DLB (N=18), FTD (N-10), CJD (N=6), and vascular dementia (N=16). Sensitivity and specificity for CSF Aβ42 in differentiating AD from non AD dementia was 74% and 62% while t-tau showed a 65% and 66% sensitivity and specificity respectively.

- Roher et al. (2009) reported on CSF Aβ42 and t-tau results from 47 AD, 43 controls and 17 other dementia and neurological cases including FTD, Progressive Supranuclear Palsy (PSP), corticobasal degenerative (CBD), normal pressure hydrocephalus (NPH) and dementia lacking distinctive histology. CSF Aβ42 differentiated AD from controls with a sensitivity of 79% and a specificity of 61% whereas performance in differentiating AD from non-AD dementias showed 58% sensitivity and 86% specificity. CSF t-tau did not significantly differentiate AD from non-AD dementias in the current cohort. However, the inconsistency of tau's utility in this report may be partially attributed to the types of other neurological diseases included in the classification such as NPH and CBD. In general NPH and CBD would not be confused with AD in a clinical setting. In addition, samples were obtained during the post-mortem period and tau may be relatively unstable due to extensive proteolytic processing and vulnerability to caspase and calpain cleavage potentially rendering the CSF tau variants undetectable using mid-domain immunnoassays characteristic of the Innogenetics assays.

- In a study by Shaw et al. (2009) using autopsy confirmed CSF samples from the University of Pennsylvania cohort, ante-mortem CSF samples were obtained from 56 autopsy-confirmed cases of AD and 52 cognitively normal (non-autopsy) elderly subjects. CSF biomarkers distinguished pathologically confirmed groups with high sensitivities and specificities. Use of CSF tau yielded the best positive likelihood ratio while CSF Aβ42 yielded the best negative likelihood ratio. Comparison to other forms of dementia was not described in the report.

- In a study by Tapiola et al. (2009), ante-mortem CSF samples were obtained from 79 patients with clinically diagnosed AD-dementia, 29 other dementias (including FTD, DLB, PD and VAD) and 15 other neurologic illnesses (including PSP, CJD, CBD). CSF Aβ42 levels correlated inversely with pathological brain amyloid load. CSF tau levels correlated with tangle load as assessed by transentorhinal (1-2) Braak staging. Use of Tau/CSFAB42 showed highest positive likelihood ratio and lowest negative likelihood ratios in comparison to Braak or neuritic plaque (NP) staging).

- De Meyer et al. (2010) utilized a mathematical model examining binomial distributions to derive CSF cutoffs for AD from a subset of the Institute of Born-Bunge autopsy sample set. Sensitivity for autopsy confirmed cases was reported as 93% for CSF Aβ42. Sensitivity and specificity based upon binomial cutoffs of clinical diagnosed samples was estimated at 91% and 62% respectively for CSF Aβ42. No values were reported for CSF t-tau although a ratio for CSF

Aβ42 and phosphorylated tau were reported. Sensitivity and specificity of AD vs control comparisons was within ranges reported earlier by Engelborghs et al. (2008).

- Brunnstrom et al. (2010) from the University of Lund in Sweden reported on a small autopsy confirmed cohort consisting of 8 AD and 35 other dementias including VAD, CJD, DLB, FTD and one subject with primary cerebral lymphoma and one case with multiple system atrophy. Elevated CSF t-tau and low CSF Aβ42 were found in 12 of the 43 subjects. Five of the 8 AD patients showed the stereotypical pattern of low CSF Aβ42 and high tau. A follow-up CSF test resulted in 6 of the 8 showing positive CSF biomarkers results in the AD autopsy group. Biomarker performance in general was much lower in this study due in part to the low numbers of AD cases and to the high prevalence of CJD.

- Lastly, in a study by de Jager et al. (2010), ante-mortem CSF was analyzed from 177 autopsy confirmed AD and 63 autopsy confirmed non-AD cases from the United Kingdom OPTIMA cohort. The non-AD cases included subjects who were retrospectively identified as presenting with a memory complaint and were classified by NINCDS-ADRDA criteria as MCI or controls. In AD subjects, CSF Aβ42 was decreased and t- tau increased compared to non-AD cases. Use of CSF Aβ42 alone resulted in 80% sensitivity and 85% specificity with inclusion of tau showing sensitivity at 92% and specificity at 61% (BMS calculation).

There were a total of three autopsy studies that compared CSF biomarker levels to either neurofibrillary tangles (as defined by Braak staging) and/or to the number of amyloid plaques (Strozyk et al., 2003; Engelborghs et al., 2007; Tapiola et al., 2009). Two of these autopsy studies were not included in the table summary as no data was provided describing sensitivity and specificity performance. In the Honolulu-Asia aging study by Strozyk et al. (2003) a correlation between CSF Aβ42 and amyloid plaque number was identified. Data was collected from 155 male autopsy confirmed participants of whom 95 were non-demented (i.e. autopsied controls), 30 AD, 22 vascular dementia, and 8 other dementia cases. There was a significant inverse correlation between brain neuritic and senile plaque burden and CSF Aβ42 levels suggesting low CSF Aβ42 levels correlate well with increased amyloid brain burden. The second study comparing CSF biomarkers to neuropathological features was one of the few negative studies reporting no relationship between CSF Aβ42 and t-Tau and senile plaques or late stage Braak pathology (Engelborghs et al., 2007). In this study, the method of classifying Braak staging differed slightly from those reported by Tapiola et al. (2009). In addition, ELISA results from the Innogenetics Innotest kits were generally lower than other reported studies suggested some assay issues. Later follow-up studies from the sample group (Engelborghs et al., 2008; Koopman, et. al, 2009) did report good correlation with CSF biomarkers and autopsy confirmed diagnosis suggesting some of the discrepancies may have been due to a low N and a reclassification of the diagnosis.

In summary, of the fourteen available studies reviewed, one study was excluded based on a very small N of 6 (Le Bastard et al., 2010). Of the remaining 13, two focused on correlations with brain pathology rather than diagnosis and did not provide sensitivity or specificity information. One study (Engelborghs et al., 2007) reported no correlation between CSF and autopsy confirmed pathology. However, subsequent updates from this same group reported reasonable concordance between CSF

biomarkers and autopsy confirmed diagnosis (Engelborghs et. al, 2008) suggesting the negative study may have been an outlier. The 11 remaining studies were summarized and performance compared based upon sensitivity, specificity and likelihood ratios.

The most common non-AD dementias likely to be confused with AD include DLB, VaD and some cases of FTD. Thus, the ability to differentiate these types of non-AD dementia can be valuable. Based upon a survey of the autopsy literature, CSF Aβ42 alone did not always differentiate AD from other non-AD dementias (Clark et al., 2003; Bian et al., 2008). Use of either CSF Aβ42 or CSF tau alone provided a modest improvement in likelihood ratios (Clark et al., 2003, Grossman et al., 2005, Bian et al., 2008, Shaw et al., 2009, Roher et al., 2009, de Jagar et al., 2010). However, the combined use of CSF Aβ42 and t-tau improved both specificity and positive likelihood ratios. For example, the highest positive likelihood ratios ranging from 7.7 up to 23 were observed when CSF Aβ42 and t-tau were used in combination (Engelborghs et al., 2008, Bian et al., 2008, Tapiola et al., 2009) suggesting use of both CSF Aβ42 and CSF t-tau biomarkers can improve the probability that patients included in AD clinical trials are indeed positive for AD pathology.

Autopsy studies focusing on the correlation between CSF Aβ42 levels to amyloid plaque load and CSF t-tau to neurofibrillary Braak staging were also very compelling. There were good correlations between low CSF Aβ42 levels and amyloid plaque burden (Strozyk et al., 2003, Tapiola et al., 2009). Tapiola et al., report good correlations between CSF t-tau levels and entorhinal stage (1-2) Braak staging. Finally, CSF biomarkers show good correlation with amyloid brain burden as measured by PET imaging (see below). In summary, autopsy literature reports support the position that both CSF Aβ42 and t-tau can provide evidence of AD pathology in ante mortem cases and use in clinical trials will likely improve the probability that enrolled patients exhibit AD specific pathology suitable for amyloid target modulation.

(2) Data comparing CSF biomarker levels in clinically diagnosed AD dementia to controls or to clinically diagnosed non-AD dementia

The number of studies examining the sensitivity and specificity of CSF Aβ42, t-tau or a combination of the two in differentiating clinically diagnosed AD from controls and AD from clinically diagnosed non-AD dementias is extensive and has been summarized in numerous reviews and meta analysis (Frankfort et al., 2009, Prvulovic and Hampel, 2011, Sunderland et al., 2003). In 2003, a meta analysis was published describing CSF biomarker performance in over 40 articles. The meta analysis confirmed that in AD, CSF Aβ42 levels are low and t-tau levels are high (Sunderland at al., 2003). According to the author, cutpoints of 444 pg/mL for CSF amyloid1-42 and 195 pg/mL for CSF tau gave a sensitivity and specificity of 92% and 89%, respectively, to distinguish AD patients from controls, which is comparable with rates with clinical diagnosis. Meta-analyses of studies comparing CSF amyloid and tau levels in AD participants and controls confirmed an overall difference between levels in these 2 groups. When comparisons of CSF Aβ42 levels are made to other types of non-AD dementias, the ability to differentiate AD from non-AD dementia using CSF Aβ42 alone can sometimes be challenging as CSF Aβ42 ranges in DLB, FTD and VaD can overlap ranges observed in AD (Brunnstrom et al., 2010).

In 2011, van Harten et al., published a systematic literature review summarizing the utility of CSF t-tau and p-tau in differentiating DLB, FTD, VaD, CJD from AD and from controls. The meta analysis reported a Cohen's delta on the effect size and details on the sensitivity and specificity of the utility of CSF tau. Average Cohen's delta was -1.03 when comparisons were made between DLV and AD suggesting ante-mortem CSF t-tau levels were lower in DLB than in AD. Average sensitivity in the DLB vs AD comparison was 73% (62%-84%) and specificity was 90% (85%-95%) based on 208 DLB and 473 AD cases.

Comparisons of CSF t-tau levels in FTD vs AD were also described (van Harten et al., 2011). Cohen's delta was -0.87 and when early stages of AD were included, Cohen's delta was -2.34 suggesting CSF t-tau levels are generally lower in FTD than in AD. Sensitivity in the FTD vs AD comparison was 74% (66%-82%) and specificity was 74% (66%-81%).

A similar analysis was also conducted for VaD and CJD relative to AD with sensitivity and specificity at 73% (60-86%) and 86% (80-94%) for VaD and 91% (86-96%) and 98 % (97-100%) for CJD, respectively. When CSF phosphorylated tau was included, sensitivity and specificity improved for VaD and FTD. It should be noted that elevations in phosphorylated tau have been rarely reported in CJD.

Clearly, CSF Aβ42 or CSF t-Tau alone has value in differentiating AD from non-AD dementias based upon current literature review. However, literature reports in mild-moderate AD and in the predementia literature support improved performance when CSF Aβ42 and t-Tau are used in combination.

In summary, autopsy, cross-sectional and longitudinal reports in AD, non-AD dementia and control cases provide significant and extensive evidence to support the premise that low CSF Aβ42 and high t-tau are reflective of AD neuropathology. Published autopsy literature strongly supports the notion that patients with low ante-mortem CSF Aβ42 and elevated t-tau have greater probability of exhibiting a significant number of amyloid plaques and neurofibrillary tangles consistent with AD pathology. The key to improved specificity and greater positive likelihood ratios appears to be the combined use of CSF Aβ42 and t-tau. When used in combination, low CSF Aβ42 and high t-tau reflect an AD pathology that is not commonly observed in other dementias or other neurological disorders. Thus, patients with a pathologic CSF signature are highly likely to have underlying neuropathology of AD and are therefore more likely to benefit from amyloid-modulating therapies. As such, extensive published literature from many independent groups exists which supports this CSF biomarker signature for qualification for use in clinical trials of amyloid targeted therapies to enrich patient populations and enhance the probability of positive study outcomes.

Question 2

PET-Amyloid Imaging: In clinical studies of amyloid targeted therapies in mild to moderate dementia of the Alzheimer's type, are there sufficient data to support the use of PET-

amyloid imaging as a biomarker for enrichment, by excluding patients who are unlikely to have underlying AD pathology?

Applicant's position

Over the last decade, PET imaging has allowed the quantitative and qualitative assessment of amyloid burden in living subjects. Multiple radiotracers binding to brain amyloid have been successfully used (e.g., 18F Florbetaben, 18F Florbetapir, 11C PiB, and 18F PiB). Scientific support for the use of PET-amyloid imaging to enrich clinical trials of amyloid targeted therapies in patients with mild-to-moderately severe AD more likely to have underlying AD pathology comes from the following lines of evidence:

(1) Agreement of ante-mortem PET-amyloid imaging of amyloid burden with post-mortem autopsy diagnosis [Cohort 4 of Systematic Review];

(2) Convergent agreement between PET-determined amyloid burden and a CSF profile indicative of AD pathology [Cohorts 2 and 3 of Systematic Review];

(1) Longitudinal Ante-mortem PET-amyloid imaging compared with post-mortem autopsy: A systematic review was conducted to assess the performance of PET-amyloid imaging as a diagnostic tool. This was done by searching for studies that reported the correlation between PET-amyloid imaging and histologic *post mortem* assessment of the presence of AD pathology in the brain. A total of 2 studies met these criteria and were reviewed in Study Cohort 4. Data from the two studies suggest a strong correlation between pre mortem PET amyloid imaging and post mortem presence of AD pathology.

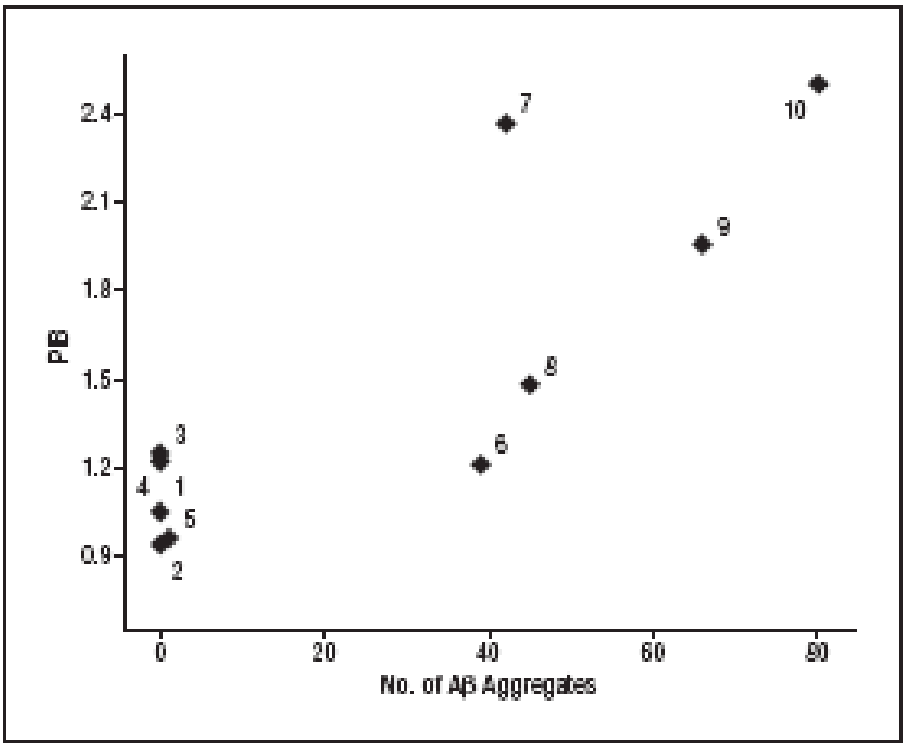
Clark et al. (2011) reported on the performance of florbetapir. Florbetapir has been shown to effectively identify the presence of Aβ aggregates (plaques) in the brain (Clark 2011). In a pivotal study, subjects with a variety of degrees of cognitive dysfunction nearing the end of life underwent PET-florbetapir scanning and consented to post-mortem autopsy (Clark 2011). The first 29 autopsy cases demonstrated that ante-mortem Florbetapir-PET imaging (qualitative assessment) was concordant with post-mortem assessment of Alzheimer's pathology in 96% of cases. Quantitative assessment of the standard update volume ratio (SUVr) cut-offs yielded 100% agreement. In addition, 74 healthy young subjects without evidence of cognitive impairment had Florbetapir-PET scans; there were no cases of elevated amyloid burden. Correlation coefficients (*r*) of pre-mortem (using SUVr) and post-mortem (using quantitative histopathologic assessment of plaque burden) assessments were very consistent across the various measurement methods, ranging from 0.68 to 0.78.

Sojkova et al (2011) assessed ante mortem 11C PiB binding (distribution volume ratio; DVR) with post mortem histopathologic assessment in 6 elderly subjects from the Baltimore Longitudinal Study (1 with dementia and 5 without). One subject who met criteria for Probable AD (CERAD criteria) had the highest mean cortical DVR (1.59). Other subjects' cortical DVR ranged from 0.96 - 1.42. There was limited agreement between NP load and mean cortical DVR. Focused evaluation of the precuneus, a site of early amyloid deposition, revealed that increases in the precuneus DVR over 1.2 reflected increasingly abnormally high levels of amyloid on post mortem assessment and a dichotomous cut-

point of 1.2 fully separated patients with normal from abnormally elevated amyloid burden in the precuneus.

An additional publication (Leinonen et al., 2008) that did not fulfill inclusion criteria of the systematic review merits attention. In this study from Finland, 10 patients with normal-pressure hydrocephalus (age 66 - 75 years) underwent 11C PiB imaging and had histopathologic assessment of frontal cortex biopsies. The ratio of cortical to cerebellar 11C PiB binding was markedly elevated in all 5 subjects who had an abnormally elevated amyloid load (see Figure 3).

Figure 3: Scatterplot of Carbon 11-labeled Pittsburgh Compound B ([11C]PIB) Uptake in the Right Frontal Cortex



Aβ indicates the number of β-amyloid (clone 4G8) aggregates in the right frontal cortical biopsy specimen (count of diffuse and dense aggregates independent of size in a visual field). The diamonds are labeled by case numbers indicated in the publication.

Source: (Leinonen et al., 2008)

The results of these two studies combined with the additional supporting publication provide evidence that PET amyloid imaging may be used as a means of identifying subjects with mild to moderate dementia with underlying AD pathology. In addition, current work is ongoing to further establish the correlation of ante mortem PET-amyloid binding with post mortem histopathologic assessment for 18F Flutemetamol (NCT01165554 , n=100) and 18F Florbetaben (NCT01020838, n=232) to ultimately support approval for their use in ruling out the presence of AD pathology.

(2) Agreement between PET-determined amyloid burden and a CSF profile indicative of AD pathology: In studies examining both CSF biomarkers and PET-amyloid in broad populations (AD, MCI, and healthy elderly), there was a strong correlation between amyloid burden and CSF Aβ42 concentration as well as t-tau:Aβ42. The Systematic Review identified 9 relevant studies, summarized in Table 3 that assess the agreement of PET-radiotracer binding with CSF profile in subjects with AD-dementia (Cohort 3) and MCI (Cohort 2). In addition, the table summarizes data from ADNI that BMS examined with a focus on the population with mild AD-dementia as well as baseline data from an ongoing study in Predementia AD (CN156018). Since the cut-off for the Systematic Review we have identified an additional publication meriting summary (Weigand 2011) that examines the correlation of PiB SUVr and CSF Aβ42 concentrations.

Overall, these results indicate a strong inverse association between PET PiB retention and CSF Aβ42 concentration. While associations between amyloid-PET binding and CSF tau concentrations are less strong, the use of t-tau:Aβ42 (Fagan 2011) or p-tau:Aβ42 (Koivunen 2008) ratios has been shown to enhance the agreement.

Literature on the repeatability of amyloid-PET imaging has shown that there is an average of 90-97% overlap in regional 11C-PiB binding in AD subjects between scans (Tolboom et al., 2009b). Similar results have been obtained with 18F amyloid-PET ligands such that Flutemetamol has shown 96-99% test-retest reliability in scans with a seven-day interval (Vandenberghe et al., 2010). Florbetapir (AV-45) has shown absolute test-retest reliability of 94-96% (Pontecorvo et al., 2009) and Florbetaben has an average test-retest reliability of 93% in AD subjects (Rowe et al., 2009). When comparing results from 11C- and 18F-based compounds, there are correlation coefficients ranging from 0.89-0.92 in locations of regional ligand binding. Patterns of regional amyloid-PET binding in the AD brain closely co-localize to known regions vulnerable to atrophy and metabolic dysfunction such as the precuneus, posterior cingulate and frontal cortex. Disruptions in these cortical regions are known to contribute to memory impairment (Buckner et al., 2005). High replicability in these cortical regions across multiple subjects and different amyloid tracers demonstrates the utility of these agents as biomarkers in clinical trials. These data provide insight into the high test-retest replicability for clinical use that closely follows known patterns of amyloid deposition in Alzheimer's disease.

Overall, the literature suggests that elevated amyloid burden, as determined by PET-amyloid imaging, increases the probability that patients classified as AD by NINCDS/ADRDA criteria do indeed have existing amyloid pathology. In addition, elevations of PET-amyloid burden reflect similar information as pathologic CSF profile and, hence, both can be used to reduce the heterogeneity in clinical trials on populations with mild to moderately severe AD.

Table 3: Summary of Literature Review of Studies on PET-PiB Imaging in Patients with AD dementia, Healthy Elderly Controls (HC), Mild Cognitive Impairment (MCI) and Other Dementias

Study	Year	N	PET	CSF	Correlation	Concordance	Comment
Fagan	2006	24 (AD 4; HC 18; non-AD 2)	PiB	Aß42	n/a	100%	Correlations were not reported for any measure. Among a population of healthy controls, and mild AD patients, those with positive PIB binding had the lowest CSF Aβ42 level and those with negative PIB binding had the highest CSF Aβ42 level. Data suggest that a specific plaque-associated alteration in Aβ42 metabolism is involved in the PiB/CSF Aβ42 association, and suggest that in vivo amyloid imaging, as well as CSF Aβ42 measures may have utility as antemortem AD biomarkers.
Koivunen	2008	37 (15 MCI; 22 HC)	PiB	Aß42	ns	54%	54% of PIB-positive subjects showed AD-type (<450 pg/ml) Aβ42 values. The corresponding figures showed 69% for t-tau, 63% for p-tau and 67% for the Aβ42:p-tau ratio, indicating a moderate to strong correlation between amyloid binding and CSF analytes. Correlations of PiB to CSF Aβ42 concentrations were not significant. It is worth noting that in a later report by this group (Koivunen 2011) using similar PET methods, a higher definition of abnormal cortex-to-cerebellum ration (1.5) was offered. Optimized cut-points would have suggested concordance in 11 of 15 subjects (Aβ42 <450 pg/ml) and cortex-to-cerebellum ration > ~1.45).
Jagust	2009	55 (AD 10; HC 11; MCI 34)	PiB	Aß42	-0.73	91%	Dichotomous categorization showed substantial agreement between PIB-PET and CSF Aβ1-42 measures (91% agreement, k=0.74), modest agreement between PIB-PET and p-tau (76% agreement, k=0.5). Regression models showed that PIB-PET was significantly correlated with Aβ42, t-tau, and p-tau181p.
			PiB	t-tau	0.42	55%	
Tolboom	2009a	37 (AD 15; 10 HC; 12 MCI)	PiB	Aß42	-0.72	n/a	For global 11C-PiB binding, significant correlations with CSF levels of Aβ42 and tau were found across groups. Linear regression analyses showed that, adjusted for regional volume, age, sex, and diagnosis, global 11C-PiB uptake had an inverse association with Aβ42 CSF levels.
			PiB	t-tau	0.58	n/a	
Grimmer	2009	30 (AD)	PiB	Aß42	-0.48	est 87%	All patients showed a positive [11C]PiB scan demonstrating amyloid deposition. Linear regression analysis revealed a significant inverse

Table 3: Summary of Literature Review of Studies on PET-PiB Imaging in Patients with AD dementia, Healthy Elderly Controls (HC), Mild Cognitive Impairment (MCI) and Other Dementias

Study	Year	N	PET	CSF	Correlation	Concordance	Comment
							correlation between the overall [11C]PiB uptake and CSF Aβ42 levels. Voxel-based regression and regional correlation analyses did not attain statistical significance after correction for multiple comparisons. Numerically, correlation coefficients were higher in brain regions adjacent to CSF spaces. Only 5 patients had CSF Aβ42 concentrations in the normal range and only 2 patients had normal 11C-PiB binding -- with graphs suggesting these latter two patients overlapping with the former (hence estimated 87% agreement).
Forsberg	2010	58 (AD 37; 21 MCI)	PiB	Aß42	-0.46	n/a	Significant correlations between PiB and Aβ42, t-tau and p-tau were observed in most brain regions when including full cohort. No significant correlations were observed between 11CPIB retention and the CSF biomarkers when the AD patients were analyzed separately (p>0.05). Among MCI subjects (Forsberg 2008), PiB correlations to Aβ42 (r, 0.64 - 0.74) were greater than to t-tau (0.51 - 0.64) -- both with significant nominal p-values.
Galvin	2010	31(HC, AD, unspecified dementia)	PiB	Aβ42	n/a	n/a	Among 10 subjects with clinical AD, CSF and PiB showed 70% agreement. Similar agreement among entire sample.
Degerman Gunnarsson	2010	10 (AD)	PiB	Aβ42	n/a	100%	PiB binding strongly inversely related to low CSF Aβ42 (p = 0.01). CSF and PiB assessment of pathologic amyloid burden agreed in all patients (6 pathologic;4 non-pathologic). Correlations between PiB and CSF Aβ42 were significant (although values were not reported). Similar correlations with t-tau and p-tau were reported as not significant.
Fagan	2011	103 (14 AD; 89 HC)	PiB	Aβ42	-0.71	n/a	ROC curves demonstrate higher AUC for t-tau:Aβ42 ratio (0.94 - 0.96) than for Aβ42 concentrations alone (0.89 - 0.93)
				Aβ42: t-tau ratio	0.73	n/a	

Table 3: Summary of Literature Review of Studies on PET-PiB Imaging in Patients with AD dementia, Healthy Elderly Controls (HC), Mild Cognitive Impairment (MCI) and Other Dementias

Study	Year	N	PET	CSF	Correlation	Concordance	Comment
FOLLOWING DATA NOT INCLUDED IN SYSTEMATIC REVIEW -- UNPUBLISHED OR PUBLISHED AFTER CUT-OFF							
CN156018 (ongoing)	2011	64 (all MCI)	Florbeta pir	Aß42 & t-tau	n/a	89.1%	In this interim analysis on baseline data from an ongoing study a subset of patients with cognitive impairment underwent both spinal taps and PET amyloid scanning prior to randomization. Concordance between PET florbetapir scanning (qualitative read) and pathologic CSF (either Aβ42<200 or t tau:Aβ42 ratio ≥ 0.39) was 89.1%, with an observed agreement statistic Kappa of 0.73 (95% confidence interval of 0.55 - 0.92). Sixty-six percent and 23% of subjects were either positive or negative on both biomarkers, respectively. Five subjects were positive only on PET radiotracer imaging while 2 subjects were positive only on CSF biomarkers. [Preliminary data]
Weigand (ADNI)	2011	41 (10 AD; 22 MCI; 9 HC)	PiB	Aβ42	0.77 (R^2)		Regression model of log-transformed PiB binding and CSF Aβ42 concentrations (with ApoE status as covariate) yielded R^2 of 0.77. ADNI sample with CSF Aβ42 alone shown to have similar modeled distributions (e.g., probability density) as PiB-studied population with measured amyloid burden.
BMS ADNI Analysis		9 mild AD	PiB	Aβ42 t-tau		100%	Pathologic 11C PiB binding (i.e., SUVr > 1.5) was concordant with pathologic CSF (i.e., either Aβ42 < 200 or t-tau:Aβ42 ratio $\underline{>}$ 0.39) in all 9 mild AD patients for whom data could be analyzed

Abbreviations: AD, Alzheimers Disease dementia; MCI, mild cognitive impairment; HC, Healthy Controls; n/a, not assessed

Based on the coordinators' reports the CHMP gave the following answers:

Qualification opinion of the use of CSF AB 1-42 and t-tau signature and/or PET-amyloid imaging (positive/ negative) as a biomarkers for enrichment, for use in regulatory clinical trials – in mild and moderate of Alzheimer's disease

Summary

The purpose of this "qualification" procedure is to assess CSF AB 1-42 and t-tau signature and/or PET-amyloid imaging positive/ negative as a biomarker for enrichment can be considered a marker of amyloid pathology in subjects with cognitive deficit compatible with early Alzheimer's disease.

The potential value of the proposed marker in other settings (e.g. in subjects without clinical diagnosis of AD for other reasons) or for other purposes (e.g. as a criterion for the diagnosis of a condition/disease -namely Alzheimer's disease- in a particular subject or the usefulness of repeated measurements to assess the effect of therapeutic interventions -as a marker of efficacy-) are not considered here.

CSF biomarker signature based on a low Aβ1-42 and a high T-tau can be useful to identify patients with clinical diagnosis of mild to moderate AD who are at increased risk to have an underlying AD neuropathology, for the purposes of enriching a clinical trial population.

The one contemplated in this procedure is to "enrich" recruitment into clinical trials aimed at studying drugs potentially slowing the progress/conversion to severe (AD) dementia of the included patients. Impractically large numbers of subjects and/or duration of follow-up would be required and the trials would be unfeasible or inefficient.

Scientific discussion

Accepting the value of the biomarker to "enrich" recruitment is, probably, less demanding than assessing its value in other potential uses (see above) as less accuracy in the prediction is required than e.g. to include a particular individual into a diagnostic category. It has to be considered that, in the end, the rate of patients spontaneously converting in the control arm of the trial (whether accurately predicted or not) will be known at the end of the trial so that the consequences of some out of target prediction would not be as crucial as the same inaccuracy would be to establish a relevant diagnosis in an individual subject.

The data on which the Sponsor base their request for the biomarker to be accepted as qualified derive from a systematic review they have conducted after searching the literature for longitudinal studies

evaluating PET imaging or CSF AB 1-42 and t-tau signature in predicting conversion to severe AD dementia from a clinical mild & moderate AD.

The conclusions are mainly obtained via a "voting" procedure (the majority of studies report that……) but although it can be accepted that a true meta analysis would, probably, have been unfeasible given the heterogeneity of the studies, further attempts to obtaining global estimates may well be justified.

However, some discussion with the Sponsor was needed, both to clarify some aspects of the systematic review and its internal and external validity and to explore whether a more in depth analysis of the retrieved data could justify a more precise statement than simply accepting the vague view that using CSF or PET as a biomarker would "somewhat" enrich recruitment into clinical trials within the considered context. If the review is finally considered valid, this is the type of statement that would be supported by the current analyses.

Based on the co-ordinators' report the Scientific Advice Working Party determined that the applicant should discuss the following points, before advice can be provided:

SAWP/CHMP question

Please provide, if available, an estimate of the negative predictive value (NPV) for CSF in mild to moderate AD.

Applicant's position

During the June 29 clarification meeting with the Scientific Advice Working Party (SAWP), BMS was asked to provide estimates of the negative predictive values (NPVs) for the cited literature in support of the use of CSF biomarkers in clinical studies in mild to moderate Alzheimer's disease (AD). The request for NPV data was in direct response to utilization of CSF biomarkers as exclusion, rather than inclusion, criteria. BMS subsequently contacted authors from the four major independent groups reporting autopsy-confirmed diagnosis of AD with ante-mortem CSF Aβ42 and T-tau data. These groups included the University of Kuopio, Finland (Tapiola et al., 2009), the Institute of Born-Bunge, Anthwerp Belgium (Engelborghs et al., 2008; Koopman et al., 2009), the University of Pennsylvania, US (Shaw et al., 2009) and the Oxford Project to Investigate Dementia and Aging (OPTIMA) group at Oxford, UK (de Jager et al., 2010). The University of Kuopio provided a re-analysis with AD versus non-AD dementia using comparison to Braak stages and neuritic plaque (NP) neuropathological criteria to define the relationship to CSF biomarkers. This re-analysis excluded the subset of other neurological disorders included in the original paper based upon low likelihood of having such a population enrolled in a typical mild-moderate AD clinical trial. The investigators from the Institute of Born-Bunge provided additional data and the University of Pennsylvania expanded on the original AD vs. Control data to include AD vs. non-AD dementia using a frontal temporal dementia (FTD) specific cohort. The University of Oxford (OPTIMA) provided the missing tau/Aβ42 ratio data.

It should be noted that NPV values provide information on the probability that a patient with a negative CSF test result is truly free of AD pathology, and positive predictive value (PPV) provides information on the probability that a patient that is positive on the CSF test truly has AD pathology. The NPV and PPV results must be viewed with caution as accurate NPV and PPV values are highly dependent upon disease prevalence in the population being examined. It is currently unknown what the true prevalence of pathologically confirmed dementia of the AD type is in the context of clinical trial enrollment. Thus, interpretation of the NPV can be problematic in the absence of the known prevalence of the disease under question. Unlike NPV and PPV, likelihood ratios can be calculated without knowledge of disease prevalence. Likelihood ratios can be a useful index in understanding how much the CSF biomarkers are improving the odds that enrolled dementia patients truly have tau and amyloid pathology. Positive likelihood ratios provide an understanding of how much the odds of actually having a disease increase when testing positive. Conversely, negative likelihood ratios provide information on how much the odds of having the disease decrease when testing negative. In a general rule of thumb, positive likelihood ratios (LR) between 2-5 generally provide moderate improvement over current standard diagnostic workup, whereas positive likelihood ratios greater than 5 are perceived to provide significant improvement over current standards. Table 2 in BMS's original submission reports that 10 out of the 11 studies showed that CSF biomarkers had at least comparable LR+ (2-5) as a clinical diagnosis; some biomarkers with certain comparison group did have significant LR+ (>5).

In order to put the data into context, the sensitivity, specificity, NPV, PPV, and likelihood ratios for a probable AD diagnosis using current National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria vs. autopsy confirmation is again provided (Chiu et al., 2003). Note that the NPV was 45% for probable AD using NINCDS-ADRDA, a common inclusion criteria for clinical trials in mild-moderate AD. Utilization of CSF biomarkers vs. autopsy based diagnosis (Koopman et al., 2009; Shaw et al., 2009; de Jager et al., 2010), either alone or in combination improved NPV performance compared to performance based upon a clinical diagnosis of probable AD alone (Chiu et al., 2003). Improvements were highest when comparisons were made using AD vs. controls. When comparisons were made using AD vs. non AD dementia, the performance was not as high. Nevertheless, performance was still better than use of NINCDS-ADRDA criteria alone. Specifically, NPV of AD vs. non AD dementia ranges to 50-88% for CSF T-Tau and to 58-89% for tau/Aβ42 when compared to NPV values (45%) for NINCDS-ADRDA criteria alone. CSF Aβ42 alone showed the NPV values ranging from 44-87%. When examining performance based on specific neuropathological criteria (e.g. Braak staging or neuritic plaque numbers), NPV values were improved using T-Tau (77%), Aβ42 (82%) and the ratio of Tau/Aβ42 (92% Braak, 89% neuritic plaques), suggesting direct comparisons to neuropathology may provide a more accurate interpretation of the correlation.

In summary, the use of CSF T-Tau and Aβ42 together improve NPV in AD vs. Non AD analysis when compared to NPV values based upon clinical diagnosis alone. Thus, CSF T-Tau and Aβ42 can improve the likelihood that enrolled patients do indeed have AD pathology in clinical trials targeting amyloid and tau pathology.

SAWP/CHMP question

Please provide, if available, an estimate of the negative predictive value (NPV) for PET amyloid in mild to moderate AD.

Applicant's position

During the June 29 clarification meeting with the Scientific Advice Working Party (SAWP), BMS was asked to provide estimates of the NPVs for the two cited articles (Clark et al., 2011; Sojkova et al., 2011) examining performance of amyloid PET in autopsy confirmed cases to support the use of PET amyloid biomarker in clinical studies in mild to moderate Alzheimer's disease (AD). The number of subjects in the Sojkova et al., (2011) was too small to calculate NPVs. However, data from Clark et al, were re-calculated to provide NPV data as follows (see Table 4).

Table 4: Clinical and Outcome Values for 35 Participants with a Postmortem Evaluation

			Florbetapir-PET Imaging		Autopsy Reference Standard				
								AD Diagnosis	
Clinical Diagnosis Category	Age at Death, y	Cause of Death	SUVr	Median Visual Reading	β-Amyloid IHC	NPS	Braak Stage[18]	CERAD	NIA/Reagan Institute
ODD	87.4	Esophageal cancer	0.81	1	0.02	0	2	No	Low likelihood
AD[b]	82.8	Congestive heart failure	0.87	0	0.15	0	3	No	Low likelihood
MCI	92.2	Congestive heart failure	0.87	0	0.01	0	4	No	Low likelihood
HC	62.5	Respiratory arrest	0.88	0	0.01	0	1	No	Low likelihood
HC	85.9	Respiratory failure	0.88	0	0.01	0	1	No	Low likelihood
HC	84.6	Lung cancer	0.91	1	0.01	0	1	No	Low likelihood
MCI	86.2	Cardiac arrest	0.92	1	0.03	0	3	No	Low likelihood
HC	99.9	Heart failure	0.92	1	0	0	3	No	Low likelihood
HC	62.1	Infection	0.93	0	0.01	0	1	No	Low likelihood
ODD	104.3	End-stage dementia	0.98	0	0.49	1	1	Possible	Low likelihood
HC	70.1	Prostate cancer	1.00	0	0.47	1	1	Possible	Low likelihood
HC	93.2	Acute MI	1.00	1	1.11	0	0	No	No AD
HC	85.7	Hepatic cancer	1.00	1	0	0	3	No	Low likelihood
ODD	73.9	Advanced PD	1.07	0	0.01	0	3	No	Low likelihood
MCI[b]	48.0	Respiratory and renal failure	1.09	1	0	0	1	No	Low likelihood
HC	55.9	Prostate cancer	1.09	0	0.04	0	1	No	Low likelihood
ODD[b]	78.5	Acute respiratory failure	1.17	2	3.63	2	5	Definite	High likelihood
AD	81.5	Respiratory failure	1.20	3	7.01	3	5	Definite	High likelihood
AD	76.3	AD	1.20	3	5.27	2	5	Definite	High likelihood
ODD	88.7	Cardiac and respiratory arrest	1.21	3	1.42	3	5	Definite	High likelihood
AD	88.1	AD	1.23	1	4.85	2	5	Probable	Intermediate likelihood
ODD	67.9	Pick disease and stroke	1.34	4	6.69	2	5	Definite	High likelihood
AD	72.1	AD	1.36	3	5.31	3	6	Definite	High likelihood
AD	91.8	Acute MI	1.37	3	9.11	2	5	Definite	High likelihood
AD	55.5	Cardiac and respiratory arrest	1.38	3	4.67	3	6	Definite	High likelihood
AD[b]	79.8	AD	1.38	4	7.92	2	6	Definite	High likelihood
AD	89.2	Pneumonia	1.39	3	1.48	2	3	Definite	Intermediate likelihood
AD	88.2	Respiratory failure	1.40	3	3.42	2	5	Definite	High likelihood
AD	86.8	AD	1.45	4	3.27	1	4	Probable	Intermediate likelihood
AD[b]	86.5	AD	1.56	3	5.39	3	5	Definite	High likelihood
AD	60.0	Unknown	1.57	4	9.44	3	6	Definite	High likelihood
AD	69.3	Respiratory failure	1.63	4	5.61	2	5	Definite	High likelihood
AD	92.3	AD	1.64	3	1.11	1	4	Probable	Intermediate likelihood
AD[b]	84.6	AD	1.66	4	8.62	3	6	Definite	High likelihood
AD	91.7	AD	1.91	4	5.38	2	4	Probable	Intermediate likelihood

Abbreviations: AD, Alzheimer disease; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; HC, cognitively healthy control; IHC, immunohistochemistry; MCI, mild cognitive impairment; MI, myocardial infarction; NIA/Reagan Institute, National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease; NPS, neuritic plaque score; ODD, other dementing disorder; PD, Parkinson disease; PET, positron emission tomographic; SUVr, semiautomated quantitative analysis of the ratio of cortical to cerebellar signal.

[a] Participants are ordered by increasing florbetapir-PET SUVr score.

[b] Indicates participant was in the interim analysis (n=6).

Source table from Clark et al., 2011

There were a total of 35 autopsy-confirmed AD cases with pre-mortem amyloid PET imaging using the ligand Florbetapir. A positive autopsy diagnosis was based upon a Consortium to Establish a Registry for Alzheimer's disease (CERAD) rating of probable or definite and a National Institute of Aging (NIA) and Reagan classification of high-intermediate likelihood. A visual amyloid PET test rating between 2-4 was classified as positive on the amyloid PET-test.

Sensitivity was 95% and specificity was 100%. Negative predictive values for amyloid PET based on data from Clark et al., (2011) was 94% and PPV was 100%. Caution should be taken as the N is quite small. In summary, the NPV derived from Clark et al., (2011) are in good agreement with the NPV data observed using both CSF T-Tau and CSF Aβ42.

SAWP/CHMP question

The Applicant will need to clarify the plans of labelling at the time of the MAA. Is the Applicant suggesting that the use of their drug would be limited to patients that have a positive test in PET and/ or CSF biomarker and a diagnosis of the Mild or Moderate AD?

Applicant's position

The applicant's view that the biomarkers proposed for qualification are for the enrichment of clinical trials only and not as a diagnostic was stated. It was indicated that the clinical trial section of the SmPC (§ 5.1) will be expected to reflect the clinical diagnosis of the population studied as well as the use of biomarkers. The applicant expressed its' belief that biomarker testing on all patients with a clinical diagnosis to exclude a small fraction is likely to be too prescriptive and that the decision to test should be physician and patient/caregiver driven. However, it was concluded that a definitive position on the indication statement would be dependent on the benefit/risk profile of a given medication.

The SAWP raised the question of whether the applicant had any plans to do any clinical trial in a broader population, i.e., including biomarker positive and negative patients, to see if both populations could benefit from treatment as this would speak to generalizability of the data.

The applicant acknowledged the concern and replied that the current plan is to exclude subjects who do not meet the biomarker positive eligibility criterion. Furthermore, it was noted that the qualification procedure was for the use of the biomarkers for enrichment of clinical trials and not as an outcome measure.

In addition, the applicant was asked about the possibility of applying the CSF biomarkers in more than a dichotomous way (i.e., analysis of patients by varying degrees of biomarker positivity).

The applicant responded that the biomarkers are being used for clinical trial enrichment and not as an outcome measure or to predict which patients will respond to treatment. However, as the CSF biomarkers are continuous variables, application of more than two categories could be considered in the analysis and could provide additional information in terms of disease severity and/or extent of disease pathology.

SAWP/CHMP question

Can the applicant give standardization suggestions for both Biomarkers?

Applicant's position

The main points presented by the applicant to address this issue are summarised below:

CSF standardization:

- CSF biomarker standardization issues are the same as those already presented for the qualification of CSF for pre-dementia AD. The issues are well defined and are being addressed in consideration of pre-analytical, analytical and post-analytical methodologies.
- Best practices are being developed by the pre-competitive collaborations including the Alzheimer's Disease Neuroimaging Initiative (ADNI), the Alzheimer's Association Global Consortium for Biomarker Standardization and the AD Biomarker Standardization Initiative (ABSI), and will be applied.
- A position paper is planned to support implementation of best practice recommendations for CSF standardization.

PET amyloid imaging standardization:

- PET amyloid standardization issues related to image acquisition and analysis are well defined.
- Best practices are being developed by the manufacturers, academic community and sponsors of clinical studies, and will be applied.
- There is an important role for the core imaging laboratory to address issues of quality control, rater training and analytical standardization. This will address consistency and reliability in the PET measures.

Discussion on CSF standardization

The SAWP enquired whether the applicant had any data on CSF samples stability over time.

- The applicant reported that there are very good 2-year data from the manufacturers and up to 5-year data from the key opinion leaders confirming that CSF samples (considering both Aβ and tau determination) are very stable over time. Short term test-retest data are also widely available and consistent with long-term stability data.

A question on the cut points was raised by the SAWP as to what was meant by defining cut points for a specific "intended purpose" and whether these cut points will be the same for the pre-symptomatic stage as for MtM AD.

- The applicant informed SAWP that the cut points may be different at different stages of disease and that the samples required to derive them will be specific for the population specified in the intended use. In addition, the applicant confirmed that once cut points are set, they will be held constant within the trial.

Discussion on PET standardization

The SAWP asked whether the applicant was envisaging the core imaging laboratory doing the rating of all the images or doing only QC rating, and whether the data to be presented in an MAA will therefore come only from the core imaging laboratory or also from all the sites.

- The applicant clarified that the data from all sites will be transmitted to the core imaging laboratory, which will do the rating of all the scans so that, in the end, all the study data will come from the core laboratory.
- Nevertheless, the applicant cited a very recent study sponsored by Avid Radiopharmaceuticals showing that an on-line training of previously PET amyloid imaging-naive nuclear medicine physicians can successfully ensure appropriate rating at the individual sites.

The SAWP asked if there are conditions that could be associated with a scan which was atypical for PET amyloid, notably a scan with a single positive region or other distribution pattern atypical for AD.

- The applicant responded that single areas or atypical distribution patterns do occur, although infrequently, and subjects with such patterns could still meet the criteria for study inclusion as demonstrating amyloid positivity. (The applicant further noted that all patients would have previously received a clinical assessment and diagnosis and that the PET scan was being used for clinical trial enrichment). Analysis could be undertaken with individuals having such atypical patterns.

With regards to a specific question on Down's Syndrome, the applicant clarified that Down's Syndrome will be clinically excluded from the study although recognising that these subjects, as they age, will develop an amyloid positive pattern similar to that of the AD. This led to a broader discussion of the need to interpret amyloid PET within the clinical context.

CHMP opinion

CSF biomarker signature

- CSF biomarker signature based on a low Aβ1-42 and a high T-tau qualifies to identify patients with clinical diagnosis of mild to moderate AD who are at increased risk to have an underlying AD neuropathology, for the purposes of enriching a clinical trial population.
- Collection, handling and measurements of all CSF samples should be performed according to Good Clinical Practice and to the specific international standards for these measurements.
- The concurrent assessment of other qualified biomarkers in mild to moderate AD would be highly desirable and of greatest value.
- CSF biomarker signature based on a low Aβ1-42 and a high T-tau is not qualified as diagnostic tool or outcome or longitudinal measure.

PET biomarker signature

- Amyloid related positive/negative PET signal qualifies to identify patients with clinical diagnosis of mild to moderate AD who are at increased risk to have an underlying AD neuropathology, for the purposes of enriching a clinical trial population.
- Collection, handling and measurements of all PET signals should be performed according to Good Clinical Practice and to the specific highest international standards for these measurements.
- The concurrent assessment of other qualified biomarkers in mild to moderate AD would be highly desirable and of greatest value.
- Amyloid related positive/negative PET is not qualified as diagnostic tool or outcome or longitudinal measure.

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