2015

Revolutionizing Alzheimer's disease and clinical trials through biomarkers

Niklas Mattsson
*Lund University*

Maria C. Carrillo
*Alzheimer's Association*

Robert A. Dean
*Eli Lilly & Co., Inc.*

Michael D. Devous Sr.
*Avid Radiopharmaceuticals*

Tania Nikolcheva
*F. Hoffman-La Roche*

*See next page for additional authors*

Follow this and additional works at: [https://digitalcommons.wustl.edu/open_access_pubs](https://digitalcommons.wustl.edu/open_access_pubs)

**Recommended Citation**

Mattsson, Niklas; Carrillo, Maria C.; Dean, Robert A.; Devous, Michael D. Sr.; Nikolcheva, Tania; Pesini, Pedro; Salter, Hugh; Potter, William Z.; Sperling, Reisa S.; Bateman, Randall J.; Bain, Lisa J.; and Liu, Enchi, "Revolutionizing Alzheimer's disease and clinical trials through biomarkers." *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring. 1,4. 412-419. (2015).*

[https://digitalcommons.wustl.edu/open_access_pubs/4775](https://digitalcommons.wustl.edu/open_access_pubs/4775)
Authors
Niklas Mattsson, Maria C. Carrillo, Robert A. Dean, Michael D. Devous Sr., Tania Nikolcheva, Pedro Pesini, Hugh Salter, William Z. Potter, Reisa S. Sperling, Randall J. Bateman, Lisa J. Bain, and Enchi Liu
Abstract

The Alzheimer’s Association’s Research Roundtable met in May 2014 to explore recent progress in developing biomarkers to improve understanding of disease pathogenesis and expedite drug development. Although existing biomarkers have proved extremely useful for enrichment of subjects in clinical trials, there is a clear need to develop novel biomarkers that are minimally invasive and that more broadly characterize underlying pathogenic mechanisms, including neurodegeneration, neuroinflammation, and synaptic dysfunction. These may include blood-based assays and new neuropsychological testing protocols, as well as novel ligands for positron emission tomography imaging, and advanced magnetic resonance imaging methodologies. In addition, there is a need for biomarkers that can serve as theragnostic markers of response to treatment. Standardization remains a challenge, although international consortia have made substantial progress in this area and provide lessons for future standardization efforts.

Keywords: Alzheimer’s disease; Biomarkers; Amyloid; Tau; PET; Imaging; MRI; Cerebrospinal fluid; CSF; Blood biomarkers; Clinical trials

1. Introduction

A biomarker is a characteristic that can be objectively measured and evaluated as an indicator of a normal or pathologic process, or as a measure of response to therapy [1] (Biomarker Working Group 2001). Biomarker research has revolutionized the understanding of Alzheimer’s disease (AD) and is in the process of transforming the design of AD clinical trials. Until recently, AD was only imprecisely diagnosed in life using clinical assessments during the dementia stage or at time of death by neuropathology. Nonetheless, substantial progress over the past decades in
developing cerebrospinal fluid (CSF) and imaging biomarkers has shown that AD brain changes can be detected and used for diagnosis and prognosis of AD [2,3].

As these biomarkers have been included in observational studies of AD, better understanding of the biochemical and pathologic changes of AD has occurred. This has led to confirmation of the hypothesis [4,5] that AD is a disease progressing from preclinical to early and then late clinical stages, and which is now emphasized in novel research diagnostic criteria incorporating biomarkers [6]. Previously, drug developers focused on the dementia stage of the disease. This has now radically changed as clinical trials move toward earlier stages of AD, before extensive neurodegeneration has occurred [7–9], and even to secondary prevention before symptom onset [10–12], when disease-modifying treatments are likely to have maximal effect. Biomarkers play a key role in the design of these trials, both for inclusion of subjects with AD pathology and to track biological effects of drugs. Yet even though it is a widely held belief that AD biomarkers can be used for diagnosis, prognosis/prediction, and to monitor the effects of therapy [1,13], in the absence of an effective treatment to slow progression of AD (and the underlying pathogenic processes), the link between biomarkers and effect on disease cannot be established.

Data from many studies all over the world, including the Alzheimer’s Disease Neuroimaging Initiative [14], its worldwide partners (WW-ADNI) [15], and the Dominantly Inherited Alzheimer’s Network (DIAN) [16], have done much to delineate the temporal changes in biomarkers over time and clarify their relationship to cognition and function. Yet despite the field’s growing acceptance of the need for biomarkers in drug development, the belief that biomarkers could improve clinical trial design and the success of those trials was shaken by recent mixed clinical trial results. The phase III bapineuzumab trial, in particular, went of those trials was shaken by recent mixed clinical trial re-

2. Biomarkers as enrichment tools for clinical trials

Further critical examination of the bapineuzumab and solanezumab studies suggested several possible reasons for the negative trial results. One contributing factor is that some of the enrolled trial subjects may not have AD [21]. Clinical criteria for patient inclusion in each program resulted in study populations with a significant percentage of participants without evidence of brain amyloid by positron emission tomography (PET; ~7 and 36% amyloid negative in apolipoprotein E (APOE) ε4 carriers and noncarriers, respectively) [22]. Using amyloid biomarkers to enrich for trial subjects who are amyloid-positive—and thus presumably on the AD trajectory—may improve the ability of future trials to detect a treatment effect especially for anti-amyloid therapies. Indeed, data from several studies have shown that among cognitively normal elderly, those who are amyloid-positive are at greater risk of decline compared with those who are amyloid-negative [6,23–26]. In the placebo arms of both the bapineuzumab and solanezumab studies, which enrolled subjects with mild-to-moderate AD, amyloid-positive subjects had significant decline on both cognitive and functional measures, whereas the amyloid-negative subjects did not [22]. Importantly, the effect of amyloid pathology on longitudinal memory decline may be greater in APOE ε4 carriers compared with APOE ε4-noncarriers [27].

Disease severity may be another factor that contributed to the negative trial results. In comparison with subjects with mild disease, those with more advanced clinical disease may have far more advanced neurodegeneration. The modest impact of treatment on the underlying pathology and markers of the pathology may not be sufficient to translate to a clinical benefit. In the bapineuzumab studies, even individuals with the largest reported decrease in amyloid still had elevated values in the AD range and although significant treatment differences were observed between bapineuzumab and placebo, the change from baseline values in the bapineuzumab groups ranged ~0%–10% (with reductions in the CSF P-tau concentration and inhibited further accumulation of brain amyloid by PET) [17–19]. Finally, it is possible that the presence of copathologies (for example tau, vascular, Lewy body, or transactive response DNA binding protein 43 [TDP-43] pathology) may influence cognitive trajectories independent of amyloid pathology [28,29] and impact trial results.

Many trials currently underway or planned are therefore enrolling subjects in earlier stages of disease and using amyloid biomarkers, either amyloid PET imaging or CSF Aβ42 levels, to enrich for trial subjects thought more likely to
benefit from therapy, as reported at this Roundtable (Table 1). Only the TOMMORROW trial of the mitochondrial targeting agent pioglitazone (an approved antidiabetic prescription drug) uses a genetic enrichment strategy based on TOMM40 and APOE genotype and age to identify normal individuals at risk of developing mild cognitive impairment over a 5-year period.

Several considerations are raised with the use of amyloid PET imaging or CSF as an inclusion criterion to be addressed for trials of anti-amyloid therapies:

- How practical is it to require amyloid positivity by PET or CSF as an inclusion criterion in a large (global) trial?
- Is there an advantage of one amyloid test over the other (CSF vs. PET)?
- Is it possible to establish standard cutoffs for PET or CSF amyloid assessments to differentiate between normal and pathologic state?
- Will standard cutoffs differ based on the stage of disease, for example as discussed in Lim et al. (2015) [30]?
- Are quantitative PET reads required, or is a visual read sufficient?
- Are the different radiotracers interchangeable, or is it necessary for all screens to be performed using a single radiotracer?
- Are different CSF assays interchangeable? Can novel development of reference standard procedures and materials overcome variability problems?
- How can between-site variability for PET scans or CSF biomarker measurements be overcome?
- If a subject has had a previous amyloid biomarker measurement that indicates amyloid-positivity, can this historical data be used to satisfy the inclusion criterion?

- What are the labeling implications of having amyloid positivity as an eligibility criterion? Will the amyloid tests be considered companion diagnostics?

### 3. Supporting a disease modification claim with biomarkers

In the US Food and Drug Administration (FDA) draft guidance on developing drugs for early stage disease, disease modification is defined as a “direct effect on the underlying disease pathophysiology.” The guidance goes on to consider the possibility “that a claim of disease modification could be supported by evidence of a meaningful effect on a biomarker in combination with a clinical benefit” [31]. In its Guideline on Medicinal Products for the Treatment of Alzheimer’s Disease and Other Dementias, the European Medicines Agency defines disease modification as “slowing or arrest of symptom progression of the dementing process,” and like FDA, suggests that “Ideally proof of a disease modifying effect would require demonstration of clinically relevant changes... [and] supportive evidence for a change in the underlying disease process based on biological markers,” [32].

Based on research from the past several decades and more recent data from larger observational studies such as ADNI, Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL), and DIAN, a consensus is emerging that AD begins decades before dementia onset [5,33] and is heralded by successive changes in markers of the underlying disease processes [4,34]. Several biomarkers are known to be relevant in AD and have been studied intensely [35–38]. Hippocampal atrophy rates show high correlation with cognitive change [39]. However, by its very nature, magnetic resonance imaging (MRI) does not

### Table 1

<table>
<thead>
<tr>
<th>Phase/duration</th>
<th>Population</th>
<th>Sample Size</th>
<th>Regions</th>
<th>Primary Biomarker Screen</th>
<th>Takeda (TOMMORROW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3 y</td>
<td>Amyloid+; 65–85 yo; normal cognition</td>
<td>1000+</td>
<td>USA, Canada, Australia</td>
<td>Amyloid PET (Amyvid)</td>
<td>None</td>
</tr>
<tr>
<td>2.0 y</td>
<td>Amyloid+; 55–85 yo; mild and pAD</td>
<td>1500+</td>
<td>Global</td>
<td>Amyloid PET or CSF Aβ42</td>
<td>Amyloid Aβ42</td>
</tr>
<tr>
<td>Ph1b/2.0 y</td>
<td>Ph2b/1.5 y</td>
<td>~160</td>
<td>USA, North America, EU</td>
<td>Amyloid PET (Amyvid)</td>
<td>CSF Aβ42</td>
</tr>
<tr>
<td>Ph2b/1.5 y</td>
<td>Amyloid+; mild and pAD</td>
<td>Adaptive; up to 800</td>
<td>North America, EU, Japan</td>
<td>Amyloid PET (Amyvid) or CSF Aβ42</td>
<td>rs10524523, APOE</td>
</tr>
<tr>
<td>Ph3/1.5 y</td>
<td>Amyloid+; early AD (mild and pAD)</td>
<td>2100</td>
<td>Global</td>
<td>None</td>
<td>age (Risk algorithm)</td>
</tr>
<tr>
<td>Ph3/1.5 y</td>
<td>Amyloid+; mild AD</td>
<td>1960</td>
<td>Global</td>
<td>Amyloid PET (Vizamyl)</td>
<td></td>
</tr>
<tr>
<td>Ph3/2.0 y</td>
<td>55–85 yo; MtM AD</td>
<td>1500</td>
<td>Global</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ph3/2.0 y</td>
<td>Amyloid+; 50–85 yo; pAD</td>
<td>(1) 700; (2) 1000</td>
<td>Global</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ph3/2.0 y</td>
<td>Amyloid+; (1) pAD</td>
<td>3800</td>
<td>North America, EU, Russia, Australia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer’s disease; MCI, mild cognitive impairment; PET, positron emission tomography; CSF, cerebrospinal fluid; y, years; yo, years old; pAD, prodromal AD; MtM, mild-to-moderate.
measure a single molecular entity, thus atrophy could reflect not only neuron loss but other neurodegeneration-associated events including loss of axonal tracts/dendrites, loss of other cell types (e.g. astrocytes, microglia), inflammatory processes, and associated alterations of interstitial fluid volume [37,40].

Other available biomarkers that reflect pathogenic processes of the disease include CSF levels of total tau (T-tau) and P-tau, which are related to cortical axonal degeneration and tangle pathology [41,42]; CSF Aβ42 and amyloid PET imaging, which correlate negatively and positively, respectively, with amyloid plaque load at autopsy [43]; and fludeoxyglucose (FDG)-PET, a measure of glucose metabolism, which may partly reflect synaptic dysfunction [44].

However, even if a biomarker has been shown to be relevant to AD, its utility as a theragnostic biomarker is not guaranteed. For example, although biomarkers such as CSF T-tau/P-tau and volumetric MRI are clearly affected in observational studies of AD, the effect of a disease-modifying therapy on these biomarkers may be influenced by multiple factors, such as the mechanism of action of the therapeutic or related disease processes. Thus, each candidate biomarker must be evaluated empirically for response to therapy.

For example, although both bapinezumab and solanezumab are monoclonal antibodies against the Aβ42 peptide, there were differences in which AD relevant biomarkers responded to treatment. These differences may be due to differences in binding properties of the antibodies, i.e., soluble versus aggregated amyloid [45–47], and illustrate the fact that not all AD relevant biomarkers may serve as theragnostic biomarkers in all trials and that multiple factors will influence their utility. An additional point is that the observed biomarker changes in response to therapy were small to modest and not accompanied by a slowing of decline in cognition and, thus, do not support a disease-modifying effect. To date, it is unknown to what degree treatment-induced change in a biomarker would be sufficient to test the underlying therapeutic hypothesis. Thus, it is premature to judge the theragnostic value of any biomarker that has, to date, only been modestly perturbed by any treatment.

Furthermore, differentiation between utility as biomarkers of target engagement versus theragnostic response may be needed. For an anti-amyloid treatment, an amyloid marker may be used to assess target engagement, but use of a downstream neurodegenerative marker, such as a marker of tau biology, may be required for therapeutic monitoring. Similarly, for a therapeutic agent targeting tau pathology, measurement of CSF tau levels or tau PET imaging might prove useful for trial enrichment and/or target engagement, but other markers might be required to characterize treatment effect on the downstream pathology. Thus, novel biomarkers need to be discovered and/or developed that can characterize other aspects of the neurodegenerative process. Importantly, when analyzing biomarkers, whether new or old, longitudinal studies are needed that provide evidence of change over time.

4. Standardization

For biomarkers to be used in clinical trials, standardization of sample and data collection protocols and analytical methods is essential. Particularly, the ability to combine data across time and study sites is crucial. Perhaps more importantly, the ability to reduce or control variability introduced by the measurement methodology improves the potential to detect and measure biological changes resulting from therapeutic intervention. Standardization can promote comparison across studies and ease the transition from research use to use in a regulatory environment (pivotal clinical trials) and in clinical practice. Here, we will review standardization efforts for CSF and imaging biomarkers.

Currently there are several commercially available assays to measure CSF biomarkers (Aβ42, P-tau, and T-tau), some of which have Conformité Européenne (CE)-marking in Europe, but none of which have been cleared by FDA. Although performance of the different assays to differentiate between normal and pathologic is similar, the absolute values reported by them for any particular analyte are different [48]. There are several different possible sources of preanalytical and analytical variability of CSF biomarker measurements [49]. Standardization efforts for CSF biomarkers to address preanalytical and analytical issues have been initiated by the Alzheimer’s Association (AA) under the Global Biomarkers Standardization Consortium [50]. To date, the lack of standardization across available assays has required use of assay/laboratory-specific cut points to convert numerical data from each method into clinically meaningful categorical results. The standardization programs described previously aim to enable a universally acceptable cut point to differentiate normal from pathologic states using CSF biomarker results generated across time, assays, and laboratories.

Quantitative analyses from amyloid PET images acquired from multicenter studies require standardization of protocols to reduce measurement variability and error with attention and emphasis on different aspects when conducting cross-sectional or longitudinal comparisons. Standardization efforts involve three main areas of focus: scan acquisition, image processing, and image analyses. A comprehensive review of these topics is recently detailed [51]. With the introduction of multiple tracers, each of which with slightly different characteristics, comparison of results across studies has become even more challenging. The Centiloid Project proposes to calibrate all amyloid imaging tracers according to a unified and standardized numerical scale [52] and would theoretically allow results from studies using different tracers to be combined and compared.

Standardization of methods for structural MRI measures of hippocampal volume has been undertaken by the European Alzheimer’s Disease Consortium (EADC)-ADNI Hippocampal Harmonization Protocol project, with funding from the AA and six medical device, diagnostics, and pharmaceutical companies. This project has developed a harmonized protocol for estimating hippocampal volume through
manual segmentation [53], which addresses issues relating to acquisition of images (e.g., different manufacturers, sequences, positioning of the patient), variability in algorithms, readers, and patient characteristics.

5. Pattern and time course of biomarker changes in AD

The hypothetical model of dynamic biomarkers proposed by Jack et al. [4], in which a gradual, ordered, and successive alteration in AD biomarkers, beginning with amyloid pathology, then tauopathy, and later neurodegeneration, preceding clinical symptoms, and eventual dementia, recapitulates the key tenets of the amyloid cascade hypothesis [54,55]. Although recent data support many aspects of this model [2,16,26,56–58], some do not and the model has been updated accordingly [34]. The main impetus for the change is due to the evidence that medial temporal lobe tau pathology occurs in normal aging independent of amyloid [59,60]. Furthermore, data support that amyloidosis and neurodegeneration (tauopathy) may initiate independently but that abnormal (high levels) amyloid and tau interact with amyloid accelerating the downstream neurodegeneration [61]. Thus, although the model provides a framework for the general trend of biomarker changes at a population level, more work is needed to understand possible individual differences in the ordering of biomarker changes [34].

6. Novel biomarkers

Roundtable participants agree on the need for additional novel biomarkers for other aspects of AD pathophysiology, including neuroinflammation, neurodegeneration, synaptic dysfunction, and other associated neuropathologies (e.g., α-synuclein) and comorbid conditions. Although CSF biomarkers provide the most direct biochemical access to the brain with few confounds from other organs [62], blood-based biomarkers are desired for large-scale screening. Novel imaging biomarkers, including tau imaging [63–67], advanced MRI methodologies [68,69], and new neuropsychological testing protocols, have also shown promise in providing a more comprehensive picture of the progression of dementing diseases [70–73].

A number of novel CSF biomarkers have been identified for staging and potentially tracking AD, including visinlike protein (VILIP)-1 [74,75], YKL-40 [76] and heart-type fatty acid binding protein (HFABP) [77], among others. Novel biomarkers may also be helpful in differentiating AD patients with copathologies.

Ideally, to enrich studies for subjects who are likely to have AD pathology, low cost and minimally invasive biomarkers optimized for sensitivity may be part of a screening funnel that can reduce the number of subjects to be evaluated by a more costly/invasive amyloid test (PET or CSF). Blood-based assays are currently in development for a variety of analytes, including various species of Aβ and tau, α-synuclein, and TDP-43. Furthermore, proteomic studies have identified hundreds of potential markers of inflammation, oxidative stress, mitochondrial dysfunction, neuronal and microvascular injury, and metabolic processes. Investigators have constructed several multianalyte panels to detect, classify, and predict disease progression, although these findings require replication [78–80]. Other approaches such as microRNA and plasma exosome analyses are also being investigated for utility in identifying individuals likely to have AD pathology [81–83]. An international working group of experts in the field has been formed to address the challenges that have hampered development of blood-based biomarkers [84].

7. Conclusion and steps forward

The Roundtable concluded that more longitudinal studies on biomarker trajectories, specifically evaluating change in individuals, are needed to confirm or modify the findings of cross-sectional studies that have dominated the field to this point. In addition, there was widespread support for increased effort and focus on studies linking neuropathology to biomarkers, and a suggestion that additional public-private funding may be needed to achieve this. Specifically, it would be valuable to assess and compare postmortem samples from patients involved in different clinical trials. Finally, novel biomarkers that reflect other disease processes downstream of the initiating AD pathologies would not only increase our understanding of AD but can potentially provide needed tools for next-generation AD therapies in development.

**RESEARCH IN CONTEXT**

1. Systematic review: There have been many reviews on Alzheimer’s disease (AD) biomarkers in the last 5 years, but this article is unique that the Alzheimer’s Association Research Roundtable provides an unparalleled forum in which the leading experts on Alzheimer’s disease from pharmaceutical industry, nonprofit organizations, and governmental organizations can collaborate on moving the field forward by identifying areas of research that are most critical to achieve a successful next generation AD therapeutic.

2. Interpretation: This article summarizes the foremost areas of work in the view of industry drug developers to achieve a successful next generation AD therapeutic.

3. Future directions: Continued work in the Alzheimer’s Association Research Roundtable (AARR) precompetitive space will allow optimization of resource use across different sectors.
References


Buchhave P, Minthorn L, Zetterberg H, Wallin AK, Blennow K, Hansson O. Cerebrospinal fluid levels of beta-amyloid 1–42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. Arch Gen Psychiatry 2012;69:98–106.


