Measuring Target Effect of Proposed Disease-Modifying Therapies in Alzheimer's Disease

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Summary: Alzheimer's disease (AD) is the most common cause of dementia and is an increasing public health problem. Because of the severity and increasing prevalence of the disease in the population, it is urgent that better treatments be developed. Active research efforts over the past several decades have produced a vast knowledge base regarding AD natural history, pathology, and key biological mediators involved in pathogenesis. As knowledge of the biomolecular mechanisms of AD has increased over the past several decades, there has been a growing consensus on the pathophysiology of the disease. These scientific advancements have led to proposals for disease-modifying therapeutic interventions that promise to significantly alter the course of AD. The translation from preclinical models to human studies requires therapeutic biomarkers to increase the likelihood of success. This review covers the current methods and technologies used in the therapeutic translation of proposed disease-modifying therapies for AD.
Key Words: Alzheimer's disease, Alzheimer, treatment, biomarker, amyloid, amyloid-β, CSF, cerebrospinal fluid, therapeutic, kinetics, production, clearance, PET, PiB.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia and is an increasing public health problem. It is currently estimated to afflict 5 million people in the United States, with an expected increase to 13 million by the year 2050.[1] Alzheimer's disease leads to loss of memory, cognitive function, and ultimately independence, and so it takes a heavy personal and financial toll on the patient and the family. Because of the severity and increasing prevalence of the disease in the population, it is urgent that better treatments be developed.

Active research efforts over the past several decades have produced a vast knowledge base regarding AD natural history, pathology, and key biological mediators involved in pathogenesis. As knowledge of the biomolecular mechanisms of AD has increased over the past several decades, there has been a growing consensus on the pathophysiology of the disease. These scientific advancements have led to proposals for disease-modifying therapeutic interventions, which promise to significantly alter the course of AD.

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AMYLOID HYPOTHESIS

The most widely accepted hypothesis for the etiologic cause of AD is the amyloid hypothesis.[2] This scientific theory, based on multiple lines of evidence, proposes that amyloid-β (Aβ) in one or more toxic forms causes a cascade of events that are toxic to neurons and cause progressive dementia of the Alzheimer's type. Some of the earliest evidence for the amyloid hypothesis came when Aβ was sequenced from the extracellular deposits in amyloid plaques and cerebral amyloid angiopathy.[3]

Although the precise pathogenic form of Aβ is not yet known, it is clear that alteration of Aβ metabolism is necessary and sufficient to cause certain autosomal dominant forms of AD.[4-6] Mutations in one of three genes have been identified in familial AD and affect the production, ratio of species, or biochemical properties of Aβ. Mutations in either presenilin 1 or presenilin 2, which are components of γ-secretase, lead to alterations in the production of the $A\beta_{42}/A\beta_{40}$ ratio.[6] Mutations in the amyloid precursor protein gene (*APP*)[5] can affect the production of Aβ or its propensity to aggregate. Individuals with trisomy 21 (with an extra copy of *APP*) invari-

ably develop Aβ deposits by their 40s, and progressive AD. An extra copy of the *APP* locus is sufficient to cause AD in individuals without complete trisomy 21.[7] These observations provide evidence in humans that increased dosage or misprocessing of Aβ is sufficient to cause AD. Autosomal dominant AD mutations have been used in creating animal models of AD that recapitulate much of the amyloid pathology seen in the human form of the disease.[8,9] Models using multiple transgenes (including human tau) incorporate tau pathology and progressive neurobehavioral decline.[10-13]

Toxic species of Aβ

The exact species of Aβ that contributes to neurodegeneration and progressive dementia is still not clearly defined and is discussed elsewhere.[14-19] However, it is likely that several forms of Aβ contribute to the pathophysiology of AD, because Aβ plaques show clear neuritic dystrophy and surrounding inflammation,[20-22] and various oligomeric species interfere with long-term potentiation.[23,24] An important concept is that various forms of Aβ are in dynamic equilibrium, and interconversion between less toxic and more toxic forms is likely a continuous phenomenon. The insoluble pool of Aβ is typically 100-fold larger than the soluble pool, so any changes in the concentration of Aβ monomers or oligomers may be readily buffered by equilibrium with the insoluble pool. Given that the ultimate target of AD treatment is the toxic form of Aβ, a way to measure the amyloid plaques and oligomer species in the brains of humans is of critical importance. In 2004, collaborating groups from Uppsala and Pittsburgh first reported a breakthrough of imaging amyloid in living humans using positron emission tomography (PET) and the radiotracer that has come to be known as Pittsburgh Compound B or PiB.[25] This imaging technique has been validated in human AD brain and has received extensive interest from academic centers, which are now using this imaging for advancing the understanding of AD.[26,27]

Aβ as a therapeutic target

Because of the central role of Aβ in AD, intensive research has focused on the physiology and pathophysiology of Aβ in cell culture systems, animal models, and humans. Therapeutic developments aimed at modifying disease progression have primarily targeted Aβ in one of four ways: decreased production, increased degradation, increased clearance, or inhibiting aggregation or toxicity to neurons in the central nervous system (CNS). Many of the proposed pathways for disease modification in AD are described in detail elsewhere in this issue. In addition, there are excellent reviews by others that focus on proposed treatments.[28,29] The present review focuses on methods to measure responses to proposed disease-modifying therapies that target Aβ. Principles discussed here may be applied to other targets in the CNS.

Production and clearance of Aβ

Within the CNS, Aβ is produced mostly by neurons as a normal physiologic process.[30] Recent evidence indicates production of Aβ is related to neuronal activity.[31-33] Aβ is cut from the transmembrane protein APP by β-secretase and then γ-secretase enzymes and is released into the extracellular space. Extracellular Aβ is normally degraded or cleared out of the CNS, maintaining a homeostatic balance. In AD, however, Aβ levels in the brain increase 100- to 1000-fold normal levels. Increased production or decreased clearance may cause this massive increase in Aβ, but the contribution of each in AD pathogenesis is still not known.[34,35]

In autosomal dominant AD, mutations result in Aβ dysregulation and subsequent AD pathology similar to that seen in the more common late-onset AD.[36] However, the regional distribution of amyloid early in the course of some forms of autosomal dominant AD is often atypical.[37] Several animal models of Aβ deposition are based on the human AD mutations, and the pathology observed in these transgenic mice appears to be due primarily to Aβ deposition, including neuritic plaques, cerebral amyloid angiopathy, microglial activation, astrocytosis, evidence of oxidative damage, and changes in neuronal cytoskeletal proteins.[38] A study of Aβ clearance in a mouse model of AD demonstrated that the elimination half-life of Aβ in the brain interstitial fluid is 2 h in mice without AD pathology and 4 h in mice with AD pathology.[39] The altered CNS Aβ clearance rate was associated with the presence of amyloid plaques.

The levels of Aβ in the CSF reflect the balance of production in the extracellular space of the brain and clearance from the brain and CSF compartment. In diagnostic studies of AD, the level of CSF $A\beta_{42}$ is significantly decreased in AD but the level of CSF $A\beta_{40}$ is unchanged.[40-42] CSF Aβ is transported to the venous blood via the arachnoid granulations. To a lesser degree, plasma $A\beta_{40}$ and $A\beta_{42}$ have been shown in some studies to increase with age and in early AD; however, both may decrease with advancing AD, suggesting that advanced AD may alter the clearance of Aβ from the brain to the blood *in vivo*.[43]

The relative contributions of CNS Aβ clearance mechanisms in humans are not known. These mechanisms include breakdown within the CNS, transport from the brain to the blood directly (via the blood–brain barrier [BBB]), and transport from the brain to the CSF and then to the blood. Understanding human CNS Aβ production, transport, and clearance kinetics by determining the production and clearance rates of Aβ in the CNS and in blood is likely to provide novel insights into Aβ physiology and potentially the pathophysiology that leads to AD.

Measurement of Aβ production and clearance in humans

To address critical questions regarding Aβ metabolism, a method was developed that measures Aβ fractional production rate and clearance rate *in vivo* in humans. Results indicate that by administering a stable isotope labeled amino acid ($^{13}C_6$-leucine) and using high-resolution mass spectrometry, reproducible kinetics of Aβ production and clearance can be quantified in normal human research participants (FIG. 1).[44] Using this protein stable isotope labeling kinetics (SILK) technique, it has been shown that Aβ has a rapid production and clearance rate of ~8% per hour in normal control participants aged 20 to 50 years.[45] Ongoing studies are investigating how these rates may be altered by aging or presence of AD.

Aβ plaque formation

The exact mechanism of Aβ deposition in plaques is not well understood. It is clear that an increase of the longer, 42 amino acid form of Aβ ultimately leads to plaque formation.[46] However, basic questions such as whether plaques represent a central nidus of concentrated Aβ originating from a local cellular source that is diffusing outward or a convergence of extracellular Aβ condensing toward a central core remain unanswered. Two-photon microscopic studies in transgenic mice have provided some of the most revealing insights into the process of plaque formation in these mouse models, and perhaps in humans. Recent evidence has shown that plaques can form surprisingly rapidly, within 24 h, in transgenic mice and that these plaques appear to quickly stabilize in size and do not grow further.[47] This finding would seem to favor the notion that plaque formation results from a paroxysmal event, but the nature of that event is unknown.

In humans, plaque formation may be more complex. There are several histological types of plaques, and the relationship among the various forms is not clear.[48] It is commonly believed that diffuse plaques (i.e., plaques lacking a dense central core) may be an early stage that later evolves into compact, cored plaques, but this has not been proven. Dystrophic neurites, swollen with hyperphosphorylated tau, rarely associate with diffuse plaques, but frequently interdigitate in and surround cored plaques, forming the so-called neuritic plaque. A halo of astrocytic–microglial response and neuritic and axonal dystrophy radiates from the plaque core, suggesting the existence of a gradient of a toxic, diffusible species, perhaps oligomeric Aβ in equilibrium with the much more abundant (100-fold) fibrillar Aβ that composes the cored and neuritic plaque.

Measurement of Aβ plaques in humans

Prior to the development of molecular probes that bind amyloid plaques, the only way for measurement of plaques was via biopsy or postmortem analysis of tissue. PiB, FDNNP, and other tracers have been developed to detect Aβ deposits in plaques and cerebrovascular amyloid. PiB PET, the most well studied to date, demonstrates good correlation to pathological findings.[49,50] Careful correlation of *in vivo* PiB retention with histology in autopsy samples from an AD patient who had been imaged with PiB prior to death showed that PiB binds to fibrillar Aβ (whether in diffuse-appearing or compact plaques or cerebrovascular amyloid), but not to tau in tangles.[50] PiB does not appear to detect Lewy bodies.[51] Fleecy, amorphous deposits of Aβ, composing the majority of cerebellar amyloid, also is not stained by PiB.[50]

THERAPY IDENTIFICATION

One can use advancements in measuring Aβ physiology in humans to measure effects of therapies that target Aβ. Traditional drug discovery centers on a model of screening compounds for therapeutic target hits in a high-throughput system that is typically enzyme- or cell-based. Identification of these compounds eventually leads to testing of drugs in animal models of AD. These preclinical studies evaluate the effectiveness of the treatment in blocking or reversing the pathology, as well as behavioral performance. After preclinical validation in one model, more extensive validation is recommended, including testing in other models and at multiple doses. After toxicology studies are completed in animal models, the program may advance to first in-human studies. These phase I studies are typically done for toxicology purposes, to determine clinical side effects, as well as pharmacokinetics. In this translation from preclinical to clinical trials, evidence of a pharmacodynamic effect in humans is highly recommended. Phase II studies in patients with AD further test safety, pharmacokinetics, and pharmacodynamic effects. These translational steps from preclinical models to human studies are not incremental and represent a leap in therapeutic testing from nonhuman animal models to clinical studies.

Preclinical *versus* clinical

There are many differences between preclinical studies and clinical studies that may account for variable responses to the same dose of the therapy. The differences between animal models and humans may be categorized as follows:

1) Difference in species, which includes different background genetic variability,[52] brain structure and function, differences in CNS cells including neurons and glia, groups of interacting proteins, and the dynamics of Aβ.
2) Differences in the pathogenesis and pathology of AD. Animal models are most similar to the early-

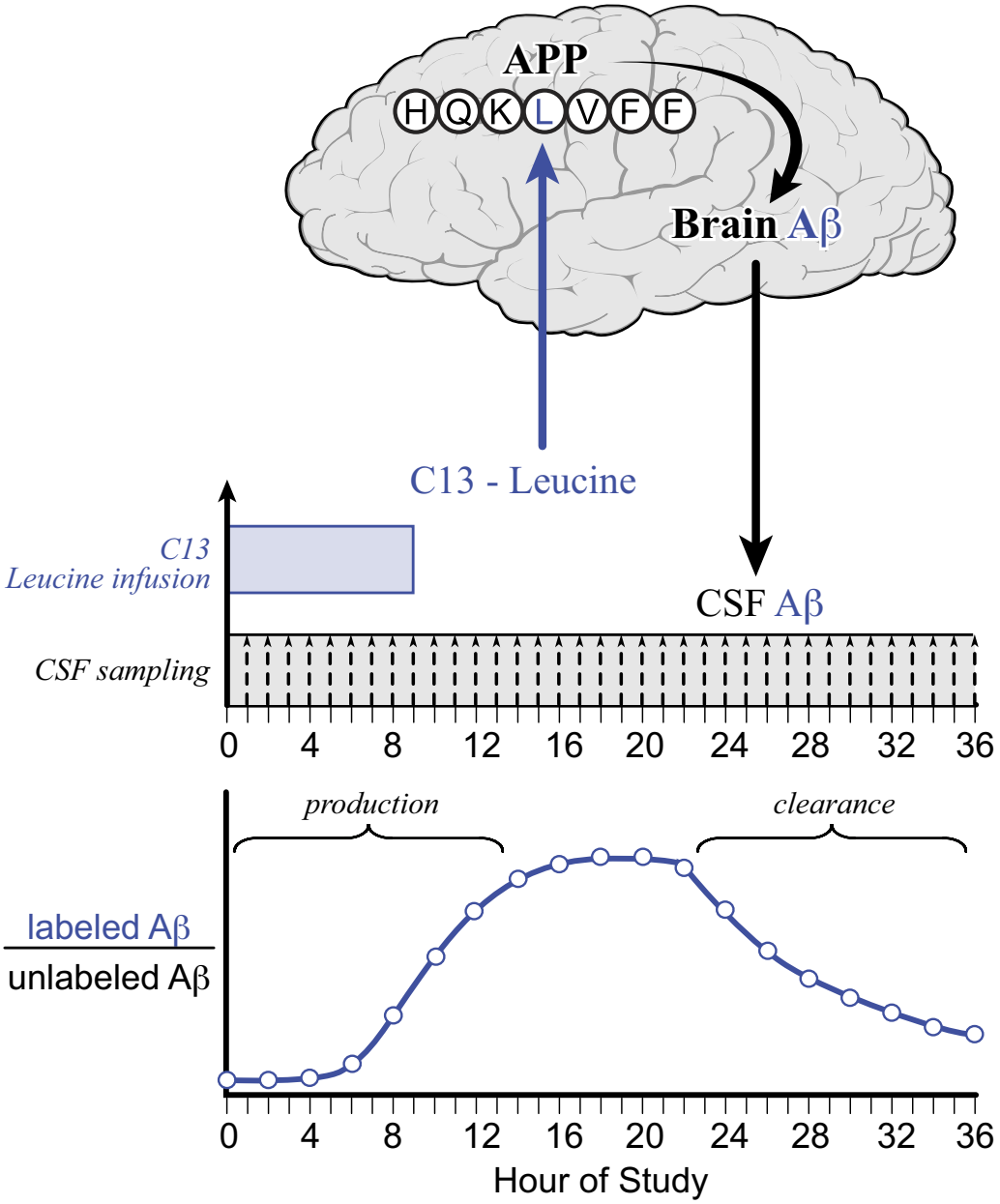

FIG. 1. Diagram of CNS protein stable isotope labeling kinetics (SILK). *Top:* Carbon-13 stable isotope label ($^{13}C_6$-leucine) is administered for 9 h, to label newly synthesized proteins as labeled amino acids are incorporated into proteins during synthesis; the partial amino acid sequence of APP/Aβ containing the labeled leucine (blue L, circled) is indicated. The Aβ is generated from APP (β- and γ-secretase) to the extracellular space of the brain, which is in direct communication with the CSF. *Bottom:* Hourly CSF samples are processed and analyzed to determine the relative amount of labeled Aβ at each hour time point. Production and clearance of Aβ from the CNS are calculated during and after labeling, respectively.

onset autosomal dominant form of AD, which comprises less than 1% of all AD, and its direct relation to the more common later-onset form of AD is unclear. It has been hypothesized that the common later-onset form of AD is primarily a defect in clearance and that the rare autosomal dominant form is primarily a defect in the overproduction of $A\beta_{42}$.[6,53-55] The pathology in animal models is not identical to in human disease; for example, Aβ plaques in mouse models do not bind amyloid imaging agents such as PiB in the same way as in humans.[26] This appears to be due to a basic difference in the structure of aggregated Aβ in mouse models. In transgenic mice, the frequency of PiB binding sites per molecule of Aβ is only 0.2% of the frequency in humans.[26] In addition, there are recent findings indicating that the most common form of AD (i.e., with PS1 mutations) may respond differentially to γ-secretase inhibitors,[56] compared with the PS1 wild type.

3) Differences in environment may influence Aβ metabolism in humans, compared with animal models. Diet,[57,58] medications,[59-61] and environmental enrichment[62-65] have been shown to influence Aβ metabolism.
4) Pharmacokinetics, the fourth category of difference between the clinical and preclinical studies, includes differences of medication compliance, administration, absorption, metabolism, excretion, and interaction with other drugs. In addition, there may be differential pharmacokinetics of the drug in the human CNS compared with the animal model CNS, due to differences in brain size, myelination, or transport mechanisms.

Because of such major differences, translation from preclinical models to clinical studies has a high rate of failure. It is therefore of critical importance that there be proper tests of the therapeutic mechanism in humans, with quantitative measurement of the therapeutic target. Proceeding forward with phase II or III studies without knowledge of whether the drug is hitting the target in humans is a potentially costly risk that may result in several years of lost progress, costs of unnecessary phase III studies, and erroneous or no conclusions at the end of clinical studies. It is critical, at early stages, to separately assess success at affecting the target and clinical response, because many pathophysiological issues remain unproven in AD. For example, it is not known for certain if clearance of Aβ will have a beneficial effect in mild-to-moderate AD. Therefore, full interpretation of a negative clinical study requires knowing whether or not the test drug affected brain Aβ but failed clinically, or whether it simply failed to have an effect on the target.

Therapeutic biomarkers of Aβ

For therapies that target Aβ, recent developments have allowed for highly quantitative and informative assays of production, clearance, and aggregation of Aβ within the human CNS. These developments are reviewed here, as well as their potential application for measuring the effect of proposed disease-modifying therapeutics that target Aβ. In addition to Aβ, other important pathways in the pathophysiology of AD may be targeted (e.g., tau, apolipoprotein E, or neuroinflammation). The principles discussed may be applied to those targets and other CNS targets, with the development of appropriate biomarkers.

Blood Aβ

Amyloid-β can be measured peripherally in the blood.[66-68] Blood measurements do not correlate with central brain Aβ levels or with CSF $A\beta_{42}$ levels, as evidenced by the poor individual predictive power of blood Aβ levels in distinguishing elderly controls from people with AD. This is likely due to several factors, including the very short half-life of Aβ in the blood compared with the central compartments and the BBB, as well as the unclear contribution of CNS Aβ to the blood. In AD, there may be a disconnect between the transport mechanisms from the CNS to the blood.[69]

CSF Aβ

Levels of Aβ in the CSF have been extensively tested as a diagnostic and prognostic biomarker for AD.[40,70-73] CSF levels of $A\beta_{42}$ are decreased by approximately one-half, relative to age-matched controls. There is now evidence that this decrease in $A\beta_{42}$ is indicative of CNS amyloid, as measured by PiB PET, regardless of the clinical state of the individual.[27]

CSF $A\beta_{42}$ is an important biomarker for AD and has been demonstrated to be approximately 80% to 90% sensitive and specific for AD.[27,42,74] Aβ levels change significantly over hours in younger normal participants,[75] but this normal pattern of variation may be disrupted in age or AD pathology. Aβ dynamics are specific. Many other proteins found in the CSF do not show similar fluctuations, including apolipoprotein E, albumin and total protein levels.[75] Multiple studies have demonstrated decreased and stable $A\beta_{42}$ CSF levels over time (months to years) only in patients with AD.[76,77] However, one study of patients with AD showed significantly greater variability in $A\beta_{40}$ levels,[76] and another showed increased variability in $A\beta_{1\text{-}42}$ in participants with subjective complaints[78] but not in patients with dementia of the Alzheimer type. The Aβ dynamics in the control group may lead to an increase in false positives if a control subject's Aβ levels decrease below the threshold for the diagnostic test.

The CSF is in direct communication with the extracellular space of the brain and appears to accurately reflect the levels of Aβ in the brain extracellular compartment, as well as the transport of Aβ. The exact reason for the decrease in CSF $A\beta_{42}$ while total brain $A\beta_{42}$ levels are increased is still under study. One hypothesis is that the Aβ plaques and cerebral amyloid angiopathy within the brain act as a sink for $A\beta_{42}$, so that it cannot be transported through the extracellular fluid to the CSF and eventually to the bloodstream.[79]

Prior measurements of CSF Aβ have been made in therapeutic trials.[80-83] Some studies indicate that certain treatments can result in a decrease in detectable Aβ, but others have not shown the predicted decrease. The choice of research participant population, sampling method, sample handling and storage, and analysis may all affect the measurements and should be accounted for in the design of studies. Because of significant fluctuation in CSF Aβ levels over hours,[75] there are concerns regarding the use of CSF Aβ levels to measure the effects of therapeutics. In contrast to static Aβ levels, Aβ production and clearance rates determined in SILK studies do

not have significant hourly fluctuations in the same samples. Measurements of CNS Aβ kinetics in the setting of therapies that decrease Aβ production or increase Aβ clearance may provide valuable information on the pharmacodynamic effects of these therapies.

Production and clearance of Aβ

The SILK methodology can be used to determine the effect of a drug on production, clearance, or the CNS soluble pool of Aβ. A recently completed study using a γ-secretase inhibitor suggests that measurement of newly generated Aβ in the CSF can demonstrate a significant dose–response relationship with five subjects in each group (unpublished data [in preparation]). Studies may be designed to measure the production and clearance of Aβ between groups. Results of a drug effect in humans may be obtained in an acute dose study, obviating the need for chronic administration studies to determine proof of action of the therapy. β-Secretase and γ-secretase inhibitors, which have the potential to decrease newly generated CNS Aβ, can be tested and their effects quantified in placebo-controlled studies using SILK. Similarly, Aβ clearance can be measured with a putative clearance-enhancing drug. Increased clearance of soluble Aβ caused by transport out of the CNS or breakdown within the CNS will be manifested as a decreased clearance rate in a CNS SILK study.

CNS amyloid imaging

To date, nine amyloid imaging agents have been tried in human studies.[84-92] Of these, only PiB and FDDNP have undergone extensive evaluation in AD and mild cognitive impairment (MCI). All of these agents appear to target fibrillary Aβ deposits, presumably because these agents have been derived from histological dyes specific for β-sheet amyloid deposits. In the context of AD, these agents primarily reflect Aβ in plaques and cerebrovascular amyloid. When tangles and plaques coexist, it would be difficult to detect a tangle-specific signal, due to the signal from the much larger Aβ pool. However, when tangles are present in isolation (e.g., in tauopathies), it is conceivable that amyloid imaging could detect a tangle-specific signal. Nonetheless, there is no direct evidence of detection of an *in vivo* signal from tangles with any existing imaging agent.

These agents have demonstrated the presence of Aβ deposits in the vast majority of clinically diagnosed AD patients, 50% to 75% of MCI patients and in 20% to 30% of cognitively normal elderly. Good correlation of *in vivo* retention with postmortem levels of Aβ has been demonstrated for PiB.[50] The test–retest variability of PiB PET is on the order of 5% to 8%, being lowest in areas with high PiB retention.[86,93] Plaque load appears to plateau by the mild stage of clinical AD.[94] The implications of this for monitoring of therapy is that, for a meaningful effect of a drug to be detected in AD with amyloid imaging, the drug should decrease the load of deposited Aβ by at least 15%. This effect would seem biologically meaningful (i.e., a signal that is two to three times the noise inherent in the measurement). From a statistical viewpoint, a decrease in PiB retention of 15% should give greater than 90% power for detecting a significant effect in a typical sample of AD patients with no more than 20 subjects.

Decreasing production or decreasing the soluble pool of Aβ may not be detectable—at least not acutely—unless the treatment ultimately shifts the equilibrium away from the deposited form of Aβ. Amyloid imaging may be best suited for therapy-monitoring of drugs intended to increase clearance in the setting of AD, and perhaps of MCI (e.g., immunotherapy). If presymptomatic or preventive treatment trials are begun in individuals who show early amyloid deposition, then it may be possible to detect the effect of drugs that slow Aβ deposition, because this is the most active phase of Aβ accumulation. Another use of amyloid imaging in therapeutic studies is to screen-in only subjects who have significant Aβ deposition, because it would make little sense to include a subject with no brain Aβ deposits in a clinical trial of an antiamyloid drug.

Downstream biomarkers

Measurements of biomarkers downstream of the target may be performed including structural MRI of the brain, electroencephalograms, magnetoencephalograms, inflammatory biomarkers, or other biomarkers not directly tied to the mechanism of action. The interpretation of these downstream biomarkers may assist in determining secondary effects, but do not inform on the treatment mechanism of action. For example, in the AN 1792 trial, Aβ immunization was associated with a paradoxical shrinkage of brain as measured by MRI.[95] The predicted response was slowing of atrophy due to beneficial effect of the immunization. MRI results of increased or decreased shrinkage have both been interpreted as potentially beneficial.

ADDITIONAL TREATMENT APPROACHES TO Aβ MODULATION

Other approaches to altering Aβ metabolism are discussed elsewhere in this issue, including RAGE inhibitors [Zlokovic,[96] this issue], and direct plaque inhibitors [Landreth et al,[97] this issue]. These may be measured by their respective pharmacodynamic activity directly in CNS system by sampling of CSF, or imaging.

Pharmacodynamic effect confirmation in humans

The results of a therapeutic biomarker study can be used to design and interpret a phase III clinical outcomes study. Specifically, evidence of a pharmacodynamic effect in the patient population of interest and possible

clinical effects are important to consider in future phase III trials. For example, if a phase II study drug results in no significant clinical change and no change in the target of that drug in the patient population, it is unlikely to have a significant effect on the course of disease in a phase III study. However, if a phase II study shows evidence of therapeutic target effect without a significant clinical benefit in a short phase II trial, it is still possible that the treatment will produce a significant clinical benefit in a phase III trial. If in a phase II study it is found that the treatment significantly affects both the pharmacodynamic measurement and clinical outcomes, it is likely that the phase III study will confirm this in a larger population set and help validate the pharmacodynamic biomarker as a potential surrogate biomarker that can be used in the future refinement of the proposed therapy, as well as other therapies. A phase III study can be designed to maximize the chances of determining clinical benefit by determining the effective route, dosage, and timing of the therapy by incorporating information on the treatment effect on the target and comparison of dose escalation from phase II studies.

Defining the therapeutic window of Aβ modulation

It is not known to what degree Aβ metabolism needs to be modulated to have clinical benefit. For example, recent studies with γ-secretase inhibitors in mouse models indicate that a 30% to 50% decrease of γ-secretase activity decreases the formation of amyloid pathology in mice when this decrease in γ-secretase activity is maintained for the life of the mouse.[98] Once Aβ deposits are present, however, decreasing Aβ production by 95% in an animal model did not reverse established pathology—but it did prevent further worsening of the amyloid plaque load.[99] An unresolved question is what degree of β-secretase or γ-secretase inhibition, if any, is necessary to improve the clinical outcomes of AD. In addition, the timing of treatment during the disease process may be a critical factor.

Ultimately, the clinical outcome is the most important parameter to patients, family members, clinicians, and regulatory agencies. Therefore, any degree of pharmacodynamic modulation in Aβ or other pathways must ultimately be tied directly to the clinical outcomes. With this knowledge, future therapeutic approaches and refinements can target the appropriate level of Aβ inhibition or clearance to maximize therapeutic efficacy while limiting toxicity. Measurements of therapeutic effects on molecular targets in phase II studies may include amyloid imaging by PiB PET[100] or another molecular imaging agent. It also may include other biomarkers specific for pharmacodynamic assessment, including the production and clearance rates of Aβ, as well as estimates of Aβ pool size in the central nervous system. It may also include enzyme-linked immunosorbent assay measurements of CSF and plasma Aβ for estimates of activity in the central and peripheral compartments and correlation to production and clearance rates.

Phase III confirmation of pharmacodynamic effect

In phase III studies, the effect of pharmacodynamic measurements should be correlated with clinical outcomes. For example, induction of anti-Aβ plaque antibodies by patients in the AN-1792 active vaccination trial was correlated with slowed cognitive decline.[101] Direct measurements of CSF Aβ concentration levels, production, clearance, or amyloid imaging, may provide evidence for successful modulation of the therapeutic target. The amount of target effect can be correlated with clinical changes, supporting development of a treatment biomarker. In addition, measurement of downstream biomarkers with electroencephalography, MRI, and cognitive and clinical testing can be performed to determine effects on atrophy, electrical activity, and cognitive performance. Such testing allows for confirmation that the drug is hitting its target and quantitates to what degree. The results of a phase III study can provide significant data to assist in the design of future treatment studies. Without the measurement of the target of the drug in phase II and phase III studies, an uninformative study may result. This may result in the loss of several years of work, with no interpretable data to advance further studies for the development of future drugs.

Logic of testing pharmacodynamic effect in phase III

In a negative phase III trial, for example, any of four conditions may exist:

1) The drug is not hitting its target, and therefore produces no effect (i.e., ineffective drug).
2) The drug was hitting its target but at a level lower than needed to have a clinical effect (i.e., insufficient potency or dose).
3) The drug was hitting its target at a level predicted to have a clinical effect, but there still was no clinical effect (i.e., incorrect target).
4) The drug was hitting its target and having a beneficial clinical effect; however, because of study design, adverse events, or serious side effects—issues not specific to the target—these benefits were not observed (i.e., confounding effects in the study).

Without information regarding a target effect, one can not distinguish among these possibilities.

Refining the treatment

One can conclude whether a given level of target modulation is sufficient to modulate the clinical disease by measuring the clinical and pharmacodynamic effects in

patients. For example, if 15% inhibition of Aβ production with a secretase inhibitor is achieved but there is no change in clinical progression, then one can conclude that future therapies need to inhibit more than 15% to have a reasonable chance of benefit. Only from well-designed trials, regardless of whether they are clinically positive or negative, will understanding of AD treatment grow to the point where highly effective treatments for AD are likely to be refined. As a case in point, extensive development in the cardiovascular field has occurred by having a surrogate biomarker such as LDL cholesterol level to predict the response to treatment. This has led to rapid development of drugs which treat hypercholesterolemia and lower the risk of heart attacks and strokes.

To summarize, measuring the therapeutic effect of therapies directly in humans can provide answers to the following questions:

1) Is the treatment hitting its target of amyloid plaques, Aβ production, Aβ clearance, Aβ CNS levels, tau, phospho-tau, or other biomolecules involved with disease pathogenesis?
2) What is the duration and magnitude of the drug effect? (This will allow for deciding dose selection and frequency of medication.)
3) How much soluble or insoluble Aβ modulation is needed for a positive clinical outcome? Is more better? Do certain patients respond more favorably than others?
4) What form of Aβ is it critical to affect: soluble Aβ, oligomeric Aβ species, insoluble amyloid plaques or some combination of these? For example, if therapies that primarily target one form of Aβ appear to be clinically beneficial but therapies that target other forms do not, this would provide strong evidence regarding which is the more toxic species.
5) What species of Aβ ($A\beta_{42}$, $A\beta_{40}$, or others) are the best targets of therapeutic intervention?

These questions can be answered with well-designed studies using direct measurements of drug targets in human studies throughout all phases of clinical drug testing.

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