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Developing new treatments for Alzheimer's disease:

The who, what, when, and how of biomarker-guided therapies

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Abstract

This synthetic review presents an approach to the use of biomarkers for the development of new treatments for Alzheimer's disease (AD). After reviewing the process of translation as applied to AD, the paper provides a general update on what is known about the biology of the disease, and highlights currently available treatments. This is followed by a discussion of future drug development for AD emphasizing the roles that biomarkers are likely to play in this process: (1) Define patients who are going to progress rapidly for the purpose of trial enrichment; (2) Differentiate disease and therapeutically relevant AD subtypes; (3) Assess the potential activity of specific therapies *in vivo* or *ex vivo*; and (4) Measure the underlying disease state, so as to (a) detect disease and assess drug response in asymptomatic patients, (b) serve as a secondary outcome measures in clinical trials of symptomatic patients, (c) decide if further development of a treatment should be stopped as it is not likely to be effective. Several examples are used to illustrate each biomarker utility in the AD context.

Overview and objectives

This paper has several objectives. These are to review the process of translation as applied to Alzheimer's disease (AD), provide a general update on what is known about the biology of the disease, and highlight currently available treatments. The above sets the stage for a discussion of future drug development in AD and how this will almost certainly require the use of novel biomarkers that are in the process of being developed.

Several definitions for biomarkers have been suggested (Schulte, 2005). For the purposes of this discussion, a biomarker is defined as a biological signature objectively measured and

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evaluated that is an indicator of a normal biologic processes, a pathogenic processes, or a pharmacologic response to a therapeutic intervention, and *that adds value to treatment development*. A biomarker might involve the analysis of a body fluid such as blood, cerebrospinal fluid (CSF), or urine for a specific molecule, or it might be a brain imaging measurement.

The process of translation

As shown in Figure 1, the process of translation begins with a description of the clinical disease, which in the case of AD is quite well developed. Ideally, knowledge about the clinical disease leads to an understanding of its biology, followed in most cases nowadays by the development of animal or cellular models of the disease. Studying the biology of the disease both in humans and in the available models helps us better understand the crucial mechanisms that are involved. Candidate therapies, typically medication therapies, emerge out of this understanding. These therapies are then tested in animal models for safety and, if the models are suitable, efficacy. Therapies that appear to be safe and have the potential to be effective are brought into human testing.

In order to test a new therapy in humans, it is necessary to be able to measure the progression of the disease. Psychiatry has traditionally relied on clinical measures (such as measures of depressive symptoms) in order to do this. In the context of AD, clinical measures are fairly well developed and include a spectrum of neuropsychological tests and rating scales [e.g., the MMSE (Folstein *et al*., 1975), ADAS-COG (Rosen *et al*., 1984), or Zarit Burden Questionnaire] that are administered to the patient or the caregiver. With the availability of such measures over the last 10-15 years, treatments have been developed that have not been very effective overall. It is becoming increasingly apparent that introducing treatments after the onset of dementia may be too late to stop, let alone reverse, AD symptoms as the brain damage may be too severe. As a result, there is a growing appreciation that it will be important to develop and test therapies in earlier stages of AD (Rosenberg, 2006), when clinical symptoms are less reliable indicators and change much more slowly. Thus, early (pre-clinical) biological measures (i.e., biomarkers) of disease activity are necessary, and will be critical to treatment development. The theme of how biomarkers may be utilized to facilitate the successful development of more effective treatments for AD is the focus here. It is important to note that while candidate therapies will likely come from the pharmaceutical industry, academic medical centers will play an important role in the process by helping develop biomarkers that allow rapid, efficient and targeted testing of drugs in humans.

Ultimately, the outcome of these efforts will be a series of new biomarkers that will allow us to answer the following key questions about treating AD patients.

- Who should we treat?
- What should we treat them with?
- When in the course of the disease should we treat them?
- How well is the treatment working?

If in the future we want to introduce therapies to patients in the earlier stages of disease, before symptoms emerge and the damage to the brain is too far advanced, it will be necessary to use biomarkers to detect *who* actually has the pathologic process of AD. Furthermore, because the etiopathogenesis of AD is heterogeneous, it is reasonable to assume that a single treatment will not be good for all patients. Thus, it will be important to evaluate *what* treatments will be most effective for different sub-groups of patients, many of which will be defined through the use of biomarkers (for example, genotype at *ApoE* or specific blood measures of oxidative damage). Using disease subtypes to guide treatment choice is well established in other diseases;

for example, antihypertensive therapy has been shown to reduce the risk of coronary artery disease in persons with essential hypertension, but not in normotensive individuals. The biomarker (blood pressure) is important for treatment choice. In addition, different treatments may be more effective at specific stages of the disease. As a result, it will also be important to determine *when* a particular treatment should be given, using biomarkers to define stage. Finally, it will be helpful to be able to establish quickly *how* well a treatment is working in a patient so that the opportunity to effect a "cure" is not lost and other treatment modalities can be tried. Biomarkers will help us to address all of these issues in order to develop and maximize the effectiveness of current and future treatments. Ultimately, the development of biomarkers will require a deeper understanding of the biology of AD, particularly of the heterogeneity and different risk pathways involved, as well as the relationship between the successive stages of the disease.

Brief overview of AD

The public health burden is staggering: about 10% of people 65 years or older and 33% of people 85 years or older have dementia, of which about 70% have AD (Hendrie, 1998). With the aging of the population it is projected that there will be up to 12 million new cases in the US and 81 million worldwide over the next 30-40 years (Brookmeyer *et al*., 1998; Ferri *et al*., 2005). The costs already are enormous, on the order of $100 billion per year in the US alone (NIH Office of Science Policy Analysis, 2006). AD is a chronic disease with an extended pre-clinical period during which the underlying disease damages specific parts of the brain but no clinical symptoms are apparent. This is followed by an early clinical period during which patients exhibit symptoms such as mild memory dysfunction and other cognitive or affective symptoms, but day to day functioning is preserved. This is often referred to as mild cognitive impairment or MCI (Gauthier *et al*., 2006). Eventually, daily functioning is affected and the onset of dementia occurs. The dementia progresses over many years and ultimately leads to death, typically about 10-12 years after initial diagnosis (Brookmeyer *et al*., 2002; Fitzpatrick *et al*., 2005). In general, AD dementia has gradual onset with slow progression; but, it is not unusual for patients to have fits and spurts or even stepwise progression through the different phases.

There is clear heterogeneity in the course of disease as it passes through the different phases above. As people with AD get older, they start to have brain changes and develop the preclinical brain disease and early symptoms at different times. The transition from asymptomatic through MCI and on to dementia also proceeds along different trajectories. There are at least two aspects to this heterogeneity. One has to do with age of onset of the various symptoms and the other has to do with the rapidity of progression through the different disease periods. Some individuals might develop AD later in life but have a steeper course of decline through the phases of the disease. Alternatively, others might have an earlier onset of disease pathogenesis but have a slower decline through one or more of the phases, and as a result develop dementia around the same age as the previous group of individuals.

The essential neuropathologic features of AD include extracellular amyloid deposition ("Abeta"), intracellular neurofibrillary tangle formation, synaptic dysfunction, blood vessel changes ("amyloid angiopathy"), and microglial activation (Selkoe, 2004; Price and Sisodia, 1994). The relationship between these factors is complex, and the different degree to which they contribute to the pathogenesis of symptoms is also a source of heterogeneity. The current hypothesis of how this neuropathology emerges is that it begins with amyloid deposition in the brain (Selkoe, 1996; Selkoe, 2001). Amyloid is generated from the amyloid precursor protein (APP), a transmembrane protein that all neuronal cells express. Within this protein is a 42 amino-acid peptide segment known as "Abeta" that is released from APP by the action of specific enzymes known as secretases. Three secretases are involved in the processing of

APP: alpha-, beta-, and gamma-secretase. If alpha-secretase cleaves first, it does so in the middle of the Abeta domain. The resulting fragments, after further cleavage by gamma-secretase, are soluble and readily cleared. If beta-secretase cuts first, there is a conformational change in the APP protein such that alpha-secretase can no longer cleave. The resulting fragments are cleaved by gamma-secretase and the full Abeta segment is released. Abeta, which is relatively insoluble and harder to clear, appears to be neurotoxic. It aggregates to form dimers and oligomers and eventually deposits into extracellular plaques. The deposition of Abeta is followed by synaptic dysfunction and eventually neuronal death. This process begins in the peri-hippocampal areas and spreads over time in a variety of directions into the cortex, affecting temporal, parietal and frontal cortex in early dementia.

Figure 2 further summarizes this hypothesis as a cascade of key events. As described above, the disease most likely starts with the miss-processing of APP and the deposition of amyloid. This leads to activation of microglia (Akiyama *et al*., 2000; Wyss-Coray and Mucke, 2002; Sastre *et al*., 2006) and, through mechanisms that we are now only beginning to understand as discussed below, synaptic injury and ultimately neuronal death. As this process spreads throughout the brain, whole neuronal systems are lost and clinical symptoms begin to emerge. Current therapies operate at the bottom part of the cascade. These treatments do not affect the underlying pathogenesis, but instead provide only temporary relief from the emerging symptoms (Rosenberg, 2005b).

An understanding of the role of inflammation in the cascade of events is still developing (Akiyama *et al*., 2000; Eikelenboom and Van Gool, 2004; McGeer and McGeer, 2003). Microglia are resident immune cells in the brain, closely related in lineage to peripheral macrophages. It appears that quiescent microglia become activated during pathogenesis and aggregate in areas around the accumulating Abeta plaque. Microglia are thought to phagocytose the amyloid and in the process release pro-inflammatory cytokines, such as Il-6, TNF-alpha, and Il-1 beta (Rosenberg, 2005a). These cytokines have at least three effects in the brain that may be relevant. They stimulate apoptosis, reduce synaptic plasticity and long-term potentiation, and inhibit hippocampal neurogenesis. These effects may be critical to how amyloid causes neural degeneration. Further, the Abeta peptide itself appears to stimulate the activation of quiescent microglia and the subsequent release of cytokines in turn accelerates APP turnover and Abeta production, leading to a positive feedback loop that may accelerate the middle part of the cascade (Akiyama *et al*., 2000).

The time frame for the progression of AD in the brain is poorly understood, but it extends over years, perhaps even decades, and there may be a considerable delay between the advancement of disease in the brain and the emergence of symptoms (Snowdon *et al*., 1996; Mortimer *et al*., 2005). This prolonged time course will likely to be critical to the choice of novel AD therapies and to the timing of these therapies.

Importantly, there is evidence that several factors modify rate of progression. These might be thought of as risk factors for faster cognitive decline or more rapid transition through the pre-clinical to the prodromal phases to dementia. For example, carriers of the ε4 allele of the *APOE* gene develop dementia earlier in life (Blacker *et al*., 1997), have accelerated cognitive decline in response to stroke (Alberts *et al*., 1995; Horsburgh *et al*., 2000) or traumatic brain injury (Teasdale *et al*., 1997; Horsburgh *et al*., 2000), and appear to decline faster even before age 60 (Caselli *et al*., 2004). Thus, *APOE* may be a timing gene (Meyer *et al*., 1998; Khachaturian *et al*., 2004), since not everybody who carries the ε4 allele develops dementia even in very late life. There is increasing evidence that several other factors accelerate the speed of decline or increase dementia risk. These include female gender (Gao *et al*., 1998), lower educational attainment (Evans *et al*., 1997; Karp *et al*., 2004), late life depression (Green *et al*., 2003; Rosenberg *et al*., 2007), personality traits including neuroticism (Wilson *et al*.,

2006), traumatic brain injury (Nemetz *et al*., 1999; Schofield *et al*., 1997), and brain vascular disease (Honig *et al*., 2003). By contrast, factors that slow the decline may include early and sustained use of anti-inflammatory medications (Szekely *et al*., 2004; Zandi *et al*., 2002; in 't Veld *et al*., 2001; Stewart *et al*., 1997), high dietary intake of anti-oxidants (Zandi *et al*., 2004; Morris *et al*., 2002), variety of leisure activity (Fritsch *et al*., 2005; Crowe *et al*., 2003), more physical activity (Podewils *et al*., 2005; Rovio *et al*., 2005), higher mental activity (Wilson *et al*., 2002), and the "Mediterranean" diet (fish, vegetables, and olive oil) (Scarmeas *et al*., 2006; Panza *et al*., 2004). The complex interplay of factors that may up- or down-regulate the cascade of events underlying AD leads to heterogeneity in the course of disease across individuals. For example, a genetic susceptibility due to *APOE* genotype may combine with specific environmental risk factors to exacerbate the rapidity with which an individual progresses along the cascade. These modifiers may act independently or they may have additive effects, as suggested by a recent study showing that metabolic syndrome and blood inflammatory markers have additive effects on cognitive decline (Yaffe *et al*., 2004). In all, there may be ***a finite set of risk profiles*** that set the parameters for the rapidity of disease progression. This is important because the various modifiers might be therapeutic targets even if a treatment does not target the primary cascade. For certain individuals, targeting these modifiers might make enough difference in the speed of progression to have a beneficial clinical effect on disease progression. One of the roles of biomarkers will be to provide biological measures that will help define different progression profiles in a therapeutically relevant fashion. This concept is illustrated later in this paper.

Brief summary of current therapeutics

Dementia Care is an established approach that combines pharmacologic and nonpharmacologic interventions to target dementia symptoms in an effort to delay progression and improve the lives of patients and caregivers. Several guidelines and approaches have been described, including the approach developed at Johns Hopkins (Rabins, Lyketsos, Steele, Practical Dementia Care 2nd Edition, Oxford Press, 2006) (Rabins *et al*., 2006). In the last few years, it has become apparent that pharmacologic treatments in Dementia Care are best thought of as being of two types. Symptomatic therapies might provide temporary relief but they do not greatly affect the rate of disease progression so that the threshold to later clinical stages of disease is always crossed eventually. By contrast, disease modifying therapies delay the progression of disease, so that this threshold is never crossed.

All of the currently available therapies — such as cholinesterase inhibitors (donepezil, galanatamine, rivastigmine), antidepressants (e.g., citalopram, sertraline), and antipsychotics (both conventional and atypical) — are primarily symptomatic therapies, although memantine may be a disease therapy (Rosenberg, 2005b). For the most part, these medications help with cognitive, neuropsychiatric, or functional symptoms in a time-limited fashion. Given the limitations of the current pharmacologic therapies, there is significant motivation to develop more effective disease modifying therapies. Such therapies would provide considerable benefits. These therapies could dramatically reduce the prevalence of AD even if they only modestly delay its onset, because of the increased mortality among the elderly. In AD, this is referred to as the 5:50 hypothesis. If the incidence could be delayed by five years, then the number of people with the disease would be cut in half (Brookmeyer *et al*., 1998; Brookmeyer and Gray, 2000). From a public health perspective, this is a substantial effect. The ultimate goal is to develop a disease modifying intervention that could be introduced at a time when people are asymptomatic or minimally symptomatic so as to prolong their period of reasonably good, non-disabled life.

There is growing evidence that certain medications approved for other indications might be modifiers of AD progression. Several of these have been or are being investigated for efficacy

in randomized trials. However, none as yet have been shown to be effective and some may even be harmful (e.g., estrogen) (Shumaker *et al*., 2003). Examples include HMG-CoA-reductase inhibitors (statins) (Sparks *et al*., 2005), estrogen analogs (e.g., raloxifene) (Yaffe *et al*., 2005; Gleason *et al*., 2001), non-steroidal anti-inflammatory agents (e.g., indomethacin) (Rogers *et al*., 1993), antioxidants (e.g., high dose Vitamin E) (Sano *et al*., 1997; Petersen *et al*., 2005), and divalproex (Profenno *et al*., 2005) (which has *in vitro* neuroprotective activity).

A number of other "designer" drugs are currently under development and/or being investigated based on the latest understanding of the AD cascade. Some of these agents target modifiers of the cascade, while others target the cascade more directly. Examples of the former are PPAR-γ agonists, such as rosiglitazone, which facilitates the uptake of glucose into cells (Watson and Craft, 2003). The rationale for their use in AD is based on the observations that *APOE* ε4 carriers may have less efficient glucose uptake into neuronal cells (Reiman *et al*., 1996), and diabetes and insulin resistance appear to be risk factors for accelerated cognitive decline (Luchsinger and Mayeux, 2004). Other examples of agents under development that target the cascade more directly are so-called beta or gamma-secretase inhibitors that aim to reduce the production of Abeta by inhibiting the action of the key enzymes in APP metabolism (Gitter *et al*., 2004; May *et al*., 2004), and immunotherapies designed to promote the clearance of amyloid by marshalling the bodies own immune system against it (Bard *et al*., 2000; Schenk *et al*., 1999). Several of these agents are currently in clinical trials for testing of efficacy in treatment of AD in humans. One trial of R-flurbiprofen, a drug that appears to modulate gamma-secretase activity and reduce the deposition of amyloid in animal models (Eriksen *et al*., 2003), has shown some benefit (Black *et al*., 2005). By contrast, another trial for an active immunotherapy that had shown great promise in animal models was discontinued early because of toxicity related to the occurrence of meningoencephalitis in a small number of treated subjects (Orgogozo *et al*., 2003). Preliminary evidence from a limited autopsy series of patients in this trial suggested the immunotherapy was effective in clearing away amyloid deposition, but the effects on cognition and function were minimal (Fox *et al*., 2005; Gilman *et al*., 2005). This could be because the trial was stopped too early due to toxicity (too short duration), the therapy was introduced too late in the course of AD (wrong disease stage), or the amyloid hypothesis is incorrect and such a therapy would not be expected to have any impact on disease progression (wrong mechanism). The results of this trial thus illustrate the many challenges of developing new AD treatments.

Biomarkers: the key to the next phases of treatment development

The ultimate goal is to develop therapies that can ameliorate the clinical burden of AD by slowing, preventing or even reversing the progression of disease. This goal has been difficult to achieve because of several important challenges. Most trials of new therapies have been in patients with clinically established dementia. While this has been a reasonable starting approach, by the time dementia has developed the disease is at a point in the cascade of events when damage to the brain may be too extensive, so that it may be too late to stop or reverse the symptoms. For example, persons with mild cognitive impairment (MCI) have cognitive symptoms but no functional impairment and are at increased risk of incident AD; these MCI patients frequently have pathologic evidence of early AD (Bennett *et al*., 2005; Petersen *et al*., 2006). Additionally, the clinical progression, although inevitable, is fairly slow and quite inconsistent, and the currently available clinical measures are not very sensitive to subtle change. As a result, the demonstration of even a modest effect of a drug on progression in a clinical trial would require large numbers of patients treated for long periods of time. This is very, very expensive.

In the last decade, several drugs have come through the pipeline showing promise in early development but which failed to prove effective in later clinical trials. The considerable time

and costs lost to these "failures" has led pharmaceutical developers to be cautious about future investments in this area. At the same time it has motivated them to seek better ways of assessing what drugs are working or how well they are working. Thus, there is a great deal of interest in developing valid biomarkers of AD because it is believed they could help towards this end. Such biomarkers could be used to target treatments in the pre-clinical phases of disease, or to target specific therapies to specific subgroups of patients who might most benefit. Furthermore, because biomarkers are more precise than clinical measures, their use in trials, even as adjuncts to clinical measures, would help reduce sample size requirements significantly (Jack *et al*., 2003). Finally, they could provide more time- and cost- efficient means for evaluating whether patients are being helped or not by the treatment.

Unfortunately, the biomarker field in AD has been somewhat confused, because it has been approached from many different perspectives and not specifically from the point of view of therapeutics. For example, in some cases biomarkers have been considered for the purpose of developing diagnostics (de Leon *et al*., 2006), while in others they have been considered for the purpose of developing surrogate measures of outcomes for epidemiologic studies (Mielke *et al*., 2006). In this regard, the field has a great deal to learn from the experience of oncology with biomarkers, in particular the appreciation that biomarkers have different roles in therapeutic development. Adopting the arguments of Paul Schulte (Schulte, 2005) there are several types of therapeutically relevant biomarkers that:

1. Define patients who are going to progress rapidly for the purpose of trial enrichment;
2. Differentiate disease heterogeneity (e.g., the use of tumor characteristics to target chemotherapies);
3. Assess the potential activity of specific therapies *in vivo* or *ex vivo* (e.g., the use of prostate specific antigen to evaluate the effectiveness of hormonal or other therapies in vivo, or the use of a patient's own cells to study drug response *ex vivo*);
4. Measure the underlying disease state, so as to
    a. Detect disease and assess drug response in asymptomatic patients
    b. Serve as a secondary outcome measures in clinical trials of symptomatic patients
    c. Decide if further development of a treatment should be stopped as it is not likely to be effective

Biomarkers that might be used to define rapid "progressors" for trial enrichment are important but not a significant challenge and will thus not be discussed in detail. The conceptual approach and methods needed to develop such biomarkers has been worked out well in the cancer field and could easily be applied to the AD setting. Several studies worldwide are examining what biomarkers, for example, CSF levels of amyloid or tau, blood proteinomics, smaller hippocampal volumes on brain MRI, or their combination, predict more rapid progression for the purpose of clinical trial enrichment.

The remaining types of biomarkers can be broadly categorized into those that are useful for identifying heterogeneous subgroups of AD patients and those that are useful for characterizing disease progression through its various stages. We will discuss the former first, and then the latter. For the discussion of the former, we will use as an example two possible subgroups of AD patients referred to as an oxidative group and an inflammatory group. In each case the group is defined based on the premise that a specific biologic process (i.e., inflammation or oxidative stress) plays a significant role in the progression of disease in that subgroup.

Basic science and post-mortem evidence suggest that oxidative stress plays a key role in the pathogenesis and progression of AD (Moreira *et al*., 2005). Oxidative stress leads to the production of reactive oxygen species that attack lipid membranes, leading to the formation of lipid peroxidation products and ultimately cell dysfunction and death. Epidemiologic evidence suggests that oral antioxidants, including vitamins E & C, may retard the progression of AD (Zandi *et al*., 2004; Morris *et al*., 2002). However, clinical trials have not been as promising and the value of these treatments for AD is uncertain (Sano *et al*., 1997; Petersen *et al*., 2005). One reason is that there may be subtypes (or "profiles") of AD in which specific mechanisms, such as oxidative stress, contribute more significantly than others to the pathogenesis in particular subgroups of patients. Previous clinical trials of antioxidants in AD have included "all comers". If there is a subgroup of AD patients in whom oxidative stress is particularly relevant (i.e. in patients with either low anti-oxidant or high lipid peroxidation levels), the beneficial effects of anti-oxidants in a trial with "all comers" may be diluted and no longer detectable.

To illustrate this further, it should be noted that it is possible to gauge the status of on-going oxidative stress in the body by measuring levels of isoprostanes and other lipid peroxidation products in the blood, urine or CSF (Pratico *et al*., 1998; Montine *et al*., 2005). There appears to be variability in such levels in AD patients. If patients with higher levels have accelerated cognitive trajectories, it may be possible to improve their cognitive trajectories by reducing these levels through antioxidant therapy. For this to be effective it does not mean that AD patients must have higher levels compared to cognitively normal individuals, but rather that a subgroup of AD patients with higher levels progress faster than other AD patients. In a preliminary study blood isoprostane levels in AD patients and controls matched for age and gender were measured (Mielke *et al*., 2007a). Noticeably, all the controls had blood isoprostane values within a narrow range, as did about half of the AD patients. By contrast, a sizable proportion of the AD patients, especially with early dementia, had substantially elevated levels. It is interesting to speculate whether the subgroup of AD patients might experience faster decline due to the elevated indicators of oxidative stress, and therefore, might especially benefit from treatment with antioxidants. While this is very preliminary, it illustrates how subgroups of AD patients might be usefully defined using this biomarker.

Another sub-group of AD patients might be usefully defined based on inflammatory status. The hypothesis here is that in the AD cascade the activation of microglia appears to play a role in how amyloid kills cells (Akiyama *et al*., 2000; Wyss-Coray and Mucke, 2002; Sastre *et al*., 2006). It may be that the microglia of different people, once activated, produce different amounts of cytokines and therefore have different pathogenic potential along this pathway. This variability might be detectable in cultured peripheral lymphocytes that are "cousins" of the microglia. Early findings suggest that the cultured blood lymphocytes of AD patients produce significantly more Il-6, a key cytokine in inflammatory responses, than controls when exposed to the antigen LPS (Carroll *et al*., 2007). As yet, this *ex vivo* model does not show similar variability in response to exposure with Abeta, but work on the model is still on-going. Again, it is interesting to speculate that if patients whose lymphocytes produce more Il-6 in response to antigen exposure manifest accelerated clinical decline, perhaps they might benefit more from targeted treatment with "anti-inflammatory" therapies. Such a model might also be used, if successfully developed, to predict whether an individual is responding to a drug treatment, by examining *in their own cultured peripheral blood cells* the effect of individual drugs on IL-6 release after antigen exposure.

Many laboratories around the world are using similar approaches to subtype heterogeneity in AD.

Moving on to discuss how biomarkers might be used to measure the status of disease progression, it is helpful to refer to Figure 3 which again shows our current understanding of the different stages of the AD cascade. Ideally, it would be helpful to have reliable biological measures of each of the stages both for the development of therapies and so as to better study how the different stages interact and unfold in the same patients. We will highlight here some of the advances towards this goal, starting at the top of the cascade and moving downward.

One of the major advances over the last several years has been the development of Pittsburgh Compound B, or "PIB" (Klunk *et al.*, 2004), a thioflavin derived radioligand for PET imaging of amyloid plaques *in vivo*. PIB binds selectively to Abeta plaques at concentrations used for imaging, and studies have shown that its binding in the brain is highly correlated with total Abeta levels. PET neuroimaging with PIB appears to be very reliable (Price *et al*., 2005), but more longitudinal studies are needed to assess how valid a measure it is of AD progression over time. A recent concern has been that amyloid deposition imaged by PIB maybe near maximal after the onset of dementia, limiting sensitivity to detect changes with "anti-amyloid" therapy (de Leon *et al*., 2004). Interestingly, it may be possible to image Abeta plaques using very high power (7 Tesla) Magnetic Resonance Imaging (MRI) of the brain (Nakada *et al*., 2008). If confirmed, this would open up an alternative imaging strategy for assessing developments in the initial stages of the AD cascade.

It would also be useful to be able to image microglia in their *activated* state. It has been suggested that the PET ligand, PK11195, sometimes referred to as the peripheral benzodiazepine receptor ligand, can do this (Banati, 2003; Cagnin *et al*., 2001; Rosenberg, 2007). Activated microglia express these receptors on their mitochondria with substantial density, and it may be that this ligand, or ones like it, will allow estimation *in vivo* of the degree of microglia activation in AD and other related conditions.

Another useful biomarker would be one that provides an indication of how rapidly neuronal cells are dying in the brain, similar to measures of CPK or troponin in the blood after a heart attack. An interesting phenomenon happens when neuronal cells die: the cholesterol in their lipid membranes is metabolized behind the blood brain barrier to a substance called 24S-hydroxycholesterol (Leoni *et al*., 2003). This cholesterol species is produced only in brain, but it readily crosses the blood brain barrier and can be measured in CSF or blood. Its level in the blood can be compared to that of 27-hydroxycholesterol, another cholesterol metabolite that reflects cholesterol turnover in the periphery to provide an index of excess cholesterol turnover in the brain and hence of neuronal degradation. Similarly, other lipid species, such as brain derived ceramides, can be measured in blood and might become specific indicators of rapidity of brain degeneration. Several cross-sectional studies have found that the ratio of these metabolites differ between AD patients and controls (Lutjohann and von, 2003). Longitudinal studies are now also suggesting that blood ceramide levels might be able to predict cognitive decline many years later (Mielke *et al*., 2007b).

Protein markers of neurodegeneration that could be measured in blood or CSF, such as synaptophysin might also usefully serve the purpose of monitoring speed of neurodegeneration (Wyss-Coray and Mucke, 2002). This would be of greatest value if short-term changes could predict longer-term clinical changes, thus allowing the assessment of therapies in shorter time frames.

A biomarker that can image neuronal systems would also be extremely useful. Diffusion Tensor Imaging (DTI) is one such type of MRI technology that may allow this (Sullivan and Pfefferbaum, 2003). DTI images the diffusion of water along white matter tracts to reveal with high sensitivity the complex anatomy of these tracts across the brain. It may be possible to use DTI to follow the degeneration of white matter tracts over time, and thus to assess the effect

of neurodegenerative diseases on brain connectivity. Early findings suggest that DTI might be a very good indicator of the degeneration of neuronal systems in AD, even before volumetric measures of grey matter structures (Mielke *et al*., 2008; Zhang *et al*., 2007).

Figure 3 also shows other leading biomarkers that are being developed and studied for every part of the cascade. These tools will be critical to advancing our understanding of the disease mechanisms in AD and to facilitating the future development of more effective treatments. Ultimately, ***the concurrent measurement*** of different biomarker modalities in the same patients may provide the greatest value to this endeavor. For example, imaging *in vivo* amyloid at the same time as imaging activated microglia and cholinergic receptors could tell us about the distribution of amyloid in relation to that of microglia activation and of receptor loss at different stages of the disease (i.e., preclinical, MCI, and dementia). This would help us test the amyloid hypothesis as well as clarify the sequence of events in the brain that are involved in symptom emergence.

Pulling this together, the hope is that the future will bring differentiation of AD subtypes as well as accelerated treatment development. Biomarkers will be the key to this effort. If properly developed, they will give us the ability to diagnose the disease in very early stages before symptoms begin, enabling us to target therapies and evaluate the value of therapies in those stages. They will also allow us to use treatments that are tailored to patients with specific risk profiles. Finally, biomarkers will give us the ability to rapidly detect whether treatments are working or if other treatments should be tried. In short, biomarkers will help us answer the following important questions when it comes to treating AD patients: Who should we treat? What should we treat them with? When should we treat them? And, how well is the treatment working?

This approach has great extrinsic merit: what we learn here will apply either directly, or as a model, to other dementias (e.g., fronto-temporal and Lewy body), to other neurodegenerative diseases (e.g., Parkinson's), and also to other psychiatric conditions such as schizophrenia, depression, and bipolar disorder. Rational drug development is the next frontier in brain diseases. We are just starting to think about biomarkers in this context and how we are going to be using them. It is likely that this new drug development business will be quite difficult and will likely take many years if not decades. It is going to take time to get "the cure", unless we get very lucky in the near future with the immunotherapies, for example, or from other amyloid manipulating therapies. In the meantime, it is critical to remember the duty to care for the patients with the disease. In fact, we currently have therapies that can make a difference and we should do our best to apply them as widely as possible.

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Conflict of interest declaration

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