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"It Ain't Over 'til It's Over"[a]—The Search for Treatments and Cures for Alzheimer's Disease

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ABSTRACT: In the neuroscience landscape, there is no condition with higher unmet medical and societal need than Alzheimer's disease (AD). There are significant opportunities to improve upon symptomatic treatments in AD, and as yet, there are no treatments to modify (slow, stop, or prevent) underlying disease progression. Our goals are to discover new symptomatic AD therapies with improved efficacy and longevity; to complete definitive studies that refute or prove the amyloid hypothesis, potentially opening multiple avenues to new therapeutic modalities; and to initiate tests of novel mechanisms that can prevent tau pathology and neurodegeneration. It's a critical time in the testing of novel AD therapeutics—let's hope we succeed.

INTRODUCTION

"It's Not the Years in Your Life That Count. It's the Life in Your Years"[b]. In the Neuroscience landscape there is perhaps no disease with higher unmet medical need than Alzheimer's disease (AD).[1] It respects no boundaries of geography, education or economic status. Today there are 5.4 million patients in the U.S. and some 35 million worldwide, and these figures are expected to increase 3−4-fold by 2050, affecting 1 in 85 people globally, which is driven in western nations by postwar baby boomers and 1960s generation Xers growing older. AD impacts the activities of daily living for patients long before they die and, as a consequence, increases the burden of care both emotionally and financially on their caregivers, who are most often close family members. In addition to the staggering human cost of AD, in the U.S. alone it is estimated that more than one trillion dollars will be needed annually to provide healthcare for AD patients. producing a societal public health economic burden that will fast become unsustainable. For these reasons. there has been enormous public and private investment into AD research, as the rewards for truly useful new therapies that can provide enhanced symptomatic relief over current standards of care (acetyl cholinesterase inhibitors and the NMDA antagonist memantine) or slow the progression of Alzheimer's neurodegeneration preserving independent living are substantial.

"The Hardest Thing in Life Is To Know Which Bridge To Cross and Which To Burn"[c]. There are no treatments to modify (slow, stop, or prevent) underlying disease progression in AD and few credible opportunities to improve upon current symptomatic treatments. Despite there being many potential novel drug discovery targets for therapy, most lack true validation. It is therefore important to select what to study on the basis of human biology or proven clinical therapy. Targets with the highest likelihood of success are supported by strong human genetic evidence, modulate pathways of known clinical pathophysiological relevance to the disease, or impact neurotransmitter systems where intervention has proven therapeutic value. Additionally, since the diagnosis of AD, particularly in the early stages, is difficult and clinical trials in AD are long, it is critical to prioritize and select targets that have biomarkers that can guide patient recruitment, pharmacodynamics, proof of biology, and dose selection to ensure clinical hypotheses are adequately tested in expensive late phase clinical trials. AD pathophysiology and symptomatology evolve from amyloid plaques to the spread of tau neurofibrillary tangles and neuronal cell death accompanied by an inexorable progression of cognitive and behavioral disturbances that impact function and independent living.[2] As a consequence, there is the potential to define diverse disease modifying and symptomatic mechanisms that could bring benefit to patients at different stages of the disease process.

Amyloid in a Tangle? Decades of research have advanced understanding of the pathogenesis of AD and spawned current therapeutic approaches that are supported by human genetics or anchored in human pathophysiology and pharmacology. The most advanced theory for the pathogenesis of Alzheimer's neurodegeneration is the amyloid hypothesis.[3] This theory is supported by early onset AD cases in which missense mutations in the amyloid precursor protein and in presenilin 1 and 2 (*APP*, *PSEN1*, or *PSEN2*) genes speed the generation of amyloid-β peptides, notably Aβ42, in the brain. The resulting imbalance between Aβ42 generation and clearance results in early amyloid plaque deposition in the brain, neuronal dysfunction and degeneration (associated with tau protein hyperphosphorylation and neurofibrillary tangle formation), and consequent accelerated cognitive decline that mimics sporadic late onset AD. More recently, protective mutations in the APP gene that slow the progression of cognitive deficits in AD have been described.[4] As a consequence, treatment strategies that aim to lower production or increase clearance of amyloid to produce therapeutic benefit have been widely pursued. This hypothesis has, however, recently been challenged by the top line failure of the two leading clinical trials of the amyloid antibodies solenuzemab and bapineuzemab to slow the decline in memory, cognition, and performance of activities of daily living and personal care. These antibodies aimed to reduce brain amyloid load either by reducing soluble amyloid in the fluids of the brain, thereby slowing its

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accumulation in plaques, or by increasing plaque clearance through immunogenic microglial activation, respectively. Are these trial results conclusive enough, however, to invalidate the amyloid hypothesis and trigger a shift to new therapeutic targets, or do they give a glimmer of hope yet signal a need to change the design of clinical trials for drugs that slow Alzheimer's progression?

"It Isn't That They Can't See the Solution; It's That They Can't See the Problem"[d]. There was no convincing evidence from early clinical biomarker studies that bapineuzemab or soleneuzemab altered soluble β-amyloid levels in the brain, so it could be argued that these agents were never likely to be the best test of the amyloid hypothesis and, thus, the outcome of their clinical trials cannot be used to discard the theory. It is therefore worth considering whether there is any data from the phase III trials of these antibodies that, even if only from a purely scientific standpoint, could be seen as support for the amyloid hypothesis. Some have found comfort in biomarker read-outs from the failed bapineuzemab intravenous trials (presented at The European Federation of Neurological Sciences, Stockholm, Sweden, September 2012) citing marginal PET imaging evidence[5] that it prevented further accumulation of β-amyloid in the brain of high risk ApoE4 gene carrying patients compared to placebo controls and, interestingly, stopped the elevation of phosphorylated Tau in the cerebrospinal fluid, a protein biomarker of neuronal loss, despite showing no effects on brain volume decline as measured with structural MRI. Interestingly, the lack of effect on free CSF amyloid level leaves open whether or not there was sufficient central target engagement. A significant number of patients showed cerebral vasogenic edema (known as ARIA—amyloid related imaging abnormalities) as a dose related side effect of the drug, perhaps as a consequence of amyloid removal from brain blood vessels. Development of the intravenous formulation of bapineuzemab has now been discontinued, but trials of the subcutaneous form are ongoing, as they are fully enrolled and the research dollars effectively spent.

The solenuzemab data (presented at the American Academy of Neurology, Boston, MA, October 2012) has been interpreted to support the amyloid hypothesis despite failing to hit its primary cognition and functional end points in each of its two phase III clinical trials in mild—moderate AD. However, in one of the trials and in pooled data across the two trials (to increase statistical power), prespecified secondary analyses showed tantalizing evidence for slowing cognitive decline in a subset of mild AD patients. The sponsor company has characterized the combined effect (42% in trial 1 and 20% in trial 2) as a relative 34% risk reduction in progression with an absolute improvement in the ADAS-cog cognition score in the same ball-park as symptomatic treatments such as the cholinesterase inhibitors. There was no effect in moderate AD patients nor any impact of ApoE status in response to treatment. Interestingly, the effect in mild patients, unlike acetyl cholinesterase inhibitors, did not appear to abate over time, supporting the proposition that soleneuzemab may have shown disease modification. Recent high level company communications indicate, however, that the ADAS-cog end point is not supported by another cognitive end point, CDR-sum of boxes, and that there was no effect on CSF phosho-tau or brain volume neurodegeneration markers, although there may be some evidence of a shift in amyloid biomarkers. Discussion of this data is promised for late October 2012 at the Clinical Trials conference on AD in Monte Carlo.

Now the fun starts! Is it a statistical oddity? What do the regulators think? Is the data sufficient to support registration? Can it, or does it, need to it be reproduced? Is the data compelling enough to bet on more amyloid based immunotherapeutics, or do alternative mechanisms now take center stage? Companies and financial markets are now anxiously scenario planning to analyze the potential impact of the solenuezemab data on their AD research portfolios and drug development pipeline valuations. What's for sure is that the answers to these questions could shape future amyloid based clinical trials and the willingness to invest further in diverse amyloid based therapeutic targets.

"You Can Observe a Lot Just by Watching"[a]. The biomarker findings from the bapineuzemab trials and the signal in mild Alzheimer patients with soleneuzemab have been interpreted optimistically to suggest that these amyloid based therapies could have been effective if given at an earlier stage in the disease before cognitive symptoms were seen and that starting treatment when patients already have signs of dementia may be too late. Perhaps the most important debate in the field of Alzheimer's drug discovery at the moment is when to start preventative treatment, whom to treat, and for how long? As a result, biomarker and clinical trial strategies are evolving rapidly.[6] An interesting test of the treat-early hypothesis that moves the start of trials back into asymptomatic populations with biomarker evidence of early AD will use creneuzemab given for 5 years to a Columbian patient cohort with a familial genetic predisposition to developing AD in middle age. This trial will also incorporate CSF and imaging biomarkers to assess the effectiveness of the drug, perhaps further validating them for use as clinical trial end points. Other early intervention efforts, such as DIAN (Dominantly Inherited Alzheimer Network—patients with *APP*, *PSEN1*, or *PSEN2* genetic mutations), will study the antibodies gantenerumab and solenuzemab and a BACE inhibitor, and antiamyloid trials in asymptomatic AD, such as the A4 trial, are also planned.

Many of today's Alzheimer drug trials are, in essence, evaluating preventative drugs in treatment trial designs and so arguably have little or reduced chance of success. Although these trials in mild to moderate AD have been the initial focus in drug development, efforts to identify patients (based on biomarkers and other risk factors such as ApoE4 status) who are at risk for dementia but still in the prodromal stage of the illness are now intensifying to see whether earlier intervention can delay the onset of dementia. Given the enormity of the problem, the drumbeat is loud on the possibility of using biomarkers as surrogate end points for Alzheimer's drug registration to speed approvals of safe new therapies that show promise in clinical trials. Some even suggest the possibility of provisional regulatory approvals with monitoring of clinical outcomes later in real time but with the big proviso that if negative, the approval is rescinded and therapeutic discontinued. To be successful, this approach would, however, need to resolve who would pay for the medicines that have only biomarker evidence of effect while clinical outcomes data is accumulated. The shift to early intervention and an emphasis on biomarkers will undoubtedly raise usual questions about the limitations of surrogates and biomarkers. How sensitive and specific are the measurements that will be used to chart progression and what is their true predictive value especially in the early stages of the disease? More studies will be required to show that PET plaque imaging, brain structural imaging, and CSF fluid biomarkers of amyloid deposition and neuro-

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degeneration such as Aβ42 and hyperphosphorylated tau can rise to the occasion. Perhaps any one of these alone is insufficient but a panel of orthogonal biomarkers that all point to decreased decline might be worth considering. The collection of this brain derived data will, however, place a considerable practical burden on the individuals who take part in these trials, perhaps making them hard to recruit. A transformative event would be the discovery and qualification of a peripheral blood based Alzheimer's progression biomarker for clinical trials.

"The Problem When Solved Will Be Simple"[e]. It has been said recently that the burden of proof is on those who continue to target amyloid, yet there are many companies who are optimistic that lowering Aβ levels in the brain will provide therapeutic benefit.[7] The first-generation of drugs preventing Aβ42 synthesis were γ-secretase inhibitors (e.g., semagecestat and avagacestat) that showed no therapeutic benefit in mild-moderate AD - and even caused cognitive worsening in some cases. Clinical toxicities that are most likely a consequence of inhibition of NOTCH signaling, an alternative substrate that is involved in cellular proliferative and differentiation processes, have also proved an intractable problem for this approach. The γ-secretase inhibitors, like the monoclonal antibodies, have so far failed to test the amyloid hypothesis, as they did not produce sufficient Aβ lowering in humans at tolerable doses. Avagecestat remains in development in a phase II trial in prodromal AD in the hope that lower, better tolerated, but less active, doses may be effective earlier in the disease. Next generation approaches to γ-secretase that are in phase 1 clinical trials aim to modulate cleavage sites (γ-secretase modulators) to favor the formation of Aβ40 over the neurotoxic species Aβ42 rather than inhibit synthesis in an attempt to circumvent the unwanted NOTCH side effects.

Currently, the most advanced amyloid programs focus on inhibiting the enzyme β secretase inhibition (BACE1) that processes amyloid precursor protein (APP) to sAPPβ and C100, with the latter being the substrate for γ secretase, which in turn releases Aβ40 and Aβ42. BACE as a target is validated genetically[4] by the observation that a protective mutation in the APP substrate (A673T) leads to decreased β-amyloid production as a result of reduced BACE1 cleavage, resulting in less AD and slowing of cognitive decline. Conversely, a gain of function mutation (A673 V) at the same site enhanced BACE1 cleavage 50-fold, increasing the incidence of early onset AD. BACE inhibition has been shown to suppress Aβ formation to unprecedented levels in animals and healthy human volunteers with a generally well tolerated clinical side effect profile and so has an improved chance over previous approaches to test the amyloid hypothesis rigorously. BACE1 clinical programs are now advancing to AD patients at several pharmaceutical companies worldwide.

"When You Come to a Fork in the Road, Take It"[a]. Increasing knowledge of the human genetics of neurological diseases continues to expand our understanding of the pathophysiology of age-related degenerative disorders such as AD. Genetic abnormalities associated with AD have provided clues for new amyloid target identification in the biology of neuroinflammation, immune clearance, lipid metabolism, and synaptic or endocytic cell membrane dysfunction. Amyloid research has been the predominant area of Alzheimer's research for the past 2 decades, but if amyloid therapies fail how do we close the gap to evaluate new approaches to prevention neurodegeneration? Will we have to wait another 20 years for the next wave of targets to move to clinical evaluation?

Some scientists say the antibody trial results so far prove that targeting amyloid was always the wrong therapeutic strategy. Others, perhaps previous supporters, are jumping off the amyloid bandwagon to join those who have long promoted alternate avenues of research, such as Tau. There is abundant Tau pathology in AD[8] that spreads from the temporal lobes through the brain to the cortex, correlating well with disease progression and cognitive decline despite there being no genetic association. Tau pathology is thought to be downstream of amyloid deposition and to be the result of soluble hyperphosphorylated Tau aggregating to form toxic neurofibrillary tangles. Emerging Tau biology[9] undoubtedly offers potential for the discovery of new targets for cerebral proteinopathies such as Alzheimer's disease. But how do we choose what to study, and how do we validate the novel targets we discover?

Validation of prospective targets is critically dependent on the development of well-behaved small molecule probes and appropriate animal assays. Indeed, tool molecules that have near druglike selectivity and activity against the target are critical to this mission. Targets need to be prioritized rigorously on the basis of the availability of target engagement (PET imaging), pharmacodynamic, and disease biomarkers that enable early decision-making and enhance the probability of successful clinical proof of concept testing. Tau genetic abnormalities are present in non-AD dementias, such as fronto-temporal dementia (FTD) and progressive supranuclear palsy (PsP), in which neurofibrillary tangles, similar to those found in AD brains, are a hallmark of brain pathology. Today it is these dementias that provide a somewhat imperfect basis for transgenic animal models to test and select potential AD therapeutic molecules in discovery. They may also provide clinical populations for rapid early proof of principle studies before advancing to trials in AD.

It is not going to get easier. Programs testing Tau hypotheses face many of the same hurdles as those directed against amyloid. What species of Tau to target: soluble or fibrillar? What modality to use: immunotherapeutics or small molecules? Whom to treat, when to treat, and for how long? New biomarker approaches are likely to be needed, and these will need to be developed alongside drug candidates. For example, novel phospho-tau imaging tracers could become read-outs for tau based drug discovery and may also be useful as true progression biomarkers for any disease modifying mechanism in clinical POC trials, unlike the amyloid PET tracers that so far appear only to identify people at risk.

"Nobody Goes There Anymore Because It's Too Crowded"[a]. Optimization of cholinergic and glutamate neurotransmission has been the target of many attempts to improve the treatment of Alzheimer's symptoms by building on the efficacy shown by acetyl cholinesterase inhibitors and NMDA antagonists.[10] The cholinergic system is critically involved in cognition and is known to degenerate in AD. The recent registration of a high dose of donepezil indicates that cholinergic activation has not yet been optimized for therapeutic benefit even if in this case there was a price to pay in terms of increased cholinergic side effects that can make the regimen intolerable for some people. Activation of brain postjunctional muscarinic M1 receptors, that are relatively conserved in AD, is thought to be a major contributor to the clinical efficacy of acetyl cholinesterase inhibitors. The

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muscarinic agonist xanomeline showed promising clinical activity but eventually failed due to lack of tolerability, probably because it lacked sufficient selectivity over other muscarinic receptor subtypes. It has proven very difficult to identify M1 receptor selective orthosteric agonists, as the acetyl choline binding site is highly conserved across all the muscarinic receptor subtypes. Novel strategies to boost M1 signaling in the absence or presence of cholinesterase inhibitors nonetheless have the potential to provide new options.

There are significant challenges to the symptomatic field. First it has proved difficult to get good lab to clinic translation. Second, replication of promising early efficacy read-outs in phase 3 trials is difficult (as exemplified by the late phase failure of the antihistamine dimebon), especially when trials cross geographies and move from populations that are relatively drug naïve to include the vast number of patients who are actively medicated with today's standards of care. Third, new therapies will have to be set in the context of acetyl cholinesterase inhibitors and memantine that will soon be generic and so will have to show improved efficacy when used alone or in combination with these agents without paying a penalty through worsening tolerability. In the past cognition in animals, usually rodents, has translated poorly to the clinic. Leading cognition strategies now increasingly use translational biomarkers to prove target engagement (e.g., PET imaging) and central pharmacodynamic activity (e.g., EEG) to navigate across species from the lab to the clinic to conduct well-defined proof of concept trials.

Summary—"You Miss 100% of the Shots You Don't Take"[f]. There is a considerable mismatch between the current advanced state of the biology in the Alzheimer's field and the prolonged time it takes the drug discovery and development process to bring novel therapeutics agents forward for definitive clinical evaluation. The current glacial pace of testing a theory every 10−20 years cannot be fast enough for a healthcare problem of this magnitude. We have to solve this dilemma. Is it through better data sharing, risk sharing, coordination, and alignment of expectations in public/private/philanthropic/academic consortia such as ADNI (Alzheimer's Disease Neuroimaging Initiative) or through big science such as the National Plan to address Alzheimer's disease (NAPA), and if so, where does the deep funding that will be needed come from in these financially constrained times?

The recent report "Aging in the 21st Century: A Celebration and a Challenge" from the United Nations population fund has warned that the world's aged population is growing rapidly, particularly in developing countries. Indeed, the number of elderly people is increasing faster than any other age group. The number of people aged over 60 is expected to grow by 200 million in the next 10 years to surpass one billion, and it will reach two billion by 2050. This demographic shift will present huge challenges to welfare, pension, and healthcare systems worldwide. Despite major advances in the understanding of AD, there has, however, effectively been a near zero return on investments made into the discovery and development of Alzheimer's therapies in the past 20 years. For all the negative press given to the pharmaceutical industry, global society is still relying heavily upon it to help find, develop, and fund solutions to the financial, societal, and personally destructive epidemic that is AD. It is naïve to think that single approaches will meet all the needs of a progressive neurodegenerative disease whose symptoms progress from cognition to disruptive behavioral symptoms. It is imperative to find ways to study a wide-spectrum of approaches to symptomatic and disease modifying therapies in the most expeditious way possible. This includes moving projects to and through the clinic faster using novel translational approaches that bridge the bench to the bedside and facilitate proof of principle testing in patients. We have to keep work focused on validating biomarkers that can identify individuals with early Alzheimer's, so that new drug candidates can be tested in patient populations where therapy can still make a difference, and on biomarkers that can help us monitor their therapeutic impact on disease progression to help speed medicines to market before more lives are destroyed. We must take chances, and for the sake of patients, caregivers, and healthcare economies worldwide, we must succeed.

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Notes

Views expressed in this Viewpoint are those of the author and not necessarily the views of the ACS.

The author declares the following competing financial interest: Full time employee of Merck & Co. Inc.

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[b]Abraham Lincoln.
[c]David Russell.
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