The Silent Epidemic of Alzheimer’s Disease: Can Precision Medicine Provide Effective Drug Therapies?

by Stephen C. Waring DVM, Ph.D and Stephen Naylor, Ph.D

Alzheimer’s disease is a complex, multi-faceted condition. Our understanding of the causal onset, diagnosis, progression, and treatment of the disease is limited and beset with confusion. It is estimated that ~35 million adults worldwide are afflicted with Alzheimer’s disease, representing less than 1% of the global adult population. However, a significant and poorly understood “risk factor” for Alzheimer’s disease is aging, and >95% of patients are 65 years of age or older. This rapidly growing segment of the global population is more prone to onset of Alzheimer’s, and represents a significant but “silent” epidemic threatening to overwhelm the world. In this work we discuss the current limitations of our understanding of causal onset, diagnosis and progression of the disease, and the paucity of effective therapeutic treatments. We consider the potential of systems biology and Precision Medicine to unravel and provide insight into the complex causal pathway and network biology of the disease, as well as facilitate safe and effective therapeutic drug treatments.

Introduction

Alzheimer’s disease (AD) is a progressive and irreversible neurocognitive disorder. Disease onset and manifestation results ultimately in loss of memory and other cognitive abilities, as well as the inability to carry out simple daily tasks. AD is one of the many forms of dementia that also includes vascular dementia, dementia with Lewy bodies, frontotemporal lobar degeneration, Creutzfeldt-Jakob disease, normal pressure hydrocephalus, Parkinson’s disease dementia, Huntington’s disease, and mixed dementia. The pathway and network pathobiology, causality and progression of AD are both complex and multi-faceted. Therefore, it is not surprising to recognize that the diagnosis and therapeutic treatment of AD are poorly understood.

We have noted previously that the current practice of patient care has had limited impact on the prevention, prediction, accurate diagnosis, and effective treatment of complex diseases. This notable lack of progress, in concert with a growing awareness of the complexity and variability of individual patients, as well as our limited understanding of causal mechanisms of onset, progression and treatment of most diseases have led to a growing demand for a paradigm shift. The clamor for change has led to the emergent growth of “P-Medicine”. The P-Medicine list of endeavors includes Personalized, Precision, Preventive, Predictive, Pharmacotherapeutic and Patient Participatory Medicine. In particular, it has been suggested that Precision Medicine appears to offer some potential in complex disease analysis and understanding. A report published by the National Research Council defined Precision Medicine as “... the tailoring of medical treatment to the individual characteristics of each patient. It does not literally mean the creation of drugs or medical devices that are unique to a patient, but rather the ability to classify individuals into subpopulations that differ in their...
susceptibility to a particular disease, in the biology and/or prognosis of those diseases they may develop, or in their response to a specific treatment. Such a development leads to the question, can Precision Medicine provide any meaningful insight into AD?

P-Medicine in the guise of both Personalized and Precision Medicine is over a decade old, and continues to be accompanied by enthusiastic expectations. However, it is unclear as to the actual impact of Personalized/Precision Medicine on even a “simple” disease such as diabetes, which is now aptly described as a global pandemic. The International Diabetes Federation estimated that ~733 million adults were suffering from this disease either in the form of impaired glucose tolerance (i.e. pre-diabetes) or diabetes in 2015. This represents ~15.5% of the adult global population. Based on the statistics the initial and tentative conclusion is that to date, the impact of Personalized and Precision Medicine on a “simple” disease such as diabetes, with a significant and well defined population base of patients has been minimal.

In stark contrast, only ~46.8 million adults worldwide were deemed in 2015 to have some form of neurocognitive disorder as defined by dementia criteria. AD is by far the most common type of dementia and accounts for ~60-80% of all cases. It is estimated that there are ~35 million adults worldwide suffering from AD, which represents less than 1% of the adult population. We consider here the expectations of Personalized/Precision Medicine advocates as they pertain to the treatment of a complex disease state such as AD. This analysis is compounded by the fact that the global AD population appears to be a relatively small, well defined group from an age-related perspective, but paradoxically also heterogeneous.

**Alzheimer’s Disease – Problems**

The impact of any disease is mitigated by our understanding of causality, prevention, diagnosis, and therapeutic treatment. The importance of safe and effective treatment is reflected in the size of the global pharmaceutical market which is projected to achieve $1.3 trillion in sales by 2016. It is noteworthy that the size of the USA pharmaceutical market in 2014 was $376.3 billion. This is greater than the other top ten markets combined as reflected by sales in Japan ($78. billion), China ($75.9 billion), Germany ($45.7 billion), France ($38.1 billion), Italy ($28.5 billion), United Kingdom ($25.2 billion), Brazil ($23.8 billion), Spain ($21.0 billion), and Canada ($20.9 billion). In addition the US pharmaceutical industry spent $51.2 billion on therapeutic research and development in 2014. We would therefore predict that if any one country was making significant inroads into the effective treatment of AD it would be the USA.

**Impact of Alzheimer’s Disease**

Unfortunately that does not appear to be the case. AD is now the sixth leading cause of death in the USA, with an estimated 5.3 million Americans now diagnosed with the disease. Every sixty-seven seconds someone in the USA is diagnosed with AD, and this is projected to change to every thirty-three seconds by 2050. It is startling to note that 96% (~5.1 million) of those US adults with AD are over the age of 65, which represents 1-in-9 older Americans (see Figure 1). Recent reports have indicated a cautiously encouraging decline in the number of new cases annually. However, while in effect leading to a lower trajectory of incidence, the impact on overall numbers with AD at any given point in time will be small due to the vast increase in numbers of individuals at risk. This is due to the fact that a large segment of the US “Baby Boomer” population (born 1946-1964) has begun to reach the age of sixty-five and is now at elevated risk for AD onset as highlighted in Figure 1. By 2030 the US population that is 65 years of age and older will constitute 20% of the total population, and it is estimated that by 2050, the number of people aged 65 and older in the US with AD will triple.

**Figure 1.** Projected prevalence of Alzheimer’s disease in the USA adult population for individuals 65 years of age and older.

The solid blocks represent the total number of adults 65 years of age and older

The Red line represents the estimated number of 65 years of age and older patients with AD.
As noted above AD is now a significant cause of death in the US, with 1-in-3 seniors dying in a given year having been diagnosed with AD or non-Alzheimer’s dementia\(^\text{17}\). When compared to major causes of death associated with breast cancer, prostate cancer, cardiovascular disease, stroke, and HIV, all of which have shown a significant decline over the past 15 years, AD is the only one of these showing a major increase over the same period as summarized in Figure 2. There is a major limitation that underlies interpretation of mortality data for AD having to do with how this information is recorded on the death certificate. For many individuals with AD, the death certificate often lists an acute complication of disease such as pneumonia as the underlying cause of death. Therefore, the impact of AD related to mortality is grossly underestimated. Indeed, while the National Center for Health Statistics of the Centers for Disease Control and Prevention (\(\text{CDC}\)) lists 84,767 deaths from AD in 2013, deaths attributed to AD among people 75 and older (65-74 data not available) in 2010 was around 500,000\(^\text{18}\). The authors concluded that these deaths would not be expected in that year if the individuals did not have AD, clearly underscoring the limitations of death ‘with’ dementia and death ‘from’ dementia. It is noteworthy that the population at risk is expected to increase over the next 25 years due to the baby boomer effect (Figure 1), and is compounded by the long duration of illness, of up to 8 years on average but as much as 20 years\(^\text{19,20}\). This significantly underscores the true magnitude of this disease in terms of an expected ever increasing number of deaths, poor health and disability. Hence our description of AD being the “silent epidemic” appears to be salient and an issue to be urgently addressed.

**Risk Factors**

The biggest risk factor for AD is age, with most people diagnosed at age 65 or older. But it is important to consider that while age is a major influence, this is not a normal part of aging. The search for other risk factors over the past 20 plus years has been rigorous, with particular interest in identifying modifiable factors that could either prevent or delay onset of disease if interventions can be delivered early enough in the process to considerably change an untoward trajectory. Numerous studies have reported factors that either increase risk or protect against risk for developing AD\(^\text{21,22}\). Many of these factors may be relevant to pathways involving amyloid processing, lipid/cholesterol transport and metabolism, neurovascular, neuroinflammatory, and neuropsychological function. Apolipoprotein E is involved in cholesterol transport mechanisms as well as injury repair in brain tissue, and has three different isoforms, \(\varepsilon_2, \varepsilon_3,\) and \(\varepsilon_4\) representing differing abilities to traffic lipids, with \(\varepsilon_4\) (\(\text{APOe}_4\)) being the least efficient. As a result, carriers of either one or two copies of \(\text{APOe}_4\) are at greater risk for developing AD compared to carriers of the other two isoforms\(^\text{23}\). An individual positive for only one allele (heterozygote) has up to a four-fold increase risk, whereas an individual with both copies (homozygote) has a more than 10-fold increase risk for developing AD\(^\text{24}\). In fact, \(\text{APOe}_4\) is the greatest risk factor for AD beyond age, and the strongest genetic risk factor reported to date. With the advent of genome-wide association studies (\(\text{GWAS}\)), a number of other genetic risk factors, all with very modest effects but potentially relevant pathogenic mechanisms, have been identified as detailed in Table 1a. To date, we now know of the three familial (early onset) genes associated with autosomal dominant inherited AD (\(\text{APP}, \text{PSEN}_1,\) and \(\text{PSEN}_2\)) accounting for less than 5% of all disease, a late onset gene (\(\text{APOE}\)) that accounts for up to 50% of disease, and several validated loci for other genetic variants that contribute to small but yet to be determined percentages of disease\(^\text{25,26}\). All represent four basic functional categories: amyloid-\(\beta\)eta relevant (production, degradation, or clearance), cholesterol metabolism, innate immunity, and cellular signaling\(^\text{26,27}\).

Non-genetic factors such as cardiovascular risk factors, hypertension, diabetes, obesity in midlife, smoking, and high cholesterol have also been reported. Whilst these risk factors may be significant for modifying upstream
A critical issue in considering the expected temporal role of various risk factors is that not all factors represent risk equally at the individual level and not all findings can be generalized to other populations. Owing to the complex and multifactorial nature of AD, the epidemiologic concept of a component causal model\textsuperscript{28} for disease is important to consider when interpreting the utility of various findings. This is not only important for prevention, but for treatment options as well. The component causal model represents a minimal set of factors that when present, explain why disease occurred at a particular point in time for a particular group of individuals. In the case of AD, this explains why many of these reported factors are neither sufficient nor necessary for disease to occur, and why there are many conflicting interpretations regarding epidemiological findings. It is not surprising that with so many underlying neurobiological mechanisms over the spectrum of aging with potential relevance to neurodegenerative changes that could lead to AD, a broad range of genetic and non-genetic factors, some direct, some as surrogates for yet to be determined processes, could be explanatory at a given point in time and have minimal relevance otherwise. For example, as stated above, APOE \( \varepsilon_4 \) is the strongest genetic risk factor for AD. However, not everyone with APOE \( \varepsilon_4 \) will develop the disease and about half of the individuals with AD did not inherit an APOE \( \varepsilon_4 \) allele. Additionally, APOE \( \varepsilon_4 \) may be an age-dependent risk factor since reports suggest that for individuals over 80 years of age other factors may be more important\textsuperscript{29-31}.

Biomarkers are also important factors, both in establishing risk and in supporting a diagnosis, assuming they have been validated and approved. Similar to genetic markers, numerous studies have reported on certain protein markers in blood (individual or in a panel) that are significantly associated with AD or with potential neurobiological pathways\textsuperscript{32,33}. However, methods, assays, handling, and tissue analyzed (plasma or serum) among other issues

---

**Table IA.** Genetic risk factors associated with AD onset.

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Lipid Transport</th>
<th>Amyloid Processing</th>
<th>Immunity</th>
<th>Inflammatory</th>
<th>Neuronal/Cellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>ABCA7</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLU</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>CR1</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>PIICALM</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>TREM2</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>SORL1</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>BIN1</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>MS4A</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

**Table IB.** Non-genetic risk factors associated with AD onset.

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Amyloid/tau Processing</th>
<th>Inflammatory/Oxidative</th>
<th>Neuronal/Cellular</th>
<th>Cognitive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vascular</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular Disease</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Cerebrovascular Disease</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>Lifestyle</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Midlife Hypertension</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Midlife High Cholesterol</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>Dietary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturated Fats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homocysteine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Traumatic Brain Injury</strong></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

Effects relevant to risk for developing AD, they may be less suitable for direct therapeutic interventions aimed at specific neurobiological pathways as shown in Table IB. However, their role in interactions with said pathways and in establishing more precise risk profiles is important to consider, particularly since drug monotherapy is unlikely to be effective.
may vary between laboratories and between multiplex platforms, making replication and validation a major challenge. Standardization efforts are underway and expected to shed more precise light on the subject in the not so distant future.  

A number of studies have established the diagnostic utility of amyloid-β and tau measured in cerebrospinal fluid (CSF) and positron emission tomography with [(18)F]fluorodeoxyglucose (FDG-PET). Amyloid-β (Aβ42) is a measure of amyloid deposition in the brain and tau (total and phosphorylated) is a measure of neuronal injury and neurodegeneration. The temporal relationship of these markers in CSF is hypothesized to reflect a significant departure from normal for amyloid followed by elevated tau. However, further evaluation in large longitudinally followed, well characterized cohorts is required to refine our understanding of this relationship. An international standardization protocol and quality control program has been developed through the Alzheimer’s Association Global Biomarker Standardization Consortium, the Alzheimer’s Disease Neuroimaging Initiative (ADNI), and the Coalition Against Major Diseases Biomarker Working Group and are now being followed by many laboratories worldwide to help support this critical area of investigation.

In addition to biomarkers in blood and CSF, perhaps the most significant markers, not only for diagnostic specificity but for profiling risk, are based on neuroimaging. Specifically they include structural magnetic resonance imaging (sMRI) and positive emission tomography with fluorodeoxyglucose (FDG-PET). Medial temporal atrophy, particularly the regions of interest (hippocampus and amygdala) relevant to short term memory processing and progression of memory impairment, are evident by sMRI early in the neurodegenerative process (pre-clinical stages). FDG-PET detects differences in cerebral metabolism, specifically cerebral glucose metabolism, in AD and mild cognitive impairment (MCI) compared to cognitively normal individuals. This is highly correlated with cognitive deficits and with progression from MCI to AD. Another imaging technique, Pittsburgh Compound B (PIB-PET) is also a significant measure of amyloid deposition with particular utility in establishing a risk profile as well as following neurobiological preclinical progression. The recent development of tau PET imaging holds great promise since this correlates more closely with neuronal and synaptic loss. Preliminary evidence with tau PET (F-T807) indicates great potential in furthering our understanding of the temporal relationship between amyloid, tau, and cognitive impairment and decline. Coupled with the CSF markers, neuroimaging represents the most significant biomarker capability identified to date. All together, they represent critical measurements that must be taken into consideration when planning clinical trials, and particularly for prevention trials designed to prevent the pathological accumulation in the first place.

Diagnosis

In spite of an avalanche of information regarding clinical and neuropathological characterization of AD over the past 30 years, the diagnosis of the disease has been predicated on the Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS–ADRDA) criteria established in 1984. These criteria considered both clinical evidence and evidence of moderate to severe AD neuropathology (plaques and tangles). With the mounting knowledge gained from state-of-the-art technologies such as imaging and high-density/high-throughput genetic and biological assays, and new information on the clinical course of the disease, a broad consensus to revise these criteria led to the National Institute on Aging-Alzheimer’s Association workgroups (NIA-AA AD Workgroup, NIA-AA MCI Workgroup, and NIA-AA Preclinical Workgroup) that reported new diagnostic guidelines in 2011. These include revised criteria for diagnosis of dementia due to AD, MCI, and the newly recognized need for preclinical staging criteria to support promising drug trials aimed at the period when they may be most effective.

Two of the most critical concepts that influenced not only the urgent need to revise diagnostic criteria, but that are captured in the various criteria, have to do with intermediate stages prior to clinical onset and non-Alzheimer’s dementias. The explosion of literature on MCI since it was first described in the late 1990s has led to a better understanding of intermediate stages, both clinical and neuropathological, in the continuum from normal aging to dementia. This has been incorporated into the criteria to account for the discordance in clinical-pathological states and to provide a better framework for establishing pre-clinical stages upstream from even MCI, much less early AD. Another significant concept influencing the need for revisions in diagnostic criteria stems from the growing body of evidence over the past decade regarding non-Alzheimer’s dementias, particularly Lewy body dementia and frontotemporal dementia, and how they are distinct as well as how they overlap with clinical features of Alzheimer’s and with neuropathological expectations.

The preclinical stage criteria is pivotal, representing a long overdue recognition that the pathophysiological processes of neurodegeneration begin well before there is clinical evidence of dementia, possibly 20 years or more. Even the well understood and accepted MCI stage, where there is already substantial neuronal loss, may be too downstream in the continuum for disease-modifying therapies which could account for the significantly high failure rate of drugs tested as well as the challenges in developing successful treatments. The hypothetical staging framework proposed by the NIA-AA Preclinical Workgroup categorizes clinically normal individuals on a risk trajectory for AD based on
the presence or absence of amyloidosis, neurodegeneration, and subtle cognitive decline (see Figure 3). Two important distinctions are the Stage 0 group with no evidence of amyloidosis, neurodegeneration or cognitive impairment and least likely to be on the AD trajectory and the newly recognized SNAP (Suspected non-Alzheimer’s pathophysiology) group suggested by the Mayo Clinic. The percentage of individuals in the Stage 0 group progressing to dementia (rather than to a higher Stage) would be expected to be less than 10% and most likely would develop non-Alzheimer’s dementia. The SNAP group, while possessing markers of neurodegenerative changes consistent with aging and non-AD disorders. Therefore, this group fills a critical gap between cognitively normal individuals and cognitively impaired individuals, and provides a more refined clinical-pathological correlation at autopsy to distinguish between aging, non-AD, and AD. As this is a new concept just now undergoing vast scrutiny across many large studies, it will be important to revisit the constructs relevant to this subgroup in light of any new findings that would impact its utility in planning for future treatment trials and prevention trials.

The recommendations of these three working groups have different relevance to clinical practice, with the criteria for both AD and MCI intended to guide clinical diagnosis as well as research, while those for preclinical AD being strictly for research purposes. An important distinction must be realized between any criteria established for a clinical diagnosis of dementia and the need for more rigorous criteria necessary for use in a research setting. The latter are designed specifically to standardize processes to accelerate scientific understanding and inform better treatment and prevention trials. For instance, the new criteria for AD incorporate biomarkers (CSF and imaging) that help to raise the level of confidence regarding a clinical diagnosis of dementia due to Alzheimer’s, but while they are not necessary and may not even be available in a clinical setting, they have tremendous relevance in establishing the diagnosis for research interests. Ultimately, the goal is to accelerate knowledge that will lead to transforming clinical care for earlier and more precise diagnosis, more effective and timely treatment, and improved outcomes for individuals at risk for developing AD during their lifetime.

While this is perhaps the most exciting and potentially transformative developments in Alzheimer’s research to come along in some time, there is much work needed to standardize the use of these criteria in large, broadly representative populations, specifically the component parts representing biomarker and clinical features. There also needs to be a commitment to updating these criteria at much shorter intervals so the relevance of new information can be included to ensure an ever increasing precision and pace that will truly advance the collective effort towards a cure. But in spite of the requisite steps that
must be completed, this does represent a very robust foundation, incorporating state-of-the-art knowledge and technology, and a timely opportunity to leverage this gain by not only looking back on what may have been missed, but in moving forward beyond just Alzheimer’s-specific investigations to foster cross-fertilization of ideas and methodologies from other disciplines and diseases. Only then will the promise of Precision Medicine relevant to AD be attainable.

**Drug Treatment**

The history of drug development for the treatment of AD is littered with missteps, misleads and mistakes. Schneider and colleagues recently reviewed this subject in some detail and highlighted the following periods of AD drug development focus:

i. Cholinergic Era (1970’s-80’s) - since the early 1970’s memory had been related to the cholinergic system, and that early work in AD causality indicated a loss of central cholinergic neurons. These findings lead to the development of the first four therapeutic agents shown in Table 2.

ii. Amyloid Cascade (early 1990’s) - the hypothesis is predicted on amyloid-beta deposition facilitates hyper-phosphorylation of tau, neurofibrillary tangle formation and neuronal death. Although the model is simple the validation and development of drug targets has been complex and the output of successful therapeutic drugs has to date been unsuccessful.

iii. Variable Clinical Trials Time Periods - the regulatory authorities struggled to determine the appropriate time period for late stage clinical trials, and included a. 1986-1996, early symptomatic trials; b. 1990-2011, six month trials; c. 1994-2010, twelve month trials; d. 2011-present, eighteen month and disease modifying trials. In summary, eighteen-month, placebo-controlled trials have become a de facto standard for trials of mild and moderate Alzheimer’s disease even though no statistically significant primary outcomes favoring the test drug have been demonstrated.

iv. Mild Cognitive Impairment Trials (2000-2005) - MCI was characterized as progressive memory impairment and could be a potential clinical target for early treatment intervention. The results of all the trials in MCI were negative, with no significant benefit on progression or onset of AD.

v. Prodromal AD Trials (2010-Present) - The advancement of trials for prodromal AD followed the intent to diagnose the disease before dementia onset and to enrich clinical trial samples in terms of increasing confidence that patients actually had AD pathology and presumably would advance to dementia after a relatively brief interval. Drug trials for such an approach are currently ongoing.

vi. Prevention Trials (1996-Present) - these trials are logical extensions of MCI and prodromal trials and address the fact that the pathology of Alzheimer’s disease is progressing perhaps two decades before the onset of the disease.

There have been only a handful of completed prevention trials, in large part because they require large patient sets, prolonged follow-up, are complicated and expensive to conduct, and until recently were hindered by the lack of compelling and safe drugs or interventions to test.

Despite the enormous efforts, our limited understanding of causal onset, diagnosis and progression of AD is reflected in the paucity of effective and safe treatments of the disease. Symptomatic but limited interventions for AD have been widely available since the 1990’s, as summarized in Table 2. Cholinesterase inhibitors (CI) such as tacrine, donepezil, rivastigmine and galantamine were developed to improve cognition and facilitate patient function and behaviour. In China, huperzine-A, an alkaloid derived from the firmoss Huperzia serrata, was also approved as a CI treatment for AD patients (used as a supplement in the USA and Europe). However, the additional subsequent clinical trials as well as clinical

<table>
<thead>
<tr>
<th>Drug</th>
<th>Year</th>
<th>MOAa</th>
<th>Indication</th>
<th>CEIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognex (tacrine)*</td>
<td>1993</td>
<td>Ci</td>
<td>Mild to Moderate AD</td>
<td>3.5</td>
</tr>
<tr>
<td>Aricept (donepezil)</td>
<td>1996</td>
<td>Cl</td>
<td>Mild to Moderate AD</td>
<td>3.5</td>
</tr>
<tr>
<td>Exelon (rivastigmine)</td>
<td>1998</td>
<td>Cl</td>
<td>Mild to Moderate AD</td>
<td>3.6</td>
</tr>
<tr>
<td>Rasadyne (galantamine)</td>
<td>2001</td>
<td>Cl</td>
<td>Mild to Moderate AD</td>
<td>3.7</td>
</tr>
<tr>
<td>Namenda (memantine)</td>
<td>2003</td>
<td>NMBe</td>
<td>Moderate to Severe AD</td>
<td>3.0</td>
</tr>
<tr>
<td>Huperazine-A</td>
<td>1994</td>
<td>Cl</td>
<td>AD (China)</td>
<td>3.0</td>
</tr>
<tr>
<td>Risperdal (risperidone)</td>
<td>2015</td>
<td>Antipsychotic</td>
<td>Aggression in AD (Australia)</td>
<td>3.6</td>
</tr>
</tbody>
</table>

**Table 2. Current therapeutic drugs approved for the treatment of AD.**

a MOA - Mechanism of action  

b CEI - CureHunter Effective Index score. It shows the top ranked drug monotherapies for the treatment of AD based upon an analysis of all relevant articles indexed by the National Library of Medicine (PubMed). Article relevance is determined using an artificial intelligence approach that combines and weights each article based upon its level of evidence and the numbers of articles containing outcome statements, clinical trial or study statements and articles with clear context of study statements. The engine provides an effectiveness index on a scale of 1-10, with 10 being the most effective (see reference 7 for a detailed description of how a drug is scored).

c Drug withdrawn from the US market in 2013 due to liver toxicity  
d CI- Cholinesterase inhibitor  
e NMDA - N-Methyl-D-Aspartate antagonist
practice data demonstrated that CI therapeutic drugs only manifested modest improvements on the Mini Mental state Examination scores for AD patients over a 6-12 month period.

The N-methyl-D-aspartate (NMDA) antagonist, memantine was the first and only approved AD therapy that acts on the glutamatergic system. Despite a significant number of clinical trials and clinical observation, the drug has no discernible effect on mild AD, and modest impact on moderate-to-severe AD patients. In addition numerous antipsychotic drugs are commonly used to treat agitation, aggression, and psychosis in patients with AD. Most are prescribed off-label, with the exception of risperidone which was recently approved in Australia for the short-term treatment (up to 12 weeks) of severe aggression in AD. Once again it has been noted that “benefits are moderate” and that serious adverse events including sedation, parkinsonism, chest infections, ankle oedema, and an increased risk of stroke and death are also possible when taking this drug.

The limited efficacy of available therapeutic drugs is well documented and is highlighted in Table 2 by the relatively low CureHunter Effective Index scores. However, there has also been considerable concern raised about the safety and toxicological effects. For example, tacrine, the first CI introduced in 1993 (Table 2), was ultimately withdrawn from the US market in 2013 due to marked hepatotoxicity. Bayer was forced to halt its trials of the CI drug metrifonate due to manifestation of respiratory paralysis in patients. More recently the use of risperidone by AD, as well as dementia patients, led the FDA to require drugmaker Janssen-Cilag, a subsidiary of Johnson & Johnson, to market the drug with a black box warning. Such a warning appears on the label of a prescription medication to alert patients, caregivers and health care providers of important safety concerns associated with that medication.

The segmented drug discovery and development efforts of AD drugs continue unabated to this day, even though our understanding of causal onset is unclear. For example there is disagreement about the role of amyloid plaques and tau tangles. It appears that they are both important in the pathology of AD, but their individual roles in causal onset is still mired in controversy. The amyloid cascade hypothesis continues to dominate drug development, with targets being developed for individual steps in the cascade: secretase inhibitors and modulators, passive and active immunization with antibodies against various epitopes of amyloid-β monomers, oligomers and fibrils, fibrilization inhibitors, anti-aggregants and other approaches. However, both anti-amyloid and tau-based approaches are currently being advanced in 18-month trials in patients with mild and prodromal Alzheimer’s disease. In addition small molecule, neurotransmitter approaches are also being actively pursued, primarily in the form of 5-HT6 receptor antagonists.

Unfortunately in recent years the development of AD drugs has become the graveyard of expensive failures. For example in 2012, Pfizer/Johnson & Johnson announced the failure of a second Phase III trial of the anti-amyloid antibody bapineuzumab. This prompted the proclamation that the “amyloid hypothesis is dead.” More recently Eli Lilly indicated that its twice-failed late-stage drug solanezumab would likely miss the clinical endpoint of significantly improving cognitive function. Since this latter candidate is also an anti-amyloid drug, it suggests that we are missing the pathological-clinical link, since if amyloid is the critical toxic component and trial patients are at an early stage clinically, an effective anti-amyloid therapy should slow disease progression.

The continued failure-rate of late stage AD candidate drugs has begat a sense of frustration and desperation on the part of all concerned stakeholders. In some cases this has led to an unusual circumstance of events. In mid-summer of 2015 Vivek Ramaswamy, a former hedge-fund manager with no biotech experience, licensed an abandoned 5HT6 antagonist from GlaxoSmithKline (GSK) for $5 million and created an AD start-up company Axovant. The drug candidate had been previously tested in 1,250 Alzheimer’s patients and healthy subjects in five different clinical studies that GSK conducted between 2008 and 2012 and then subsequently abandoned. Axovant gathered a team together and without recruiting a single patient for a pivotal study of a marginal drug designed to treat symptoms of the disease, raised $315 million in an IPO that resulted in a market capitalization of the company worth greater than $1 billion. This exasperating situation was further compounded when Pfizer announced last month that it was terminating an AD Phase II clinical trial of PF-05212377. They concluded that this 5HT6 drug would fail to meet the key endpoint and put the spotlight on the drug’s mechanism of action, raising fresh questions about the likely efficacy of other drugs that try to safeguard cognitive abilities by targeting the 5-HT6 receptor. The stock price of Axovant stock tumbled by ~50% over the subsequent week after the Pfizer announcement. The Pfizer AD drug failure, compounded by the Axovant situation clearly indicates that something has to change in terms of how we do AD drug discovery and development.

Alzheimer’s Disease – Systems Biology and Precision Medicine

The fate of AD drug candidates in the drug development process over the past twenty years stands at a remarkable 99.8% failure rate. In addition the cost of those failures to the pharmaceutical industry has been in excess of $15 billion for amyloid-beta trials alone during that same time period. Currently there are twenty-three Phase I, forty-seven Phase II and eighteen Phase III AD candidate drugs in US clinical trials. However, evaluation of the individual clinical trial drug candidates reveals that the vast majority are focused on individual druggable targets in the amyloid-beta or tau...
Genetics has been recognized as an important causal factor in AD onset acting via the dominant mutations of APP, PSEN1 and PSEN2, but occurs in less than 5% of patients. In contrast it is estimated that the risk of AD onset is 70% attributable to genetic components, and is potentiated throughout the lifetime of the individual.26 In addition the two core pathological harbingers of AD are amyloid plaque and tau neurofibrillary tangles. More recently there have been proposals that a concomitant amyloid-tau cascade mechanism is responsible for AD onset. This hypothesis proposes that changes in tau and consequent neurofibrillary tangle formation are triggered by toxic concentrations of amyloid-β.[2]

The pathways linking amyloid-β and tau in AD onset and progression50. Recently there has been considerable effort in understanding the role of the neurovascular system (NVS) and onset of neurogenerative diseases such as AD58. The NVS consists of vascular cells such as endothelium and vascular smooth muscle cells, as well as glial cells and neurons. The endothelial cells are a constitutive part of the blood-brain barrier (BBB), and the latter controls entry of all cellular and metabolites into the brain. One purported involvement of the NVS suggests the amyloid-β-rich environment in AD and increases the receptor for advanced glycation end products expression at the BBB and in neurons amplifying amyloid-β-mediated pathogenic responses. Zlokovic has suggested the role of BBB dysfunction in amyloid-β accumulation, underlies the contribution of vascular dysfunction to AD58. Finally, it has also recently been reported that AD pathogenesis is not restricted just to the neuronal compartments.
but involves significant, interactions with immunological processes in the brain. It has been suggested that mis-folded and aggregated proteins bind to receptors on microglia and astroglia cells, and trigger an innate immune response characterized by release of inflammatory mediators, which contribute to AD progression and severity. In addition glial activation which leads to neuroinflammation contributes to neurodegeneration and synaptic abnormalities leading to neuronal cell death, lending more support to the developing hypothesis that neuroinflammation is associated with AD pathology. As stated above, AD causal onset is multifaceted and temporally mediated (Figure 4), thus how do we deal with such complexity in attempting to design effective therapeutic agents?

**Systems Biology-Drug Discovery**

The emergence of systems biology in concert with the development of a suite of accompanying analytical and bioinformatics tools and technologies has facilitated the evaluation and unraveling of complex disease mechanisms. Therefore it is not surprising that we and others have suggested such an approach should ultimately find widespread use in understanding causal onset, progression and effective treatment of any complex disease such as AD. Recently Bennett and colleagues have suggested an approach to drug discovery and development for effective and safe AD drugs using a systems biology approach. They argued that any lead drug candidate must disrupt molecular networks "... that lead to the accumulation of AD neuropathology, and trigger the neurodegenerative process that leads to cognitive decline, and ultimately to the clinical manifestations of cognitive impairment and dementia due to AD." Note the emphasis on targeting a network as opposed to a single pathway such as the amyloid-beta pathway. Based on Bennett’s suggestions as well as our experiences we would propose the following broad-based systems biology approach to drug discovery:

1. **Network Biology Discovery** - multi-omics analysis at the gene, protein and metabolite level. This should include DNA methylation, miRNA, and mRNA transcriptomic data from human brain material derived from brain region at the hub of neural networks subserving cognition, as well as AD pathologic and clinical quantitative traits, to nominate genes, and therefore proteins, from networks and nodes involved in the molecular pathways leading to AD. We were the first group responsible for developing a Systems biology methods approach for integrating multi-level "omics" data derived from a mammalian system and such approaches are now relatively routine.

2. **Identification of Potential Targets** - the network biology analysis should provide a prioritized list of target genes. An assortment of analytical tools primarily employing mass spectrometry should be used to generate protein expression data which can be analyzed with standard regression techniques, pathway analyses, and structural equation models to provide empirical support for biologic pathways linking proteins to AD endophenotypes. As Bennett notes "the empirical data will be fed back to the network modeling stage to refine the network analyses. This component ensures that the candidate genes generated from the systems biology component above are translated in the human brain and that the measured protein variants themselves are related to AD endophenotypes."

3. **Functional validation** - utilization of RNAi screens to either over-express or knock down each of the selected genes in neurons. This provides an efficient, high throughput approach to generating the data required for the analysis of transcriptional networks. RNA profiles derived from each experimental condition will allow the empirical reconstruction of the molecular networks for the target, and human cell types to confirm the pathways identified in the initial integrative analyses. As Bennett notes, this component of the pipeline has several purposes: i) it will refine the networks and confirm that the genes nominated in systems biology analyses actually have the expected effects when they are disrupted on an individual basis; ii) it will identify other genes which may be involved in the network because they have similar functional consequences when their expression is perturbed; iii) it will identify transcriptional programs or "gene sets" that, in vitro, capture aspects of the function of a given pathway and can be used as outcome measures in future drug screening; and iv) it will also identify nodal points in each network, "hubs" for a given pathway that may make particularly effective targets for the disruption of a given cellular pathway.

4. **Drug Candidate Screen** - selected, prioritized molecular targets that are expressed in the aging brain and are associated with pathologic and/or clinical AD quantitative traits, are interrogated with the appropriate chemical or biological library. It is important to note that the experimental pipeline is by design driven by empiric observations from the first three stages. This should culminate in the selection of target nodal points for molecular screening which, a priori, were not preselected.

Bennett has suggested that any target should be a nodal point in a network related to AD pathology and a clinically quantitative phenotype clearly identified by integrative omic data analysis from human brain tissue. The target must be expressed in brain and also clearly demonstrated to be related to AD pathology and clinical phenotype. In addition we would argue that any consideration and scoring of the viability of the target need not simply fit into the amyloid, tau, or amyloid-tau pathway of AD. We believe that the target selection criteria should take into account the systems analysis of the causal onset involving, genetics, amyloid plaque, tau tangles, neurovascular and neuroinflammatory events that occur as a function of time.
Drug repurposing, also called repositioning, repriorizing or rescue (DRPx), refers to the process of taking a drug developed for one disease indication and applying it to another different disease indication. Similar risk factors and biological pathways can underlie seemingly unrelated diseases, opening the door for novel hypothesis-driven and data-driven repurposing strategies. While we need to accelerate the development of new drugs, the slow progressive nature of AD, the long duration of trials, the large number of patients required, the lack of comprehensive understanding of the neurobiology, and the limitations of the clinical trial enterprise make it incredibly costly and challenging and prone to high failure rates. Even existing knowledge of pharmacological effects and safety profiles of repurposed drugs can expedite early phases of clinical development, shorten drug development timelines, and reduce failure rates due to pharmacokinetic and safety issues. Repurposed agents are more than twice as likely across all disease indications to make it to market compared to de novo derived drugs. It is interesting to note one of the commercially available AD drugs memantine (Table 2) is a DRPx drug. It was first synthesized by Eli Lilly in 1968 and patented as an anti-influenza drug, but subsequently found serendipitously to have “beneficial” anti-dementia properties.

The value of a systematic approach adopted in drug repurposing efforts facilitate one-of-two predominant outcomes either i) a known candidate compound/drug interacting with a new target, or ii) known target mapping to a new disease indication. Methods and tools utilized in DRPx discovery to identify such possibilities include in vitro and in vivo (cell/organ/tissue/animal) phenotype model screening, High Throughput Screening, High Content Screening, Chemoinformatics, Bioinformatics, as well as Network and Systems Biology. These approaches are used in conjunction with available information on known targets, drugs, biomarkers of disease, and pathways/networks of disease that can ultimately lead to accelerated timelines in the discovery and development of DRPx candidate drugs.

Numerous challenges still exist to execute on cost-effective DRPx efforts since to evaluate a large number of drugs for a specific disease; it is difficult to unify the needed computational approaches because the available information for different diseases or drugs varies. For example, to use target-based methods to reposition drugs for 100 different targets, one would have to know the biomarkers or available pathways for each of those specific targets. The knowledge needed for this type of drug repositioning might be unavailable or difficult to derive from the literature or available databases. However, a number of computational systems biology DRPx companies have taken such an approach. For example CureHunter Inc (Portland, Oregon) utilizes an Integrated Systems Biology platform which effectively synthesizes all the data and knowledge acquired from “reading” the US National Library of Medicine to produce a clinical outcome database. This database is structured for autonomous prediction of new disease indications, such as AD, for any given drug, biomarker, or active biological agent. The value of such a computational systems biology approach to AD treatment is the effective utilization of pathway/network biology, large clinical datasets, electronic medical records, bioinformatic and artificial intelligence tools. This facilitates the deconstruction of complex disease processes such as those demonstrated to occur in AD (Figure 4), and then make useful predictions based on pathway/network analysis as to a druggable and viable target(s).

**Precision Medicine**

It was recently suggested that the “goal of precision medicine is to deliver optimally targeted and timed interventions tailored to an individual’s molecular drivers of disease.” As we discussed the utilization of systems biology tools in this type of endeavor is clearly synergistic and can and will facilitate such efforts. However, what specifically can precision medicine provide for patients in the form of safe and effective therapeutic treatments? As we have discussed, one of the probable reasons for the continued failure of large scale clinical trials to identify effective therapies is the erroneous assumption that AD is a single clinical disease state. Thus data that provides a risk profile of a patient with AD may be of some considerable value if it can be correctly interpreted. Precision medicine in contrast to the “one-size-fits-all-approach” of current medical practice, attempts to optimize the effectiveness of disease prevention, and treatment as well as minimize side effects for persons less likely to respond to a particular therapeutic. This is done by considering an individual’s specific makeup of genetic, biomarker, phenotypic and psychosocial characteristics. The measurement of molecular, environmental, and behavioral factors contributing to a specific disease improves the understanding of disease onset and progression as well as response to treatment. In addition, it allows a more accurate diagnosis and more effective disease prevention and treatment strategies specifically personalized to the individual.

We and others believe that precision medicine affords the following opportunities in facilitating more effective therapeutic drug treatments:

1. **Determination of Risk Assessment** - currently, a significant goal of risk assessment is to provide insight into relevant disease mechanisms and disentangle genetic from other causal events that lead to AD onset and progression. For example increased risk factors for AD onset include cerebrovascular disease, traumatic brain injury (TBI) or intellectual activity.

2. **Cerebrovascular Changes** - such as hemorrhagic...
infarcts, small and large ischemic cortical infarcts, vasculopathies, and white matter changes, increase the risk of dementia. Possible mechanisms through which stroke could lead to cognitive impairment and AD include direct damage of brain regions that are important in memory function (such as the thalamus), an increase in amyloid-beta deposition, and an induction of inflammatory responses impairing cognitive function.

ii. Detection of Latent Pathophysiologica Processes- treatment is routinely initiated after disease symptomology is clearly manifested, and is rarely initiated based on risk assessment alone. The availability of diagnostic tools to determine latent pathophysiologic processes provides opportunity for effective early-stage intervention. For example neuroimaging efforts show unusual promise in AD because of their growing ability to measure brain function at increasingly higher levels of organization, and is being considered for more widespread application.

iii. Personalized Intervention to an Individual’s Molecular Risk and Disease Pathology Profile- as discussed in detail above there are currently no effective preventive or therapeutic measures for AD. We have noted that almost all failed clinical trials performed to date have neglected the underlying clinical and molecular heterogeneity of the disease. In an attempt to address this methodological shortcoming, “a first round of ongoing trials including the Dominantly Inherited Alzheimer Network (DIAN), the Alzheimer’s Prevention Initiative and A4 trial are now incorporating information of underlying pathological mechanisms, selecting patients considered most likely to respond to the anticipated action of the therapeutic tested.” It is hoped that these trials prove to be more successful in terms of outcome of providing an effective safe treatment for AD. Indeed overall it is hoped that the impact of precision medicine via the three initiatives discussed here will provide a more insightful strategy for optimal therapeutic intervention to “prevent, stop or slow progression of AD.”

Conclusions
The “silent epidemic” gathers momentum. The essence of the crisis is due to a lack of mechanistic understanding of disease causality, onset, progression and effective prevention and treatment. We have argued in this paper that the need to evaluate AD via a systems biology perspective is imperative. The underlying heterogeneity of AD in clinical trials has until recently been misunderstood and poorly considered. This has led to a paucity of effective therapeutic treatments, and a trail of failed expensive clinical trials. In the case of precision medicine it is still a fledgling process. The ultimate facilitation of precision medicine should be to enable clinicians to prescribe effective and safe therapeutic drugs for individual patients. Whilst that is an admirable goal, it is nonetheless lofty and is often wafted around as something that is attainable “tomorrow”. The reality is somewhat different, as attested to here and described above. There is hope, but alas it continues to be accompanied by hype, which should have no part in this silent epidemic crisis. Ken Kaitin from the Tufts Center for the Study of Drug Development recently stated that “…finding newer and better medications to treat Alzheimer’s disease is one of the great scientific challenges of the 21st century. Success will ultimately lie in all stakeholders participating in the process, sharing in both the risks—and the rewards—of the effort.” Now that’s a rallying cry to get behind and continue to exploit the use of systems biology and precision medicine tools to develop safe and efficacious therapies for AD. We believe that the combined use of such an approach will help unlock the hindrances of AD complexity and facilitate the development of safe, efficacious drugs or drug combination cocktails.

Stephen Waring, DVM Ph.D, is an Epidemiologist and Senior Research Scientist with the Essentia Institute of Rural Health in Duluth, Minnesota, and Adjunct Professor of Pharmacology at the University of Minnesota, College of Pharmacy, at Duluth. His research into genetic and non-genetic risk factors associated with Alzheimer’s disease and other cognitive disorders has spanned 25 years, and includes work at Mayo Clinic (Rochester, MN USA) during the 1990s as part of the team that produced novel characterizations of mild cognitive impairment (MCI) and MRI volumetric studies of brain regions relevant to Alzheimer’s disease. He serves as the Leadership Council of the state of Minnesota ACT on Alzheimer’s initiative as well as the Medical and Scientific Advisory Council for the Minnesota-North Dakota Alzheimer’s Association.

Stephen Naylor, Ph.D, is the current Founder, Chairman and CEO of MaiHealth Inc, a systems/network biology level diagnostics company in the health/wellness and precision medicine sector. He was also the Founder, CEO and Chairman of Predictive Physiogy & Medicine (PPM) Inc, one of the world’s first personalized medicine companies. He serves as an Advisory Board Member of CureHunter Inc. In the past he has held professorial chairs in Biochemistry & Molecular Biology; Pharmacology; Clinical Pharmacology and Biomedical Engineering, all at Mayo Clinic (Rochester, MN USA) from 1990-2001. Correspondence should be addressed to him at snaylor@midmarkcap.com

Acknowledgements
We would like to thank Mr. Andrew Jackson (flaircreativedesign.com) for his considerable help and artistic capabilities in producing the figures contained in the article. In addition we would like to thank Mr. Judge Schonfeld, Founder and CEO of CureHunter Inc. for his help and advice in determining the CureHunter Effectiveness Index values for commercially available AD therapeutic drugs.