PERSPECTIVE

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Resolving controversies on the path to Alzheimer's therapeutics

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Alzheimer's disease constitutes a personal and societal tragedy of immense proportions. Since 1960, research in laboratories and clinics worldwide has elucidated many features of this insidious and ultimately fatal syndrome, and this progress has led to initial human trials of potentially disease-modifying agents. However, some of these agents have already failed. Gnawing controversies and important gaps in our knowledge seem to cast additional doubt on the ability of the field to move forward effectively. Here I discuss some of these looming concerns and offer possible explanations for the major trial failures that suggest they are not predictive of the future. Rigorous preclinical validation of mechanism-based therapeutic agents followed by meticulously designed trials that focus on the cardinal cognitive symptoms and their associated biomarkers in the mild or presymptomatic phases of Alzheimer's disease are likely to lead to success, perhaps in the not-too-distant future.

For scientists, few of life's experiences can match making a discovery that explains a hitherto unknown facet of how natural systems work. In the area of biomedicine, coupling such a discovery with its application to helping solve a human malady is particularly meaningful. The example of research on Alzheimer's disease includes many unexpected findings over the last three decades that have clarified pathogenesis and sometimes also illuminated fundamental aspects of cell biology. This interplay between basic and applied biology has attracted scientists of varying backgrounds and has led to divergent approaches to the disease. A substantial portion, but certainly not all, of the field has focused its investigative efforts on the theory that gradual accumulation of the amyloid-β protein (Aβ) in brain regions serving memory and cognition is a precipitant of the earliest symptoms of Alzheimer's disease. This theory, increasingly supported by measurements of biomarkers over the course of the disease[1], has led to human trials of diverse agents that could potentially decrease the production or aggregation of Aβ or enhance its clearance from the brain.

Despite this progress, the field of Alzheimer's research seems to have entered a period of disappointment and some doubt about the path forward. Beyond a healthy skepticism regarding the validity of the amyloid hypothesis, there has been understandable concern that several late-phase clinical trials have failed and that important questions about pathogenic mechanisms remain unanswered. The concept of redirecting the field toward other approaches is much discussed (for example, in ref. 2). In this context, we may benefit from a deeper analysis of some of the principal controversies in Alzheimer's disease research today and a careful consideration of the reasons for the recent trial failures. Here I offer a perspective on these central issues and conclude that current knowledge does provide a way forward to design and validate truly disease-modifying treatments.

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Controversies surrounding the Aβ hypothesis

The neuropathological, genetic, biochemical, animal modeling and biomarker findings that support a role for Aβ dyshomeostasis in precipitating Alzheimer's disease have been extensively reviewed (for example[3–6]; see **Fig. 1** for a summary of some principal discoveries in Alzheimer's disease research). Nevertheless, there remain several substantive concerns about this theory, upon which so much investigative effort has been based.

β-amyloid deposits in normal people. Perhaps the most frequently cited challenge to the Aβ hypothesis is the finding of some or many amyloid deposits in the brains of humans who died without evidence of dementia. Several considerations explain this apparent paradox. First, many of the Aβ deposits in such subjects are of the diffuse type, lacking fibrillar amyloid and significant neuritic dystrophy, microgliosis and astrocytosis, that is, the signs of frank cytotoxicity. Second, the levels of soluble Aβ oligomers in such brains have not been systematically quantified, yet it is these diffusible assemblies that have been increasingly associated with the degree of cognitive impairment in Alzheimer's disease. Third, the absence of subtle amnestic signs in such individuals without dementia before death has often not been rigorously established. Indeed, the advent of β-amyloid imaging by positron emission tomography (PET) has recently shown that the presence of Aβ deposits in ostensibly normal subjects is often associated with subtle cognitive deficits. Fourth, and most important, is the recognition that chronic diseases always show substantial pathology before the onset of the earliest symptoms. The occurrence of abundant atherosclerotic plaques in the coronary arteries of people dying late in life with no symptoms of heart disease or the presence of neoplastic changes in the prostates of many older men dying without clinically diagnosed prostate cancer are two common examples. No one believes that these lesions in healthy individuals have no relevance to disease. The emerging evidence of much higher rates of conversion from mild cognitive impairment (MCI) to Alzheimer's disease dementia in subjects having amyloid-positive PET scans and low $A\beta_{42}$ levels in their cerebrospinal fluid (CSF) strengthens the analogy to presymptomatic atherosclerotic lesions.

Amyloid as both a cause and an effect of Alzheimer's disease. Alzheimer's is by no means the first disorder in which scientists have

postulated a central role for amyloid accumulation. Decades ago, amyloidotic diseases were often relegated to the backs of medical textbooks and thought to represent secondary tissue reactions of unclear pathogenic importance, but that view has long since been disproven. Transthyretin-mediated amyloidosis is a compelling example of a non-brain amyloidosis in which a hydrophobic, self-aggregating protein can be the site of infrequent genetic mutations that can cause disease or, in other patients, can accumulate in its wild-type form due to other pathogenic factors. In the rare individuals with Alzheimer's disease who carry mutations in amyloid precursor protein (APP) or presenilin, accumulation and aggregation of $A\beta_{42}$ seems to be causative. We have not yet learned the causes of the invariant $A\beta_{42}$ elevation in most individuals with late-onset Alzheimer's disease. This 'idiopathic' category once included those individuals expressing apolipoprotein E4, but we subsequently learned that this normal genetic variant accelerates cerebral Aβ accumulation and precipitates a more aggressive form of the disease[7–10]. Aging (time on the planet) may be a key factor that gradually overcomes the energy barrier to aggregation of the normally secreted Aβ monomer in long-lived primates, including humans. The Alzheimer's disease field is hard at work identifying other genetic and environmental reasons for Aβ elevation, but that this search is incomplete takes nothing away from the urgency of discovering effective Aβ-lowering treatments.

It's not 'oligomers or plaques'—it's both. Another current concern is the relationship between soluble Aβ oligomers and amyloid plaques in Alzheimer's disease pathogenesis. In experimental systems, several key features of the Alzheimer's disease phenotype can be induced solely by soluble oligomers: synaptic loss (a strong morphological correlate of the degree of dementia in individuals with Alzheimer's disease), impaired hippocampal synaptic plasticity, microgliosis and even tau hyperphosphorylation/neurofibrillary change, the other hallmark pathology of Alzheimer's disease. But this bioactivity of small soluble oligomers does not mean that plaques have no role in the progressive degeneration of neurons and their processes, as there is clear evidence that diffusible, oligomeric Aβ assemblies immediately surround the plaques and are intimately associated with local dendritic spine loss[11] and neuritic dystrophy[12]. Indeed, the presence of bioactive dimers and larger oligomers 'trapped' within human amyloid plaque cores[13] strongly suggests that plaques serve as local reservoirs of a dynamic range of small oligomers (no single size of oligomer is the key toxin), and these can diffuse away from plaque cores and cause surrounding neuritic/synaptic injury.

As Alzheimer's disease begins (that is, long before symptoms appear), rising Aβ monomer levels (resulting from partially identified factors such as APP or presenilin mutations, decreased Aβ clearance by the apolipoprotein E4 variant[14] or perhaps increased β-secretase activity) promote the formation of dimers and then larger oligomers (**Fig. 2**). The dimers and other oligomers, which have exposed hydrophobic amino acids ready to accept another monomer, need to escape the aqueous environment, and they bind cell membranes and aggregate progressively to form protofibrils (~4 nm), fibrils (~8 nm) and plaques of fibrils, a process that markedly decreases their exposed surface area and can be viewed as 'protective', at least temporarily. Plaques eventually reach a maximal size and density in the brain[15,16], so that newly arising oligomers may then accumulate principally on lipid membranes; moreover, the plaques have an off-rate by which

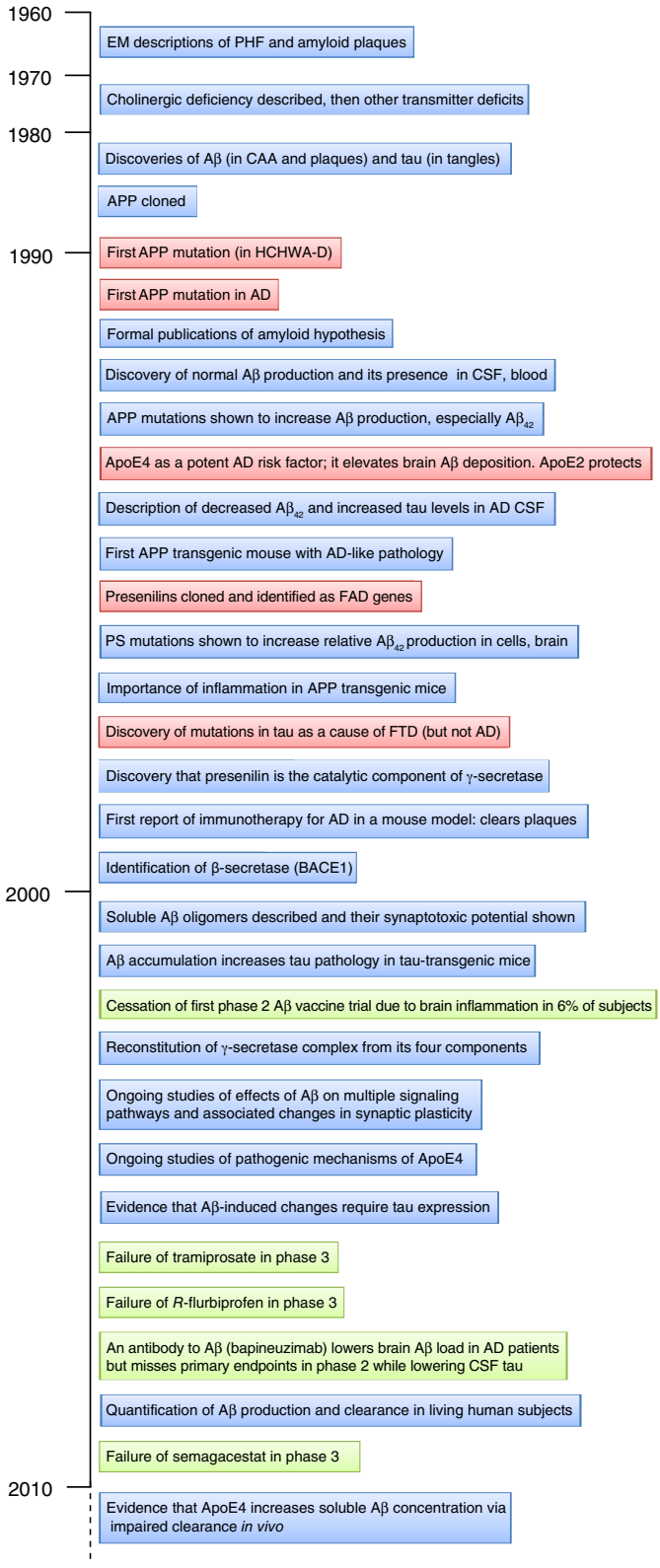

Figure 1 Approximate timeline of some principal discoveries in Alzheimer's disease research since 1960. The list is by no means exhaustive and focuses on findings deemed important for the current stage of general understanding of Alzheimer's disease (AD) pathogenesis and for the development of potentially disease-modifying agents. EM, electron microscopy; PHF, paired helical filaments; HCHWA-D, hereditary cerebral hemorrhage with amyloidosis-Dutch type; CAA, Congophilic amyloid angiopathy; PS, presenilin; tg, transgenic; BACE1, β-secretase 1; FTD, frontotemporal dementia. Red, genetic discoveries; blue, discoveries about molecular pathogenesis in cells and animals; green, clinical trials.

oligomers can diffuse away. In short, a complex equilibrium develops: diffuse and compacted plaques form, initially sequestering (but also releasing) oligomers, and some newly formed oligomers remain free to bind the hydrophobic surfaces of cell membranes, creating the opportunity for local cytotoxicity induced by the oligomers. So, an initial rise in the number of insoluble plaques in the presymptomatic phase of Alzheimer's disease (as has been well documented in the early stages of the Alzheimer's disease process that occurs in Down's syndrome[17]) is not yet associated with clinically noticeable toxicity, but, eventually, nonsequestered oligomers can begin to cause progressive synaptic/neuritic injury by 'a thousand tiny blows' and gradually overcome the brain's substantial physiological ('cognitive') reserve.

Aβ receptors: an embarrassment of riches. Given the abundance of reports that Aβ peptides of synthetic or natural origin have activity on cells, one needs to determine the signaling pathways by which these effects are mediated. Several receptor proteins have been proposed to be capable of binding various forms of Aβ, thereby inducing its cellular effects. These now include α7-nicotinic receptors, NMDA and AMPA receptors, insulin receptors, the cellular prion protein and ephrin receptors. Most but not all experiments implicating one or another of these have used synthetic Aβ peptides at high nanomolar to low micromolar concentrations, sometimes without biochemically and structurally defining their precise assembly states at the time of receptor binding. Growing evidence from numerous labs suggests that soluble oligomers, more than monomers or fibrils, are responsible for Aβ's cytotoxicity (see above). Accordingly, the exposed hydrophobic amino acids on an Aβ oligomer (which stands ready to accept another monomer or oligomer to yield a larger assembly) should make oligomers far more likely to avidly bind hydrophobic surfaces, that is, lipid membranes, rather than the largely hydrophilic ectodomains of protein receptors. Sustained binding of oligomers to membranes could perturb the fine structure of the phospholipid bilayer, and this could lead to secondary biophysical effects on the structure and function of resident transmembrane receptors that may then contribute to the resultant signaling changes. So, the initial binding of soluble Aβ oligomers *in vivo* probably involves lipids, but membrane proteins may promote, stabilize or otherwise modulate the oligomer-lipid interactions.

In any event, deciphering the cognate binding sites of the biochemically diverse, natural oligomers actually found in humans (as opposed to assemblies of one synthetic peptide (for example, $A\beta_{1-42}$) at supraphysiological concentrations) will now require purifying the endogenous Alzheimer's disease brain oligomers to homogeneity, labeling them without perturbing their structure and exposing them to primary neurons or brain slices to identify in unbiased fashion their binding sites. Without this labor-intensive biochemical approach, we cannot be certain that the several candidate receptors put forward to date are physical receptors for Aβ assemblies *in vivo*.

A separate issue is clarifying the normal function of endogenous Aβ monomers in health. A few studies have suggested that monomers can modulate the electrical activity of neurons, perhaps as part of a negative feedback loop (for example, refs. 18,19). But such studies often cannot specify precisely what form the Aβ was in at the time of neuronal binding, that is, fully monomeric or also partially oligomeric. This is particularly true if one uses synthetic $A\beta_{42}$ peptide; its two extra hydrophobic residues (alanine and isoleucine) give it a remarkable propensity to aggregate, even at low concentrations. Studies of the normal function of Aβ should instead focus on the $A\beta_{40}$ peptide, as this is by far (tenfold) the most abundant Aβ monomer under physiological conditions in young mammals. Studies that attribute normal biological functions to low levels of $A\beta_{42}$ must confirm these findings using $A\beta_{40}$. And the receptors for soluble Aβ monomers would be predicted to be distinct from those for the oligomers, which have a different structure.

Alzheimer's disease: the most common tauopathy. Some years back, the field of Alzheimer's research was characterized by an amusingly titled—but false—dichotomy much discussed in the lay press: the BAPtists versus the TAUists. This 'religious war' has been resolved by human genetic discoveries (for example refs. 20–22) and subsequent mouse modeling (for example refs. 23, 24) which showed that tau alteration occurs downstream of Aβ accumulation in Alzheimer's disease. Nevertheless, tau expression seems to be necessary for neuronal dysfunction and behavioral deficits in Alzheimer's disease and models thereof. Data from APP-transgenic mice[25,26] and neuronal cultures[27,28] have provided evidence that a marked decrease or absence of tau expression ameliorates the neurotoxic impact of Aβ, including that of soluble oligomers. One hundred years after Alois Alzheimer called attention to the two classical lesions of the disease, we finally understand that histopathology is inextricably linked with biochemistry: the subunit proteins of these two lesions (which are both types of amyloid, as broadly defined) have a decisive role in inducing dementia together. In short, the era of the TAUists versus the BAPtists is history. Current efforts to develop therapeutics that downregulate pathological features of tau (for example, hyperphosphorylation and oligomerization) should nicely complement the numerous Aβ-lowering agents that are in, or will enter, human trials. Some agents targeting tau, microtubules or both have reached phase 2 human trials. For example, a neuroprotective eight-residue peptide (NAPVSIPQ) that can bind tubulin and stabilize microtubules improved performance on two specific memory tests in subjects with MCI in a 12-week phase 2a trail, although significance on primary and other secondary endpoints was not observed (summarized in ref. 29; full results not yet published).

Are current mouse models of Alzheimer's disease adequate? The short answer is no. No engineered animal model has yet reproduced the full spectrum of Alzheimer's disease phenotypes in a manner closely similar to the human disease. But, given the strong evidence for a disease-promoting role of $A\beta_{42}$ in Alzheimer's disease, existing APP-transgenic lines do offer compelling models to study the mechanisms of amyloid accumulation and assess Aβ-lowering agents preclinically. Doubly transgenic mice that express both mutant human APP and mutant human tau can enable studies of the pathogenic relationship between these two key proteins, although Alzheimer's disease–type paired helical filaments of tau are generally not formed in rodents. Better mouse models that include gradually progressive amyloid and then tau lesion formation with associated inflammatory changes and selective neuronal loss are clearly desirable. Thus, the available mouse models are useful for studying some but not all aspects of the disease and for preclinical evaluation of treatments directed at Aβ or tau.

Why recent failures of experimental drugs are not predictive of the future

Perhaps the greatest negative influence on the perceived promise of Alzheimer's disease research arises from recent failures of certain mechanism-based therapeutic agents in phase 3 trials. But a close examination of these human trials provides no evidence that the target was the problem; rather, the specific agents were highly flawed. Tramiprosate (Alzhemed) was a putative anti–Aβ-aggregation compound, but this mechanism was not proven in its phase 2 trials, and the agent failed phase 3 without evidence that it had efficiently entered the CNS and engaged the Aβ target robustly (see **Fig. 1**). Indeed, no reports that tramiprosate clearly altered $A\beta_{42}$ levels in human CSF or plasma were published.

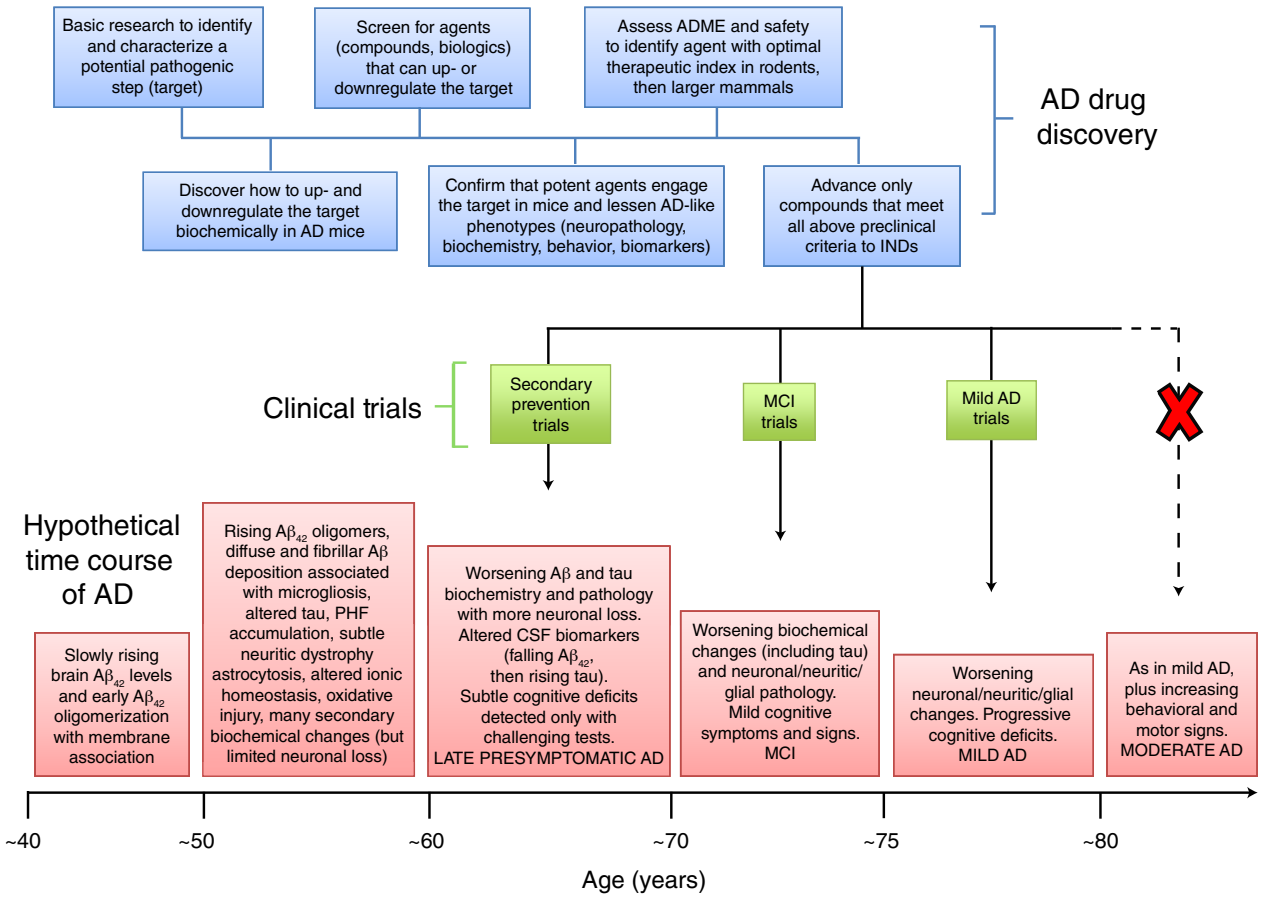

Figure 2 Intersecting disease-modifying agents for Alzheimer's disease with the course of the disease. Blue boxes, sequence of steps in the discovery of compounds or biologics appropriate for investigational new drugs (INDs) in Alzheimer's disease. Red boxes, speculative stages in the long presymptomatic and symptomatic phases of Alzheimer's disease in a hypothetical individual who undergoes Aβ buildup for one of several possible reasons (for example, presenilin or APP mutation; ApoE4 inheritance; increased BACE activity, and so on) and develops MCI by around age 70. Green boxes, clinical trial categories dependent on the stage of Alzheimer's disease. Red X, trials in moderate Alzheimer's disease not recommended.

A putative γ-secretase modulator (*R*-flurbiprofen) derived from a nonsteroidal anti-inflammatory drug failed in phase 3, but it lacked potency (~250 μM half-maximal inhibitory concentration) and did not enter the brain well[30]. Semagacestat, a γ-secretase inhibitor, had a therapeutic index of <3 (that is, the half-maximal inhibitory concentration for Aβ reduction was only two to three times lower than that for inhibiting Notch signaling). Consequently, patients in the phase 3 trial were dosed only half as frequently (once a day) as originally intended. Nonetheless, signs of apparent Notch toxicity accrued, such as gastrointestinal symptoms and skin cancer. The information released after trial cessation suggested that some treated patients actually declined in mental function. But it is very unlikely this was due to some adverse cognitive effect of lowering brain Aβ levels too much, as the agent had only been used in these subjects with mild-to-moderate Alzheimer's disease for an average of ~12 months at the time of the interim analysis, and a fall in their markedly elevated brain Aβ to below normal levels was apparently not observed (and not expected) by PET imaging. Rather, the acknowledged occurrence of adverse events, including toxicity from chronically blocking the processing of Notch and other important γ-substrates, could cause a setback of cognition in subjects with Alzheimer's disease, just as an intercurrent urinary tract infection or other systemic medical problem often makes a person's dementia appear worse. Such unacceptable Notch effects could be avoided by using γ-secretase inhibitors with much higher therapeutic indexes or γ-secretase modulators that modify the processing of APP to Aβ without blocking γ-secretase cleavage in general. It is reasonable to conclude that the three trial failures do not signify that Aβ lowering cannot work but rather that the agents chosen by these companies could not test the hypothesis adequately.

The importance of treating early. The cardinal medical principle of intervening early if one hopes to arrest a chronic disease (such as cancer, atherosclerosis, hypertension or diabetes) has been underscored by data from these and other Alzheimer's disease trials. The clinical research community has recognized that, to modify Alzheimer's disease, which has recently been redefined as a neuropathological disorder with presymptomatic and symptomatic phases, one must attempt to treat when the neuronal dysfunction is far from full blown and largely irreversible[31]. This concept is supported by preclinical research on the effects of transgene downregulation or immunotherapy or γ-secretase inhibition in Alzheimer's disease mouse models. For example, in mice co-expressing human APP and tau, hippocampal injections of an Aβ-specific antibody cleared local Aβ deposits and then ameliorated surrounding tau pathology if given at a young age, but not later on[32]. Similarly, treatment of APP-transgenic mice with a γ-secretase inhibitor arrested plaque formation and growth at 6 months but not at 10 months of age[16]. Importantly, this principle obtains in humans. *Post hoc* analyses suggest that the ApoE4-negative subjects in a small phase 2 trial of the Aβ-specific antibody bapineuzimab showed a better response compared to placebo than did the ApoE4-positive subjects[33]. ApoE4 clearly confers

a more aggressive form of Alzheimer's disease, with worse Aβ pathology and earlier symptom onset than occur in ApoE4-negative subjects[7–9]. And in a 12-week phase 2a trial in subjects with mild Alzheimer's disease (MMSE 20-26), a copper/zinc ionophore (PBT2) believed to decrease metal-mediated Aβ oligomerization improved performance on two cognitive function measures, although it missed significance on its ADAS-Cog and MMSE endpoints[34].

How should we proceed?

The profound complexity of the Alzheimer's disease process, coupled with the disappointment from some initial trial failures, has raised concerns that the field may be heading in the wrong direction. But emerging results on agents that are still in trials suggest that targeting Aβ can produce a biological effect that may represent a slowing of the disease process. For example, in a controlled, blinded study bapineuzimab was reported to significantly decrease amyloid burden (as imaged by Pittsburgh Compound B) during an 18-month treatment period, in contrast to the expected increase seen on placebo[35]. And although primary clinical endpoints were not met in the small (227-patient) phase 2 trial of bapineuzimab, patients receiving all six scheduled doses showed less decline than those on placebo on certain measures of cognitive function[33], mirroring the result of antibody responders in the aborted trial of an active Aβ vaccine[36]. And even though a postmortem follow-up of a small subset of patients from the phase 1 trial of this Aβ vaccine suggested that some subjects could undergo marked clearance of Aβ plaques but still die with advanced dementia[37], it is inconclusive, as it documented only two such subjects with this outcome from a trial that originally included 80, and residual levels of Aβ oligomers in the brains were not assessed. Corollary evidence of potential disease modification came from the finding that CSF tau levels fell significantly in the small number of bapineuzimab phase 2 trial subjects who had lumbar punctures[33], and a trend in that direction occurred in the active Aβ vaccine trial, as well[36]. Moreover, careful histopathological analyses of neuritic dystrophy, tau alterations and other Alzheimer's disease changes in postmortem brains of some active vaccine recipients provided definite evidence of disease modification[38]. If CSF tau lowering is confirmed in the large phase 3 trials of bapineuzimab now underway in 25 countries, then a therapeutic agent that can only be acting on Aβ will have lowered levels of the most validated biomarker of the neurodegenerative process in Alzheimer's disease.

Taken together, the findings reviewed above suggest a reasonable course for the near future. We should continue to attempt disease modification by targeting Aβ in various ways, using agents that show rigorous data in preclinical models and seem to be generally safe in phases 1 and 2 (**Fig. 2**). Surveys show that affected individuals and their families are highly interested in disease-modifying clinical trials and willing to accept some level of risk, given the devastating, ultimately fatal course of Alzheimer's disease. Of salient importance is testing in the mild phase of dementia—or even earlier. Individuals with MMSE scores in the 21–26 range (with normal being 30) plus CSF findings and/or amyloid imaging indicative of Alzheimer's disease are an attractive trial population. Once one agent achieves significant efficacy and safety in this population (perhaps with a product label restricted to use in mild Alzheimer's disease), trials in presymptomatic or very early symptomatic subjects, including those with MCI-amnestic type, should be pursued. I would argue that the latter trials should proceed now, even as we await the results of current trials in subjects with mild-to-moderate Alzheimer's disease. Our field should be pleased if we see any safe disease-slowing effect, even if it is modest and can only be demonstrated in mild (or earlier) phases of Alzheimer's disease. Secondary prevention trials in fully presymptomatic Alzheimer's disease, although clearly desirable, present their own special challenges[30].

Importantly, alternative disease-modifying targets, especially tau and neuroinflammation, should also be vigorously pursued. These approaches are lagging behind Aβ-directed compounds, but, in the intermediate to longer term, such agents could be used in combination with an Aβ-lowering or Aβ-neutralizing treatment. And, of course, it is important to pursue symptomatic treatments, particularly for the difficult and burdensome behavioral aberrations that are of paramount concern to caregivers.

Conclusion

The situation that the Alzheimer's research field finds itself in could no doubt be better, but it is far from bleak. We yearn for more rapid progress, but molecular research on Alzheimer's disease only began some 25 years ago, and a few rationally developed therapeutic candidates have now reached phase 3. There is room for optimism that an initial, perhaps modest, therapeutic success may occur relatively soon.

A final point: it would be fair to our patients and their families if some of the lay press coverage of issues arising in Alzheimer's disease research became more measured and less provocative. Although new findings from preclinical research or early clinical trials can seem exciting, they rarely deserve front-page coverage before their full importance for the human disorder has been established. We should resist overselling the clinical relevance of animal studies or the outcomes (positive or negative) of small phase 2 studies. Our field is in a sensitive situation: its promise is palpable, but its potential for disappointment is great.

COMPETING FINANCIAL INTERESTS
The author declares competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemedicine/.

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