Failure Analysis of Clinical Trials to Test the Amyloid Hypothesis

The amyloid hypothesis of Alzheimer disease (AD) pathogenesis has been the leading theory behind our understanding of the disease mechanism and the predominant therapeutic target for the past decade or more.1 The rationale for believing that β-amyloid (Aβ) is critical to the etiology of AD is simple and compelling. Very high levels of an amyloidogenic peptide, Aβ, accumulate in the brains of patients with AD; all genetic forms of AD are associated with increased amyloid deposition, including the early onset autosomal-dominant disease associated with mutations in presenilin 1 and 2 or the amyloid precursor protein (APP) itself. In addition, AD develops in nearly all individuals with Down syndrome, who have a triplication of the APP gene. The APOE4 polymorphism, which markedly increases the risk for late onset AD, also is associated with an increase in amyloid deposits, whereas the APOE2 polymorphism that diminishes risk for AD is associated with decreased amyloid levels. Conversely, a rare protective polymorphism in the APP gene itself is associated with decreased Aβ synthesis.2–7 Thus, a primary, causative, pathogenic link between Aβ and AD seems secure.

Nonetheless, there are some twists that complicate the picture. Aβ accumulation does not correlate well with extent of neuronal loss or cognitive dysfunction,8,9 and demonstrating direct neurotoxicity of Aβ has been difficult in most animal models, suggesting the possible existence of key intermediates between amyloidosis and neurodegeneration. The most obvious candidates as intermediates are neurofibrillary tangles that contain tau; such tangles accumulate in AD brains, but in contrast to amyloid, their levels correlate quantitatively with the severity of clinical symptoms and with the areas of the brain thought to be responsible for the specific symptoms of the disease, such as memory impairment. Despite this, Aβ has remained the primary target for a therapeutic intervention in AD, with numerous phase III trials underway or completed and designed to reduce Aβ levels using a range of strategies. Thus far, the results are either negative or inconclusive, leading many to call into question the underlying validity of the amyloid hypothesis.

Karran and Hardy10 analyze multiple clinical trials targeting Aβ that had disappointing outcomes to ask, “What went wrong?” They conclude that despite enormous investment, most trials to date have failed on grounds related to trial design as opposed to the invalidity of the underlying therapeutic (amyloid) hypothesis. This is an extremely important point, because it suggests that the amyloid hypothesis has not been refuted, which would motivate a search for new targets, but rather that trials need to be redesigned. In particular, their analysis10 raises basic questions, such as whether target engagement was measured, whether amyloid was actually neutralized, whether an adequate dose was used to achieve therapeutic endpoints, and whether excess side effects biased enrollment (or continued participation) in trials to an extent that interfered with the mechanism of action. Our inability to answer these questions satisfactorily raises the distinct possibility that failure of phase III trials of antiamyloid agents might be due to poor pharmacokinetics, problems with dosing, et cetera, and not problems with the underlying concept. A recent analysis11 from investigators at a major pharmaceutical company comes to largely the same conclusion—that failed antiamyloid trials in AD did not achieve sufficient target engagement to truly test the hypothesis that reducing amyloid in the brain would (ultimately) improve symptoms, or at least stabilize disease progression.

Another complication is the heterogeneity of the AD disease process, and the nonlinear relationship between amyloid deposition and cognitive failure12 even when dementia is ultimately driven by amyloid, making amyloidosis a problematic surrogate endpoint. Also unclear are the confounding effects in individual patients of non–AD-related pathobiology such as vascular lesions. The impact of non–amyloid-dependent cognitive impairment on clinical trials is far from trivial; even in carefully selected patient populations, as many as 20% of the subjects in AD trials do not have observable amyloid by positron emission tomography (PET), or even on subsequent autopsy.13

Despite these issues, we have nonetheless learned some important lessons from AD trials to date. For example, the ill-fated AN1792 active immunization trial did not reduce or reverse cognitive failure and had only a small effect on neurodegenerative phenotypes such as neural loss, gliosis, and accumulation of tau
tangles. However, AN1792 administration did reverse amyloid deposition (although the number of patients followed was small because the emergence of a subset of patients with meningoencephalitis made it necessary to halt the trial). This suggests an alternative model of AD in which amyloid acts as an early step in a more complex neurodegenerative cascade that becomes independent of amyloid as disease progresses. It also seems likely that such a pathological cascade occurs in the setting of multiple genetic or physiological factors that impact the probability that amyloid deposition will actually cause neurodegeneration and cognitive failure. In this scenario, even if a drug successfully engages and reduces amyloid levels, clinical improvement will be difficult to achieve once disease becomes sufficiently advanced.

We agree with Karran and Hardy that to test various aspects of the amyloid hypothesis, we need to give drugs to the right patients at the right time and then follow them closely. Specifically, we need patients in whom amyloid-dependent neurodegeneration is active, and we must account for the finding that it may be a decade before frank cognitive symptoms emerge. Amyloid levels are observed to rise in asymptomatic patients and then level off as disease becomes manifest, suggesting that the time to test antiamyloid drugs is in asymptomatic patients identified with PET scan or cerebrospinal fluid analysis. At present, the A4 trial in sporadic presymptomatic AD, the apoE4-targeted prevention trial, the Columbia PS1 kindred trial, and the DIAN Tu trial of autosomal-dominantly inherited individuals all are attempting to intervene earlier in the disease, hoping to get the time right. By focusing on individuals who are amyloid positive either by biomarkers or by virtue of strong genetic predisposition, these trials aim to select a more homogeneous group that has an amyloid-dependent cause of neurodegeneration, thus getting the right patients enrolled. More attention to pharmacodynamic readouts may help ensure that the right drugs are being used. It will be several more years before we know if these interventions alter the natural history of AD, but there is good reason for optimism. By addressing issues in the design of early trials, it seems likely that the next set of trials will put the amyloid hypothesis to a convincing clinical test, hopefully with better outcomes.

The work of Karran and Hardy demonstrates the importance of carefully analyzing failed clinical trials. There is always a tendency for certain targets to be viewed as most exciting, leading to a flurry of simultaneous, competing trials. When these trials prove complex or disappointing, something that is all too common with slowly developing neurodegenerative diseases, years of research and enormous investment are abandoned, and attention moves on to new targets. Contrast this with the norm in complex engineered systems such as civil works projects or aircraft manufacture. It is inevitable that unanticipated problems will arise with such systems (lithium batteries in Boeing 787 aircraft, for example), but engineers have a fundamental commitment to “root cause” or failure analysis as a means to understand why such problem arise and how to solve them in an effective and timely manner. It is time that we develop the methods needed to analyze failures in drug discovery with sufficient rigor so that we can avoid making the same mistake in the future. This will have the upside that future trials will be more informative about underlying hypotheses even if the trials fail, and some compounds that run into problems may be rescued. We hope that testing of the amyloid hypothesis in AD drug trials may be one place in which failure analysis proves its worth.

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**Potential Conflicts of Interest**

Nothing to report.

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**References**


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