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DRUGS

A tangled web of targets

Drugs in development for Alzheimer's disease take aim at a variety of neural mechanisms. But despite a wealth of possibilities, there have been few successes.

BY LAUREN GRAVITZ

For research into Alzheimer's disease, 2003 was a good year. The US Food and Drug Administration (FDA) had just approved memantine, the first in a class of drugs that reduces abnormal brain activity. Scientists had identified several potential targets, various academics and companies were developing therapies based on each, and the field seemed to be moving in the right direction.

Memantine is one of several drugs on the market in Europe and the United States that can slow the mental and physical decline of patients already in the throes of Alzheimer's disease. These drugs boost the activity of healthy neurons in the brain, masking the progression of dementia for a limited time (memantine, for example, seems to be effective for at least six months). But none of them can stop Alzheimer's disease in its tracks. So researchers began to shift their emphasis from treating symptoms to attacking the underlying cause of disease.

Eight years on, multiple therapies are in late-stage testing (see 'Selected drugs in clinical trials 2011'), including four that have the potential to modify the biological roots of Alzheimer's disease[1]. And yet, despite these seemingly imminent improvements in Alzheimer's therapeutics, a vague pall of scepticism hangs over the field. There is no clear evidence that these approaches will work, and many indications that they may not.

"Progress in the basic science of disease has been so substantial for the last few decades that many of us were quite optimistic," says Paul Aisen, director of the Alzheimer's Disease Cooperative Study and a researcher at the University of California, San Diego. But a sense of stasis has now set in. "We haven't had a new drug since 2003," says Aisen, "and the result of every major trial that's reported since then has been very disappointing."

A TWISTED TALE

A big part of the problem is that researchers don't know enough about the biology of Alzheimer's disease to identify the right targets. The disease is the result of a long chain of events, but some of the links in that chain are still a mystery — nobody is certain which link to cut to stop disease progression.

In a field with limited funding, the multitude of theories and possible targets has made for a difficult, albeit stimulating, challenge. "The therapeutic landscape for Alzheimer's disease is wide open — and it's wide open because we don't have a good definition of the disease, we don't have validated drug targets, and we have too many unvalidated ones," says Lon Schneider, a gerontologist and neurologist at the University of Southern California in Los Angeles.

But despite the wide variety of potential approaches, three of the four drugs in phase III trials share one main target: an improperly folded peptide called amyloid-β. In people with Alzheimer's disease, this protein fragment is sequestered into hard plaques nestled between neurons in the brain. Although few researchers doubt that amyloid-β is at least partly to blame for the disease (see 'Little proteins, big clues', page S12), many are beginning to wonder whether it is the right molecule to target.

Amyloid plaques are one of the hallmarks of Alzheimer's disease. Indeed, imaging studies have shown that plaques can start to accumulate 10–15 years before symptoms

emerge, prompting researchers to suggest that amyloid-β may be a good target for prevention (see 'Prevention is better than cure', page S15). Eliminating amyloid-β might not halt the disease, however. By the time Alzheimer's becomes symptomatic, attacking amyloid-β could have no perceptible effects.

So maybe a different type of drug is needed to halt or reverse cognitive decline. It might be better, some researchers suggest, to target another characteristic of the disease: the twisted clumps of fibrous protein inside neurons called neurofibrillary tangles. These are caused by the accumulation of a toxic form of the tau protein and correlate closely to the timing of symptom onset.

Other researchers champion wholly different approaches, ranging from brain surgery to repurposing drugs approved for a host of conditions including diabetes and arthritis. "This is a messy illness, and there are many, many ways of potentially cleaning up the mess," says Schneider. "That's what's so frustrating."

PREVENTING CLEAVAGE

The evidence pointing to amyloid-β as a cause of Alzheimer's disease seems overwhelming. Genetic studies reveal abnormal amyloid-β production in familial Alzheimer's disease, and cell-culture and animal studies implicate the misfolded protein in everything from neuronal death to behavioural and memory problems. For nearly two decades, most of the therapeutic research has focused on finding ways to reduce amyloid-β production and dissolve amyloid plaques in the brain.

But targeting amyloid-β is far from simple. "There's a slew of uncertainty about where in the disease course one would have to intervene" to target amyloid-β, says Jeffrey Cummings, director of the Cleveland Clinic's Lou Ruvo Center for Brain Health in Las Vegas, Nevada. "Its biology is very complex, the pathways for amyloid-β metabolism are multiple, and it may prove to be very difficult to work with."

Two different enzymes — γ- and β-secretase — cleave the amyloid precursor protein (APP) in two different spots, separating the short amyloid-β peptide from its progenitor. These peptides aggregate into small, stable clusters called oligomers, which then clump together to form larger plaques. Every step of the process, from the first snip to the final plaque, presents an opportunity to arrest the disease.

One approach involves γ- and β-secretase inhibitors. If γ- and β-secretase can be prevented from cleaving APP in the first place, there will be no amyloid-β. But targeting these enzymes has proven tricky, partly because γ-secretase is not specific to APP but also cleaves other proteins — including the vital protein Notch. One of the greatest disappointments in Alzheimer's therapeutics so far came last year when Eli Lilly, of Indianapolis, Indiana, abruptly halted a phase III trial of its γ-secretase inhibitor, semagacestat,

when an interim analysis revealed that the drug actually accelerated the progression of disease rather than slowing it down.

The reasons for this failure are still being investigated. "One possibility is that maybe anything you do that manipulates amyloid-β is bad for the brain," says Eric Seimers, the Eli Lilly senior medical director who oversaw the trial. "A more likely possibility, though, is that the worsening is not because we reduced amyloid-β, but because γ-secretase does something else that the brain needs." Several of the trial subjects also developed skin cancer, perhaps because of the drug's effects on Notch.

"Amyloid-β biology is very complex and it may prove to be very difficult to work with."

So what about β-secretase? It is more specific to APP than γ-secretase, and pharmaceutical companies are in dogged pursuit of drugs that inhibit it. But the enzyme's shape has turned out to be problematic. Researchers have had a difficult time creating a molecule that is large enough to inhibit the enzyme's active binding site but small enough to pass through the blood–brain barrier so it can be taken orally. Despite some early setbacks, many companies — Eli Lilly included — are continuing to target β-secretase, and a few compounds are in early stage trials.

AIMING AT AMYLOID

All eyes, however, are trained on a passive immune therapy that leaves both secretase enzymes alone and goes after amyloid-β directly. Two candidates, Eli Lilly's solanezumab and Janssen and Pfizer's bapineuzumab (originally developed by the Dublin-based company Elan), are monoclonal antibodies that work with the immune system, binding to amyloid-β and helping to clear accumulated amyloid-β peptides in the brain. Both are being tested in phase III trials on thousands of participants with mild-to-moderate Alzheimer's disease (see 'Chasing the dream', page S18).

"Probably every big company and even a number of smaller companies have products that will eliminate amyloid in an amyloid-producing mouse," says William Thies, chief medical and scientific officer at the Alzheimer's Association, a nonprofit organization in Chicago, Illinois, dedicated to patient care and research funding. "But they're not going to move them on to a phase III study until they see the results of the ongoing trials. They would like to be convinced that the amyloid hypothesis is correct."

There are some hints that it might not be — or at least that targeting amyloid-β will not work once symptoms are apparent. The 18-month phase II trial of bapineuzumab left many researchers feeling sceptical. Although imaging studies showed that the antibody decreased amyloid plaques in the brain[2], it seemed to have little, if any, effect on cognition. With vaccine studies yielding similar results, many are growing uneasy with the approach, and suggest that it might work only as a preventive measure and should be tested in people without symptoms[3]. Attacking amyloid plaques in symptomatic patients may be like cleaning up the mess inside a house after a flood: the structure remains, but all the personal effects are long gone.

Others say that plaques could be the body's way of sequestering the toxic amyloid-β oligomers. Elan, which led the field in immune approaches, has a candidate called *scyllo*-inositol. "It binds to amyloid-β at some intermediate structure, blocking its ability to form plaques and also blocking its ability to cause toxicity to neurons," says Dale Schenk, Elan's chief scientific officer.

But some worry that researchers are spending too much time and resources on something that might never pan out. "I think amyloid-β is proving to be a very intractable target," says Cummings. "The great danger to the field is that if bapineuzumab fails, some pharmaceutical companies will decide that Alzheimer's disease is too tough a target to yield stockholder value and will redirect their resources toward more tractable diseases."

TAUIST PHILOSOPHY

With the amyloid-β issue still unresolved, more researchers are looking to the second major target: tau protein.

Tau protein normally stabilizes structural elements, called microtubules, in healthy neurons. In Alzheimer's disease and other 'tau-opathies', however, tau acquires too many phosphate groups and becomes dysfunctional. It aggregates inside neurons, the microtubules collapse, and the resulting neurofibrillary tangles block neuronal signalling.

Neither amyloid plaques nor tau tangles are solely responsible for causing Alzheimer's disease, but of the two, tangles show a better correlation with clinical symptoms, says Peter Davies, director of Alzheimer's research at the Feinstein Institute for Medical Research in Manhasset, New York. "You can have a lot of amyloid in your

SELECTED DRUGS IN CLINICAL TRIALS 2011

Drug	Trial status	Mode of action	Developer
Bapineuzumab	Phase III, ongoing	Humanized monoclonal antibody to amyloid-β; targets the peptide's N-terminus	Pfizer/ Janssen
Solanezumab	Phase III, ongoing	Humanized monoclonal antibody to amyloid-β; targets the centre of the peptide	Eli Lilly
Intravenous immunoglobulin (IVIg)	Phase III, ongoing	Isolated from pooled human blood, believed to have anti-amyloid-β and anti-inflammatory properties	Baxter
Latrepirdine (Dimebon)	Phase III, ongoing	Thought to stabilize mitochrondria, thereby protecting neurons and preventing them from malfunctioning	Pfizer/ Medivation
Scyllo-inositol / ELND 005	Phase II completed, Phase III in planning	Prevents or inhibits amyloid-β aggregation	Elan
Methylthioninium chloride (Rember)	Phase II completed, Phase III in planning	Unclear; thought to inhibit tau aggregation, but may be acting as an anti-amyloid-β disaggregator	TauRx Pharmaceuticals
CERE-110	Phase II, ongoing	Adenovirus-aided delivery of a nerve growth factor gene that helps protect neurons; delivered via surgery	Ceregene
PBT2	Phase IIb in planning	Metal chelator, small molecule that inhibits tau hyperphosphorylation and amyloid-β aggregation	Prana Biotechnology
Davenutide/AL-108	Phase II completed	Microtubule stabilizer, preventing tau hyperphosphorylation and tangle formation	Allon
BMS-708163	Phase II, ongoing	Inhibits formation of γ-secretase, thereby inhibiting formation of amyloid-β	Bristol-Myers Squibb
PF-04494700/ TTP488	Phase II, ongoing	RAGE inhibitor, modulates glial activity and reduces amyloid-β plaque formation	Pfizer
Tideglusib/NP-12 (Nypta)	Phase II, ongoing	GSK-3 inhibitor, preventing tau hyperphosphorylation	Noscira

brain and be absolutely fine," he says. "If you have a lot of tau pathology, you're never fine."

Self-dubbed 'tauists', who believe that tau protein is the key to Alzheimer's disease, are studying whether interfering with the extra phosphate groups[4] or the enzyme that attaches them could slow or even reverse the symptoms of disease. "Until you can undo tau pathology and show that it undoes symptoms, you won't know for sure," Davies says.

Tau research has progressed more slowly than work on amyloid-β, partly because of scant funding and the overwhelming interest in amyloid-β, and partly because of tau's essential role in maintaining healthy cells. But a few groups have persisted, and at least one drug candidate has made it to phase II trials. In April, Madrid-based biopharmaceutical company Noscira began European efficacy trials on a compound that inhibits GSK-3, the enzyme that adds phosphate groups to tau. This is actually the second GSK-3 inhibitor to go to human testing — lithium inhibits the same enzyme, but small trials of lithium were inconclusive.

One of the most hyped therapies in the tau class is a repurposed drug. In 2008, researchers from TauRx Pharmaceuticals in Singapore made an announcement that sent small tremors of excitement through the field. They tested a modified version of methylene blue — an outdated treatment for malaria, urinary tract infections and bipolar disorder — in a dosing and efficacy trial of 321 people with mild-to-moderate Alzheimer's disease. After 84 weeks, the cognitive decline of those on the drug appeared to be 81% slower than those taking a placebo.

At the time, TauRx scientists claimed that their drug, which they call Rember, worked by preventing tau aggregation. But the company never published the data — the compound is old, with a proven safety record, so they had no regulatory incentive to do so. Their reported results still remain a mystery. An independent team of researchers found that, in animal models at least, methylene blue clears amyloid-β but has no effect on tau whatsoever[5]. Despite these contradictory interpretations, the company is seeking patents in both Europe and the United States, and announced in December that it plans to press ahead with a large-scale phase III trial.

SLOWING THE DECLINE

Alzheimer's disease is one of dying neurons — of incomplete circuits, of neural impulses left unfinished, of thoughts, memories and ideas that are lost with the dying nerves. Thus, other than those therapies that target amyloid-β or tau protein, most of the drugs in the pipeline aim to protect some of these neurons and to slow the course of disease, not reverse it.

Among the more radical approaches is CERE-110 from Ceregene in San Diego. The product delivers a gene for nerve growth factor (NGF) — a large protein that helps nurture neurons — where it is most needed. Because NGF cannot pass through the blood–brain barrier, and because the gene must be incorporated by the specific subset of neurons most affected by the disease, precise delivery requires brain surgery.

The seriousness of brain surgery cannot be overlooked, but this particular procedure takes just a few hours and no safety problems have been reported from more than a hundred operations done so far, says Mark Tuszynski of the University of California, San Diego, the researcher who devised the approach. "It won't be a cure," he says, "but the hope is that it can meaningfully enhance neurons enough to slow decline and have a useful impact on quality of life."

Dimebon, which has been used as an antihistamine in Russia since 1983, has also shown an ability to protect neurons. In 2008, a small phase II trial of dimebon in 155 people with mild-to-moderate Alzheimer's disease yielded surprisingly good results: those taking the drug appeared to improve in cognition and daily function for up to 12 months.

But the excitement over the drug's potential abated when a larger trial failed to elicit the same results. In 2010, the first large phase III trial of dimebon showed no significant difference between the test and control groups. A second phase III trial is underway. "Something like dimebon comes along, with no rational reason about why it works the way it's supposed to work, and people go gaga and suspend their ordinary scientific scepticism," says Schneider at the University of Southern California.

Alzheimer's disease hides its secrets well. So although researchers may disagree on the best approach for halting it, most agree that the current range of targets provides a good starting point. "There are lots of things in the pipeline — lots of different possibilities. And that's what we need at this time," says Thies of the Alzheimer's Association. "The reality is that nobody knows which approach will be best." ■

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