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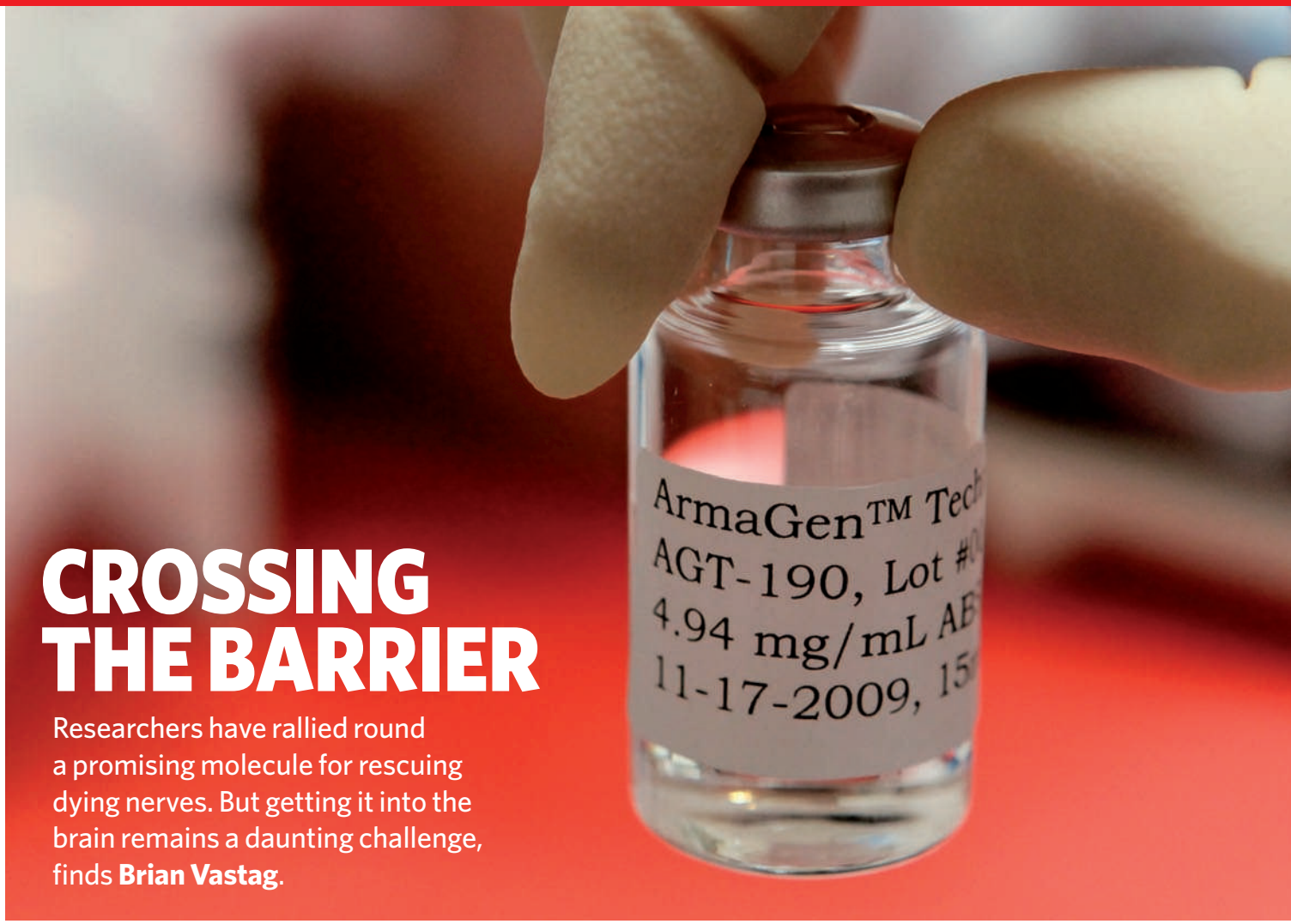

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CROSSING THE BARRIER

Researchers have rallied round a promising molecule for rescuing dying nerves. But getting it into the brain remains a daunting challenge, finds **Brian Vastag**.

Nine blocks from the beach in Santa Monica, California, a small biotechnology company occupies one bay of a single-storey commercial block, about the width of a three-car garage. Founded by William Pardridge, an endocrinologist at the University of California, Los Angeles (UCLA), ArmaGen Technologies employs just five people: call it garage-band biotech. From a small refrigerator in the cramped space, Pardridge pulls out a black plastic tray. Within are four dozen small bottles labelled 'AGT-190', a drug that Pardridge hopes will revolutionize treatment for several debilitating brain diseases.

Starting with a radical idea for sneaking therapeutic proteins into the brain, Pardridge launched ArmaGen in 2004. This year, the company is close to achieving a milestone: a green light from the US Food and Drug Administration (FDA) to carry out human safety tests of its first product.

AGT-190, says Pardridge, acts as a Trojan Horse. It sneaks across the barrier that separates blood and brain tissue and delivers its contents — a growth factor that can protect and repair neurons. A long line of researchers has heard the siren song of this naturally occurring brain protein, called glial-cell-derived neurotrophic factor (GDNF) — and for good reason. A thick stack of reports on animal studies and a smattering of evidence from initial trials in humans show that GDNF can halt the damage that follows stroke, interrupt drug addiction, and slow or even reverse the neuronal death march that incapacitates patients with Parkinson's or Huntington's disease[1].

"The excitement behind growth factors such as GDNF is that not only could they be protective, but there's the possibility for regenerating or rejuvenating some of the sick cells in the brain," says Todd Sherer, vice-president of research programmes at the Michael J. Fox Foundation for Parkinson's Research, based in New York, which has awarded about US$20 million in grants for growth-factor research.

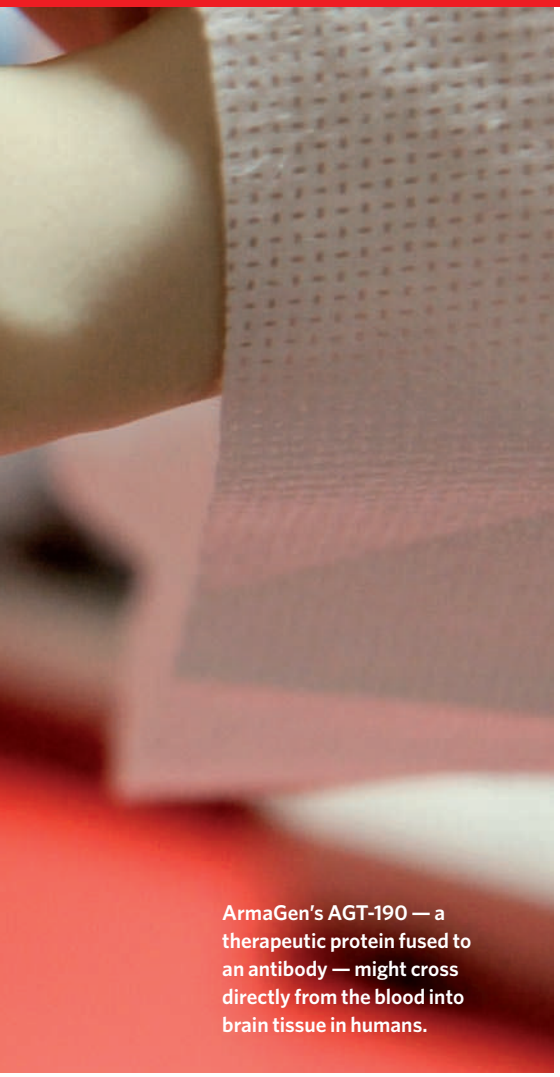
But a maddening hurdle remains: delivery. Early surgical trials in which GDNF was delivered through catheters to the brains of patients with Parkinson's disease failed to spread the protein to a sufficient proportion of damaged regions to do much good. Advancing on these techniques, companies are moving forward with more trials looking to circumvent the blood–brain barrier, but Pardridge is convinced that his technology can reliably deliver GDNF to the entire brain without the need for surgery. Still, his company is running out of funds, and there is no hint of investment forthcoming. "Nobody has succeeded at crossing the blood–brain barrier before, so why should we?" he asks, echoing the feedback he has heard from prospective investors. Pardridge — like other researchers — soldiers on, nevertheless, knowing that the pay-off could be huge. "The therapeutic potential of GDNF is just enormous," he says.

A hit-or-miss history

GDNF was a star from the start. Discovered in 1991 by researchers at Synergen, a biotechnology company based in Boulder, Colorado, GDNF dramatically revived dishes of dying neurons. When Synergen tested GDNF in monkeys with an induced form of Parkinson's disease, the treated monkeys trembled and spasmed much less than their untreated cage mates.

A year after announcing their results, in 1994, Synergen was snapped up by a larger biotechnology firm, Amgen, for about $240 million. Kevin Sharer, Amgen's chief operating officer at the time, said the company made the decision to buy Synergen after seeing the before-and-after footage of treated monkeys. As Sharer told *The New York Times*: "We looked at that movie and said, 'Buy this company'. Literally."

Amgen quickly moved GDNF into human trials. Between 1996 and 1999, 38 patients with advanced Parkinson's disease underwent surgery to place catheters in their brains. Hooked to a pump implanted in the abdomen, the catheter delivered GDNF into the fluid-filled spaces



ArmaGen's AGT-190 — a therapeutic protein fused to an antibody — might cross directly from the blood into brain tissue in humans.

between the main lobes of the brain in the hope that GDNF would migrate deeper into the brain, to the structures most affected by Parkinson's disease. This wishful strategy failed. Instead of improvement, patients experienced nausea, delusions and chest pains, the exact cause of which has still not been uncovered. Amgen halted the trial early.

After shelving further trials, the company made the protein available to interested researchers. Steven Gill, a neurosurgeon at Frenchay Hospital in Bristol, UK, partnered with researchers at the University of Kentucky in Lexington and designed a new catheter to push the drug directly into brain regions affected by the disease. Delivered under pressure, the GDNF solution continuously diffused from the tip of the catheter into the putamen, a thumb-sized structure at the base of the forebrain that degenerates in individuals with Parkinson's disease.

In 2001, Gill inserted his catheters into the brains of five patients. Although designed as a safety trial, the changes were swift and positive[2]. Over 12 months, all five patients showed improvements in standard scores of movement and motor skills. It was a "very successful trial", says Erich Mohr, chief executive of MedGenesis Therapeutix, based in Victoria, Canada, which earlier this year acquired the rights to GDNF from Amgen.

After that trial, Amgen quickly launched a larger trial to test GDNF against a placebo. But in this case, patients given GDNF fared no better than patients who received the surgery without GDNF. "That trial was basically designed to fail," says Clive Svendsen, a neuroscientist who was a consultant to Amgen on the study. Amgen chose a catheter that was thicker than Gill's, dripping GDNF into the brain rather than delivering it under pressure. Consequently, the GDNF solution simply refluxed up the outside of the catheter, says Svendsen, now director of the Regenerative Medicine Institute at Cedars-Sinai Medical Center in Los Angeles. Amgen declined to comment about the trial for this story. In 2004, amid a huge spate of negative publicity, it mothballed its GDNF project.

"GDNF has this lovely regenerative capacity and rejuvenating ability."

But shortly after, Gill and his colleagues made a tantalizing discovery. One of the patients in Gill's trial had died of a heart attack, and when the team carefully sliced the man's brain the researchers saw something amazing. Neuronal fibres had sprouted in the patient's putamen on the right side, where the catheter had been placed[3]. The man had described a huge improvement in his quality of life over the 43 months for which he received the drug. To those in the field, the message was clear: his brain had been healing.

New surgical trials

GDNF isn't the only drug that has run up against delivery troubles. The blood–brain barrier has been an enormous — often unacknowledged — show-stopper in drug development. Almost none of the hundreds of potential drugs for treating brain disorders can penetrate the tight mesh of endothelial cells lining the blood vessels in the brain. This barrier protects the brain and keeps most large molecules out of the cerebrospinal fluid that bathes neurons. Still, the tantalizing hints of effectiveness seen in some trials convinced researchers that GDNF was worth pursuing. Although Amgen had little interest in GDNF between 2004 and 2010, researchers continued to seek other strategies for getting the protein into the brain.

Now, several biotechnology companies are launching a new round of surgical trials. One project will test next-generation catheters — similar to Gill's design — for delivering GDNF protein. A second will, as early as next year, implant neural stem cells that have been programmed to produce GDNF into the spinal cords of patients with the degenerative disorder amyotrophic lateral sclerosis. And one study, already under way, is delivering viruses that carry the gene encoding a growth factor that is closely related to GDNF through a brain-implanted catheter.

Ceregene in San Diego, California, is the company taking this gene-therapy approach. Jeffrey Ostrove co-founded the biotechnology company in 2001 and chose a growth factor called neurturin, which acts much like GDNF, as a potential treatment for Parkinson's disease. The team at Ceregene packages the gene encoding neurturin into a gutted virus, and then infuses the virus into the patient's putamen under pressure. There, the virus delivers the gene to brain cells, which in turn should pump out the growth factor, perhaps indefinitely, says Ostrove.

But results released in 2008, from the company's trial in 58 patients with advanced Parkinson's disease, were disappointing. A year after surgery, patients who had received the altered virus fared no better than patients who received sham surgery and no virus. Both groups improved about equally on standardized symptom scores. (For unknown reasons, sham surgery for Parkinson's disease produces a strong placebo effect.) Despite raising some $70 million, Ceregene laid off 30 of its 50 staff, and Ostrove pondered shuttering the programme.

Then, donated brains from two trial patients who had died suggested a path forwards. In the trial, surgeons had infused the virus in eight locations across each patient's putamen. Yet Ostrove says that just 15% of the putamen expressed the neurturin gene — the delivery problem again. Persuaded that neurturin might still work if its delivery could be improved, this June the Michael J. Fox Foundation gave

Ruben Boado of ArmaGen collects a sample from a bioreactor used to grow therapeutic proteins.

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Ceregene $2.5 million to support another trial, in which surgeons will infuse virus into both the putamen and the substantia nigra, a structure in the midbrain. The company is also increasing the viral dose fourfold. Twenty-six patients will undergo the revised procedure over the next few months, and the same number will receive sham surgery.

Back-door protein

ArmaGen is taking a different approach to breaching the blood–brain barrier. Rather than going around it, with all the risks that brain surgery entails, Pardridge wants to enter through a biological back door. Pardridge has been studying the barrier since 1970. After about a decade, he discovered a potential way in, an insulin receptor. Pardridge showed that insulin receptors in the capillaries that feed the brain are transporters — grabbing molecules of insulin and pulling them into the brain tissue.

Over the next 15 years, while at UCLA, Pardridge developed a monoclonal antibody that latches onto part of the brain's insulin receptor without interfering with insulin binding. The receptor pulls both the insulin and the antibody through the blood–brain barrier, says Pardridge. He published this 'Trojan Horse' antibody design[4] in 1995, and then set to work engineering it as a vehicle for therapeutic proteins.

Pardridge and Ruben Boado, a molecular biologist also at UCLA and ArmaGen, stitched the gene encoding the antibody together with the gene encoding GDNF and, after several painstaking years, worked out how to scale up production of the hybrid protein, AGT-190.

Animal studies funded by ArmaGen show that the Trojan Horse approach works: it gets GDNF into the brain[5]. A large meeting poster hanging in Pardridge's office displays the results: sections of a rhesus monkey brain stained a lurid blue. (Rhesus monkeys, unlike other monkeys or mice, have a blood–brain barrier very similar to that of humans.) When the researchers injected the antibody into the animals' veins and then looked for it in the brain, they found it everywhere. About 2% of the AGT-190 injected into veins arrives in the brain. That's about the same as for antidepressants and other traditional, small-molecule, brain drugs that can cross the blood–brain barrier unassisted.

Pardridge says there is a fatal flaw in surgical, catheter-based delivery. The zone immediately surrounding the catheter is blasted with the drug, whereas tissue more than seven or eight millimetres away receives almost none. "Unless the region of the brain you're trying to reach is the size of a pinhead, a transcranial delivery system isn't going to work," he says.

The scientists planning the catheter-based GDNF trials disagree. Ostrove thinks that Ceregene's new surgical protocol will deliver GDNF to 25–30% of the putamen — and he predicts that this will be sufficient coverage to reverse the progression of Parkinson's disease. Mohr is confident that MedGenesis Therapeutix's next-generation catheter will push GDNF into a larger proportion of the structure than earlier catheters did.

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Garage-band biotech: ArmaGen's commercial space in Santa Monica is close quarters.

When asked about ArmaGen's strategy, Ostrove, Mohr and other scientists involved in the surgical trials argue that the Trojan Horse will be felled ultimately by the very trait that Pardridge touts most: it hits the entire brain. "You don't want GDNF to go all over," says Svendsen, pointing out that high doses of GDNF can cause neurons to make connections that they shouldn't. It could result in the same side effects as seen in Amgen's first human trial, which essentially bathed the brain in GDNF.

Pardridge argues that the doses of GDNF delivered by AGT-190 will be much lower than those in the Amgen trials. But whether the Trojan Horse approach is safe remains an open question, because no one has received the drug.

That could change as early as this October, when 12 healthy volunteers in Kansas are slated to receive three doses of AGT-190. Pardridge doesn't know what will happen after that. Even if all goes well, his company is almost out of money.

ArmaGen is $1 million in the red on AGT-190. A $3-million National Institutes of Health (NIH) grant funded the drug's development, and the company has spent a total of $4 million on the project, including $1.5 million on the monkey study. Large biotechnology companies, big drug makers and venture capitalists alike have all rebuffed Pardridge's entreaties for investment. He has a stack of grant applications that he has submitted to the NIH; it is nearly half a metre high. Most have been rejected.

Venture capitalists, too, "are very risk averse, right now", says Casey Lynch, a biotechnology analyst and president of the Neurotechnology Development Foundation, based in San Francisco, California. It is just one of several impediments for Pardridge, including the expense of producing biological drugs, as opposed to small molecule drugs, which can be synthesized chemically.

So Pardridge is stuck. He has developed a new approach to delivering a promising brain drug. He has compiled thousands of pages of data required by the FDA. And by the end of this year, he may know whether AGT-190 is safe to use in humans. But that might be the end of the line. A larger study of the drug's efficacy would cost at least $15 million, and Pardridge has no idea where to get this money.

In many ways, GDNF is stuck, too. It is unclear whether surgical procedures, even if effective, will be practical for a condition such as Parkinson's disease, which affects millions of people worldwide, not to mention the many other indications for which people have been eyeing it. The costs and dangers associated with putting a hole in someone's head or spine make it prohibitive for all but the most severe cases, those unresponsive to other treatments.

And yet, the development of GDNF as a treatment will probably continue, because its brain-healing properties are too tantalizing to pass up. "It has this lovely regenerative capacity and rejuvenating ability," says Svendsen. "But how it's going to work across these different diseases, which all have different mechanisms, I don't think we'll find out until after clinical trials." ■

Brian Vastag is a freelance reporter in Washington DC.

1. Airaksinen, M. S. & Saarma, M. *Nature Rev. Neurosci.* **3**, 383–394 (2002).
2. Gill, S. S. *et al. Nature Med.* **9,** 589–595 (2003).
3. Love, S. *et al. Nature Med.* **11,** 703–704 (2005).
4. Pardridge, W. M., Kang, Y. S., Buciak, J. L. & Yang, J. *Pharm. Res.* **12,** 807–816 (1995).
5. Pardridge, W. M. & Boado, R. J. *Pharm. Res.* **26,** 2227–2236(2009).