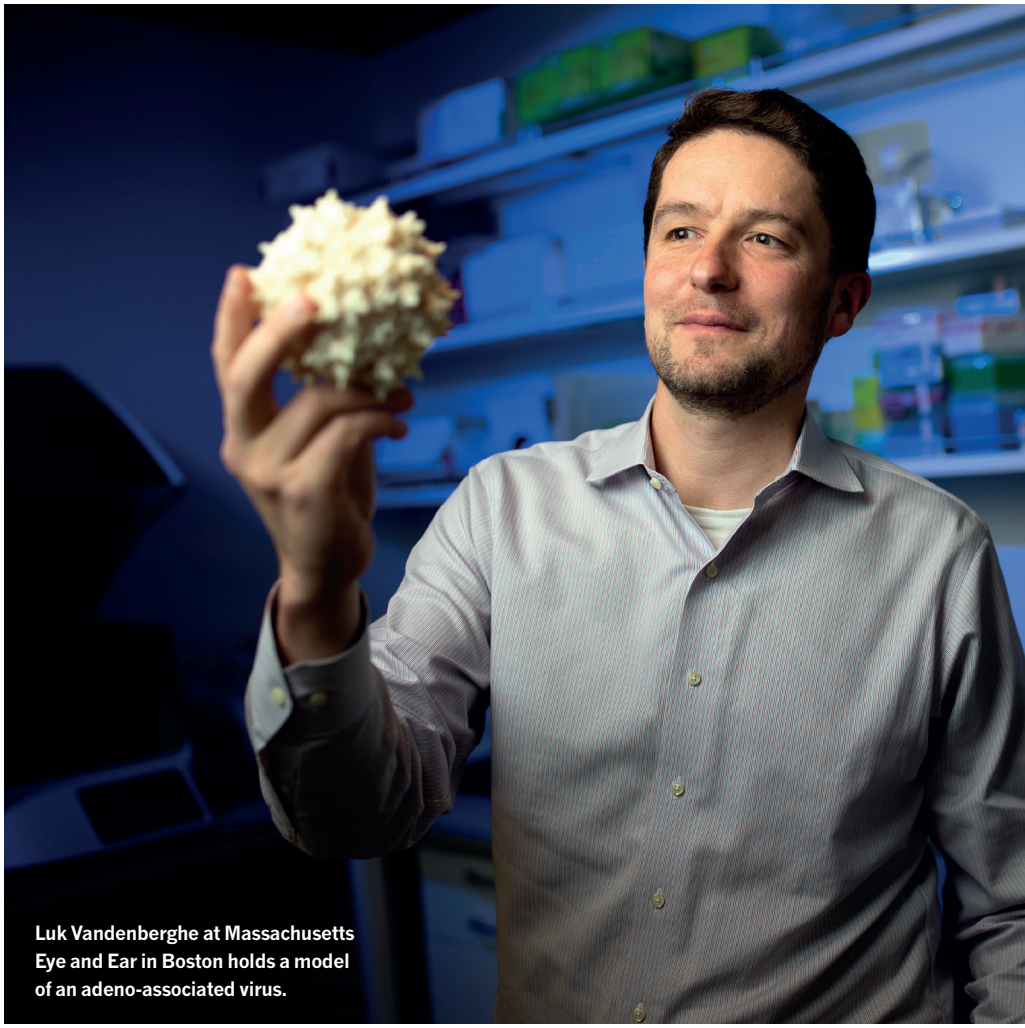
THERAPEUTICS

Special delivery

By tweaking a virus's shell, Luk Vandenberghe thinks he can transport genes into cells much more efficiently and cost-effectively.

BY NEIL SAVAGE



Luk Vandenberghe at Massachusetts Eye and Ear in Boston holds a model of an adeno-associated virus.

ARAM BOGHOSIAN/MASSACHUSETTS EYE AND EAR

Luk Vandenberghe walks over to a shelf in his office and picks up two fist-sized objects. One is a more complicated version of a Rubik's Cube, with 20 individually coloured sides instead of the standard 6. The other is an off-white glob of hard plastic produced by a 3D printer. It's studded with bumps, dimples and repeating triads of vaguely pyramid-like shapes, 20 in all.

Both are models of an adeno-associated virus (AAV), a favourite vector among clinicians for delivering genes to cells. Vandenberghe, a bioengineer who directs the Grousbeck Gene Therapy Center at Massachusetts Eye and Ear in Boston, is trying to work out what effect all those tiny structures have on the behaviour of the virus. His aim is to manipulate them to improve the vector's ability to deliver genes without, in essence, messing up the colour pattern on the Rubik's Cube — or in this case, the icosahedron.

Vandenberghe completed his doctorate on the structural basis of AAVs in 2007 at the Catholic University of Leuven in Belgium, and later went on to become an associate professor at Harvard University in Cambridge, Massachusetts. Through a mix of computational modelling and DNA synthesis, he has been trying to solve the problems that arise from using natural AAVs for gene therapy, and has founded three companies to bring his technologies to market. One of them is using an unusual non-profit approach to tackle the economics of developing gene therapy for extremely rare diseases.

Naturally occurring AAVs have become a workhorse of gene therapy. They infect human cells without causing illness, and different variations of the virus target different cell types — so selecting the right virus is essential for getting replacement genes to cells where they are needed. Vandenberghe and his colleagues have so far identified more than 140 natural variations of the virus[1].

But scientists would like to fine-tune AAVs to improve their specificity and the efficiency with which they penetrate tissue. The goal of AAV research over the past two decades has been treatments that use lower doses and do not affect off-target tissues.

Researchers are also trying to solve another problem. Because the viruses circulate in the wild, many people have been exposed to them and have developed immunity. That puts therapies that rely on AAVs out of reach for many patients. Estimates for the number of people with immunity vary widely, Vandenberghe says, from 20–90%. Some of that variation is due to geography; the viruses are more prevalent in Africa, for instance, than in the United States.

Bioengineers think they can achieve large changes in the function of AAVs by altering the capsid — the protein shell of the virus. For instance, capsid differences are the reason why one naturally occurring AAV targets liver cells with up to 100 times the efficiency of another. "Unfortunately, we still don't know exactly what it is that makes one virus go to the liver 100-fold better than the other," Vandenberghe says. Scientists also don't fully understand how a change in one part of the virus might affect the structure in another part, in much the same way that moving a red square on a Rubik's Cube might put a green square on another face out of place. "What we're trying to do is exactly solve that Rubik's Cube dilemma," says Vandenberghe. "That's not trivial on a cube, and it is certainly not trivial on an icosahedron."

LEARNING FROM HISTORY

To learn more about how structure affects function, Vandenberghe and his team decided to reconstruct the evolutionary history of AAVs. In 2015, he and his colleagues fed the protein sequences of 75 AAV variants isolated from human and non-human primate tissues into an evolutionary computer simulation and reconstructed the sequences of nine possible ancestors of modern AAVs[2], the oldest of which they named Anc80. Vandenberghe is not claiming these are the actual forms of previous generations of viruses, but that isn't the point, he says. "We didn't quite care. What we really wanted to do was find inroads into this structural problem that we had."

On the basis of the sequences, the researchers synthesized the ancestral viruses and examined their characteristics — and Anc80 proved to be especially interesting. When injected into mice, the virus was able to penetrate all of the hair cells in the inner ear and most of the hair cells in the outer ear, something no previous virus had accomplished. In 2017, Vandenberghe and his colleagues used Anc80 in mice to treat a genetic

disorder called Usher syndrome that causes deafness and visual impairment[3]. Excited by the potential of such a vector, Vandenberghe and his colleagues founded a company, Akouos, in Boston to develop treatments for hearing loss. In August, the start-up secured US$50 million in a first round of investment.

Vandenberghe's team is also collaborating with Selecta Biosciences in Watertown, Massachusetts, which wants to develop gene therapies using Anc80. Vivet Therapeutics in Paris is licensing the vector for use in developing treatments for inherited liver disease. And Lonza in Basel, Switzerland, is licensing the technique for making the virus so it can manufacture the vector for drug-makers. Back in 2011, before the Anc80 work, Vandenberghe also co-founded GenSight Biologics in Paris to develop treatments for rare inherited retinal diseases; the company currently has two drugs in clinical trials.

Creating better vectors is the key to expanding gene therapy, says Eric Kelsic, a systems biologist in the laboratory of molecular engineer George Church at Harvard University. Kelsic is taking a data-driven approach to capsid engineering. He selects an amino acid from the protein sequence of an AAV and systematically switches it with each of the other 19 amino acids in existence in turn to see what changes. Then he moves on to the next amino acid in the sequence and repeats the process. "With this approach, we know what the effect is for every possible individual change," he says. Using machine learning, he predicts what will happen when single-amino-acid changes are combined, then synthesizes promising sequences and tests the AAVs in mice or non-human primates.

Kelsic and Church have founded a company, Dyno Therapeutics in Cambridge, Massachusetts, to create vectors this way. Kelsic predicts that even for tissues such as the brain that can already be targeted with AAVs, more-efficient viruses will lead to improved therapies. The greater achievement, however, will be the ability to target organs that are currently hard to treat, such as the lung and kidney. "As we improve delivery further it will enable new therapies which just aren't possible today," he says.

A DIFFERENT BUSINESS MODEL

The companies that these researchers have founded follow the standard for-profit model used by most biotechnology start-ups. But Vandenberghe is taking a different approach with Odylia Therapeutics, a not-for-profit company he founded in February. Odylia aims to develop therapies for what Vandenberghe calls "ultra-rare" genetic causes of blindness, which he defines as those that affect 3,000 or fewer people in the United States. The firm is supported financially by Massachusetts Eye and Ear and the Usher 2020 Foundation in Atlanta, Georgia, a charity focused on curing the sight loss caused by Usher syndrome. One of the charity's founders, Scott Dorfman, who has two children with Usher syndrome, is chief executive of Odylia.

So far there is only one available gene therapy for blindness. In late 2017, the US Food and Drug Administration (FDA) approved voretigene neparvovec (Luxturna) for the treatment of eye disease caused by a mutation in the *RPE65* gene, which normally produces a protein in the thin layer of cells at the back of the eye. As a proof of concept, the treatment shows that gene therapy can be used to cure eye disease. But mutations in more than 200 genes have been linked to hereditary eye diseases, and Vandenberghe says that there is little appetite in the pharmaceutical industry for developing individual therapies to correct many of the other genes.

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It can cost millions of dollars to develop a drug and take it through clinical trials, and if a disease is rare, it may not make economic sense for companies to pursue a treatment for it. That is a particular issue in gene therapy, in which people are often cured with a single dose rather than a life-long drug regimen. The doses required for eye diseases are tiny because the retina is a relatively small organ, and some retinal diseases are so rare that it's possible that a single batch of the drug could treat the entire patient population in the United States, Vandenberghe says.

A WIDER CONCERN

The question of how to develop gene therapies for rare diseases is of great concern to the US National Institutes of Health, says P. J. Brooks, program director at the institute's Office of Rare Diseases Research in Bethesda, Maryland. "When people discuss business models around treatments for rare diseases, the basic assumption is that there is a business model," he says. "But for some of these diseases where there's a very small patient population, there may not be one." Brooks says Odylia is the first company he has heard of to try this non-profit approach.

The idea, Vandenberghe says, is to find economies of scale by sharing resources and scientific and commercial expertise across the development of a range of drugs that are similar to one another. If the same group of people develops the drugs, designs the clinical trials and produces the materials, there should be less duplication of effort, he notes. Vandenberghe also hopes that after creating two or three successful treatments, the company will be able to provide data to convince the FDA that there are enough similarities between the drugs to enable them to use experience with one drug to help establish the safety and efficacy of another. It is also possible that Odylia will take development of a drug far enough in this model that a for-profit company will decide to buy it and complete the work, providing funding for Odylia while reducing the pharmaceutical company's costs and risks.

If Odylia does bring a drug to market, it will probably be sold at cost, Vandenberghe says. That could still be expensive, but possibly less so than if it had been developed the usual way. There is also a chance that if a drug candidate gets through phase I and II clinical trials, the FDA could allow it to be provided on a compassionate-use basis without a final clinical trial, or that most patients could be treated as part of an open-ended trial.

If the model is successful, it could be extended to other rare, single-gene disorders and perhaps provide insights for developing gene therapies for more common conditions. "Maybe this is one of those areas where industry can acknowledge that this is indeed non-competitive," Vandenberghe says. Ideally, he says, that would set up a happy scenario. "We can all come together around some of these common goals, apply them to ultra-rare diseases, and then take those lessons to the more commercial world afterwards." ■

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2. Zinn, E. *et al. Cell Rep.* **12**, 1056–1068 (2015).
3. Pan, B. *et al. Nature Biotech.* **35**, 264–272 (2017).