

WIDE

Leah Byrne uses directed evolution to create more useful species of viral vectors for gene therapy. SHOWN HERE: A new variant of an adeno-associated virus she created.

ANGLE

BYRNE MAKES GENE THERAPY CLICK

BY CARA MASSET

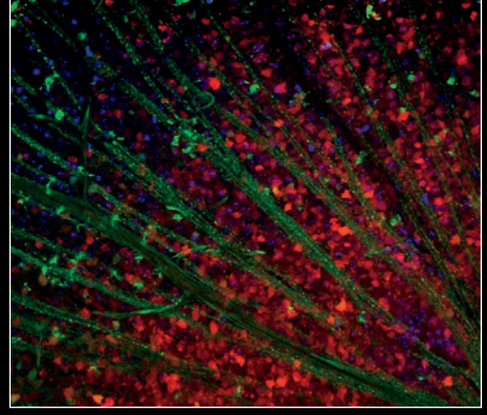


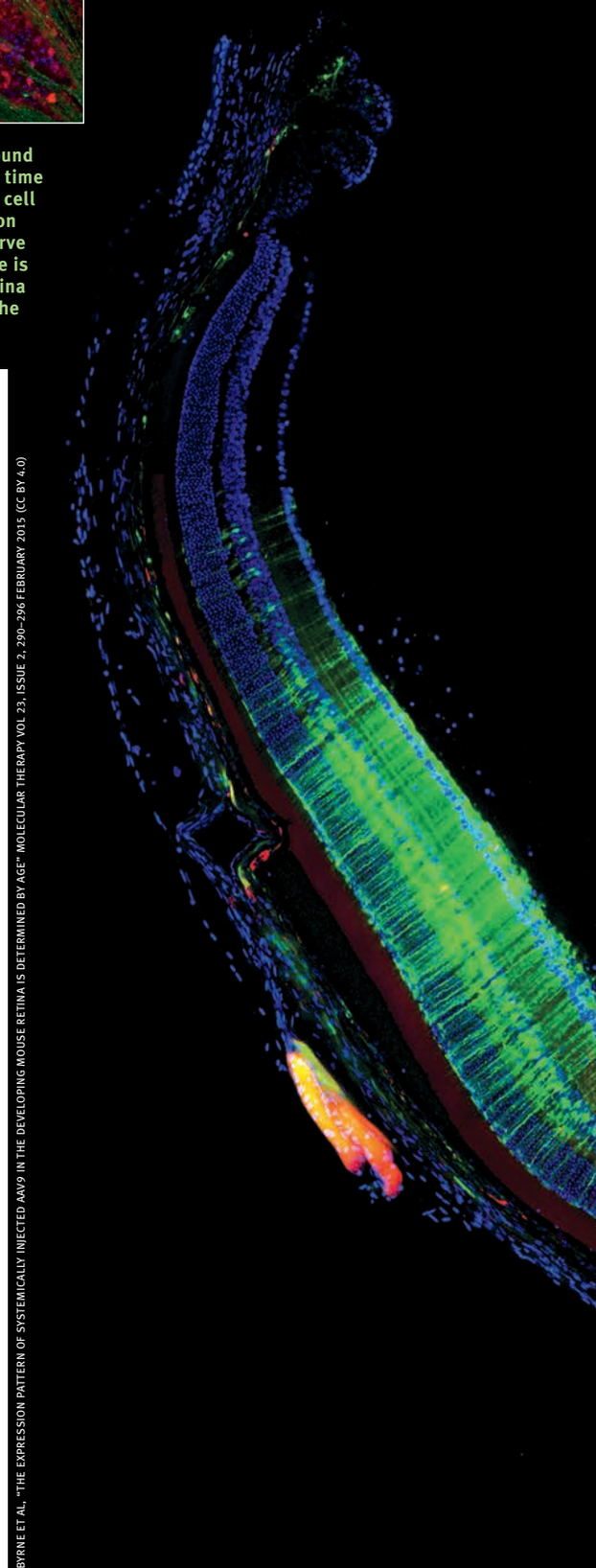
Leah Byrne

Black and white images of 20-nanometer particles beam out from a computer screen in a structural biology lab in Biomedical Science Tower 3. University of Pittsburgh scientist Leah Byrne examines the viral particles she engineered. Did she get it right this time? Or will she have to go back upstairs to her ophthalmology lab on the 10th floor to re-engineer the particles? The virus she's creating—a variety of adeno-associated virus (AAV)—is a partner in her plan to cure blindness.

If the scientist and her virus continue to succeed, they may write a good portion of the industry operating manual for gene therapy. Many at Pitt and elsewhere are looking to Byrne to turn the holy grail of gene therapy from a good idea into a viable treatment for any heritable neurological disease.

PORTRAIT COURTESY BYRNE LAB. ELECTRON MICROSCOPY IMAGE COURTESY JAMES CONWAY AND LEAH BYRNE.





ABOVE AND RIGHT: Byrne and colleagues found that barriers to gene therapy develop over time with a certain vector they engineered. The cell types infected by AAV9 varied depending on age. Here, axons (green) near the optic nerve express the AAV9-GFP vector after a mouse is 1 day old, and cells (red) in the central retina express another AAV9 vector by the time the mouse is 5 days old.

BYRNE ET AL., "THE EXPRESSION PATTERN OF SYSTEMICALLY INJECTED AAV9 IN THE DEVELOPING MOUSE RETINA IS DETERMINED BY AGE" MOLECULAR THERAPY VOL 23, ISSUE 2, 290–296 FEBRUARY 2015 (CC BY 4.0)

For starters, Byrne is working toward giving sight to patients without vision and preventing patients with degenerative diseases from losing theirs.

The intent of gene therapy—whether it be for retinal blindness, arthritis, or sickle cell anemia—is to transport needed genetic material into cells that aren't functioning properly because of faulty DNA. Give them new genes and—*click!*—eyes will see, joints will swing, and blood will flow. That's the idea, anyway. In practice, it's not easy to do.

We've been covering the promise and challenges of gene therapy in *Pitt Med* magazine since our inception in 1999. When we interviewed Pitt's "gene-therapy point man," Joseph Glorioso, back in 2005, the microbiology and molecular genetics professor predicted that the next advances would involve engineering vectors, i.e., the transporters of genetic material. "Genes already know what to do, but the problem is how to get them there," he said. Glorioso has had success modifying the herpes simplex virus as a vector (without carrying disease), including one vector he patented that targets tumors. It has been partially licensed by Oncorus; clinical trials are expected to start in early 2019.

Byrne, a PhD who joined Pitt in 2017 as assistant professor of ophthalmology, is an expert at engineering AAV vectors. The adeno-associated virus, now in vogue for gene therapy investigations, was identified by Pitt emeritus microbiology professor Robert W. Atchison in 1965. (Byrne regularly cites Atchison's *Science* report when she gives research talks—the Pitt connection is a happy coincidence.) AAVs are ideal as vectors because when they infect cells, they drop off genetic material without causing illness. Scientists like Byrne can engineer millions of AAV individuals that are capable of all sorts of feats. Plus, AAVs are amenable to structural changes.

"It's a highly malleable virus," Byrne says. "You can actually change the way that the virus infects cells. You can change what kinds of cells it infects. You can change how efficiently it infects those cells. That can enable new gene therapies and more successful outcomes for patients."

AAVs are especially good at delivering genes to the retina, the layer of neurons at the back of the eyeball that transmits visual information to the brain.

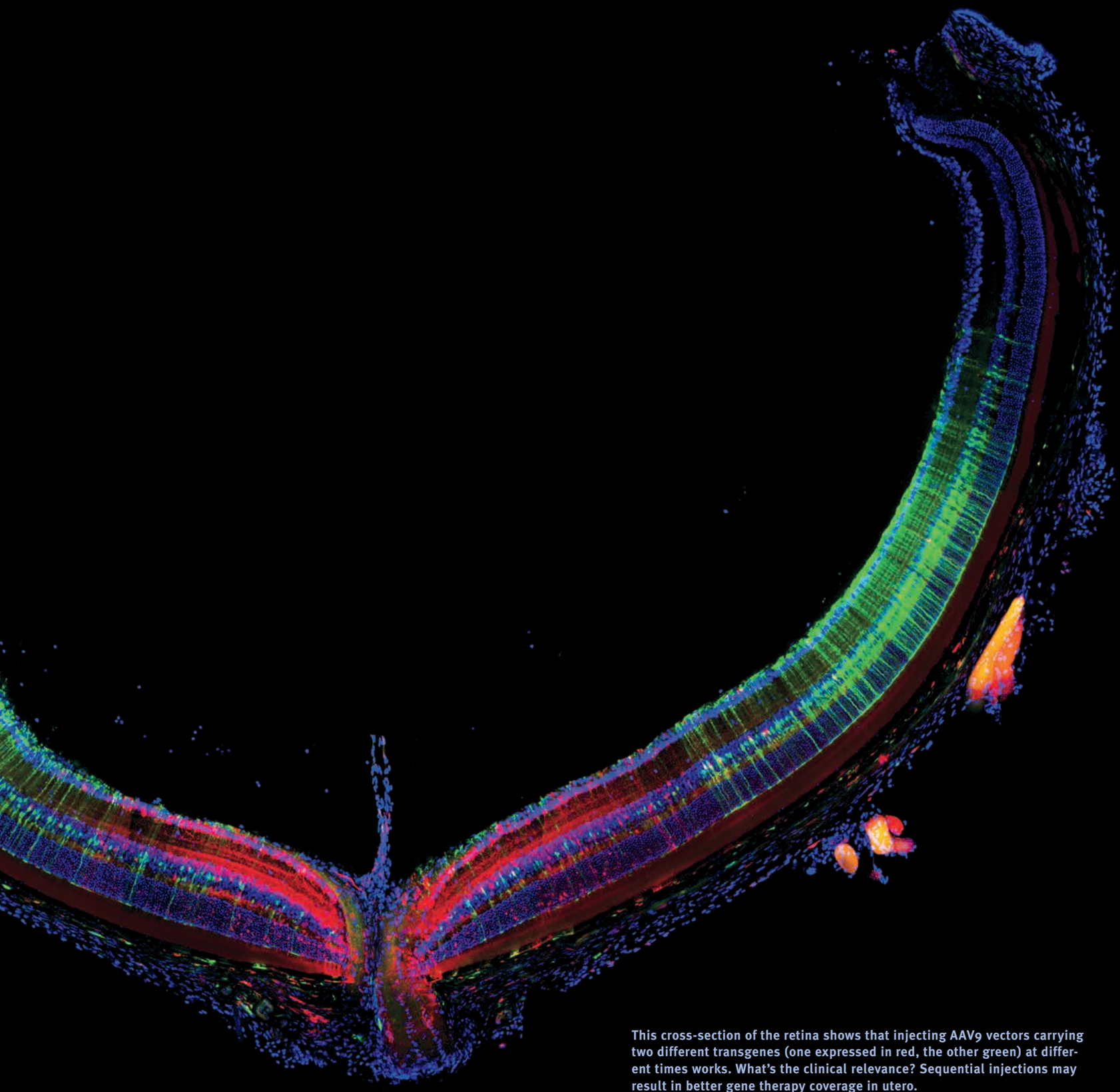
As a PhD student at the University of California, Berkeley, Byrne helped to engineer an AAV named 7m8. Mighty 7m8 is capable of traveling from the gel-like middle of the eye, known as the vitreous humor, into the retina, as Byrne demonstrated in rodent and large animal models.

The hope is that this will work safely in humans, as well. If so, it means that rather than undergoing retinal surgery—and risking retinal detachment—patients with retinal diseases could instead receive injections in the vitreous humor. Byrne and her Berkeley colleagues patented 7m8, which has since been licensed by Adverum Biotechnologies. In the fall, the company started clinical trials of intravitreal gene therapy injections for patients with wet age-related macular degeneration, a leading cause of vision loss in patients older than 60.

When the research on 7m8 was originally published in *Science Translational Medicine* in 2013 with Byrne as a first author, it was recognized by National Institutes of Health Director Francis Collins. He posted a blog entry, "Glowing Proof of Gene Therapy Delivered to the Eye," and included an appropriate glowing image—credited to Byrne—that showed that 7m8 had delivered its genetic cargo to all layers of the outer retina.

It wasn't the first time Byrne had been credited for a stunning image. Byrne is a photophile who still prefers to shoot on film. The first laboratory of sorts that she built was a darkroom at her family home in Ohio. She began college as an art major interested in photography, then a neuroscience class changed her course. She ended up graduating from Hamilton College with a bachelor's in neuroscience instead.

In her early 20s, Byrne "traveled the world for a while doing science," working as a research assistant at labs in Sweden, Oregon,



This cross-section of the retina shows that injecting AAV9 vectors carrying two different transgenes (one expressed in red, the other green) at different times works. What's the clinical relevance? Sequential injections may result in better gene therapy coverage in utero.

and Lebanon. On her world science tour, Byrne examined how the brain controls food intake; designed molecular biology tools for the diagnosis of mitochondrial diseases; and studied the neuroscience of addiction, neuropathic pain, and schizophrenia.

It wasn't until Byrne landed in California and started her PhD at Berkeley that she decided to specialize in the eye. It was perhaps natural for a photographer to be drawn to the eye, particularly the retina, where images are projected, akin to film in a camera. "It was exactly the right science for me," she says of working in the laboratory of John Flannery, who pioneered the use of AAV vectors for gene therapy for retinal disease. "I loved the engineering side of it. I loved the translational side of it. I loved the imaging."

As a PhD student, Byrne studied the mechanisms of inherited retinal dystrophies, a group of diseases involving mutations in more than 200 genes, all of which can cause blindness. She created gene therapies for retinitis pigmentosa, X-linked retinoschisis, and macular telangiectasia type 2. She developed therapies using 7m8 and then stayed on at Berkeley as a postdoctoral fellow to continue making new tools for gene therapy.

As a postdoc, Byrne mastered an approach called directed evolution to screen for AAV vectors that are best suited for large animal eyes. AAVs that work in mouse models can only take translational research so far, Byrne explains. Rodents have thin retinal membranes, and they lack foveae—the part of the retina that allows people to fixate clearly on an object—so research outcomes don't always translate well to human eyes. Large animal eyes are closer to those of humans. Primates (including humans) have foveae, and canines have streaks that are similar. Both have thick retinal membranes.

To figure out which vectors would excel in large animal eyes, Byrne pooled millions of AAVs and put them through a process of evolution in a controlled laboratory setting. (If you've read about directed evolution recently, it may have been in coverage of the 2018 Nobel Prize in Chemistry, awarded to the inventors of the approach, including Pittsburgh native Frances Arnold.) Byrne injected canine and primate models with pools of AAVs, then allowed the viruses to compete against one another to see which ones were more capable of reaching the retinas. She repackaged the winners, set up a second competition, then recovered the most successful variants again. She continued the process for six rounds of selection. At the end, she and her colleagues identified and patented the top winners of the evolutionary tournament. The research, soon to be published, not only described the new vectors. It confirmed the importance of creating specific vectors for canine and primate models. "The viruses we evolved didn't infect mice very well, indicating that there are significant differences between the retinas of small animals, in which gene therapies are often tested, and large animals" like dogs and primates, Byrne notes. This insight has led Byrne to create techniques in her new lab that will result in gene therapies that she's confident will work in people.

During her years as a Berkeley trainee, Byrne's wanderlust also took her to Paris for a year to conduct research at Institut de la Vision. It was there that she met José-Alain Sahel, founding director of the institute who became chair of Pitt's Department of Ophthalmology in 2016. They worked together on a new gene therapy for cone-rod dystrophy, a retinal disease that typically onsets in childhood and leads to vision loss over time.

Sahel's team had identified a protein for keeping cones and rods, the light-sensing cells in the retina, healthy. Byrne successfully employed AAV strains, including 7m8, to handle the delivery logistics. They demonstrated success of the gene therapy in mouse models. Clinical trials are expected to begin soon in France, followed by trials in Pittsburgh. (In more photogenic news, when the research hit the *Journal of Clinical Investigation* in 2015, the journal referred to one of the images as a "Scientific Show Stopper.")

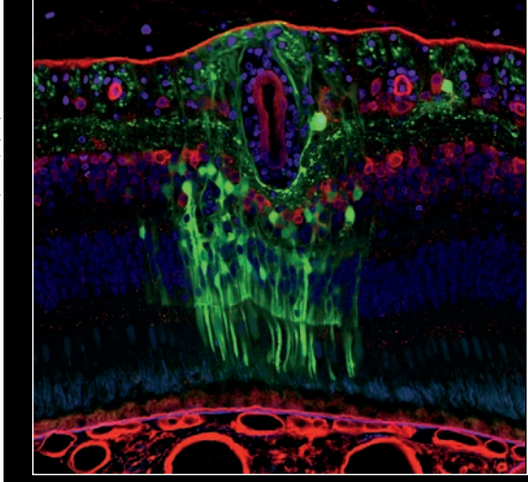
Sahel says he was impressed by Byrne's work from the beginning. "She's both a deep thinker—very focused, very well-organized—and at the same time she doesn't create any noise. She's just focused on what's important."

Byrne says she feels fortunate to be mentored by a "true world leader." When Sahel recruited her to Pitt, she was eager to become part of the expansion of the University's vision research that he's leading. For instance, he spurred an agreement, signed in 2017, between Pitt's School of Medicine and three research institutions in France: the Université Pierre et Marie Curie of the Sorbonne Universités, the Institut National de la Santé et de la Recherche Médicale (which is much like the our National Institutes of Health), and the Centre National de la Recherche Scientifique. The organizations are banding together for research, clinical trials, and joint academic conferences.

This fall, UPMC announced that in spring 2019 it would break ground for a new vision and rehabilitation hospital at UPMC Mercy in Pittsburgh's Uptown neighborhood. Sahel, an MD, will be moving both clinician and research teams there, replicating a model he set up in Paris. Sahel says having patients, doctors, and scientists in the same place is the most efficient way to make progress in bench-to-bedside research. "You have to ask the right questions. And the right questions come from patients," he says.

Byrne is looking forward to moving into the new building. At the moment, her lab is motoring along in Biomedical Science Tower 3, pursuing the most creative ideas she can dream up. "Dr. Sahel is supportive of me doing the most ambitious work possible," she says. "If they made mugs for World's Best Ophthalmology Chair, I'd get him one."

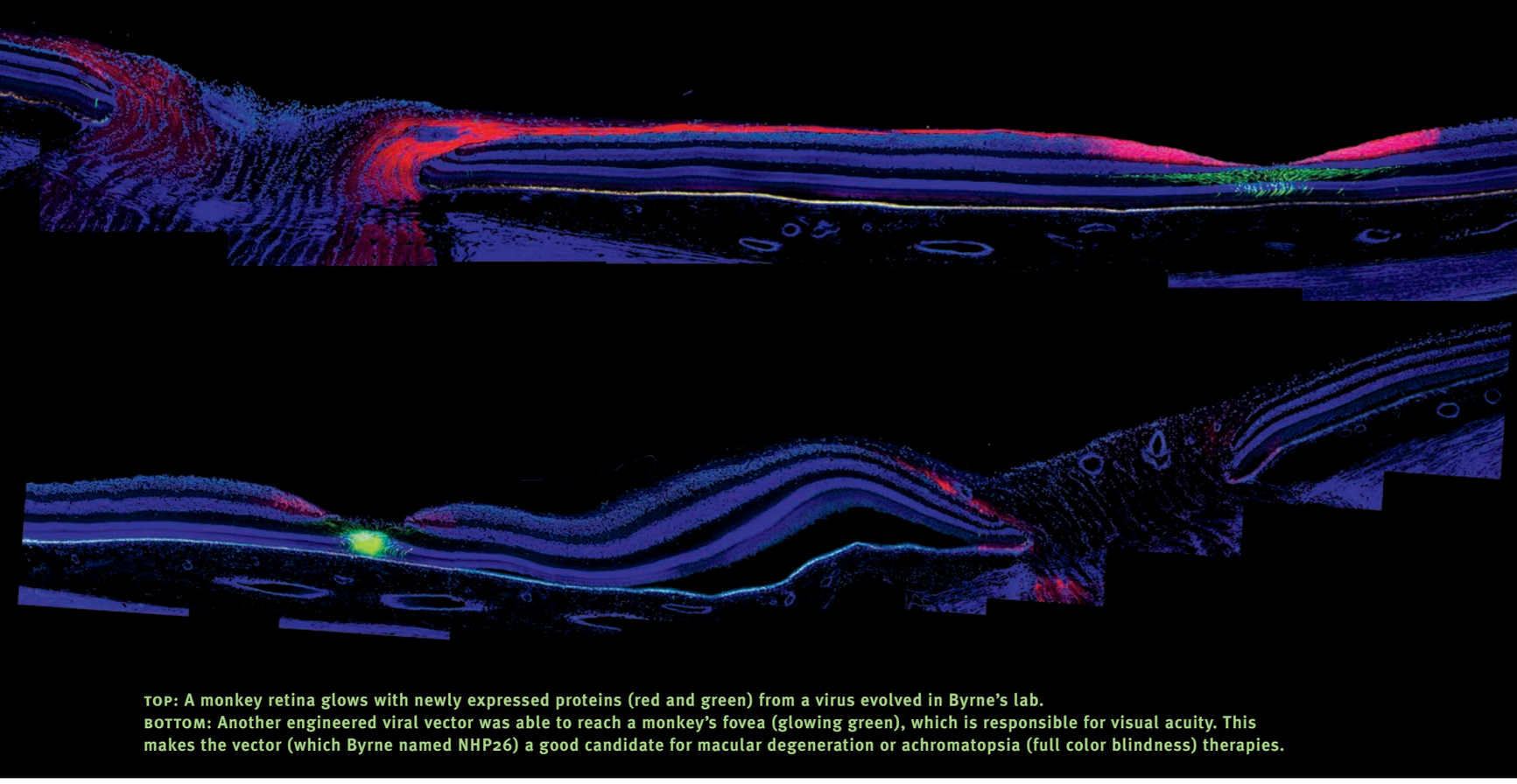
Ambitious Project No. 1 is using high-throughput screening to create a "complete" dictionary of AAVs that can infect every single cell type in the primate retina and brain. In the past, it has taken years to develop a vector for a single target, but Byrne has developed a method to speed up the engineering process; and she can track the behaviors of millions of new viruses in thousands of cells simultaneously. To make this happen, she's working with Pitt and Carnegie Mellon University colleagues as well as the UPMC Genome Center (which just opened in



DALKARA D. ET. AL. "IN VIVO-DIRECTED EVOLUTION OF A NEW ADENO-ASSOCIATED VIRUS FOR THERAPEUTIC OUTER RETINAL GENE DELIVERY FROM THE VITREOUS." SCI TRANSL MED. 2013 JUN 12;5 (189).

ABOVE: The NIH's Francis Collins called this "Glowing Proof of Gene Therapy Delivered to the Eye." Green shows a protein made from genes successfully delivered to all layers of the outer retina of a monkey with Byrne's 7m8 vector.

COURTESY LEAH BYRNE LAB



TOP: A monkey retina glows with newly expressed proteins (red and green) from a virus evolved in Byrne's lab.
BOTTOM: Another engineered viral vector was able to reach a monkey's fovea (glowing green), which is responsible for visual acuity. This makes the vector (which Byrne named NHP26) a good candidate for macular degeneration or achromatopsia (full color blindness) therapies.

the fall). "We're making a toolbox of viruses that will be available to the research community," Byrne explains. "So, for example, if a researcher needed to target photoreceptors and glial cells in the retina, or any other cell type, they could find the optimal virus for that combination of cells in the online database we are constructing." Byrne received an Individual Investigator Award in 2018 from the Foundation Fighting Blindness to fund the research.

As part of the dictionary project, Byrne has also introduced RNA-tagging, essentially a barcode that can track how much genetic material makes it into cells during gene therapy. It's an advance from the tracking process she used as a postdoc. The barcode will speed up the process of determining which vectors are most effective.

Ambitious Project No. 2 is figuring out ways to deliver large genes (including the gene that encodes CRISPR-Cas9—the hot gene-editing tool that directly rewrites the genome) into retinal neurons. Transporting CRISPR-Cas-9 into the retina could open up all sorts of solutions. Here's the problem: It's too big to fit in an AAV trunk with other molecules that would also need to be delivered for therapy. AAVs are itty bitty things—about 25 nanometers in diameter with room for about 4.7 KBs of cargo.

Byrne and her team have come up with a strategy for splitting genetic cargo, delivering it with engineered dual vectors, and then reassembling the genes inside cells. The approach would be useful not only for delivery of CRISPR-Cas9, but large genes that are involved with diseases like Stargardt disease (the most common form of retinal degeneration in children). Last year, she received a Career Development Award from Research to Prevent Blindness in support of the work. She also submitted a patent application. If awarded, it will be Byrne's sixth patented technique.

Besides splitting cargo in two, Byrne's team is also exploring if they can engineer AAVs with bigger trunks, capable of fitting more cargo. That's why, soon after Byrne set up her Pitt lab, she began collaborating with structural biology professor James Conway to use electron microscopy to zoom in on the AAVs she's developed. She heads down to the basement microscope from her 10th floor lab so she and Conway can get a good look at viruses after she creates them. She wants to see how structural changes influence AAV behavior.

Soon, she'll also tap into the live-video scopes at Pitt's Center for Biologic Imaging to see the AAVs in action.

Fingers crossed that her team can come up with a structure that increases the cargo capacities of the AAVs by, say, switching from a traditional sedan trunk to a hatchback.

"What she's going to do is to the benefit of all of us," Sahel says of Byrne.

He's not just talking about the field of vision care. Peter Strick—scientific director of the University of Pittsburgh Brain Institute, where Byrne also serves on the faculty—calls Byrne an "institutional resource" who is cross-trained and multidisciplinary. (She has secondary appointments in neurobiology and bioengineering.)

"Her skills have been applied to the visual system, but if she solves the problem for one set of neurons [in the retina], it's likely to be a general solution," Strick, who is also chair of neurobiology, says.

He has connected her with Brain Institute researchers studying decision-making who need help delivering genes throughout the brain, as well as scientists working on gene therapy for post-polio syndrome.

Beyond Pitt, Byrne spent 2017–18 as an associate scientific advisor for *Science Translational Medicine*, writing summary articles about promising gene therapy research for skin regeneration and for melanoma, sickle cell anemia, and other diseases. "That was so much fun," she says. "It gave me the chance to read in depth in research areas outside the eye."

The UPMC Vision and Rehabilitation Hospital is set to open in 2021. Once Byrne is situated there, she'll work with clinicians who will help her, she says,"better understand what the needs of patients are and how my research fits in."

And that will clarify where to focus her lens next. ■