ENDPOINTS NEWS

# Exclusive: Big-name academics and investors are quietly preparing a slate of new (epi)genome editing companies

by **Jason Mast** on November 16th, 2021

A few weeks after Jennifer Doudna introduced CRISPR/Cas9 genome editing to the world, one of her old students decided to take the central part of the biology-altering invention and kill it.

CRISPR/Cas9, as the name implies, is a two-part system: a string of letters called a guide RNA, that says where to cut the DNA. And an enzyme, Cas9, that does the cutting. Often compared to molecular scissors, it was the first system that allowed researchers to cut DNA with ease and precision, promising potential cures for genetic diseases such as sickle cell and cystic fibrosis.

Stanley Qi

But Stanley Qi, a biologist who had just left Doudna's lab to start his own at UCSF, wanted to make what amounted to a molecular clamp. Rather than slice out a disease-causing mutation, his clamp would latch onto the gene and smother it into silence.

It was simple. Over a couple of months, Qi engineered a Cas9 that bound to DNA but couldn't cut it. Qi named it dead Cas9, or dCas9. He thought it could be a powerful tool for both research and therapies.

"Genome editing is very powerful," he said in a recent interview. "But it can't do everything."

Amid the hype and heated battles that followed Doudna and Emmanuelle Charpentier's first paper, you'd be forgiven for thinking genome editing could. Top labs raced to replicate their success in human cells and investors poured millions of dollars into companies that would, in the coming years, bring treatments for cancer and several genetic diseases into the clinic. Although Qi wondered about therapies, his dCas9 was widely considered an (admittedly powerful) research tool and nothing more.

All that has changed in just the last two years. Burnished by a series of technological breakthroughs and confronted by the limits of classic CRISPR genome editing, big-name academics and blue-chip investors have quietly launched a series of companies that will try to change how a patient's genes are expressed without actually having to rewrite a patient's DNA.

These approaches target a patient's epigenome, the system of folds and tags in DNA that help govern which genes are turned into proteins and how much. The idea is that by editing the epigenome, you can manipulate a patient's cells with greater deftness and precision than breaking or rewriting the DNA itself. More a dimming dial than flicking a light switch.

In theory, that could be safer. And it could allow drug developers to do things that are more difficult to do with genome editing, such as to treat certain genetic disorders, make numerous edits inside a cell, make reversible edits, or even push new fronts in regenerative medicine.

“Gene editing is basically a binary event,” said Charles Gersbach, a professor of biomedical engineering at Duke. You either cut the DNA in X number of cells, or you didn’t. “Epigenome editing — and this is both a feature and a bug — it’s not binary, right. You can actually dial in how much you want to turn the gene on or turn the gene off, or how [long] you want to turn the gene on or off.”

The most prominent of the new companies is Chroma Medicine, a Cambridge, MA biotech that Atlas Venture and Newpath Partners have been incubating based on work MIT’s Jonathan Weissman published in *Cell* in April. The paper has been widely regarded as the capstone of a decade of epigenome editing work and, according to Pitchbook, the biotech raised a $101 million Series A in October. Well-known genome editing academics David Liu, Keith Joung and Luke Gilbert are co-founders.

They’re joined by Tune Therapeutics, a Seattle-based biotech founded by Gersbach and UC Berkeley professor Fyodor Urnov and backed, per Pitchbook, with $40 million in Series A funding. Luigi Naldini, an Italian professor whose 2016 paper led to Weissman’s work, raised $7 million from Sofinnova for a company called Epsilen.

And Qi, a decade later, has not one company, but two. The second, called EpiCrispr, just closed its own undisclosed Series A. Although virtually all in stealth mode, they’ve become an open secret throughout biotech.

All of them join Sangamo, the world’s oldest gene editing company, which just signed epigenome editing deals with Novartis and Biogen worth up to $3 billion combined to go after various neurological conditions.

People had always used dCas9 in the lab, said Prashant Mali, a genome editing professor at UCSD. “But in terms of actually taking it to the clinic, I would say it’s probably right now that it is really coming to the fore,” he said. “There’s a lot of interest.”

The question now will be how fast these companies can turn a lab tool into a medical one. And how many of the field’s grand ambitions are actually attainable.

”In the beginning, it was such a hard sell. No one really understood the promise and potential of epigenome editing,” said Christina Trojel-Hansen, a biotech entrepreneur and co-founder of Tune. “But that is changing rapidly.”

**Christina Trojel-Hansen**

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**The epigenome is often defined as the way the genome learns from experience**.

Little annotations accumulate on top of our DNA over generations. It's like an old family recipe book filled with scrawled comments and cross-outs from Grandma and then Dad and finally your own updates for current brands and cooking styles. The marks tell cells to express more or less of certain genes depending on the environment, such as how much food was available or the trauma one faced.

In that sense, epigenome editing is "basically teaching genes," said Urnov. A military crash course, really, meant to instantly instill what generally only decades or eons can.

The idea, he notes, is actually older than gene editing. Urnov is now perhaps best known as the most quotable man in gene editing and Doudna's "gene-editing wizard" colleague in Walter Isaacson's *The Code Breaker*. But he started as a postdoc in the NIH in the '90s helping isolate the first molecular machines cells use to control gene expression.

In 1991, a Darwin-bearded biologist named Carl Pabo isolated the structure of human proteins called zinc fingers that can bind to DNA and proposed they could be re-engineered to target and repress specific sequences for therapies. Urnov's advisor Alan Wolffe agreed and, with his gene regulation expertise, shut down his prestigious lab in 2000 and moved to California, to build epigenome editors at Sangamo Therapeutics.

Wolffe passed suddenly in 2001. Sangamo would put the first epigenome editor, a treatment for

diabetic neuropathy, in the clinic in 2005.

It was the first and last epigenome editor anyone tested in humans. In 2003, a handful of academics showed that, with some tweaks, you could use the same technology to cut precise sections of DNA and edit genes. Overnight, the entire field switched focus to what became known as zinc finger nucleases.

The human genome had just been sequenced and the epigenome's wide-ranging role in human health wasn't yet understood. Meanwhile, genome editing held the promise of curing diseases caused by a single mutation, such as sickle cell disease or severe combined immunodeficiency disorder.

"I don't want to say I forgot about epigenome editing. That's not what happened," Urnov said. "It's just that the range of opportunity of genome editing in terms of specifically repairing mutations that cause severe disease became the more urgent one to develop."

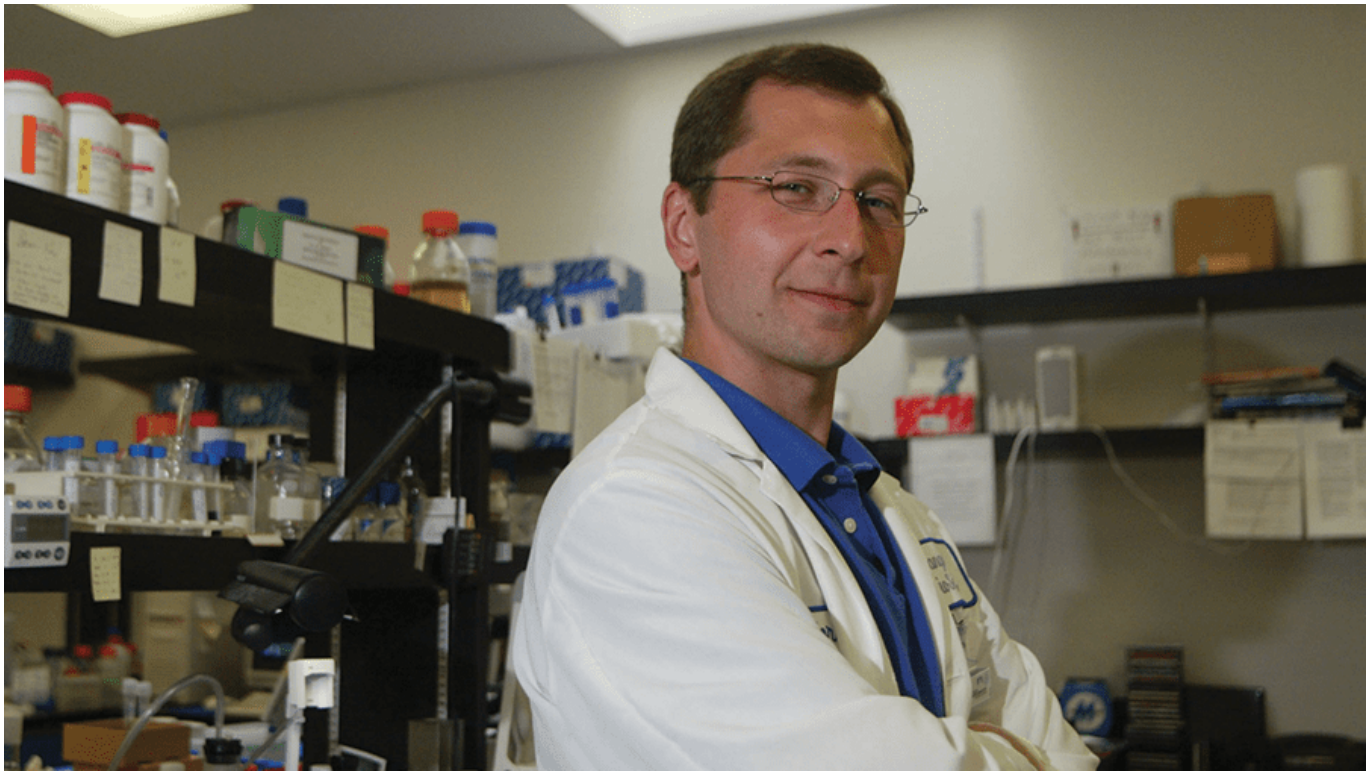
The focus on gene editing only continued with the advent of CRISPR. Qi's invention, dCas9, was seen largely as a tool to better understand how different parts of the genome function. What happens if we silence *this* sequence in a cell in a dish? What if we activate *that* one?

You can use CRISPR/Cas9 to do that too. But by cutting the DNA, you might damage other parts of the genome. dCas9, or CRISPR-interference as it became known, was far defter.

"I think it was initially thought of, at least from my vantage, as a discovery tool," said Jerel Davis, managing director of Versant Ventures, the first investor in CRISPR Therapeutics.

The idea that you could change how genes are expressed was put "on the backburner" by most of the field, said Gersbach.

**Fyodor Urnov at Sangamo Biosciences in the mid-2000s**



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**Not everything can be treated with gene editing, though.** Take chronic pain, for example, one of the most common ailments in the US and one of the hardest to treat without addictive opioids. In 2006, geneticists studying a Pakistani boy who could walk on coals without pain found a gene, called Nav1.7, critical to the sensation.

Nine years later, Ana Moreno, a PhD student in Mali's lab, was scrolling through papers late one Sunday night, when she stumbled upon the Nav1.7 paper and realized it could be a good target for epigenome editing.

You don't want to knock it out entirely — people need pain to survive; many people born with Nav1.7 mutations sustain injuries and don't survive to adulthood. But if you could just smother the gene a little, you could have a powerful one-time infusion for patients with no other options but opioids.

"We were seeing pharma companies being sued, a lot of people dying," Moreno said. "Like, what if we could do repression for this gene? Because we don't want to edit it, we don't want to completely eliminate the pain."

Moreno and Mali published their proof-of-concept earlier this year, showing you could give mice long-lasting pain relief by developing specialized dCas9s and zinc fingers targeted to Nav1.7. The two founded a company around the tech called Navega and Moreno, now CEO, is raising a Series A.

**It's one of several advancements that have quietly helped inch epigenome editing closer to the**

**clinic over the last decade.** They've given researchers newfound precision in handling a system that has long remained more inscrutable than the genome itself.

"There's a lot more basic biology and engineering that had to be done to take it to a point where we feel more comfortable with the system," said Mali. "It's a much more complex genetic surgery."

Moreno's approach has clear drawbacks. Most notably, it only works as long as dCas9 remains expressed inside a cell, clasped onto the Nav1.7 sequence. During that time, the body might mount a debilitating immune response to the foreign protein. It also might wear off over time. That's in contrast to gene editing, where you only need a one-time genetic surgery.

In 2016, though, a pair of scientists at the San Raffaele Telethon Institute for Gene Therapy in Milan, Naldini and Angelo Lombardo, showed that you could permanently alter the epigenome with a single strike. They called it "hit-and-run" epigenome editing.

To explain: dCas9 is only one part of an epigenome editor. It mostly functions like the GPS. To actually suppress or activate a gene, researchers fuse it with one of several dozen proteins they know can somehow — the exact mechanism is often unclear — modify the epigenome. Generally, it either adds chemical tags onto the sequence of DNA or changes the proteins that DNA is wrapped around, exposing or hiding certain regions.

Many, albeit not all, of these proteins are repurposed weapons. They come from proteins humans evolved to suppress viruses that inserted themselves into our DNA over millions of years of evolution.

Naldini and Lombardo showed that with the right three weapons, you can get DNA in human cells to permanently silence themselves. They stayed silent not only in that cells' lifetime but in daughter cells.

"The genome editor goes away, but the gene expression state stays," said Urnov. "That's exciting."

Naldini, who declined an interview, has built a company off this work. But initially, it was too cumbersome for therapies. You needed to give multiple different proteins and it was rarely efficient.

It also shared a flaw with most epigenome editors like Moreno's: Although proponents touted the fact that it wasn't permanent, like a DNA break, no one actually had a way to reverse it.

Around the same time, though, DARPA, the Pentagon's R&D arm, launched a pair of projects called Safe Genes and PREPARE meant to keep the US at the forefront of gene editing. They funded a variety of Gattaca-esque ideas— discovering proteins that could protect soldiers from accidental or intentional misuse of gene editing technology, for example — but among them was a program to make a reversible gene editor.

Because it's DARPA, one idea was to make soldiers temporarily radiation-proof. But they also saw it as a potentially valuable tool for therapeutics.

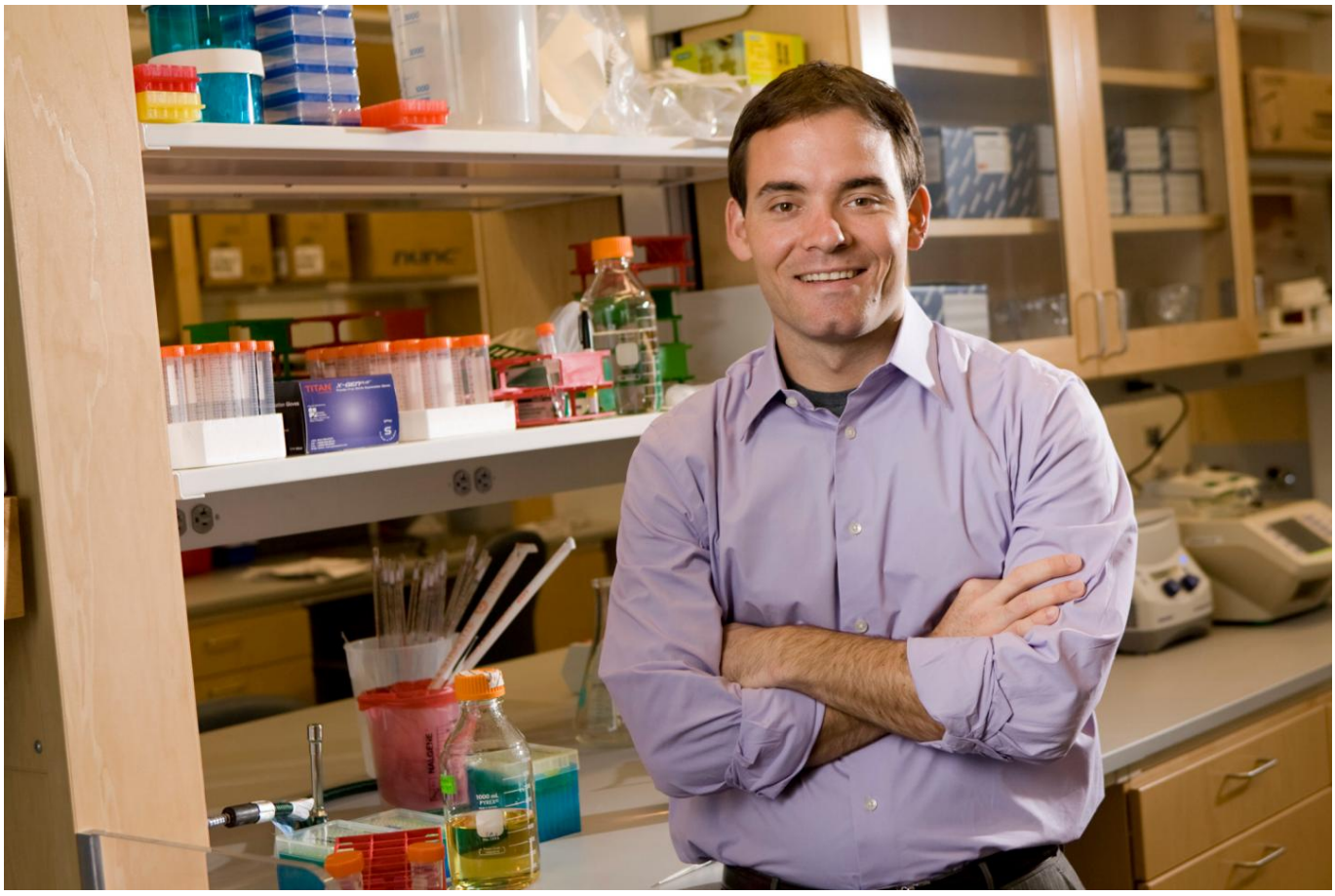
They wanted tools to "to control, counter or reverse these editors because if you think about it from the perspective of a warfighter it's the same tool to be used for both therapeutic purposes for them, but also in the case of accidental harm or misuse," said Anne Cheever, head of the Safe Genes program. "So ultimately it was an approach to look at various ways to control editors. And this was a very promising one."

DARPA funded UCSF professors Luke Gilbert and Jonathan Weissman, who was also a co-author on Qi's dCas9 paper. Earlier this year, the pair unveiled what they called CRISPRon and CRISPRoff. Fusing different parts of the proteins from the Naldini-Lombardo work, they were able to permanently silence genes and in human cells, and then reverse the change.

It was the culmination of more than a decade of ambitions and incremental breakthroughs.

"It's a tour de force of engineering," said Mali. He compared it to the evolution of the car, from Model Ts to a Ford today. "So I think it's about making the technology robust. And I think that's just very, very powerful."

**Charles Gersbach**



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**Weissman deferred an interview request to Chroma Medicine CEO Catherine Stehman-Breen while the company is still in stealth.** Atlas partner Kevin Bitterman declined an interview, saying in an email only "epigenetic editing represents a transformational advance in genomic medicine" and they were "enormously optimistic about the potential."

Weissman, though, has talked about where he'd like to see the technology applied, often comparing it to other "CRISPR 2.0" approaches like base and prime editing. The easiest is diseases that can be treated by knocking out a single gene.

The first generation of CRISPR companies also saw these conditions as the lowest hanging fruit. One, Intellia, showed positive data targeting one this year. But Weissman argued epigenome editing would be even safer, allowing you to turn off the pathogenic sequence without breaking the DNA and hoping the cell repairs itself properly.

"Why would you want to change your DNA? Why would you want to make double-stranded breaks if your goal is just to turn off one gene?" he said on a podcast in July.

Other researchers want to use it in areas where it would have significant advantages over gene editing. Weissman said it could silence the genetic stutter that causes Huntington's and other so-called triplet repeat disorders.

Another example is haploinsufficiency disorders, diseases where a patient only has one copy of a gene and thus only half the amount of the protein they need. These include neurodevelopmental diseases, forms of dementia and Marfan syndrome. An epigenome editor could dial up the single gene's expression to compensate.

Ultimately, though, many of the field's hopes lie much higher up in the tree. One of the key advantages of epigenome editing is that you can safely multiplex, i.e. hit multiple targets in a single cell.

You can do this with CRISPR and zinc finger nucleases too, of course. But each time you cut DNA, you run the risk of causing unwanted damage. An Allogene cell therapy trial is now on hold over fears that its nucleases led to "chromosomal abnormalities" in one cancer patient.

The risk/benefit can make sense when treating terminal patients, said Urnov. But if you want to use CRISPR for more common and chronic disorders, as many in the field do, safer approaches might be needed.

"Imagine a setting, for example, where we need to multiplex the control of several genes in something like the liver, or the brain, or the blood. And can we do that with double strand breaks? Yep," he said. "But as we transition to things that are chronic, we want to have an increased mindfulness about safety."

Multiplexing opens a variety of possibilities. Trojel-Hansen founded Tune with Gersbach and Urnov while she was CEO at Oscine, a company trying to engineer a type of brain cells lost in many neurodegeneration patients that Sana bought out in 2020.

She notes that you can use it to change the identity of certain neural or immune cells. You can also use it for regeneration; scientists have known for years that changing epigenetic markers can make cells in organs such as the heart proliferate after damage. Tune will focus in part on doing that in the central nervous system.

Yet epigenome editing will face many of the same hurdles as other CRISPR systems, said Harvard geneticist George Church, who published some of the first dCas9 work with Mali in 2013. Neither Moreno's nor Weissman's approach fit into the AAV viruses often used for delivering genetic therapies. Lipid nanoparticles are also an option but are difficult to deploy to much of the body.

"Whether its lipid nanoparticles or AAV, you still have to get delivery," he said. "And that's the problem with almost all systems."

Church said that when it comes to changing cell states, or regeneration, his work has shown proteins called transcription factors can work better than epigenome editing approaches. He founded a company, GC Therapeutics, around it.

Other investors see potential in haploinsufficiency disorders and other monogenetic diseases, but are skeptical of the field's loftier ambitions. Davis, the Versant investor, said he followed Urnov and Gersbach's work closely and believed they'd find the "killer applications."

But he noted researchers who have regenerated tissues in animals often had to target five or six genes. How could you do that in a patient?

"The potential is super exciting, (but) the use cases are few and far between," he said, speaking broadly about the field. "I think it's gonna take a long time for that kind of thing to happen."

Gersbach has now worked on the epigenome for over a decade, ever since his attempts at mechanical, electrical and chemical approaches to regeneration failed. He designed his own dCas9 around the same time Qi did.

He acknowledged the approach comes with uncertainty. It's not only unclear how you would deliver to multiple targets, it's also unclear in every case what would happen when you do. Regeneration is not like sickle cell disease, where if you fix a single mutation, you're virtually guaranteed to fix the disease. The same is true for most common and chronic disorders.

But, Gersbach argued, the tool was too powerful to just use on genetic diseases.

“Some of these things have more biological uncertainty than sickle cell, absolutely. But I think that’s also where the potential is,” he said. He imagined if 50 years from now, historians look back and see that CRISPR was only used for a few rare diseases and a couple cancer therapies. “That would be disappointing, right?”

*The article has been updated to clarify DARPA’s role in advancing Weissman and Gilbert’s work.*

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