STAT

Friday, May 19, 2023

BIOTECH

In its first tough test, CRISPR epigenome editing cuts cholesterol levels in monkeys

By Megan Molteni

A defanged form of CRISPR, which doesn't slice or nick DNA, but rather alters the epigenome — the layers of chemical coding that sit on top of DNA and control the activity of genes — has aced its first substantive test.

When researchers used CRISPR "epigenome editing" to dial down a cholesterol-associated gene in monkeys, the animals' blood levels of heart-disease-causing LDL, or "bad" cholesterol, plummeted by more than 50%, Jennifer Kwon, senior scientist at Tune Therapeutics, announced on Friday at the American Society of Gene and Cell Therapy meeting in Los Angeles.

The results, from Tune's experiments in five cynomolgus monkeys, are the first published data showing successful CRISPR epigenome editing in a non-human primate; in the past few years, there have been similar successes in mice. It's exciting news for Tune, which was founded in 2021 and is located in Durham, N.C., and Seattle, but also for the other young startups in this field trying to develop a new class of precision medicines that can write or erase epigenetic marks involved in human disease.

Proponents of this approach say it offers an advantage over other forms of gene editing by not cutting or nicking open DNA, which can introduce undesirable and potentially problematic mutations.

"This Tune data looks beautiful," said David Segal, a longtime gene-editing researcher at the University of California, Davis who was not involved in the study. "It does really look like they were able to silence that gene in a very long-term way and they were able to see clinical benefits from that."

Tune targeted a gene that monkeys and people share: PCSK9. In 2006, scientists discovered that mutations in the gene lowered LDL and slashed the lifetime risk of coronary heart disease by 88%. The favorable glitch isn't exactly rare; about 1 in 50 people carry at least one disabled copy of PCSK9. Drug companies rushed to copy the effect, building monoclonal antibodies and bits of interfering RNA to block PCSK9 and prompt liver cells to suck LDL out of the blood. Versions of these drugs made by Regeneron, Amgen, and Novartis have been on the market since 2015.

It's also the same gene that Verve Therapeutics, a Boston-based company led by superstar cardiologist Sekar Kathiresan, is trying to permanently disable using a form of CRISPR known as base editing. Developed at the Broad Institute in the lab of David Liu, the technology makes single letter changes to a gene without cutting both strands of DNA — lessening the risks of unintended edits and off-target mutations. Verve's base-editing tech is licensed from one of Liu's startups, Beam Therapeutics.

In 2021, Verve reported that one-time editing of the PCSK9 gene in the liver of monkeys lowered blood levels of the resulting PCSK9 protein by 89% and dropped LDL cholesterol levels by 59%, reductions that endured as far out as six months. Based on the strength of that data, Verve began dosing a few patients with the one-time treatment, VERVE-101, in a Phase 1b clinical trial in New Zealand last July. (A similar planned trial in the U.S. has yet to begin, after the FDA placed a hold on it in December.)

Tune's epigenome editor appears to work just as well, even if it works a little bit differently.

Rather than making changes to the DNA directly, it is engineered to bind to a spot slightly upstream of the PCSK9 gene, called the promoter region. It's sort of the on-switch for the gene — a landing pad for all the enzymes required to initiate its transcription. That's where Tune's scientists sent their epigenome editor (enclosed in a lipid nanoparticle). And to that promoter, it added methyl groups, chemical tags of carbon and hydrogen that stick out and recruit bulky proteins that make it harder for that pit crew of transcription factors and enzymes to assemble and get gene expression up and running.

In the three macaques that received the PCSK9-targeting epigenome editor, via intravenous infusion, blood levels of the PCSK9 protein fell by 75% and stayed that low for the first four months of the study, which is still ongoing. Over that same amount of time, the monkeys' LDL cholesterol levels had dropped 56%.

"We saw four months' durability of PCSK9 repression after a single transient delivery," Kwon told STAT ahead of her ASGCT talk. "We're thrilled to see it. It was about as good as we could have expected."

The first epi-editors date back to the late '90s, but they were difficult to deliver and quickly supplanted by gene editors like zinc finger nucleases, TALENs, and then CRISPR. One of

the researchers who stuck with them was Charles Gersbach, a biomedical engineer at Duke University. In a 2015 Nature Biotechnology paper, his lab showed that it was possible to open and close the structure of DNA in a targeted way, turning on genes that would otherwise be inactive.

He began talking with another longtime genome editor, Fyodor Urnov, of the Innovative Genomics Institute, about forming a company around the technology. In December 2021, their Tune Therapeutics emerged from stealth with $40 million and licenses to the library of epigenome editors developed by Gersbach's lab over the previous two decades.

The company's latest data represent what experts like Segal call "true epigenetic editing," meaning that the desired changes in gene expression remain, even after the tool used to make them has been degraded by the cell. For his 2015 paper, Gersbach used adenosine-associated viruses, or AAVs, to shuttle his epigenome editors to cells, which worked well. But AAVs can integrate into the genome, leading to long-term production of gene-silencing machinery, which makes it impossible to tell if the editing stuck or if it's still ongoing. It also carries the risk of triggering dangerous side effects.

"With true epigenetic editing, you want to have transient expression of the tool, hopefully not long enough to cause an immune response, and then you can change the epigenetics in enough cells to have a clinical effect," Segal said. "And that's basically what their [Tune's] data showed."

This kind of epigenetic editing was also on display at ASGCT by Tune's chief competitor in the epi-editing space, Chroma Medicine. On Thursday, its head of research Aron Jaffe presented data showing the company had achieved 99% silencing of the PCSK9 gene out to five months in mice.

Vic Myer, Chroma president and chief scientific officer, credited the advance to work the company has done to optimize the epi-editor, as well as screen for guide RNAs that send it to the most effective gene-silencing locations. "We believe our single-dose epigenetic editor can disrupt the current chronic care model for hypercholesterolemia by providing effective LDL-C lowering that is durable for the lifetime of the patient without compromising their genomic integrity and introducing unnecessary genotoxic risk," he told STAT via email.

Chroma launched just a month before Tune, with plans to commercialize a system for controlling gene expression called CRISPRoff, published in Cell in 2020 and led by MIT scientist Jonathan Weissman and Luke Gilbert of the University of California, San Francisco. That system took many of the components first used to silence specific genes via DNA methylation — published in a groundbreaking 2016 paper by Italian researchers Luigi Naldini and Angelo Lombardo — and packaged them into a single protein that has proven very effective at shutting off gene expression for the long-term.

Last year, Chroma added Naldini and Lombardo's scientific firepower when it acquired their Milan-based company Epsilen Bio. It has raised $260 million, making it the most richly funded epi-editing firm on the block. At ASGCT, Chroma executives announced that the company is initially focusing on PCSK9 as well as a hepatitis B program and that it has already begun non-human primate studies of its own.

At this time, Tune is not disclosing what diseases or target genes it might pursue in its first push toward the clinic. Kwon told STAT that there could be a niche for epi-editing even in the crowded PCSK9-blocking market — somewhere between drugs that have to be regularly consumed and a one-time therapy that requires a permanent change to a person's DNA.

But she really sees this data as a broad proof of concept for any liver-targeted epigenetic editing, Kwon said. "There are a lot of different options that we can go down for that."

Where epi-editing might ultimately have the edge over other tools is in controlling more than one gene at a time, which is difficult to do safely with DNA editing. Any time multiple breaks are introduced into the genome at once, the risks of bits of chromosomes getting lost or rearranging go way up.

Both Tune and Chroma also presented data in human T cells, showing that they could silence the expression of multiple genes simultaneously, without causing problematic structural changes to their DNA, highlighting its potential for engineering newer, more sophisticated classes of CAR-T and other cell therapies.

Some researchers have noted that it might even be possible with epi-editing to boost or shut off gene expression of T cells for short periods of time, as a sort of safety switch. But just how long various epigenome alterations last remains one of the biggest open questions facing the field.

Epi-editing harnesses the natural coding system cells use to differentiate one kind of cell from another. A neuron has the same DNA coiled inside its chromosomes as a liver cell. What makes one spark while another sucks up fat is the pattern of chemical tags added early in development to turn various genes on or off. That kind of silencing lasts over a person's entire life, so mimicking it should produce similar effects. But going against that is this idea that that particular cell wanted that particular cell to be on.

"There might be transcription factors or other forces that will try to counteract the kinds of changes we make," said Segal. Or it could be that cell's don't have some kind of lasting will written into their genetic code and that by rewriting it with epigenetic editing, that code is just changed forever. "Right now we don't know which one of those it is."