ENDPOINTS NEWS

Exclusive: After four decades, one researcher's radical HIV cure finally gets its shot

by **Jason Mast** on February 17th, 2021

In the downtime between experiments, Kamel Khalili and his mentor traded their wildest ideas for curing HIV. It was the mid 1980s and Khalili was a postdoc at George Khoury's National Cancer Institute lab, where he studied links between viruses and tumors, then one of the hottest fields in cancer research. But the epidemic was raging through New York and San Francisco, mounting the largest public health threat in decades. A couple buildings over, Robert Gallo was sequencing the virus for the first time. It felt impossible to stay away.

There was one idea Khalili couldn't stop thinking about. HIV posed a unique challenge in part because it integrated itself into human DNA, coiling away from the body's defenses. Khalili had spent his grad days tinkering with one of the earliest tools biologists invented to manipulate DNA inside a living cell. In fact, the first experiment he was assigned to when he moved from Tehran to Philadelphia as a 27-year-old graduate student was to take a bacteria-infecting virus and use it to inactivate a gene inside *E. coli*.

He wondered if he could do the same to the HIV genes inside a patient's cells — an idea, he knew, was as ludicrous as it was elegant.

"Obviously the tool wasn't there," Khalili recalls. The tools they had struck DNA randomly, subjecting the recipient to a scattershot of genetic artillery. "You'd kill the patient."

Then in 2012 came CRISPR, the so-called molecular scissors that could cut DNA wherever a researcher wanted. Khalili, who had risen to run a vast neuroscience department at Temple University without ever giving up on his youthful whim, rushing to examine a new gene editing tool whenever it appeared, got to work. Within a year and a half of Jennifer Doudna and Emmanuelle Charpentier's Nobel-winning *Science* paper, he submitted a manuscript to *The Proceedings of the National Academy of Sciences* that would send ripples through the HIV field.

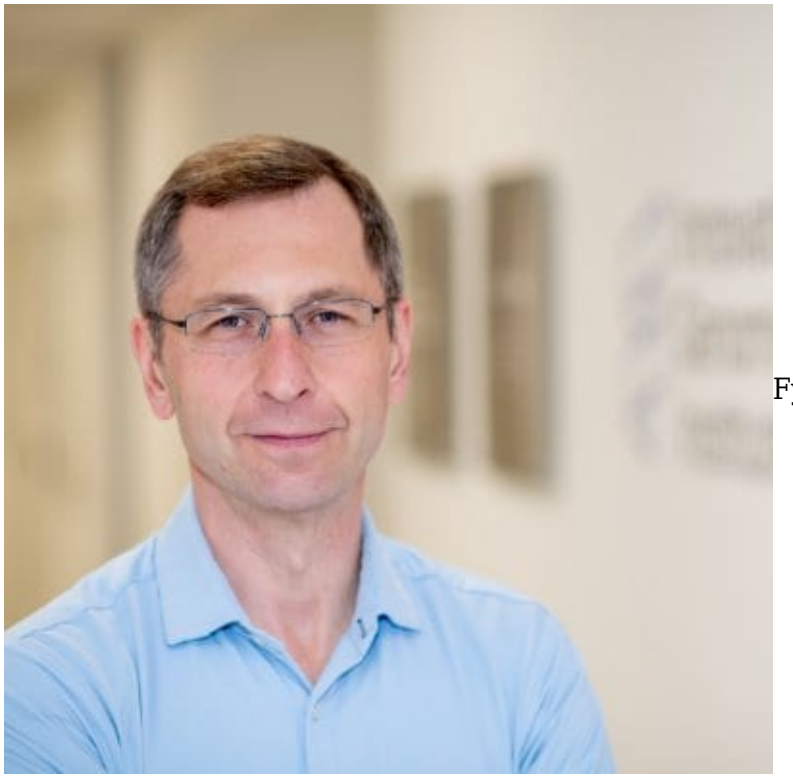
He had taken CRISPR and used it to excise HIV out of human DNA. When he put it in HIV-infected mice a few years later, about a third were cured.

"I've been working on HIV for close to 40 years," says Khalili. Now 69, he has an easy, avuncular laugh even over Zoom and large expressive eyebrows, though colleagues tell me he can have a far harder edge in the lab. "For the first time we realized a cure was possible."

In November, Khalili gave the first evidence the approach could work in monkeys. Investors are now getting on board. Excision BioTherapeutics, the company founded around Khalili's work, announced today a $50 million Series A to push the therapy into the clinic. If it works as intended, it could provide a long-sought single-shot cure for a virus that now infects 38 million people worldwide and set the stage for a similar approach for other chronic viruses, such as herpes and hepatitis B.

In a field already burned countless times, outside researchers are understandably cautious: CRISPR is a powerful tool, they say, but trying to excise all the viral DNA lurking in a patient's cells is like trying to plumb an oil spill; there's always a bit left, tucked in hard-to-reach places. Still, many were impressed he got this far, having discounted the approach years before. And they broadly agreed that Khalili may have invented one of the weapons for the multi-pronged assault they think will one day take down the virus.

The first test will come later this year, when a group of patients will be injected with Khalili's therapy and, eventually, taken off the anti-retroviral medicines that have kept their infections at bay. If the infections don't return, they will owe it to an immigrant scientist, who flung himself into a crisis much of his adopted country ignored and for four decades never let go of a brash and maybe even brilliant idea, even when the tools didn't exist and the NIH told him it was impossible.



Fyodor Urnov

Skepticism still runs high, just not as high as when Khalili first started sketching out the idea to friends and colleagues on napkins nearly a decade ago.

“We have to be very careful,” says Fyodor Urnov, a gene editing expert at Cal-Berkeley who worked on early HIV gene editing studies. “But I think, overall, the field can move from hypothetical to realistic optimism.”

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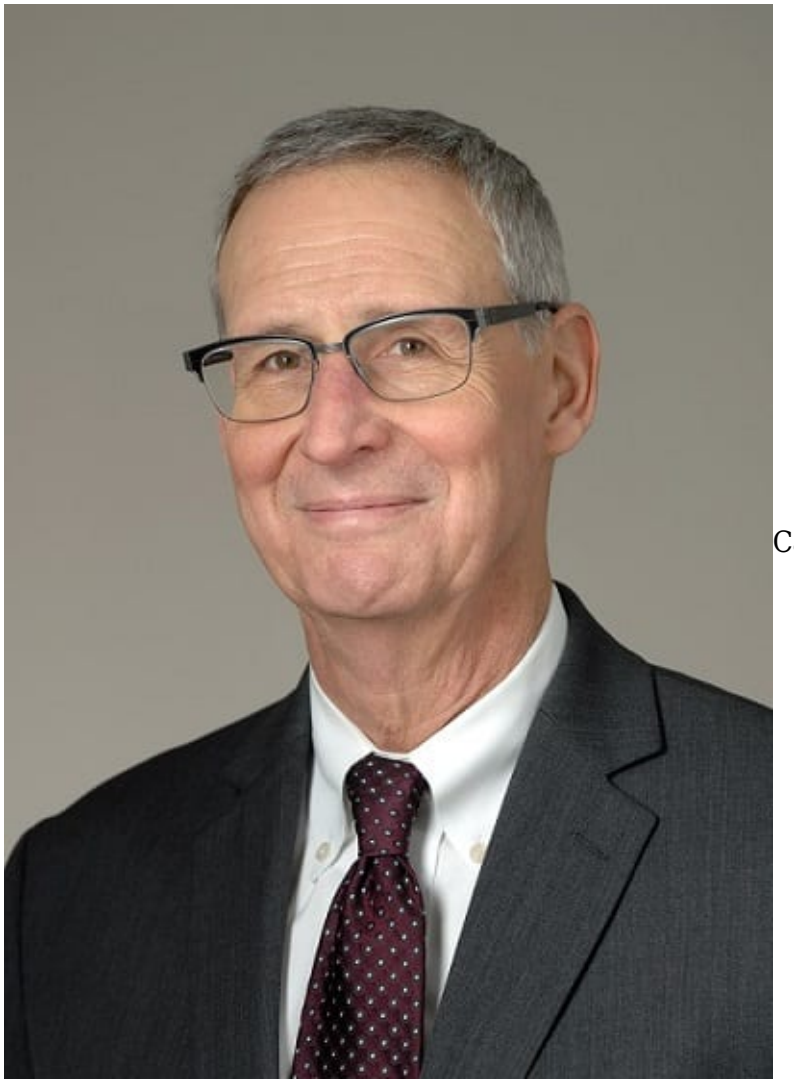
Excision isn’t the only company trying to apply CRISPR to HIV. Last year CRISPR Therapeutics received a grant from the Bill & Melinda Gates Foundation to do early lab work on a gene therapy that, by IV infusion, could knock out the receptors HIV uses to enter cells.

Gene editing approaches are gaining traction in part because the hunt for a cure has reached an impasse. In the late ’90s and early 2000s, anti-retroviral drugs brought AIDS under control for the first time, saving millions of lives, but they fell short of a panacea. Stop taking your daily pills and the infection returned. The pills were not without side effects, either, including nausea, diarrhea and long-term consequences researchers are still documenting.

In 2008, researchers at a Boston AIDS conference reported on the “Berlin patient,” a middle-aged man who was cured of HIV after receiving a bone marrow transplant for leukemia. Doctors knew the therapy wasn’t remotely scalable. It had nearly killed the patient, later identified as Timothy Ray Brown. But it told researchers for the first time in three decades of fitful progress that a cure was possible.



Carl Dieffenbach

The 12 years since have brought the opposite: a series of dead-ends for scientists' most promising approaches.

"We're in this — I think purgatory is a good way to describe it," says Carl Dieffenbach, who runs AIDS research at the National Institute for Allergy and Infectious Disease. "We need breakthroughs."

Depending on which HIV researcher you ask, they might tell you a cure is likely in the next 10 years, or a long shot in the next 40."We've now had 10 years of vigorous efforts and it's proven to be incredibly challenging," says Daniel Kuritzkes, head of infectious disease of Boston's Brigham and Women's Hospital. "It's possible that in 30 years we'll have some means of achieving a sustained remission, at least, even if not necessarily a cure. But I couldn't give you odds."

The problem is the same one that captivated Khalili in his NIH days: HIV tucks itself into DNA, forming a permanent reservoir beyond the reach of both traditional drugs and the immune system.

Daniel Kuritzkes

To get at the reservoir, scientists settled on two strategies that would make Sun Tzu proud: “kick-and-kill,” a way of coaxing out the hidden cells and then subjecting them to a full-on immune assault; or “lock and block,” a way of permanently burying the reservoir by, for example, screwing with the genetic circuitry that allows it to translate back into virus. But no one’s ever had a major breakthrough; a 2019 paper cast doubt on whether kick-and-kill could ever work.

Gene therapy, although generally talked about for rare disease, has long provided a third route, going back to some of the earliest gene therapy companies in the 1990s. The first time gene editing was used in humans was for HIV. In 2008 Carl June, the University of Pennsylvania immunologist who would later become famous for curing cancer patients with the first CAR-T therapy, extracted T cells from HIV patients, used zinc finger nucleases to cut out the CCR5 receptor that HIV leverages to enter cells, and then reimplanted the T cells.

Lynn Pulliam

The CCR5 approach has largely dominated gene-based approaches to HIV, including CRISPR's new effort, and understandably so. The Berlin patient and, later, the London patient were cured because they received bone marrow transplants from people who naturally lacked the receptor. Still, it currently involves a stem cell transplant, subjecting largely healthy patients to a laborious and intensive procedure that is not readily scalable. And, as more patients received it over the last decade, one thing became clear: They are not, in most cases, being cured — adding it to a long list of demi-remedies.

"They just weren't working, they weren't working,' says Lynn Pulliam, who runs an HIV lab at the University of California, San Francisco. In that environment, even ideas as radical as Khalili's felt tryable, however implausible many scientists found it. "It was one of the last techniques that someone had pulled out."

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Robert Gallo, center, who helped discover the HIV virus while at the NIH in 1984, the year Khalili joined

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Khalili, born in 1951, grew up in a different Tehran. Ruled by the Shah, an American-backed dictator, it hosted scientific conferences where the West's top molecular biologists flew in to present and where a teenage Khalili learned about the latest advancements in a field just beginning to acquire the tools to mess with life's central code. When Khalili flew out to the University of Pennsylvania to interview for a graduate position with Joseph Gots, the two sat in his faculty office and talked about Persian food and Iranian history. Gots knew the country well.

Khlili applied for a PhD at both Penn and the University of Miami, where he would have become a marine biologist — part of some quixotic, save-the-fish dream he still wonders about. He even visited their facility. But Penn had the best molecular biology program in the country and his wife, a similarly prodigious student named Shohreh Amini, also landed a spot there. They arrived in 1978 and Gots put him to work inactivating *E. coli* genes.

The Shah's government collapsed five months later. Khalili watched the unrest from his TV set in Philadelphia, and then the rise of an Islamic cleric as a new dictator. "I didn't like it, and I said that's not the place I wanted to do work," he says. "So I stayed in Philadelphia."

He poured himself into his graduate work, hoping that their research in gene inactivation would chart a path toward neutralizing the genes that cause cancer. He then moved to Khoury's lab at the National Cancer Institute, where he started working on what, before his CRISPR work, he become most famous for: how a common infection called JC virus can whip and warp cellular machinery, driving a rare form of brain cancer.

It was an auspicious time to join the NCI. The same year, 1984, Robert Gallo isolated a retrovirus in AIDS patients, giving a source for the mysterious epidemic that had broken out among gay men and IV drug users. As with Covid-19, scientists from other disciplines slowly poured in to tackle the emerging threat. Some of the biggest names in the history of virology were studying it: names like David Ho, Tony Fauci, and David Baltimore.

At the same time, stigma confined the disease to an American margin. And other problems — any problem — seemed more tractable than a fatal, rapidly mutating virus that snakes itself directly into human DNA. "A lot of people didn't even want to touch it," Pulliam recalls.

Khalili tweaked his research to cover the crisis, studying how JC virus interacts with HIV and how HIV affects the nervous system. He patented small molecules to block the virus from integrating into DNA. "I was always thinking about how I could get into HIV," he says. "It was a much bigger problem."

He returned to Tehran only once, accepting an invitation in the early '90s to give a series of workshops on gene transfer at the Pasteur Institute of Iran. But the city had changed. Instead, he fell in love with Philadelphia, going to Thomas Jefferson University after the NCI and then starting his own lab at Temple.

He made friends quick in his adopted country, impressing other scientists with a quick mind and an eagerness to share ideas that others jealously guarded. Jocular yet urbane, he invited fellow conference attendees to single-malt scotch tastings in Edinburgh or to sip limoncello in Italy. And when out-of-town colleagues stopped by Philly, he'd whisk them around to his favorite Italian restaurants. After the Eagles finally made the Super Bowl in 2018, he flew to Minneapolis to watch them win in person.

He had a harder edge in the lab, colleagues say. He kept his office meticulously clean. Until Covid-19 interrupted, he held hour-long review meetings on Saturdays, occasionally frustrating grad students. You could screw up the experiment but he'd grow frustrated if you didn't have an explanation, or didn't follow his instructions, or didn't keep up with the literature. It wasn't a place for people who needed a pat on the head every time they did well.

Rafal Kaminski

“You always had direction and you always know what you’re doing,” says Rafal Kaminski, who worked with Khalili for 16 years as a graduate student and professor. “The only problem is he always wanted so much.”

It worked, though. Brian Wigdahl, chair of microbiology and immunology at Drexel University, says he built one of the best neuroscience departments in the country, despite no formal training in neuroscience. Kaminski, who joined with a degree from a prominent Polish university but little lab experience, credits Khalili with teaching him everything he knows. He became a “luminary” in the field, says Steven Jacobson, a neurovirologist at NIH, founding its journal and international society.


Brian Wigdahl

Beyond neurovirology, too. His interests were polyglot, and he added collaborators as he chased new

issues. “He will move into new areas and you don’t even know he’s there,” Wigdahl says. “He jumps on things faster than anyone I know.”

That included gene editors. In the 1990s, researchers started engineering new DNA-sniping enzymes called zinc finger nucleases and TALENs that offered improvements on the tools they had in the ’80s. Khalili rarely spoke about it, but he examined each for potential in HIV, before rejecting them as too cumbersome or imprecise.

Then in October 2012 when Kaminski was about to fly back to Poland to defend his PhD thesis, Khalili pulled him outside and told him he had a special project for him when he returned. It was three months after Doudna and Charpentier’s paper but before Feng Zhang would show CRISPR could edit human cells.

“I looked at him like oh my God, what is going to happen now?” Kaminski says. “But I said, OK.”

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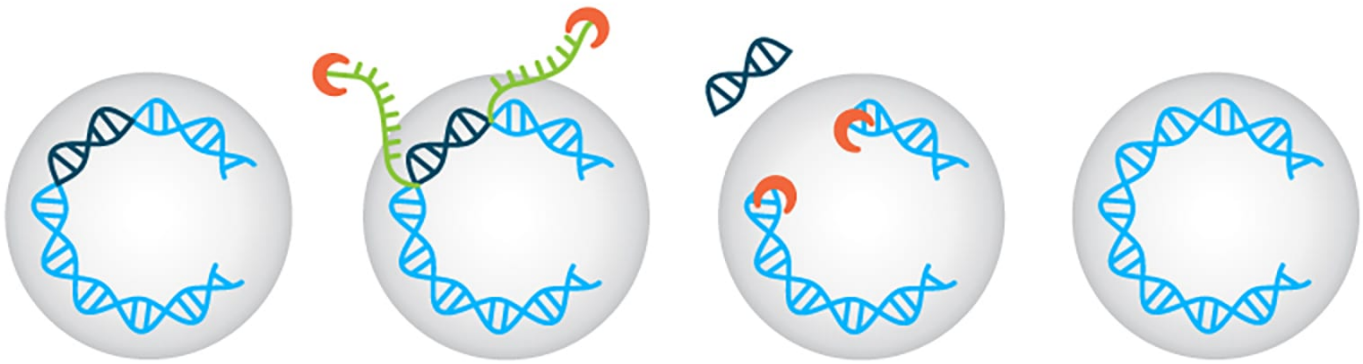
In the months before PNAS published his paper, Khalili couldn’t stop talking about it. Wigdahl said he saw him sketch it for colleagues on napkins: a whir of enzymes, RNA and human viral DNA. Pulliam remembered him being schoolboy-giddy over martinis one night, comparing it to a groundbreaking paper she had published in the ’80s, showing AIDS dementia wasn’t caused by the virus itself.

The paper published in July, showing for the first time that a gene editing tool could eradicate viral DNA, also known as latent HIV or provirus, from cell cultures. In fact, it was one of the first times in three decades of AIDS research that anyone had been able to get at the reservoir at all.

(From left to right). 1. In all patients, HIV integrates into human DNA, forming a permanent reservoir beyond the reach of drugs or the immune system. 2. In Khalili’s approach, guide RNAs lead DNA-cutting Cas9 enzymes to key sites on the viral DNA. 3. The Cas9 enzymes cut slice the HIV DNA, which falls away 4. The DNA repairs itself, forming a healthy strand, with most or all of the HIV removed. (Illustration – Adriana Maricari, Endpoints News)



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CRISPR consists of essentially two tools: a DNA-cutting enzyme called Cas and a guide RNA that tells Cas where to cut. When Doudna, Charpentier and Zhang first showed the system, reverse-engineered from bacteria, could edit human DNA, speculation centered around whether researchers could now repair genes behind rare and fatal disorders like cystic fibrosis or Duchenne muscular dystrophy. Fixing a gene, though, requires a third tool, one scientists are still struggling to master: a replacement strand of DNA for the cell to put at the site of the break.

It's much easier to simply cripple a gene. Every CRISPR-based therapy now in the clinic uses this approach, including Vertex's sickle cell treatment. It's what made HIV an attractive option.

In the 3 billion-letter chain of human DNA, latent HIV sits swaddled between two 634-letter, repetitive strands that contain the instructions for translating HIV DNA back into the virus. Khalili's team used software to identify critical sections of these so-called long-terminal repeats. They then made sure targeting these sections wouldn't interfere with healthy gene expression.

HIV shapeshifts easily, allowing it to overcome individual antivirals and, potentially, individual cuts to its genome. To avoid that risk, Khalili's team designed multiple different guide RNAs and decided to try and slice entire chunks out of the provirus. They successfully cut a small portion, and "then we got a little bit more ambitious," Khalili says. They cut out of an immune cell a complete, 9,709-letter stretch of HIV DNA. In a patient, it would amount to surgery by IV, slicing DNA out of cells like tumor from tissue.


Howard Gendelman

"That got everyone interested," says Howard Gendelman, who runs his own HIV and neurovirology lab at the University of Nebraska Medical Center. Gendelman and Khalili had been rivals for decades, two titans of a small field who crossed paths for the first time at the NCI in 1987.

"We were at the same level, at the same time, at the same age, studying the same very thing," he says. "Two poles of the same polarity repel."

Gendelman, though, had developed one of the world's best mouse models for HIV, the next logical place for Khalili to test his model. And after the intercession of another well-known HIV researcher — who called Gendelman at his daughter's engagement party and screamed at him for several minutes to

get over himself and work with Khalili because the two had complimentary technology — they started collaborating.

They wrote a grant proposal, as they had each successfully done hundreds of times in their careers. But an NIH committee rejected their application.

“They thought it was basically impossible,” Khalili says.

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Khalili and Tricia Burdo, who designed the studies showing the approach could work in monkeys. (Joseph V. Labolito/Temple University)

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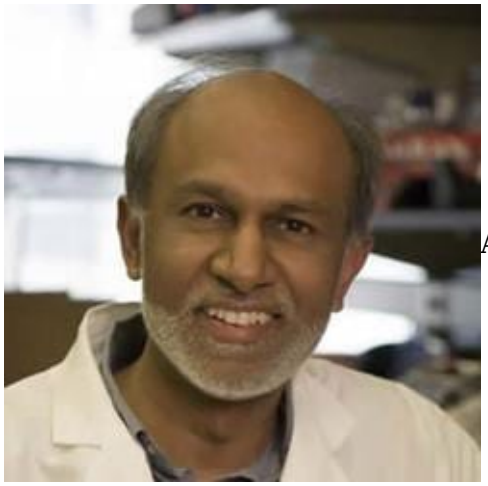

Avindra Nath

Khalili was not the first researcher to propose gene editing HIV. A team at Kyoto University published a study cutting latent HIV with CRISPR nearly a year before Khalili did, although ultimately with much less success. References in the literature go back decades. “The idea was out there,” says Avindra Nath, head of neurovirology at NIH. “No one thought it would work.”

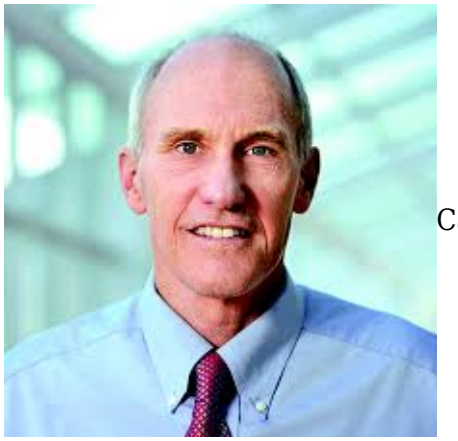
That included members of Khalili’s own team. Jonathan Karn, a Case Western AIDS researcher whom Khalili enlisted for his expertise in measuring the HIV reservoir, remembers hearing the pitch and telling Khalili that he thought it had no chance of working. Khalili told him that’s why he wanted Karn on the team.


Jonathan Karn

There were numerous scientific and technical hurdles to CRISPRing HIV, most of which the PNAS paper didn’t begin to scratch. Even now, with early monkey data, several remain unaddressed.

Karn was particularly concerned with delivery. Gene therapies are generally in hollowed out viruses, called a vector. Researchers, though, only injected CRISPR directly into humans for the first time last year, when Editas launched a trial in the eye, a largely self-contained organ that has long been one of the easiest places to do gene therapy. And it was unclear until recently whether AAV9, the hollowed-out virus most CRISPR companies use to deliver their therapies, could go to T cells, home to most of the HIV reservoir.

Hitting T cells, though, is just the first step. In an average patient, only about 40 in every million T cells contain latent HIV. Other cell types with latent HIV are scattered throughout the body. And HIV does not integrate into the same spot in every cell.


Carl June

Khalili and Gendelman had to hit bullseyes within bullseyes. Still, when they tried it in mice, cobbling together funding from other sources, they were able to excise enough HIV to prevent the infection from returning. NIH funding followed and in November, they showed that they could wipe out between 38% and 95% of latent SIV — the simian equivalent of HIV — in three monkeys.

“It’s finding the needle in the haystack and then cutting it out,” says Carl June, the immunologist who pioneered the CCR5-knockout approach. “It’s amazing they could do it all.”

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June is impressed with the approach, but he doesn’t believe it will bring a cure. He’s not alone. Several researchers tell me that Excision may be able to knock out some, maybe even most, of the reservoir. But if they can’t get all of it, then the infection will simply come roaring back. And like nearly all current gene therapies, it can’t be dosed twice: You only have one shot to make it work.

Sharon Lewin

“It depends on how effective it is in eliminating every infected cell,” says Sharon Lewin, director of the Peter Doherty Institute for Infection and Immunity in Melbourne. “And that would be a big ask.”

No one, though, has ever made a dent in the reservoir. Perhaps you need to deplete it entirely; perhaps you just have to knock it down enough. Two out of the seven humanized mice they treated in their study appeared to be cured, even though they hadn’t excised 100% of proviral DNA. And Khalili pointed out that the Berlin patient still had latent HIV, just not enough that his immune system couldn’t control it. “Do you really need to hit every single cell?” he says.

Khalili has been ignoring critics for a decade, but he’s still hedging his bets. In the past eight years, his CRISPR work has gone from a little discussed project between him, Kaminski and a postdoc to one that encompasses most of Khalili’s lab and grants, with 10 graduate students or lab workers, a handful of senior investigators and universities across the country. Khalili’s latest grant proposal is to team with outside experts on an approach both June and Lewin said was promising: combining their technology with an immune-boosting molecule that would help the body take care of the cells CRISPR doesn’t reach.

It’s part of a group of technologies June says are bringing a cure within reach. Just a year younger than Khalili, June has been studying HIV for nearly as long and he says the problem has changed in recent years: from developing new tools to implementing current ones. His CCR5-knockout approach, for example, still holds significant promise when combined with immune boosters or vaccines, particularly if researchers can figure out a way of doing the cell transplant without chemotherapy.

Dieffenbach, the NIAID HIV chief, speculated you could combine either approach with a vaccine Vir just put into Phase I, which is meant to train T cells to attack the virus.

“The future will require multiple hits,” he says. “It won’t ever be one thing.”

Implementing won’t be easy, though. Because most patients have their infection under control, the safety bar to test a new treatment on humans is higher than it is for most cancers or rare diseases. Excision Bio, for example, has the best chance of hitting all latent cells by using high doses of the AAV9 vector that carries their therapy, but high doses have shown dangerous side effects in a few rare disease gene therapy trials. They’ll instead launch Phase I this year with a lower dose than Khalili used in his monkey studies, though they could still raise the amount in future trials.

Success would bring its own hurdles. AAV can’t yet be manufactured at anywhere near the scale to reach the 38 million people with HIV worldwide, so a cure would initially further already unequal access to medicines. Still, June said he was confident that companies would solve that question for both Khalili’s approach and his own cell therapy approach. Pharma companies have too much incentive.

“Those will happen within a decade,” June says. “There’s billions of dollars being invested in these issues because of gene therapy and cell therapy for cancer. That didn’t exist years ago.”

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Steven Deeks

Even if it works not everyone would be eligible. Khalili only showed CRISPR can work on patients who already have their HIV under control, leaving out the patients most in need of a cure. “It’s a big ethical dilemma,” says Steven Deeks, an HIV researcher at UCSF.

Deeks, who signed a collaboration with Excision shortly after our conversation, rattled off numerous reasons why Excision could fail, including the potential to disrupt human DNA alongside HIV DNA, cautioning that they would have to move extremely slow. Still, he said, it could help millions of patients who struggle to take daily meds or face stigma when doing so. And it would offer an advantage over other approaches that try for a “functional cure,” leaving HIV in the body but inert.

“What everyone wants is a complete cure, they don’t want to have any of the virus left,” Deeks says. “This is one of the most promising ways of achieving that ultimate goal.”

It would be a massive achievement, one that Khalili and virtually every HIV researcher have been dreaming of for decades. But one that a few now say is within reach.

Fyodor Urnov, the Berkeley gene editing expert, offered a space analogy and a litmus test for the feasibility of any scientific endeavor: What tools already exist and what have to be invented?

“We want to go to Mars? Well kind of the pieces are there, we just need to do it,” he says. “We want to go Alpha Centauri? It’s a fundamentally different technological challenge where some things are just currently impossible.”

An HIV cure, he says, is now like reaching Mars. And Khalili, despite all the failures of the last four decades, is confident he can be the next Neil Armstrong. What are the odds he’s right?

“I don’t know, I think it’s possible,” says Lynn Pulliam. “Yeah, I think it’s possible, I do. I think it’s possible.”

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