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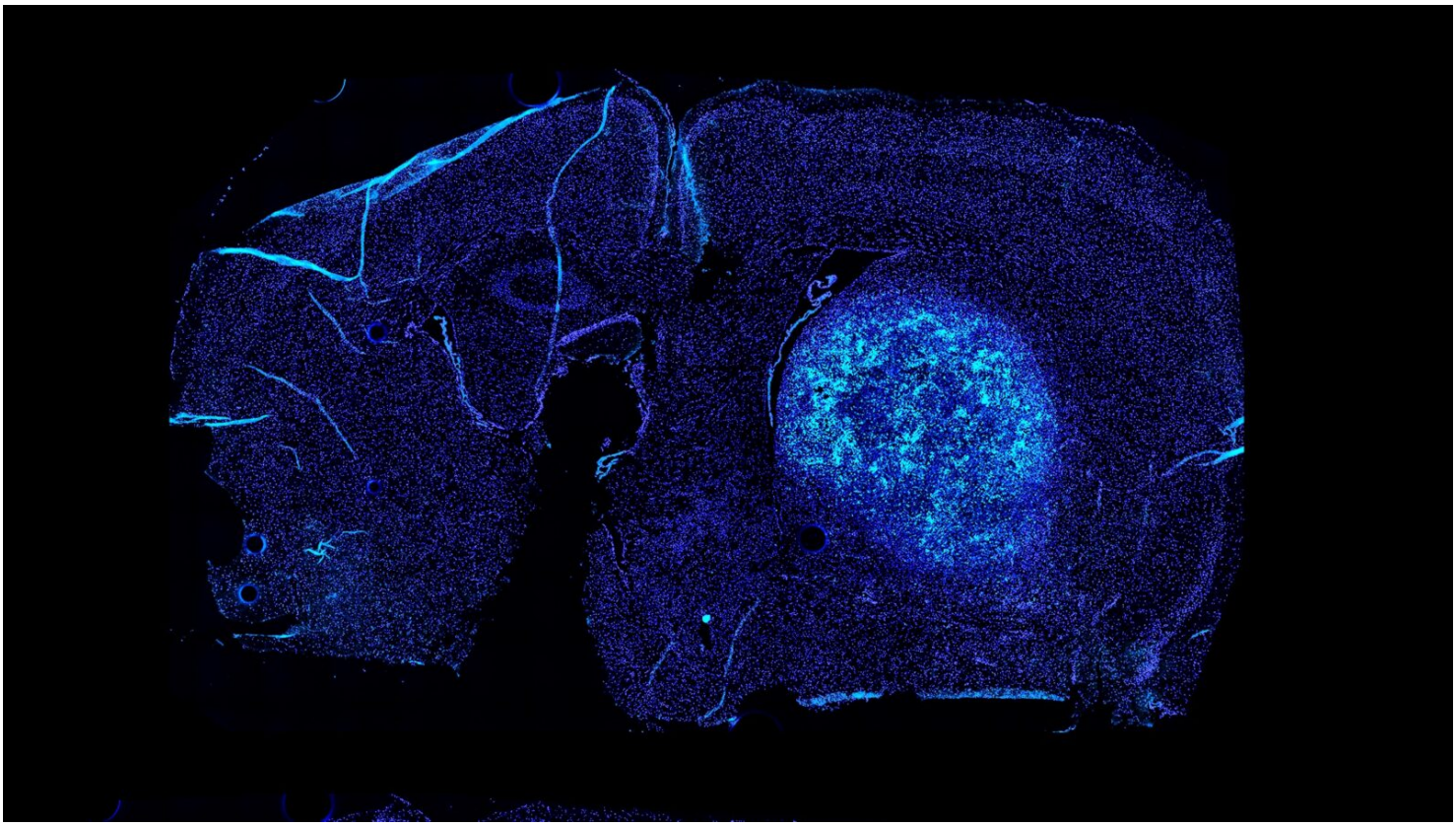
Glowing tumor-munching cells, captured on video, show the promise of a new approach to CAR-T cancer therapy

By Megan Molteni April 28, 2021



SynNotch-CAR T cell therapy is able to precisely target glioblastoma brain tumors. In this image, T cells selectively activate (cyan) only when they reach the brain. *Wendell Lim and Payal Watchmaker, UCSF*

Wendell Lim stared at the video playing on his computer screen in amazement. In the inky dark, a yellow blob pulsed: a brain tumor lit up with fluorescent tags. From the edges of the frame, T cells glowing blue crept toward it. When they reached the blob, a switch seemed to flip, and the blue cells turned green.

Lim is a biophysical chemist at the University of California, San Francisco. But what he really does is hack cells. For the last five years, his lab had been working on a new version of CAR-T therapy, re-engineering human immune cells to attack a particularly deadly form of brain cancer. Now those souped up cells had been pumped into a mouse with a human tumor growing in its brain. Lim was watching

his armies of fluorescent cellbots lay surgical siege to the Frankencancer on a video feed captured from inside the animal's skull with a pair of lasers.

As the cells swarmed the tumor, its yellow lights began to flicker out one by one. "You could see the T cells just munching away at it," he said.

The experiments are more than just entertainment though. Lim's and a UCSF colleague's research, published Wednesday in a pair of papers in the journal Science Translational Medicine, provide proof that a cutting-edge technology aimed at treating a broad range of cancers has cleared a major, albeit early hurdle. In particular, their new approach turned CAR-T therapy into an effective weapon against solid tumors — the ones that afflict the majority of cancer patients.

CAR-T therapy, short for chimeric antigen receptor T cell therapy, works by genetically reprogramming a type of white blood cell — T cells — to locate and eradicate malignant cells in the body. In recent years, the Food and Drug Administration has approved four CAR-T treatments. And they have saved the lives of many patients suffering from unresponsive blood cancers like leukemia. But solid tumors have proven a more elusive foe. Against them, CAR-T has thus far disappointed in clinical trials.

But that was the *old* CAR-T. Lim is among a growing group of scientists betting that a new generation of smarter T cells can get the job done: T cells like the ones he'd watched hunt down and wipe away brain tumors in mice. Or like the ones designed by his UCSF colleague Kole Roybal. With a few molecular tweaks, Roybal's group successfully deployed the same system against lung and ovarian cancer cells in the lab.

Clinical trials are likely still years away, but if the approach works in humans, it would mark a turning point with implications for millions of cancer patients.

The work in mice overcomes some of the key stumbling blocks that have thus far stymied attempts to turn T cells loose on solid tumors, said Stephen Gottschalk, who chairs the department of bone marrow transplantation and cellular therapy at St. Jude Children's Research Hospital and was not involved with either study.

“The approach here is unique because it’s building synthetic signaling circuits within cells to completely reengineer them,” he said. “And the investigators have shown in a very relevant preclinical model that the approach really works.”

The studies’ beginnings trace back to 2015, when Roybal, an immunologist-slash-synthetic biologist, was a postdoc in Lim’s lab. Lim had long been tinkering with the genetic programs of bacteria and yeast, prodding them to take on new identities and functions. When CAR-T started to take off, he saw an opportunity to do the same thing with T cells. That first generation of cell-based immunotherapies worked by bestowing T cells with new surface proteins called chimeric antigen receptors (the CAR in CAR-T), allowing them to better recognize and latch onto proteins poking out of cancer cells. But what happens next relies on a T cell’s natural activation program. And in many patients, that drive to kill kicks into overdrive, sparking an indiscriminate assault on healthy cells and leading to dangerous side effects. Lim thought he could tune T cells to be safer and more predictable.

That effort got supercharged when Roybal built a cellular component called synNotch — a receptor that spans the T cell’s outer membrane. When it binds its target, say a protein on the outside of a tumor, a little piece breaks off and makes a beeline for the cell’s nucleus, where it toggles other genes on or off. SynNotch endowed T cells with a new layer of biological logic analogous to an electronic circuit. Instead of just kill-IF-certain-cancer-specific-antigens-are-present, now you could instruct T cells to kill IF they sensed cancer cells AND they were in the right organ. Or IF they sensed cancer cells AND NOT other, healthy tissues. All of a sudden, T cells were much easier to customize and control.

SynNotch formed the basis of a new company Lim and Roybal started in 2016 called Cell Design Labs. The following year, it was bought by Gilead and its immunotherapy subsidiary, Kite, for $567 million. Since then, Kite has begun recruiting for a clinical trial of its solid tumor CAR-T candidate, but it has not yet moved past Phase 1.

Around the time that Lim and Roybal were working on SynNotch, a neurological surgeon named Hideho Okada arrived at UCSF from the University of Pittsburgh.

While there, Okada had discovered a molecule that T cells could use to recognize gliomas — a kind of brain tumor that emerges from malignant neural cells, as opposed to metastases from other tissues like the breast or lung. He had worked with "the cancer slayer" Carl June, one of the preeminent pioneers of CAR-T, to treat the first glioma patients with their own supercharged T cells. And the treatments worked remarkably well. At first.

The patients' tumors shrank. Their symptoms improved. But then, months later, the tumors came roaring back. When they looked closer, Okada and his colleagues realized the surviving gliomas were made up entirely of cells that lacked that signature marker molecule he had found. After more investigation, they learned that their target had been too narrow.

Tumors are little cellular ecosystems unto themselves. And only about half the cells that make up the average glioma carry this marker. The ones that didn't escaped the CAR-T onslaught and grew back. "From that trial we learned that CAR-T cells can, in fact, safely pass the blood-brain barrier to migrate to brain tumors and eliminate some of those bad cells," said Okada. "But the next question was, how do we overcome this heterogeneity issue?"

He got his answer in 2015. Shortly after arriving at UCSF, he heard Lim giving a talk about the SynNotch system that Roybal was just starting to develop. Okada approached Lim after the lecture about using the new tool to go after brain tumors. The two labs teamed up. Lim's group began designing different versions of SynNotch circuits for human T cells. Okada's built a model in which to test them, eventually settling on grafting bits of gliomas taken from cancer patients into the brains of mice. After five years of experimenting, they had a system that worked.

As described in the new paper, synNotch CAR-Ts wiped out brain tumors that first-generation CAR-Ts had missed, with no signs of dangerous side effects. And after 5 months, the mice were still tumor-free. "We tend to treat disease by sticking a wrench into the machinery of the human body," said Lim. "I think this will represent the first case where something that's operating like a biological system is being used to combat the biology of disease."

The trick was to bring together two types of antigens with different flaws into a single circuit. Molecules like the one Okada had discovered were too specific. Other targets were too broad — they might be found in brain tumors, but they were also expressed in other organs, like the liver and kidney. Sending in an indiscriminate T cell raiding party could unintentionally damage those healthy tissues. But a series of SynNotch sensors linked together provided the solution.

The first switch was targeted to Okada's molecule, the one that's only found in gliomas. "It's like a beacon to tell the T cells they're in the tumor," said Lim. Once activated, the second switch flips, directing the T cells to attack any nearby cells carrying the much more common molecular tag. Together, the complementary instructions knocked out all the glioma cells without causing any collateral damage.

And that combo turned out to be applicable beyond just brain tumors. Roybal started his own lab at UCSF in 2017, where he's continued to develop thousands of new and better versions of SynNotch. In the second paper, his team put some of those to work against difficult-to-treat cancers such as mesothelioma, which is caused by asbestos exposure, and ovarian and pancreatic tumors. Their engineered T cells recognized and killed these malignant cells without harming healthy tissues.

One surprising finding from both studies was that the SynNotch circuits preserved T cells' strength, said Roybal, who was an author on both papers. A common challenge with CAR-T treatments is the issue of T cell exhaustion. Because their kill switch is turned on all the time, researchers think they suffer from burnout faster than their non-engineered counterparts. SynNotch puts T cells on standby until they spot a tumor. So they're fresher when they get there. "They can fight longer without getting exhausted," said Roybal.

That's a big deal, says Gottschalk. And one of the reasons why he's eager to see the approach tested in humans. But CAR-T cells encounter other difficulties besides premature burnout. Tumors are living, signaling tangles of cells capable of recruiting other kinds of immune cells to suppress any T cells sent there on a mission. But in the mouse model used by the UCSF researchers, those resident

immune cells aren't around; they had to be blasted out of the mouse so they wouldn't reject the brain tumor transplants.

"So it's still an open question whether or not these T cells will require further enhancements against the inhibitor immune cells present in solid tumors," said Gottschalk.

According to Okada, experiments to address that question are starting in his lab. At the same time, he and Roybal and Lim, who is also the director of UCSF's newly launched Cell Design Institute, are trying to raise $5 million to $10 million to advance their T cells into clinical trials. They'd like to apply for FDA clearance to launch human studies in the next year. But with most pharma players more focused on improving the economics of CAR-T for blood cancers, they don't expect to find an industry partner anytime soon.

That's one of the reasons Okada is trying his best to temper his optimism. He also knows the immunotherapy field has seen its share of successes in animal experiments that later fizzled in human testing, especially for brain cancers. And yet, he can't help feel like this time might be different.

"I've been doing this for 25 years, translating lab discoveries into clinical trials, and the robustness of this data is far better than anything I've ever seen," he said. "Absolutely, this is the most excited I've ever been."

At least one pharma firm seems interested in Arsenal Bio, a startup Roybal launched with another UCSF colleague, Alex Marson, in 2019. The 100-person company is focused on combining Roybal's talents with Marson's — which is making lots of DNA edits to T cells using CRISPR. (Both scientists also receive funding from Sean Parker's immunotherapy moonshot.) Earlier this year, Bristol Meyers Squibb invested $70 million in Arsenal in exchange for the option to obtain an exclusive worldwide license to develop and commercialize any promising candidates the company discovers for fighting solid tumors.

Arsenal CEO Ken Drazan told STAT that the company is moving forward with a newer version of Roybal's latest circuits as part of its partnership with BMS. He

said the latest research out of UCSF is an important illustration of the slow but steady progress academia is making toward understanding why solid tumors don't respond to currently available treatments. "That database of mechanisms and functions is growing every day," he said. "That's the fuel for a company like Arsenal, which is trying to make complex products that can address the complexity of these tumors."