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JULY 2024 : VOLUME 20 : ISSUE 4 | PUBLISHED SINCE 2005

New Drugs to Heal the Splitting of the Mind

Drugs for treating schizophrenia have focused on the same mechanism of action for more than 70 years. With the FDA expected to approve a new drug in September, that may finally change.

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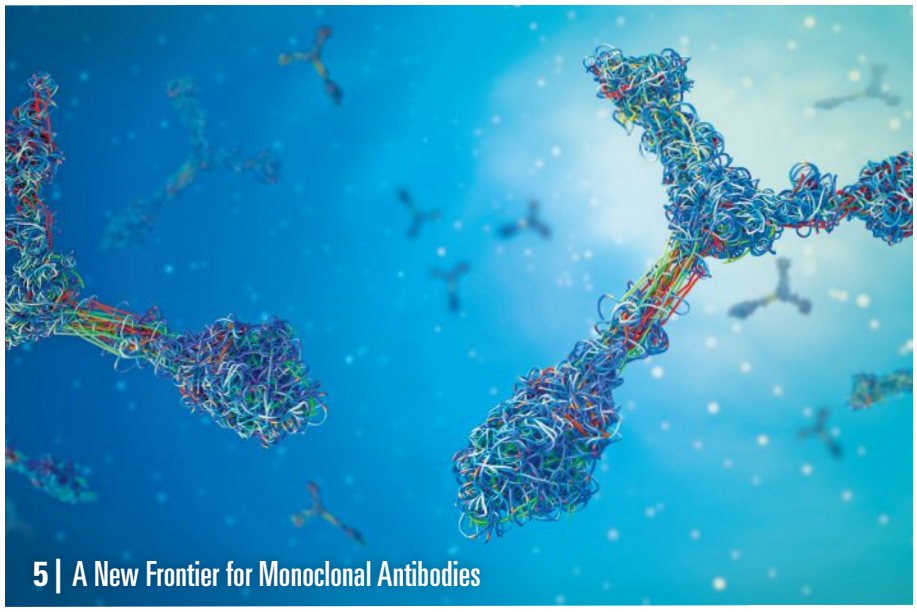

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Standing on Shaky Shoulders

Research misconduct poisons the well of scientific literature, but systemic changes to the current "publish or perish" culture will help.

I DISTINCTLY REMEMBER THE DAY I SAW A WESTERN BLOT BAND stretched, rotated, and pasted into another panel. Zoomed out, it looked like a perfectly normal blot; the imposter band sat amongst the others like it had always been there.

Sitting at a long table with the other graduate students on my training grant, I watched as our professor showed us example after example of images from published scientific papers that had been manipulated to embellish the data. I really appreciate that course and the other research integrity courses I took during my research training for teaching me and my peers how to spot bad science and what to do when we encounter it. It made me a better scientist when I was in the lab, and now, it makes me a better journalist.

When bad science infiltrates the publication record, researchers unwittingly build their own research programs around shaky science. Not only does this waste researchers' time and money, but it affects real people's lives. When allegations of misconduct began swirling around the basic science that led to the development of Cassava Science's Alzheimer's disease drug simufilam, the treatment was already in clinical trials. Now, five of those papers have been retracted, and while the drug is in Phase 3 trials, many scientists are waiting to see how the results pan out with the drug's mechanism of action now under question.

In recent years, technologies such as ChatGPT have emerged that take scientific misconduct to a whole new level. In fact, the popular blog Retraction Watch now has an entire section on its website devoted to papers and peer review reports that have evidence of ChatGPT use, and the documents featured there are likely only a fraction of the ones out there. But even so, somewhat "old fashioned" examples of misconduct like plagiarism, improper copying and pasting, and plain old lying are still rampant in the scientific literature.

Stephanie DeMarco, PhD
ASSOCIATE EDITOR

While artificial intelligence tools may have upped the ante for improper conduct, they've also made it easier for those who are looking for misconduct to spot it. More instances of research misconduct are being reported and making news headlines every year. Prolific research misconduct sleuth, Elizabeth Bik has practically become a household name for her ability to spot shoddy science, and newcomers such as Sholto David uncover more suspect papers as well. But they can't catch everything.

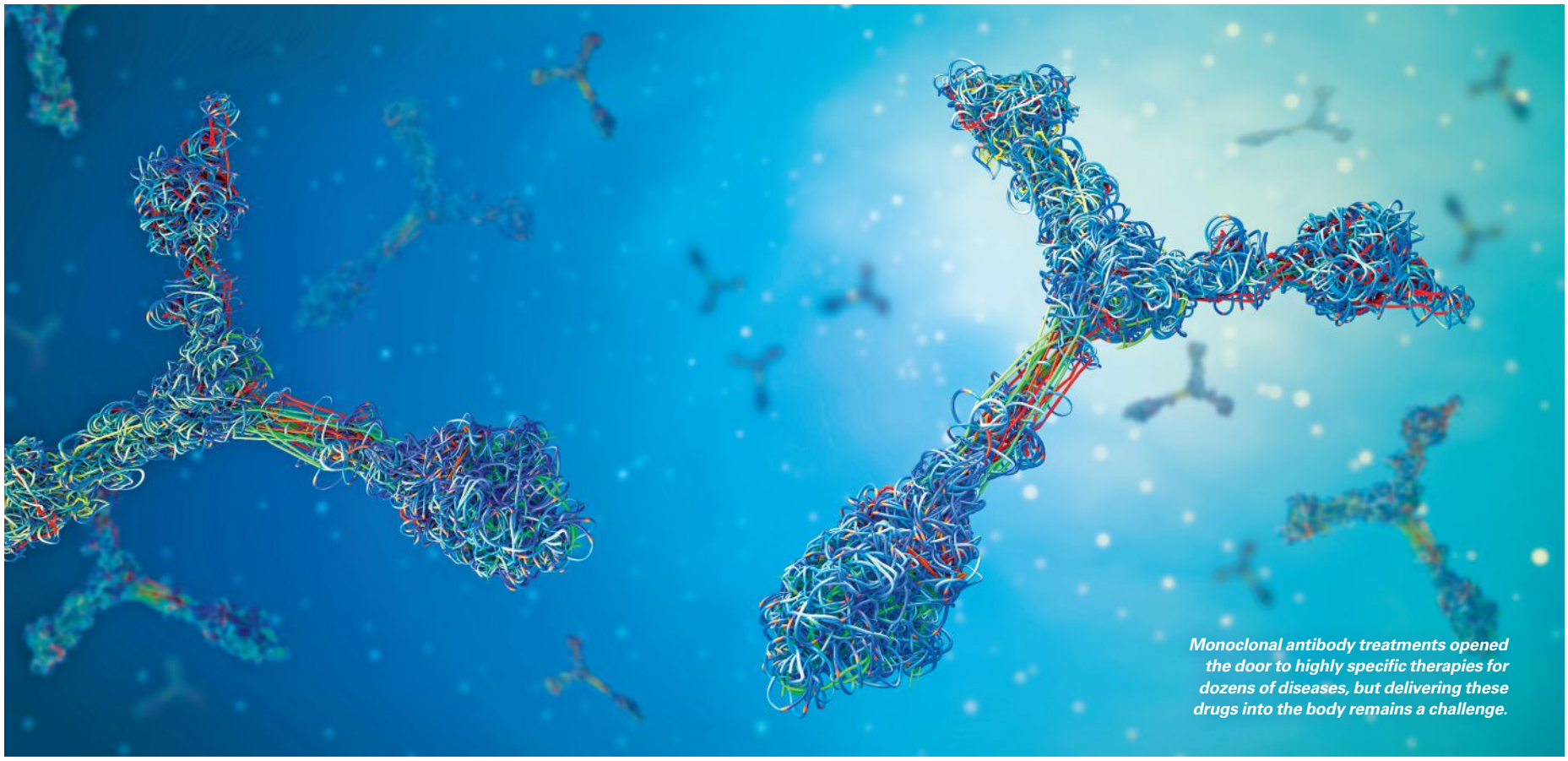
Preventing research misconduct starts before someone collects even a single piece of data. It begins with laboratory leaders who foster an honest research environment and encourage openness among all those who work there. Research integrity courses like the ones I took in graduate school help, but finding ways to change the "publish or perish" culture rampant in academic research will likely benefit science the most. A couple ways to do that would be to incentivize replication studies and research ethics training. Systemic changes that make doing ethical research easier will improve outcomes for everyone from the researchers doing fundamental research that will lead to new drugs to those who will benefit from the treatments. ■

DDN (USPS 024-504) is published 6 times a year by LabX Media Group, 1000 N West Street, Suite 1200, Wilmington, DE 19801. Periodical postage paid at Berne, IN and additional mailing offices. Publisher assumes no responsibility for unsolicited material or prices quoted in the magazine. Contributors are responsible for proprietary classified information. For single copy sales and paid subscriptions, please contact Subscriptions Services to place an order: Single copy prepaid is $7 (US/Canada), $10 International, plus postage and handling. *DDN* print subscriptions are distributed in North America free of charge to qualified drug discovery professionals; qualified professionals outside North America will receive the digital edition. Print subscription rates for those not qualified or living outside of North America must be prepaid (US/Canada $49, International $89) by credit card or check drawn on a U.S. bank. Publications mail agreement no. 41401058 return undeliverable Canadian addresses to PO Box 503, RPO West Beaver Creek, Richmond Hill, ON L4B 4R6. POSTMASTER: Send address changes to *DDN*, PO Box 2015, Skokie, IL 60076.

clinical trials

A New Frontier for Monoclonal Antibodies

New research aims to make antibody drug delivery as precise and efficient as the antibodies themselves.



Monoclonal antibody treatments opened the door to highly specific therapies for dozens of diseases, but delivering these drugs into the body remains a challenge.

CREDIT: ISTOCK.COM/ CHRISTOPH BURGSTEDT

BY MARILYN PERKINS

AT THE TURN OF THE 20th century, physician Paul Ehrlich at Institute of Experimental Therapy had a grand vision for the future of medicine. While researchers were beginning to understand how some diseases spread through tiny particles like viruses and bacteria, it remained unclear how to treat these microbe-based conditions. Based on his early research of the immune system, Ehrlich raised the possibility of a "magic bullet": a new type of laser-focused treatment that killed only noxious microbes and spared healthy cells.

Ehrlich's 1909 discovery of Salvarsan as a treatment for syphilis became the first example of a magic bullet, and over a century later, medicine continues to search for precise treatments to ever more complicated conditions. Today, monoclonal antibodies are another manifestation of the magic bullet Ehrlich envisioned. These laboratory-made proteins can bind to substances in the body such as bacteria, viruses, or cancerous cells and efficiently treat dozens of conditions with minimal off-target effects. But many monoclonal antibody treatments share a certain flaw: the treatments may be magic bullets, but aiming the gun remains a challenge.

Roughly 70 percent of monoclonal antibody therapies are still delivered intravenously (IV), an administration route that's inconvenient at best and ineffective at worst (1). Now, some researchers are reimagining how to deliver monoclonal antibodies with the hope of improving their efficacy and expanding the reach of these revolutionary therapies.

CREDIT: ISTOCK.COM/ FATCAMERA

Healthcare providers infuse 70 percent of monoclonal antibodies intravenously, a technique that leaves ample room for improvement.

The problem with antibodies

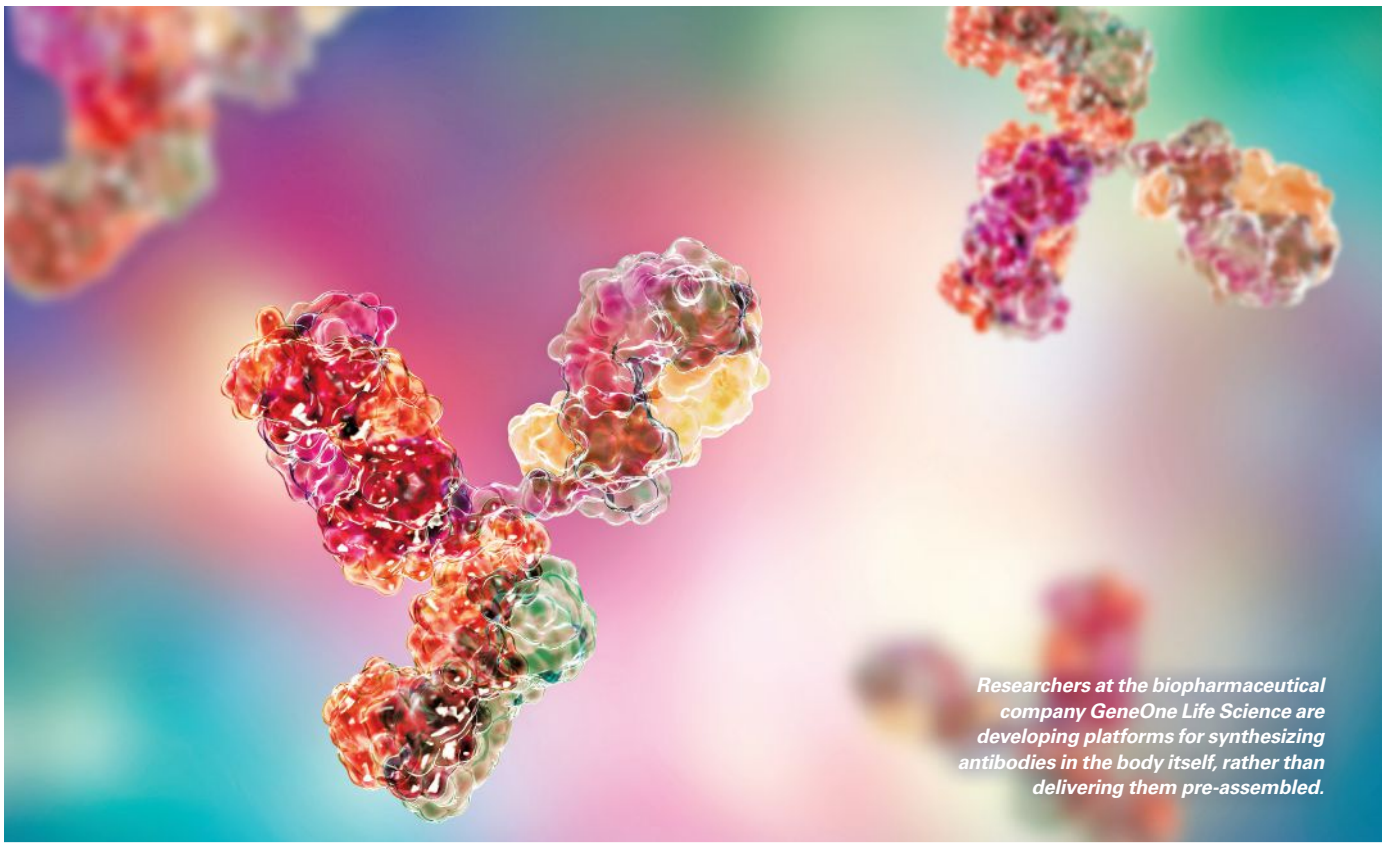
The first monoclonal antibody, Muromonab-CD3, was approved in 1986 to prevent kidney transplant rejection. Since then, the Food and Drug Administration (FDA) has approved more than 100 monoclonal antibody therapies to treat conditions including cancer, Alzheimer's disease, autoimmune disorders, and infectious diseases. On the whole, these treatments have proven successful; the FDA approves up to a dozen new therapies every year, and the long half-life and favorable safety profile of monoclonal antibodies makes them particularly effective (2).

Compared to conventional drugs, antibodies are sprawling and unwieldy, preventing them from getting into the body easily. These

> "Despite the widespread availability of naloxone, we're still seeing an all-time high of fentanyl related overdose deaths. Our goal is to add another therapy to the armamentarium, to attack these potential overdoses upstream."
>
> *– Andrew Barrett, Cessation Therapeutics*

Y-shaped molecules consist of multiple polypeptide chains that bind to specific targets and recruit a myriad of immune system players. This complex structure can result in a size 1000 times that of a single aspirin molecule (3).

The IV delivery route presents multiple problems: First, because full-body infusion is by nature imprecise, it requires large volumes of antibody, increasing associated costs and systemic side effects. Second, in conditions affecting the central nervous system, such as Alzheimer's disease or stroke, IV administration is particularly ineffective, with only 0.1 percent of an antibody solution able to cross the barriers that separate the brain and spinal cord from the general circulatory system (4). And finally, patients using these therapies need to visit clinics every few weeks, sitting for hours at a time for each infusion — a burden that may prevent some people from getting the care they need.



Researchers at the biopharmaceutical company GeneOne Life Science are developing platforms for synthesizing antibodies in the body itself, rather than delivering them pre-assembled.

CREDIT: ISTOCK.COM/ DR.MICROBE

Helping hard-to-reach patients

This barrier to patient access is the reason that Andrew Barrett, chief scientific officer of Cessation Therapeutics, wants to design a preventive antibody for fentanyl overdose that can be injected subcutaneously rather than infused intravenously.

US deaths from synthetic opioids exceeded 70,000 in 2021, with the majority of those deaths attributable to fentanyl (5). While naloxone (Narcan) has become a valuable tool to reverse opioid overdoses, Barrett envisioned a new prophylactic drug for high-risk populations, preventing both the psychoactive effects and dangerous respiratory depression associated with fentanyl.

"Despite the widespread availability of naloxone, we're still seeing an all-time high of fentanyl related overdose deaths," said Barrett. "Our goal is to add another therapy to the armamentarium to attack these potential overdoses upstream."

Barrett recently published a study in *Nature Communications* detailing new delivery methods for CSX-1004, an anti-fentanyl antibody currently being tested intravenously in a clinical trial (6,7). Barrett said that the company is pursuing subcutaneous injection — the most popular antibody delivery route after IV — because IV infusion simply isn't realistic for their patient population. Opioid addicts at high risk for overdose may not have time to sit for long infusions or may quickly change their minds, and most harm reduction clinics don't have the resources to support IV infusions. He wants to develop a treatment that people can complete in minutes, not hours.

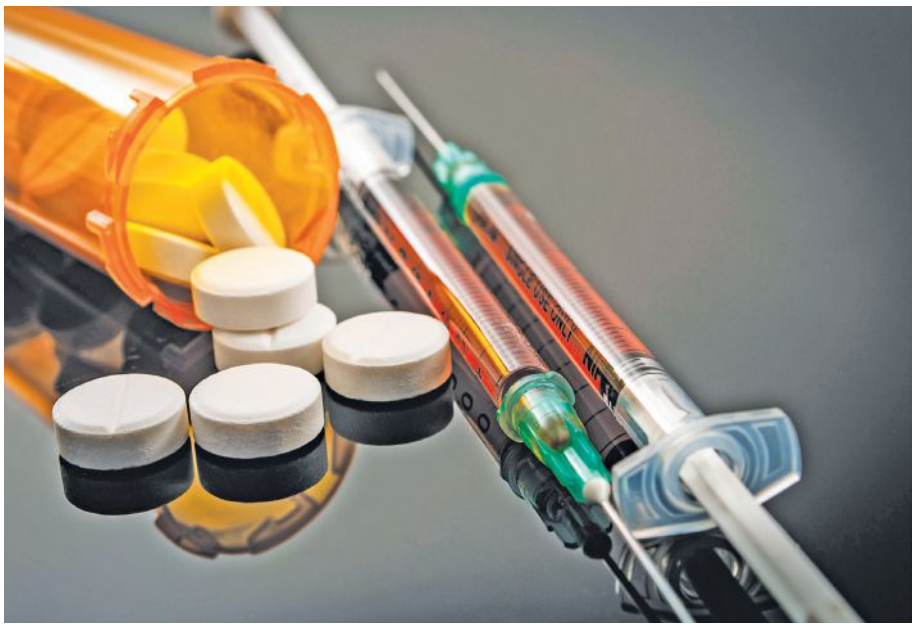
"If someone were saying they want to make a change now and would accept a very, very quick subcutaneous injection, we think the access to that treatment would expand dramatically," said Barett.

Barett's published research showed that a subcutaneous injection of CSX-1004 protected both mice and nonhuman primates from fentanyl-induced respiratory depression for up to four weeks (6). Human formulations for subcutaneous injection are already underway, with plans to take the research into clinical trials as soon as possible.

Straight to your head

For researchers using antibodies to treat the central nervous system, IV infusion means losing 99 percent of antibodies at the blood-brain barrier and blood-cerebrospinal fluid barrier, which is one potential reason so many monoclonal antibody treatments for Alzheimer's disease elicited lukewarm results, said Martin E. Schwab, a neuroscientist at the University of Zurich. "We

> "This platform is basically a way of producing monoclonal antibodies by your body itself."
>
> — *Kar Muthumani, GeneOne Life Science*



While naloxone has become a valuable tool to reverse opioid overdoses, Cessation Therapeutics is developing an anti-fentanyl antibody.

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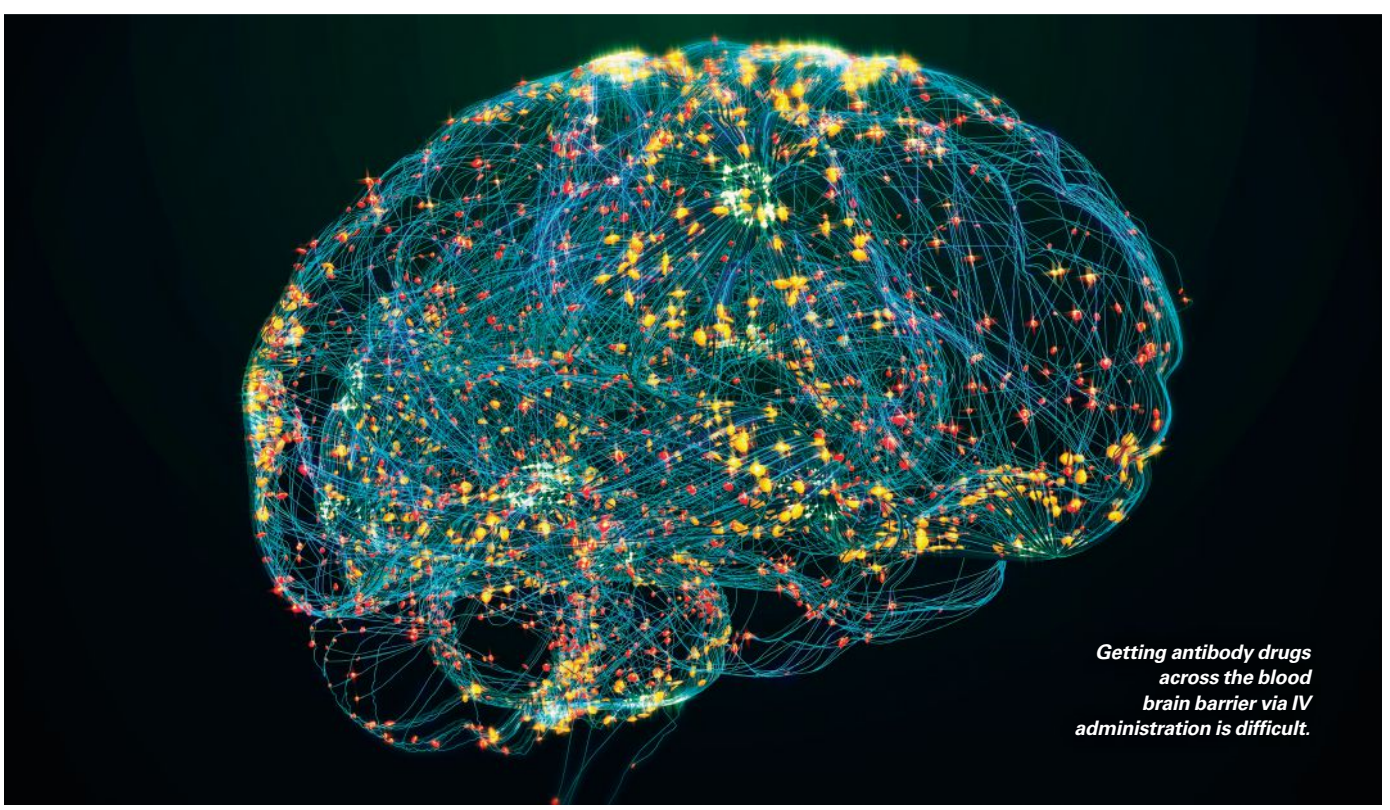

Getting antibody drugs across the blood brain barrier via IV administration is difficult.

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would very much like to have a better way of application than intravenous," he added.

Schwab spent decades studying Nogo-A, a molecule that blocks nerve growth after injuries like spinal cord damage and stroke. His team is developing a monoclonal antibody for Nogo-A that could aid recovery for these conditions. In a completed but still unpublished clinical trial of patients with spinal cord injuries, Schwab said that the team found success with an anti-Nogo-A antibody administered intrathecally, directly to the spinal cord (8).

Still, Schwab admitted that intrathecal delivery can cause uncomfortable headaches, and it requires specialized personnel and equipment. That's why he's also testing an intranasal route for his anti-Nogo A antibody, using a nasal spray that puffs antibodies straight into the sensory neurons of the nose. In a study published in *Proceedings of the National Academy of Sciences* exploring the technique, he reported that over two-thirds of rats treated with an intranasal antibody after stroke regained mobility in their forelimbs, whereas only roughly one-third of the control group recovered (9).

While these initial results are promising, Schwab said a major hurdle for this method is that researchers still don't understand exactly how it works. They believe that antibodies may cross through the sensory cells of the nose and into the brain through a process called transcytosis, but those mechanisms remain unclear. Schwab said that until he and other researchers understand the process better, he's not ready to take a gamble on a costly and time-intensive clinical trial.

Still, he believes that more direct, patient-friendly delivery methods are the future of antibody treatment for neurological disorders. If not intranasal application, Schwab thinks that other experimental approaches like Roche's Brainshuttle could soon make monoclonal antibody delivery to the central nervous system much more efficient.

"I would be ready to replace intrathecal application as soon as a really good method comes along," said Schwab. "My gut feeling at the moment is that one of these two techniques is going to make it."

Antibody recipes

A few researchers have an entirely different vision for delivering monoclonal antibodies. Rather than manufacturing these antibodies in a lab and delivering them into the body pre-assembled, some are developing platforms for synthesizing antibodies in the body itself.

> "We would very much like to have a better way of application than intravenous."
>
> *– Martin E. Schwab, University of Zurich*

Kar Muthumani, chief scientific officer of the biopharmaceutical company GeneOne Life Science, believes that DNA- or RNA-based antibody synthesis could be particularly advantageous in the fight against infectious diseases. When Muthumani witnessed the outbreak of Chikungunya virus in India beginning in 2004, he realized that even if a vaccine was developed, it couldn't work quickly enough to protect people who had already been exposed. Muthumani understood that to curb these sorts of outbreaks, health officials needed a treatment that imparted immunity within days and could be administered in a low-resource setting.

Muthumani and colleagues are working on a synthetic nucleic acid-based delivery platform for monoclonal antibodies. Using an intramuscular injection of DNA, mRNA, or a viral vector, the platform gives the body the genetic instructions for creating a monoclonal antibody. The muscles then become a living "antibody factory," eventually circulating the therapeutic antibodies throughout the bloodstream.

"This platform is basically a way of producing monoclonal antibodies by your body itself," explained Muthumani. He tested a DNA-based delivery system for monoclonal antibodies against Zika virus in a mouse model and found that those antibodies protected against the virus within days (10). He also found success using a similar DNA delivery technique to treat HER2+ breast cancer in mice (11).

Muthumani is particularly interested in DNA-based techniques for monoclonal antibody synthesis as they don't require cold chain storage, making them most accessible for managing infectious diseases in low-resource settings. The company is developing the platform in tandem with a handheld intramuscular injector device called GeneDerm that allows for easy delivery of the genetic material. Muthumani said that the team still needs to translate the work into larger animal models and humans, but he's optimistic about what a genetic delivery platform represents for the future of monoclonal antibodies.

In a 2018 study, researchers found that the average price for a year of monoclonal antibody treatment was $96,731 (12). While high prices stem from myriad factors, the World Health Organization (WHO) reported that immense manufacturing costs are a major driver of the price for monoclonal antibodies, and high prices prevent these life-saving drugs from being fully utilized in developing countries. While the research is still in its early stages, genetic delivery platforms for monoclonal antibodies could usher in a future where antibody drugs are cheaper and more accessible.

This evolving field holds the potential to fulfill Ehrlich's vision by making monoclonal antibodies not only precise but also more broadly available. As these technologies advance, the way doctors and researchers administer antibody treatments could shift dramatically, opening the door to more accessible care and broadening the effect of these critical therapies. ■

Martin E. Schwab's team aims to deliver antibodies using a nasal spray.

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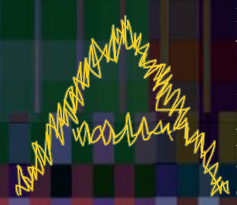
New Drugs to Heal the splitting of the Mind

Drugs for treating schizophrenia have focused on the same mechanism of action for more than 70 years. With the FDA expected to approve a new drug in September, that may finally change.

BY ALLISON WHITTEN, PHD



ANTIPSYCHOTIC DRUGS were discovered by accident. In the early 1950s, psychiatrists at the Sainte-Anne Hospital in Paris realized that chlorpromazine, a drug originally developed as an antihistamine, successfully treated patients suffering from hallucinations and delusions (1). This serendipitous discovery quickly led to an explosion of various antipsychotic drugs prescribed to treat schizophrenia around the word. Over the next two decades, researchers revealed that these drugs work primarily by acting on the dopamine 2 (D2) receptor to reduce dopamine in the brain (2).

More than 70 years later, all of the currently approved antipsychotic drugs still target D2 receptors. Unfortunately, these drugs cause a wide range of unpleasant and harmful side effects and do not treat the full range of schizophrenia symptoms. As a result, studies show that up to 70 percent of patients stop taking their antipsychotic medications (3,4). Despite a clear need for novel classes of antipsychotic drugs, no other options exist for patients.

That could finally change this year if the FDA approves KarXT, an antipsychotic drug developed by Karuna Therapeutics (now a Bristol Myers Squibb company), that mimics the neurotransmitter acetylcholine and binds to muscarinic acetylcholine receptors 1 (M1) and 4 (M4).

"September 26th of this year, [KarXT] will be approved. I don't have any doubt about this because the efficacy and tolerability is established," said Christoph Correll, a psychiatrist and psychopharmacologist at the Zucker School of Medicine at Hofstra/Northwell. Correll was involved in the placebo-controlled clinical trials that demonstrated KarXT's safety, tolerability, and efficacy in patients with schizophrenia (5-7). "This is huge. It's the first time in seven decades, since the discovery of antipsychotics, that we have a different way into the brain to treat schizophrenia," said Correll, who has consulted for Karuna Therapeutics.

The encouraging results of KarXT may have breathed new life into the field. Other non-D2 antipsychotics are also being tested in clinical trials, including additional drugs targeting muscarinic acetylcholine receptors. "If KarXT breaks through as expected, you wonder, would this be the first, and then a couple others come through once you break through that wall?" said Daniel Foster, a pharmacologist at the University of South Carolina who was not involved in the development of KarXT. "It's super exciting,"

"The idea that [KarXT] is going to be the first ever non-dopamine based treatment — hopefully — it's amazing to think about," said Andrew Miller, the inventor of KarXT and now an advisor to Bristol Myers Squibb. "I hope that there's a bunch of other medicines that are launched behind this … People respond differently to different treatments for reasons that we perhaps don't really understand, and so there is just a benefit to having options that are available."

Split of the Mind

More than 100 years ago, schizophrenia got its name from merging the two greek roots "schizo" (split) and "phrene" (mind). Today, psychiatrists are quick to point out that people with schizophrenia do not have a split mind in the sense of split personality disorder. "It's one person," said Correll, but "the feeling and the acting and the thinking are not in sync."

Psychiatrists often break down schizophrenia symptoms into three domains: positive, negative, and cognitive. The positive symptoms get the most attention, including hallmark signs of psychosis like hallucinations, delusions, and disorganized speech and behaviors. Negative symptoms refer to the absence of a behavior, like flat affect, asociality, and anhedonia, while cognitive symptoms refer to impairments in memory, attention, reasoning, and processing speed.

So far, the antipsychotic medications that block dopamine are much better at treating the positive symptoms, but not the negative or cognitive ones. "The overall outcome hasn't really improved that much in the sense that, can patients go back to work? Can they build a family? Can they live independently? In that regard, it's still disappointing," said René Kahn, a psychiatrist and neurobiologist at the Icahn School of Medicine at Mount Sinai.

On top of not effectively treating the negative and cognitive symptoms, these drugs also have undesirable side effects. Depending on the drug, these include weight gain that can lead to diabetes, brain fog, sexual dysfunction, motor impairments that can turn into tardive dyskinesia, and depression. "Patients are really looking for something else," said Correll. "We need novel mechanisms of action because we have all these unaddressed needs of patients."

The Magic Ingredient

Like its antipsychotic drug predecessors, KarXT also has a serendipitous past. Eli Lilly developed xanomeline, the muscarinic agonist component of KarXT, to improve cognition in Alzheimer's disease (AD). When they ran a clinical trial with the drug in the late 1990s, they discovered that the drug had antipsychotic effects for patients with hallucinations and delusions associated with AD (8). However, xanomeline also caused side effects like diarrhea, vomiting, and sweating because the drug binds to acetylcholine receptors outside of the brain too. Despite its positive antipsychotic effects, Eli Lilly shelved it and moved on.

About ten years later, a novel idea for xanomeline took root in Miller's mind. He thought of blocking the cholinergic side effects in the rest of the body using trospium, an antimuscarinic drug for treating overactive bladder already on the market. KarXT, which combines xanomeline and trospium, was born. Since trospium does not cross the blood brain barrier, KarXT has one medication that targets the seat of psychosis in the brain and one that controls unwanted side effects in the body.

At first, the concept for KarXT was just an idea on a piece of paper, Miller recalled. Now, the results of the clinical trials demonstrate its efficacy as a novel antipsychotic that appears to be tolerated well and may potentially treat the negative and cognitive symptoms better than prior medicines, although further studies will be needed (5-7). In terms of side effects, the addition of trospium to KarXT appears to mitigate the intolerable cholinergic side effects seen with Eli Lilly's previous drug. Researchers still observed gastrointestinal side effects, but Miller noted that these were usually mild and often resolved after about two weeks of treatment. They did not see the side effects associated with other dopamine-blocking antipsychotic drugs.

"Our data suggest that it has a very robust and potentially broad efficacy profile, and importantly, a side effect profile that is very different than existing treatments," said Miller. "We're optimistic that this could present KarXT as a unique class of medicine, the first in the unique class that doesn't interact directly with dopamine and is focused on this muscarinic agonist pharmacology."

If KarXT is approved in September, psychiatrists will finally have another option to prescribe to patients. "It is obviously nice to have something different than the classical dopamine 2 antagonists or a partial dopamine agonist. But on the other hand, since I've been in this business for a while, … let's see how the drug will do once it's on the market," said Kahn.

Activating Acetylcholine

After researchers discovered that the early antipsychotic drugs like chlorpromazine work through lowering dopamine, the dopamine hypothesis of schizophrenia took hold in 1966 (9). The hypothesis posits that schizophrenia is caused by overactive dopamine pathways, and it's had a strong influence on the field of schizophrenia research ever since. "That locked us into the idea that schizophrenia is a dopamine excess condition, and we need to block dopamine in order to treat it," said Anthony Grace, a neuroscientist at the University of Pittsburgh. "[KarXT] points to the idea that you don't need to block dopamine in order to get an effective drug."

Rather than blocking D2 receptors to lower dopamine levels in the brain, KarXT targets the muscarinic receptors that bind acetylcholine. Of the five muscarinic acetycholine receptors, KarXT targets M1 and M4. Based on studies so far, Miller explained that KarXT's modulation of the M1 receptor, which boosts acetylcholine signaling, seems to confer cognitive benefits due to M1 receptor expression in the regions of the brain important to learning and memory like the hippocampus and executive function in the prefrontal cortex. On the other hand, Miller said that modulation of the M4 receptors results in the antipsychotic effects because of their expression in brain areas related to perception.

Even though muscarinic agonists like KarXT do not block dopamine receptors directly, researchers believe that the beneficial effects result from their ability to regulate dopamine levels. "Muscarinic receptors do have some downstream effects in the prefrontal cortex dopamine circuitry, but you don't see muscarinic receptors expressed in the dopamine pathway to the motor cortex," said Miller. "We don't have effects in that pathway. We don't have effects in the pituitary gland, which is another dopamine pathway that leads to, for instance, the prolactin and hormonal changes associated with existing treatments." Thus, the ability to selectively modulate dopamine levels in some regions of the brain but not others, especially in areas where dopamine may be too low, could play a role in the improved side effect profile of KarXT.

Muscarinic agonists may also regulate dopamine in a way that's more efficacious for treating schizophrenia. "The deficits in the hyperactivity of dopamine in schizophrenia are thought to be in the release side of things. And so, by changing the release of dopamine, you might have different effects than blocking the receptors later that are postsynaptic to the release," said Foster.

After KarXT, additional drugs targeting muscarinic acetylcholine receptors to treat schizophrenia could be on their way to FDA approval. The companies Cerevel, Neumora, and Neurocrine Biosciences all have clinical trials ongoing with drugs that target the M4 receptor (10-12). Though all these drugs act on M4, their mechanisms differ; KarXT and Neurocrine Biosciences' drugs are agonists, while Cerevel and Neumora's versions are positive allosteric modulators. "An agonist has the foot on the gas pedal the whole time … whereas an allosteric modulator fits there and amplifies the signal," said Correll, who added that the amount of amplification depends on the biological availability of muscarinic receptors, but it preserves the naturally occurring signal temporally and spatially.

Different mechanisms could mean more options for patients. In addition, Correll emphasized that these drugs may work better for at least some patients than traditional D2 antipsychotics because of their influence on multiple neurotransmitters. Muscarinic acetylcholine receptors regulate dopamine levels not only through acetylcholine, but also through the interactions of acetylcholine with the neurotransmitters gamma-aminobutyric acid (GABA) and glutamate (13). "This neural network finetuning is a novel approach that hopefully will really give us outcomes that we haven't seen for some patients," said Correll.

CREDIT: KARUNA THERAPEUTICS

Andrew Miller came up with the idea to add trospium to xanomeline to create KarXT, which could become the first drug in a new class of antipyschotics for treating schizophrenia.

CREDIT: CHRISTOPH CORRELL

As a psychiatrist and researcher involved in clinical trials to test new drugs for schizophrenia, Christoph Correll hopes that more treatment options will soon be available for patients.

"This is huge. It's the first time in seven decades, since the discovery of antipsychotics, that we have a different way into the brain to treat schizophrenia."

- Christoph Correll, Zucker School of Medicine at Hofstra/Northwell

CREDIT: UNIVERSITY OF PITTSBURGH PUBLIC RELATIONS DEPARTMENT

Anthony Grace uses animal models to study the neurobiology of schizophrenia and the mechanisms of action of antipsychotic drugs.

Schizophrenia Drugs of the Future

Besides drugs targeting muscarinic acetylcholine receptors, other classes of antipsychotic drugs that go beyond the classic D2 receptor approach have also entered clinical trials. Trace amine-associated receptor 1 (TAAR1) agonists are thought to act inside neurons to decrease the synthesis and release of dopamine and potentially decrease the firing rate of dopamine neurons (14,15). In 2020, Sunovion Pharmaceuticals, now part of Sumitomo Pharma America, ran a Phase 2 trial showing positive results with a TAAR1 agonist (16). But in 2023, results from two Phase 3 clinical trials from Sumitomo Pharma and Otsuka Pharmaceutical showed that the TAAR1 agonist did not perform better than placebo, although the placebo effect was also high (17).

Correll hopes that they decide to run one more trial. "We need more drugs with novel and different mechanisms of action for schizophrenia, and this is so safe," he said. "It would be wonderful if they just did one more study and did it right, controlling for the placebo effect and maybe also finding the right dose."

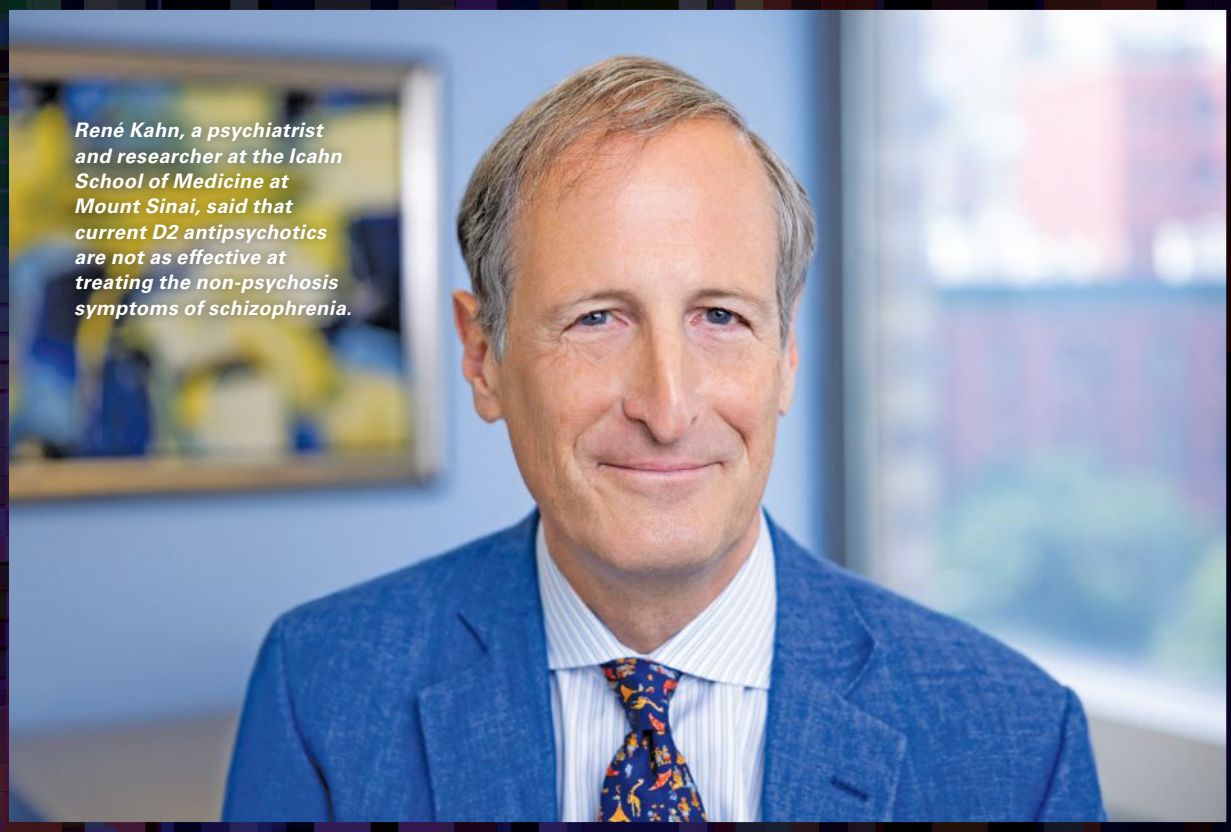
As another example, Merck has a Phase 2b clinical trial ongoing to test a phosphodiesterase 10A (PDE10A) inhibitor, which works to normalize activity of the nigrostriatal dopaminergic pathway (18). Yet, past PDE10A inhibitors failed in clinical trials, which has been a common outcome for schizophrenia drugs and psychiatric drugs more generally.

"A big issue in the field now is how so many pharmaceutical companies have dropped psychiatric drug development because of so many failures," said Grace. "[KarXT] is going to give them an outline of how they can get back into the field without all of the issues that they had before."

"We faced a very high level of skepticism from investors and potential partners," said Miller. "I really do hope that one of the things that we can be a part of is a resurgence in activity and interest because the need has always been there."

Correll is now getting emails from patients asking when KarXT will be available since they have not responded well to current therapies. "At least for subgroups of patients, this can be foundational and really not just incremental," he said.

As for the future of drug development for schizophrenia, Miller said that the field should learn from its often-accidental past discoveries. "This field of developing new treatments has been guided by these serendipitous human findings," he said. "We really need to embrace that from a research perspective and look for things that also may tell us where new treatments might lie."



René Kahn, a psychiatrist and researcher at the Icahn School of Medicine at Mount Sinai, said that current D2 antipsychotics are not as effective at treating the non-psychosis symptoms of schizophrenia.

CREDIT: MOUNT SINAI HEALTH SYSTEM

Daniel Foster studies muscarinic acetylcholine receptors with the aim of developing therapeutics for psychiatric disorders.

CREDIT: YUMIN CHOI

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milestone

THE ORIGINS OF oncolytic viral therapy

BY YUNING WANG, PHD

Since their discovery in the 1890s, viruses have intrigued scientists as potential cancer-killing agents. Early observations and experiments once excited them, but technical limitations soon dampened their hope. It wasn't until recent breakthroughs in genetic engineering that researchers began to harness viruses' full potential as targeted cancer therapies, turning these risky pathogens into lifesaving remedies.

EARLY 1900s — Remission to infection

Around the turn of the 20th century, physicians noticed that patients with cancer who contracted natural viral infections occasionally experienced temporary remissions. One of the earliest documented cases, reported in 1904 by George Dock, a physician at the University of Michigan, was about a female patient with myelogenous leukemia (1). After a bout of what was assumed to be influenza, her previously enlarged liver and spleen shrank to nearly normal size, and her elevated leukocyte count dropped more than 70-fold. The remission lasted for several months before her death a year and a half later.

In another case in 1912, physician Nicola De Pace described significant tumor regression in patients with cervical cancer who received rabies vaccines containing an attenuated strain of the rabies virus (2). These reports sparked curiosity among scientists to understand how viruses might interact with cancer in laboratory experiments.

In 1922, microbiologists Constantin Levaditi and Stefan Nicolau at the Pasteur Institute were working on a new vaccine against smallpox using the vaccinia virus. When they inoculated the virus into epithelial tumors in mice and rats, they discovered that it exhibited a selective affinity for the tumors, proliferating more rapidly in cancerous tissues than in normal ones. Levaditi and Nicolau described the tumors as acting like "a sponge attracting viral replication" (3).

CREDIT: BENTLEY IMAGE BANK

George Dock, a physician at the University of Michigan, reported one of the earliest cases of tumor regression during natural viral infections, noting that such an unusual response might hold "something of therapeutic value" (1).

LATE 1940s–1950s — First insights into viral oncolysis

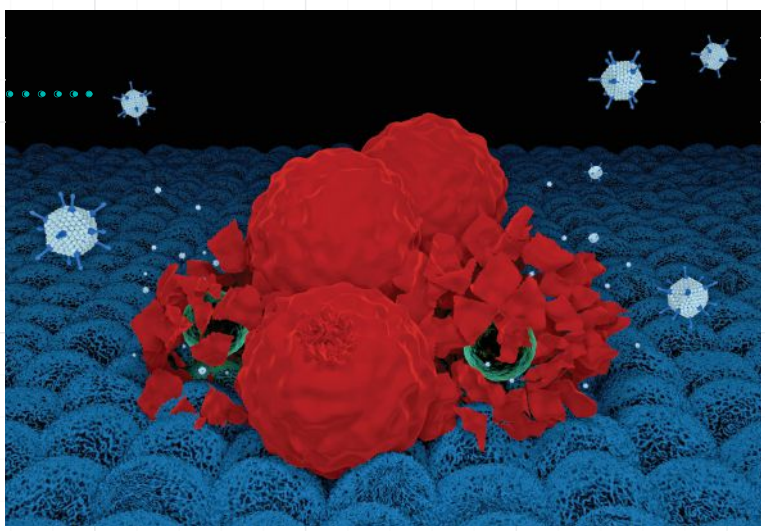
The early clinical observations of tumor regression induced by viral infections led to a wave of research in both laboratory and clinical settings between the late 1940s and 1950s. Alice Moore, a researcher at Memorial Sloan Kettering Cancer Center, implanted a transplantable mouse cancer, known as sarcoma 180, into a group of mice. She inoculated these mice with the Russian Far East encephalitis virus and monitored tumor growth and viral presence in the mouse tumors, brains, and blood.

In line with Levaditi and Nicolau's findings in 1922, Moore observed that the virus preferred tumor tissue over other tissues. Her microscopic examinations revealed that, in some cases, the virus completely destructed mouse sarcoma 180 tumors. When Moore transplanted these virus-infected tumors into healthy mice, the tumors failed to grow, suggesting that the virus had eradicated the cancer cells.

In 1949, Moore published her findings (4), demonstrating viral oncolysis in living animals for the first time. More studies on the oncolytic activity of other viruses using animal models followed. These studies laid the foundation for understanding the detailed mechanisms by which viruses selectively infect and kill cancer cells.

"In a normal cell, there is a whole set of antiviral machinery that recognizes viruses and signals the immune system to clear them quickly," explained Howard Kaufman, the president of Ankyra Therapeutics, a company that develops cytokine-based immunotherapies for cancer. "Cancer cells have defects in this antiviral machinery. That's why they're more susceptible to viruses."

During the 1950s, looser ethical guidelines for medical research allowed physicians to take bold approaches. They administered live viruses, such as hepatitis B virus, West Nile virus, and adenovirus, directly to patients with cancer (5–7). While some patients exhibited temporary tumor regression and symptom relief, others had no improvement or even severe, fatal viral infections. It became clear that viruses with greater tumor specificity and safer profiles were needed. Without the tools to precisely control and manipulate these viral properties, many researchers abandoned the field, until breakthroughs in genetic engineering emerged decades later.



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Alice Moore, a researcher at Memorial Sloan Kettering Cancer Center, first demonstrated that viruses could kill tumor cells in living animals.

1990s — Engineering a better virus

In the 1990s, molecular cloning techniques became standard for generating recombinant DNA, allowing researchers to insert and delete genes within an organism's genome. This brought about a resurgence of interest in oncolytic viruses.

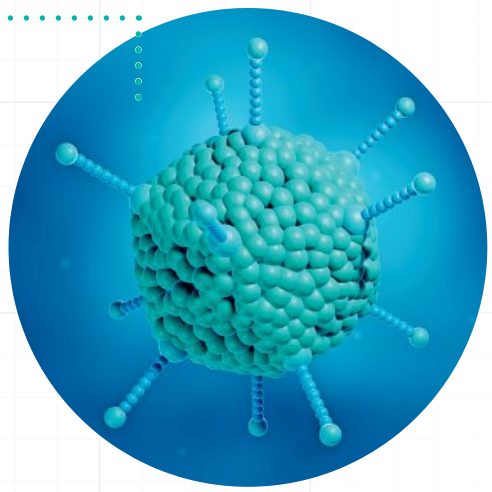
In 1994, David Kirn, now chief executive officer and cofounder of 4D Molecular Therapeutics, completed his fellowship in medical oncology at the University of California, San Francisco, and was looking for a research project. With an interest in virology, Kirn interviewed with Onyx Pharmaceuticals, a newly founded company developing novel cancer therapies.

"They said there was this idea of using viruses against cancer," Kirn remembered. "I'd always been torn between my love of virology, infectious disease, and oncology. So, for me, it was the perfect fit."

Kirn became Onyx Pharmaceuticals' tenth employee and started working on creating new viruses that could selectively target cancer cells. "There were no engineered viruses that had been in the clinic before," Kirn said. The only related research he found was by Robert Martuza, a neurosurgeon at Massachusetts General Hospital, who had genetically modified a herpes simplex virus by deleting the thymidine kinase gene in 1991. With this modification, the virus replicated only in rapidly dividing tumor cells and inhibited tumor growth in mice (8).

Inspired by Martuza's study, Kirn worked with adenovirus, deleting its E1B gene to create a modified virus named ONYX-015. The E1B gene encodes a protein that binds to and inactivates the tumor suppressor p53 protein, preventing p53-mediated apoptosis and allowing viral replication in infected cells. Without the E1B gene, the ONYX-015 virus cannot inactivate p53 and cannot replicate in normal cells. However, it can replicate efficiently in p53-deficient tumor cells.

In 1997, Kirn and his team published results showing ONYX-015's remarkable antitumor effects in mice with a substantial reduction in tumor size and complete regression in 60 percent of the tumors (9). While presenting these encouraging results, Kirn and his team were



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Using genetic engineering, David Kirn and his team at Onyx Pharmaceuticals created ONYX-015, a genetically modified adenovirus that selectively targets cancer cells.

CREDIT: DAVID KIRN

David Kirn led the clinical trials of the first engineered oncolytic virus, ONYX-015.

1996–EARLY 2000s Navigating clinical challenges

Beginning in 1996, Kirn and the Onyx Pharmaceuticals team partnered with researchers from multiple institutions to carry out clinical trials for ONYX-015. "There were a lot of questions about how to use a therapeutic that amplifies in the human body," Kirn said. "There were also questions about how best to deliver the viruses."

To address these challenges, Kirn designed and implemented a novel clinical research and development approach. They began by injecting the treatment directly into patients' tumors to assess safety. Once proven safe, they progressed to injections into body cavities, arteries, and finally veins. The studies started with patients with advanced, incurable cancers and then included those with premalignant conditions (10).

"We treated over 400 patients with ONYX-015, exploring different tumor types and all routes of administration from intratumoral to intraperitoneal and intravenous," Kirn said. "As a physician, I was allowed to inject the first patient ever treated with an engineered oncolytic virus. The patient did quite well. That was incredibly exciting."

In one of the Phase 2 trials, 37 patients with head and neck cancer received intratumoral injections of ONYX-015 in conjunction with two chemotherapy agents. The results exceeded those observed with chemotherapy alone, with 63 percent of patients experiencing significant tumor shrinkage and 27 percent achieving complete tumor regression (11). "We thought it could be dangerous. But we found the opposite. It was very, very safe," Kirn said. "We didn't see significant toxicities. Patients got a little bit of a flu-like syndrome. That was it."

However, ONYX-015 showed limited efficacy as a single agent. It failed to induce tumor regression in patients with deeply seated pancreatic, colorectal, and ovarian tumors (10). Consequently, further development of ONYX-015 was halted in the early 2000s. "I think the rest of the history of the field has been based on that ONYX-015 data," Kirn said. "That's when people started trying new viral species that might be more potent and arming them with transgene payloads."

2015 The first FDA approval

As the ONYX-015 program led by Kirn concluded, Kaufman worked as a physician scientist at Columbia University Medical Center. There, he created a recombinant vaccinia virus expressing a tumor antigen to treat metastatic melanoma and demonstrated its potential clinical benefits in a Phase 1 trial (12).

Kaufman's work caught the attention of Robert Coffin, a virologist who had recently founded a biotechnology company called BioVex. "Rob Coffin found my poster at a science meeting and said, 'We have an oncolytic herpes virus, and it looks like you're interested in this. Would you want to work with us?'" Kaufman recalled. "That was my introduction to T-VEC."

Talimogene laherparepvec (T-VEC) was a new oncolytic virus Coffin was developing. As a genetically modified herpes simplex virus, T-VEC had two key genes removed via recombinant DNA technology to prevent it from replicating in healthy cells and evading the host immune response. Additionally, Coffin engineered the virus to express granulocyte-macrophage colony-stimulating factor (GM-CSF).

"GM-CSF was known to recruit dendritic cells and help them to mature," Kaufman said. "To get a systemic immune response, a very strong T cell response is needed." Coffin anticipated that by expressing GM-CSF, which prompts dendritic cells to present tumor antigens to T cells, T-VEC would trigger an immune response against cancer cells.

Kaufman led the Phase 2 trial of using T-VEC to treat melanoma intratumorally and published the results in 2009, which showed a 26 percent overall response rate (13). "A 20 or 30 percent response rate for melanoma at that time was really good. The only available therapy worked about 10 to 15 percent of the time," Kaufman said. Encouraged by these results, Kaufman went on to design and lead a randomized Phase 3 study. This larger trial involved over 400 patients and yielded a similar response rate (14).

In 2015, after years of testing and trialing, the FDA approved T-VEC as the first oncolytic viral therapy. This new treatment option has since led to increased survival rates for patients with melanoma. "I remember telling one of my first patients who was going into the study, 'Are you ready to make history?'" Kaufman said, "She had a complete response and is still free of tumor today. She had a little kid at the time. I recently got an email from her — the kid is in college now."

CREDIT: HOWARD KAUFMAN

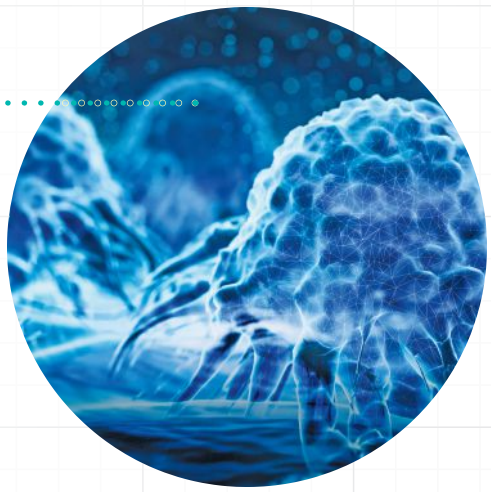
Howard Kaufman led the Phase 2 and 3 trials for treating melanoma with T-VEC, which became the first FDA-approved oncolytic viral therapy.

2016–PRESENT Unlocking new avenues

The approval of T-VEC generated excitement in the viral therapy field and led to further exploration of novel therapeutic strategies and combinations. Researchers found that oncolytic viruses have a unique ability to convert cold tumors, which typically evade immune system attacks, into hot tumors that are more susceptible to immunotherapy. In numerous ongoing clinical trials, researchers are investigating the efficacy of oncolytic viruses in combination with immunotherapies like immune checkpoint inhibitors, which have shown promising results and improved patient outcomes.

"We're now getting better at designing the viruses upfront for better lytic activity against cancer," Kaufman said. "We're also adding better payloads into the virus." These payloads include a variety of transgenes such as cytokines and immune modulators that enhance the virus's ability to stimulate the immune system. For example, some researchers are incorporating genes encoding interleukin-12, a potent cytokine that boosts the activity of T cells and natural killer cells, while some are arming viruses with the ligand for cluster of differentiation 40, an immune stimulator that enhances B cell activation (15).

Researchers are also exploring more effective delivery methods, such as intravenous administration, for oncolytic viruses. Building on the development of ONYX-015, Kirn has been creating new viral therapies that can safely circulate through the bloodstream to effectively target metastatic and distant tumors. "The next step is to unlock the full potential of oncolytic virus therapy," Kirn said.



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Researchers are exploring better virus designs and more effective delivery methods to target difficult-to-treat cancers such as metastatic tumors.

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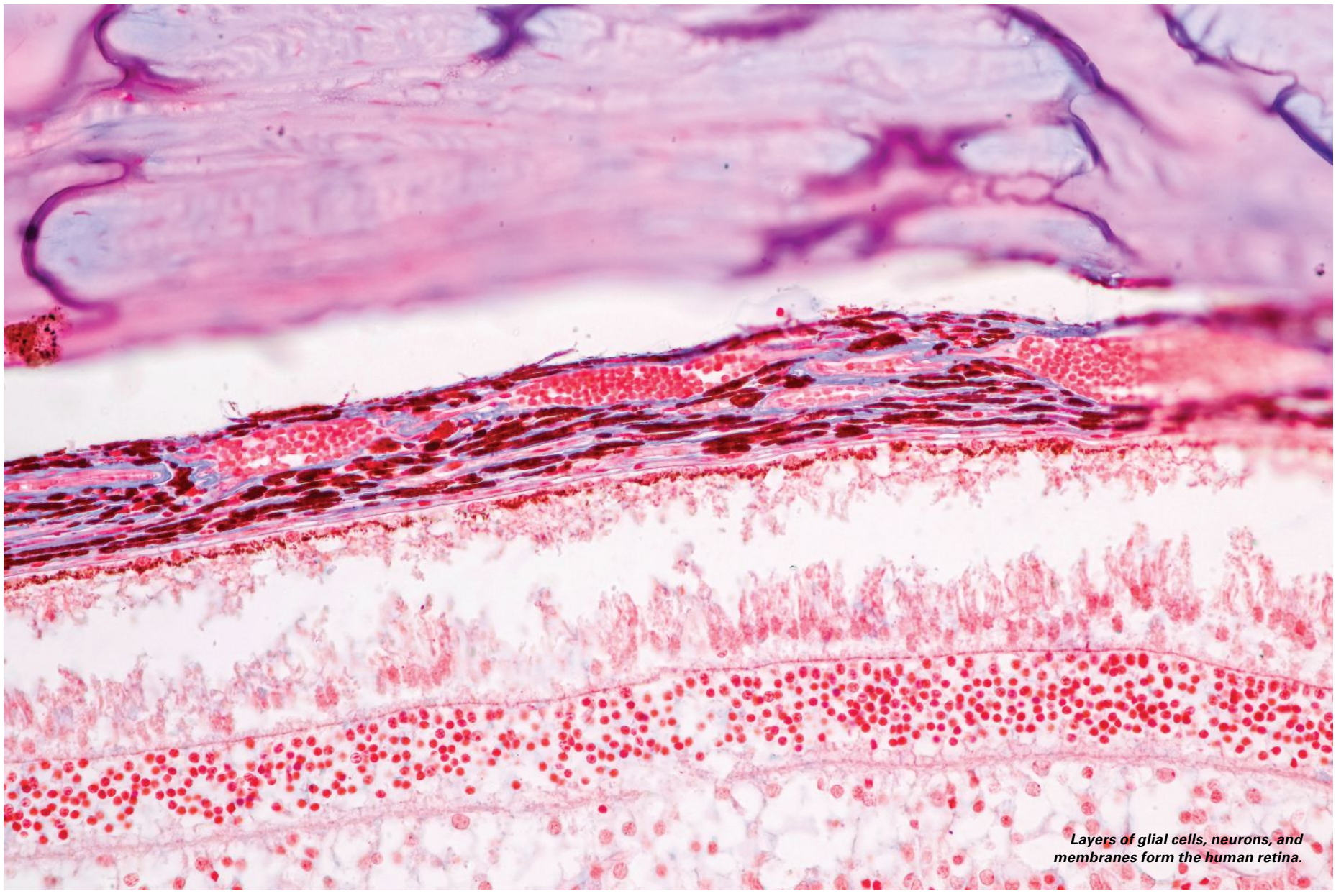
ophthalmology

Mini-Retinas Model Human Disease in a Dish

Researchers use retinal organoids to screen drugs and hope to transplant them into the eyes of people with blindness in the coming years.



Layers of glial cells, neurons, and membranes form the human retina.

CREDIT: ISTOCK.COM/SVETAZI

BY RACHAEL MOELLER GORMAN

THE BRAIN EXTENDS FROM the skull and touches the outside world in only one place: the phyllo-like layers of retinal cells that line the back of the eye. Like their brethren in the brain, these delicate light sensors cannot be replaced; if they die, vision is lost. As humans live longer lives, ocular diseases such as age-related macular degeneration, diabetic retinopathy, and retinitis pigmentosa are more common, yet physicians have little to offer patients who have lost their rods, cones, and other retinal cells.

Back in 2006, Valeria Canto-Soler, then an eye researcher at Johns Hopkins University, used animal models and cell cultures to investigate the retina, but these models were disappointingly inadequate. Two-dimensional cell cultures could not approximate intricate 3D retinas packed with layers of discrete cell types living in a rich physical environment, and animal models did not accurately represent retinal anatomy and human disease pathogenesis.

> "The cells are actually talking to each other. They are a lot happier and healthier if you grow them together." *– Valeria Canto-Soler, University of Colorado*

Then, unexpectedly, there was "a revolution in the field," said Canto-Soler. Cell biologists Kazutoshi Takahashi and Shinya Yamanaka at Kyoto University introduced four factors into mammalian cells and turned back time, reversing differentiation and allowing adult fibroblasts to become any cell in the body. They called these induced pluripotent stem (iPS) cells (1). "That's the time when I started to think about the possibility of using human iPS cells to generate a 3D tissue that would resemble as close as possible the retinal tissue," said Canto-Soler.

Motivated by the lack of adequate treatment options for patients with retinal degenerative diseases, Canto-Soler started her own research group at Johns Hopkins University to try to address this unmet need. She and her team got to work using iPS cells to generate 3D retinal tissues. They studied and manipulated iPS cells for years, while other research groups made strides with embryonic stem cells.

Canto-Soler's group kept pushing. Finally, they hit the jackpot: In 2014, they published

the first paper showing that iPS cells could self-assemble into a 3D retinal organoid with all the cell layers in the right order and photoreceptors sensitive to light (2).

"That's the time when I started to envision a bigger program, not just my lab," she said. "A team of investigators that would have complementary expertise, that will know the things that I didn't know… Can we translate these into retinal transplants to restore vision in patients who are blind because they have lost their photoreceptors?"

Building the team: Disease models and drug screening

At the same time as Canto-Soler worked on her iPS cell research, a philanthropic family led by Diane Gates Wallach and her brother John Gates, committed to fund new research in regenerative medicine, with a special interest in age-related macular degeneration (AMD). The family patriarch and former CEO of the Gates Rubber Company, Charles Gates Jr., had suffered from AMD and blindness at the end of his life, so they developed the Gates Center for Regenerative Medicine at the University of Colorado.

Once the technology for easily creating iPS cells was developed, "we could think more aggressively about how to harness that technology and apply it to the development of a therapy for macular degeneration in humans," said Mark Petrash, an eye disease researcher at the University of Colorado and soon-to-be associate director of the Gates Center. The Gates donors would match funds raised by Naresh Mandava, an ophthalmology researcher and chair of the Department of Ophthalmology at the University of Colorado School of Medicine, to create an ocular stem cell program.

Once they raised sufficient funds, Petrash and Mandava started a search for a director for their new program, and Petrash flew to Johns Hopkins University to interview Canto-Soler. "I made a trip up there to meet her to have dinner and explain what our vision was," he said.

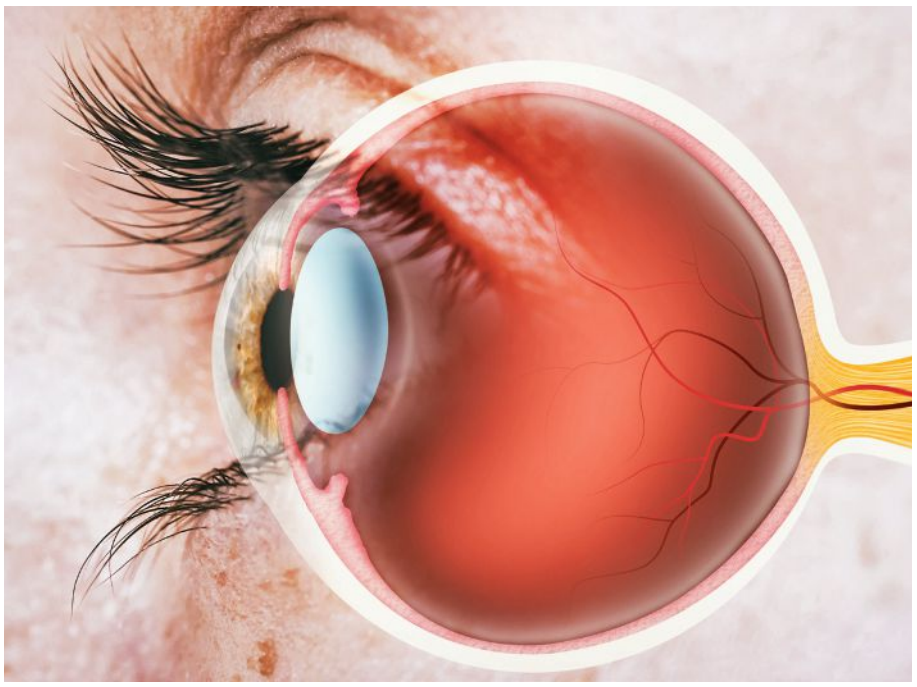
After they met, Canto-Soler said, "Their vision was almost like a mirror vision of mine."

In July 2017, Canto-Soler moved to Colorado and launched CellSight, which is the University of Colorado Department of Ophthalmology's Ocular Stem Cell and Regeneration Research Program. Canto-Soler wanted to bring together a team of people from several disciplines, including basic researchers through clinicians to work independently on their own projects, but also to come together to solve bigger problems, with the hope that someday they could cure blindness. The program recruited retinal researchers Joseph Brzezinski, Natalia Vergara, Miguel Flores-Bellver, and vitreoretinal surgeon Marc Mathias. A new lab facility was built, and the researchers started generating retinal organoids from iPS cells and finetuning their protocols.

An organoid takes as long to form in the lab as a retina takes to grow during normal human development in utero. When complete, each organoid is only one to two millimeters in diameter, and they grow better when they're with other organoids. "The cells are actually talking to each other," said Canto-Soler. "They are a lot happier and healthier if you grow them together."

Although the cells that the researchers differentiated into retinal cells self-assembled into 3D structures containing most layers of the retina, one vital layer, the retinal pigment epithelium (RPE), required additional tweaking. When it was grown from iPS cells, it didn't position itself correctly relative to the photoreceptors, so Flores-Bellver developed a new protocol to grow a functional RPE monolayer, and then the team built a 3D complex with the RPE correctly positioned facing the photoreceptors and the rest of the retinal organoid layers. Concurrently, Brzezinski investigated how the retina develops embryonically. He has also researched the developmental mechanisms that lead to the many retinal cell types forming at the right time, proportion, and place.

> "We dream of developing a transplant program to try to give vision back to these patients."
>
> *– Natalia Vergara, University of Colorado*



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Light shines through the pupil onto the retina, along the back of the eye.

"[Now, retinal organoids] are at a stage where they have begun to be common practice in our field. Many labs are routinely using them for disease modeling," said Kapil Bharti, an ocular and stem cell researcher at the National Eye Institute, who is not involved with CellSight.

In 2023, Johns Hopkins University, China Medical University, and University of Maryland researchers used CellSight retinal organoids as one of several models to study how oxidative stress affects retinas (3). "We used them as a tool, a very helpful tool, but in conjunction with human tissue, animal models, and cell-based models," said Akrit Sodhi, the retinal researcher at Johns Hopkins University who conducted this study. "No one tool, the organoids included, is a perfect tool. It's very effective in filling in some of the gaps in our understanding using other models."

Researchers in Vergara's group also created retinal organoids from patients with familial forms of Alzheimer's disease using cells that contained mutations in the amyloid precursor protein gene. They found that organoids made from these patient samples resembled healthy organoids but also accumulated more Aβ and pTau, the main hallmarks of Alzheimer's disease (4).

Sodhi said that retinal organoids work well as disease models because researchers can manipulate them. But there are also disadvantages. The organoids are immature and have no vasculature, which could be a problem since most retinal diseases involve alterations to blood vessels. In addition, all the pertinent cells may not be present. Some cells die early because certain synapses don't form, while other cells develop later. "You reproduce a lot of what's missing in, say, cell culture or animal models," said Sodhi. "It's not a perfect model, but it's definitely a huge step forward."

CellSight uses retinal organoids to screen drugs, especially those that may prevent photoreceptor loss in retinal degenerative diseases. Vergara will use her Alzheimer's disease retinal organoid model to evaluate drug candidates. She is also collaborating with researchers at Boston Children's Hospital to help a patient with a rare mutation that leads to untreatable photoreceptor degeneration. The group developed an antisense oligonucleotide therapy to fix the mutation in the patient's RNA so the protein is produced normally, and Vergara's group is making retinal organoids using the patient's own cells to see if the experimental treatment will work.

Vergara is optimistic that the approach will give the patient's doctors valuable knowledge about treatment efficacy. "We are very excited because it will help us establish a pipeline for the potential treatment of other rare blinding diseases," she wrote in an email. "There's currently no cure for these diseases, and because they are rare, there's little incentive for big companies to develop innovative treatments."

Retinal organoid transplants?

In addition to disease models and drug screening, the other long-term goal for retinal organoid research is developing retinal transplants for people who have severe retinal degeneration and have lost their sight. "We dream of developing a transplant program to try to give vision back to these patients," Vergara said.

Other researchers have begun to study transplanting just the 2D RPE layer of the retina into patients. These patients have early-stage disease, and their retinas are mostly functional. Later stage disease, in which more cells have degenerated and sight is lost, would require a larger transplantation of all the retinal layers. At this stage, the researchers hope to transplant a retinal organoid plus the RPE.

Mathias is testing retinal transplants generated from retinal organoids in pigs since their eyes are similar to human eyes. Mathias, his colleagues at Johns Hopkins University, and the CellSight team have created a surgical kit for delivering their retinal transplants into pigs by working with a medical device company to create the tools necessary; they have completed proof of concept studies and hope that these surgical techniques can be translated into humans (5).

"We have this amazing ability to grow mini-retinas, or retinal organoids in the lab, that mimic the retina in the eye," said Mathias. "The challenge is getting those into the tissue where it needs to heal a disease process and integrate with the remaining cells that are healthy … [and] are connected back to the brain." He hopes that it will be possible to test such a transplant in humans within the next six years.

There are many challenges ahead. Experts caution that the transplants derived from retinal organoids must survive within the host retina after transplantation and adapt to the vascular network, while neurons will have to establish synapses and communicate with each other and also with the optic nerve and the brain. The transplants also need to survive the potentially unhealthy retinal environment in which the previous retinal cells died. This is a complex undertaking, and some experts caution that retinal organ transplantation could be many, many years away.

Canto-Soler is grateful for the opportunity, no matter how long it takes. "It was like a magic moment! When you have a vision, you don't know how you are going to bring it to life, and someone knocks on your door and says, 'Hey, we have the same vision. We have what you need, and would you like to work with us?" said Canto-Soler. "Our hope is over the next five years or so, we may be able to demonstrate that it's a safe procedure that has potential for benefiting patients and eventually being able to reach clinical trial to test in patients. That's still a long way ahead." ■

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Seeing Color

People with achromatopsia have never seen color. Restorative gene therapies have had mixed success, leaving researchers wondering why.

BY STEPHANIE DEMARCO, PHD

WHAT IS THE COLOR RED? It's the shiny skin of a Fuji apple and the bright paint on a firetruck. It's the hue of a hummingbird's favorite flower and the pigment in an often worn lipstick. But when it comes down to defining it, what is red?

For people who have normal color vision, the answer is both obvious and hard to describe. It's the color next to orange on the color wheel or the top one on the rainbow. But for those who have only seen the world in different shades of gray, colors are almost impossible to define.

This is the case for people with achromatopsia, a recessive genetic condition in which none of their cones, the cells responsible for color vision, function properly (1). Most people with achromatopsia have a mutation in the *cyclic nucleotide gated channel subunit beta 3 (CNGB3)* or *alpha 3 (CNGA3)* gene, so researchers wondered if delivering a healthy copy of the defective gene via gene therapy would help those with achromatopsia see the world in all of its colors.

The results across different clinical trials have been mixed. Some people see some improvement, but others don't. So, when Ayelet McKyton, a neuroscientist at the Hebrew University of Jerusalem, received a message from a patient whose vision hadn't improved after receiving one of the gene therapies a few months before, she wasn't sure what to think. In the message, the patient included a photo of a bedspread with red flowers on it. "She said, 'Wow, this looks glowing. Can you tell me what color it is?' And then I told her it's red," said McKyton.

This was the first time that one of these patients had been able to distinguish any sort of color, even if what she saw was not how those with typical color vision see red. McKyton then asked other patients who had received the same gene therapy in the clinical trial about what they saw when presented with something red.

"One told me that it's like it's on a different level from the screen. It's like a different dimension," McKyton said.

While current gene therapies for achromatopsia don't seem to restore color vision, they certainly do something. Vision scientists are now taking a closer look at patients who have received these gene therapies for achromatopsia to see if they can figure out the effect they have on vision, why they are more effective in some patients than others, and how to identify the patients that are most likely to respond to treatment.

CREDIT: ISTOCK.COM/BOTINA INNA

Achromatopsia patients who received a restorative gene therapy see the color red as something that glows.

> "She said, 'Wow, this looks glowing. Can you tell me what color it is?' And then I told her it's red."
>
> *– Ayelet McKyton, Hebrew University of Jerusalem*

CREDIT: ISTOCK.COM/MELODYANNEM

What is red? It's one of the colors that decorates the world for people who have color vision.

Eye plus brain equals vision

To turn light into sight the eye relies on two types of photoreceptors in the retina: rods and cones. Rods are responsible for night vision, while cones work in daylight. Cones allow us to recognize faces, read a book, drive a car, and most of all, they detect colors. There are three different types of cones, which detect short, medium, and long wavelengths of light.

People with achromatopsia, however, only have rods to work with. Due to mutations in *CNGB3, CNGA3*, or other related genes, their cones are present but not functional. This makes it very difficult for them to see during the day.

"They're very, very sensitive to light because your rods only work in very dim light, so if you have only rod vision, you have a kind of photophobia. It's like everything is always glaring, even under fairly dim conditions," said Bevil Conway, a neuroscientist at the National Eye Institute.

There is also a special region of the retina called the fovea that only contains cones and is responsible for high-resolution vision. This means that people with achromatopsia have somewhat grainy and low-resolution vision. Their eyes can also make uncontrolled and repetitive movements, called nystagmus, and of course, they have no color vision at all.

Vision doesn't only rely on the eyes. "Vision is eye plus brain," said Michael Hoffmann, a biologist at Otto-von-Guericke University, Magdeburg. He explained that if the eye is a digital camera, the brain is the computer that allows humans to understand the image. The visual inputs from the rod and cone cells travel to the optic nerve, which sends the signal out of the eye and into the visual cortex in the brain where the information is processed.

Studies on related visual conditions showed that there is some plasticity when it comes to this eye-brain connection. For example, in amblyopia or lazy eye, one eye has blurrier vision than the other. But if doctors perform surgery to fix the lazy eye during childhood, the visual cortex can still develop properly, and the child will have restored vision. But if doctors wait too long to treat this, then even with surgery, the brain can't interpret visual information from the lazy eye.

"You need to fix the eye, but the brain has to learn how to see," McKyton explained. If something like amblyopia could be treated and lead to better vision, maybe achromatopsia could be too.

A glowing red

The 2017 approval of Luxturna, a gene therapy for a rare form of inherited blindness, sparked joy and anticipation in the world of vision science. Not only was it the first gene therapy for a genetic disease ever approved by the Food and Drug Administration, but it also gave people sight. With established animal models, a clear genetic cause, and low risk of an adverse immune response to an AAV gene delivery vector in the retina, achromatopsia seemed like a good target for gene therapy (2).

Research groups around the world initiated Phase 1/2 clinical trials to investigate the safety and efficacy of the achromatopsia gene therapies. These included trials led by researchers at the University Hospital Tübingen, two trials sponsored by the Applied Genetic Technologies Corp, and two trials led by MeiraGTx.

Because achromatopsia is rare — one in 30,000 people has it — these trials were small, each consisting of 11 to 32 patients (3). While earlier studies had already reported that the therapies were safe for patients, the treatments' effectiveness was less clear. One study reported small improvements in visual acuity, color vision, and contrast sensitivity in the eye treated with the gene therapy, and another, which McKyton helped lead, also showed only minimal progress in photoaversion and acuity in the treated eye (4,5).

"We didn't see any improvement, but they said that something had changed," McKyton said. "We're not saying that they can distinguish between colors. We don't know that yet, but we know that they can see red differently than they do gray."

She and her colleagues realized that they weren't picking up this subtle change in the patients' color vision because the typical clinical tests for measuring it are not well suited to pick up a small change like this. So, the researchers decided to design new tests to better understand what individuals who received the restorative achromatopsia gene therapy saw (6).

"The challenge was to listen to their reports carefully in order to design a good experiment," McKyton added. The team needed to convert subjective descriptions into something quantifiable.

After talking with some of the people who had received the achromatopsia gene therapy, she and her team designed three different tests to better understand their new red color perception. The first test measured how the patients perceived a color's inherent lightness.

Imagine a color image edited to look black and white. A lot of colors will still look different from one another because colors have inherent differences in lightness. For someone with full color vision, red appears to be a bright color, but because rods can't detect the long wavelength of red light, red often looks black in grayscale images. McKyton and her team hypothesized that after the gene therapy, that red would look lighter to patients, but that was not the case for the three adult patients they studied. For their fourth and final participant, a seven-year-old child, it was a different story.

"For the child, the red did become lighter, and also the other colors became more like controls. So, the child improved more than the three adults," said McKyton.

Once the team established how the participants perceived the lightness of different colors, they performed a color detection test. The researchers placed a red colored stripe among gray stripes of different intensities. "They will have to use the color attribute in order to find it, and then we saw that definitely, with the untreated eye, they cannot see it at all. With the treated eye, they can see it," McKyton said.

The team also used this method to test participants' abilities to detect yellow and cyan. Among the four people, two detected only red; one saw red and yellow; and the last adult distinguished red, yellow, and cyan. Finally, in the last test, the researchers tested the participants' color saliency, essentially how quickly they could identify a red circle among gray circles meant to distract the eye.

CREDIT: ISTOCK.COM/PHOTOGRAPHER ANDILLUSTRATOR

Cones are responsible for high-resolution color vision, allowing people to see the bright red skin of an apple.

"For us, color is a very, very intense attribute. It pops out to us very fast. It doesn't matter how many distractors we have; if everything is in grayscale, and one thing is red, we will catch it immediately," McKyton explained. The participants, however, had to search for the red circle before they finally spotted it, meaning that they have very low color saliency compared to people with normal color vision.

Conway, who was not involved in the study, was intrigued by the variability in color detection among the four participants. He reasoned that it might be due to different levels of uptake of the gene therapy by the cone cells in the retina or that the population of cones varied from person to person.

"Patients who have this achromatopsia, their cones aren't working, but their cones progressively die over the course of the patient's life," Conway said. "They don't develop normally and then just sit there waiting for a date to the ball. They gradually, progressively get fed up and then give up the ghost."

To better understand how the brain responds to the red glowing sensation seen by gene therapy trial participants, McKyton would like to take a closer look at their brains. "We're really, really hoping to make them feel in the MRI this feeling of glow sensation, and to see whether it happens in color-specific areas or in different areas [of the brain] because maybe the color-specific areas did not develop until now," she said. "Maybe their color areas were never used for color. Maybe they're reassigned to something else as well, and if they are, can we reverse it after a [gene therapy delivery] surgery?"

Diving into the visual cortex and other brain areas in people with achromatopsia

> "We're not saying that they can distinguish between colors. We don't know that yet, but we know that they can see red differently than they do gray."
>
> *– Ayelet McKyton, Hebrew University of Jerusalem*

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When achromatopsia gene therapy recipients told Ayelet McKyton that after receiving the gene therapy they saw the red pedestrian light signal differently, she and her team wanted to find out what exactly they could see.

and those who received these gene therapies will be the next step in understanding how the visual system works in these patients as well as figuring out the best ways to potentially restore their color vision more fully.

Focusing on the fovea

When it comes to understanding how achromatopsia patients might be able to gain color vision, one of the key structures to explore is the fovea. Because the fovea is so important for high-resolution and color vision, the brain devotes a lot of resources to interpreting foveal signals in the visual cortex.

"The visual cortex organizes the information of the outer world in a systematical map," Hoffmann explained. With achromatopsia, the visual cortex can't receive information from the fovea because the cones there don't work. Hoffmann and his team wondered what happens to the region of the visual cortex that would normally interpret foveal signals in achromatopsia patients. Would it get remapped to process visual signals from other parts of the retina, or would it simply sit silently in the brain with no visual processing activity?

"This is important," Hoffman added. "If you want to switch on the activity again with gene therapy in this part, you rely on this cortex to function."

To investigate how the part of the visual cortex that processes foveal information acts in achromatopsia, the researchers performed functional magnetic resonance imaging (fMRI) on 17 people with achromatopsia and 19 people without achromatopsia (7). To their surprise, they found that this region of the visual cortex was not remapped to convey signals from any other part of the eye. Instead, the fMRI showed that these regions were silent in people with achromatopsia.

"It looks different from the visual cortex that gets input, and interestingly, it looks like

CREDIT: SARAH KOSSMANN/UMMD OVGU

Michael Hoffmann studies the visual system in people with achromatopsia.

the cortex we find in people who are born blind," Hoffman said.

With no visual input from the fovea, the region of the visual cortex that processes information from the fovea failed to develop normally. Because of that, Hoffmann explained, "We don't know, but we would assume that this makes it more difficult for the gene therapy to improve the vision."

That doesn't mean that researchers should give up hope on gene therapies for achromatopsia. Rather, they may just need to treat people sooner.

> "Vision is eye plus brain."
>
> *– Michael Hoffmann, Otto-von-Guericke University, Magdeburg*

The visual cortex matures during the first ten years of life, so if clinicians deliver achromatopsia gene therapies in children, they may be able to restore acuity and color vision when the brain is still capable of figuring out how to interpret those signals. This hypothesis aligns with McKyton's finding that the gene therapy was more effective in the young achromatopsia patient than in the three adult participants in her team's analysis (6).

Hoffmann would like to develop an anatomical MRI-based screening tool to identify patients who still have a malleable visual cortex and in whom a restorative gene therapy would be most effective.

Hoffmann hopes that what he and other vision scientists learn from studying achromatopsia will help them better understand the visual system as a whole and other related visual conditions. "One thing we would like to understand," he added, is "how you see the world with different eyes." ■

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An Immunological Window into the Brain

Researchers discovered an immunological connection between the eye and the brain that could lead to new therapeutics for central nervous system diseases.

BY ALLISON WHITTEN, PHD

TO PROCESS THE COMPLEX VISUAL WORLD, THE EYE connects to the brain through the optic nerve. Light enters the eye and quickly converts into electrical signals that flow along the optic nerve until they arrive at the occipital cortex. Scientists believed that this was the only direct connection between the eye and brain. Yet, when Eric Song, an immunologist and ophthalmology resident at Yale School of Medicine, and his colleagues began investigating where the drugs from eye injections end up, they found a previously hidden immunological nexus between the eye and the brain (1).

Song's team showed that the optic nerve also serves as a lymphatic drainage system that leads to shared immune responses between the eye and brain. Specifically, a compartment at the back of the eye drains from the optic nerve into the same lymph nodes in the neck that collect cerebrospinal fluid. The finding unlocks new opportunities to explore therapeutic treatments for diseases of the eye and central nervous system.

"This really opens the door to stop thinking about the eye and the brain as a signaling privileged organ and isolated organ, but an organ system that really interacts with each other through an immunological pathway," said Song.

In the new work, Song's team discovered that the lymphatic vessels that exist along the optic nerve sheath are conserved across diverse species in humans, primates, pigs, and even zebrafish. In mice, the researchers injected viral and bacterial intravitreal immunizations at the back of the eye and demonstrated that the immune responses were similar to those from local immunizations in the brain. They also showed that effective immune responses to brain tumors in mice could be generated via intravitreal immunizations.

"As you can imagine, we're not going to go around immunizing people into the eye just to have a protective immune response in the brain. So, we thought about in what kind of scenario … can we take advantage of this new finding?" said Song. They decided to test whether they could dampen immune responses that limit the effectiveness of retinal dystrophy gene therapy over time. By blocking the lymphatic signaling on the optic nerve in mice, they successfully reduced the immune response and improved the efficacy of gene therapy.

Now, Song's team is studying how the optic nerve sheath lymphatic system influences diseases of the central nervous system and how they can manipulate it for therapeutic benefits. "The lymphatic system is an interesting system because there's actually no pharmacological agents or drugs that can stimulate lymphatics," Song said.

The new results also invite investigations to probe whether the eye can alert the brain to incoming threats. "This is really the beginning of trying to understand how the eye can be a sensor for things that are happening in the central nervous system beyond just light sensing," said Song. "It's really exciting to know that there's still a lot to be discovered … It's right under our noses." ■

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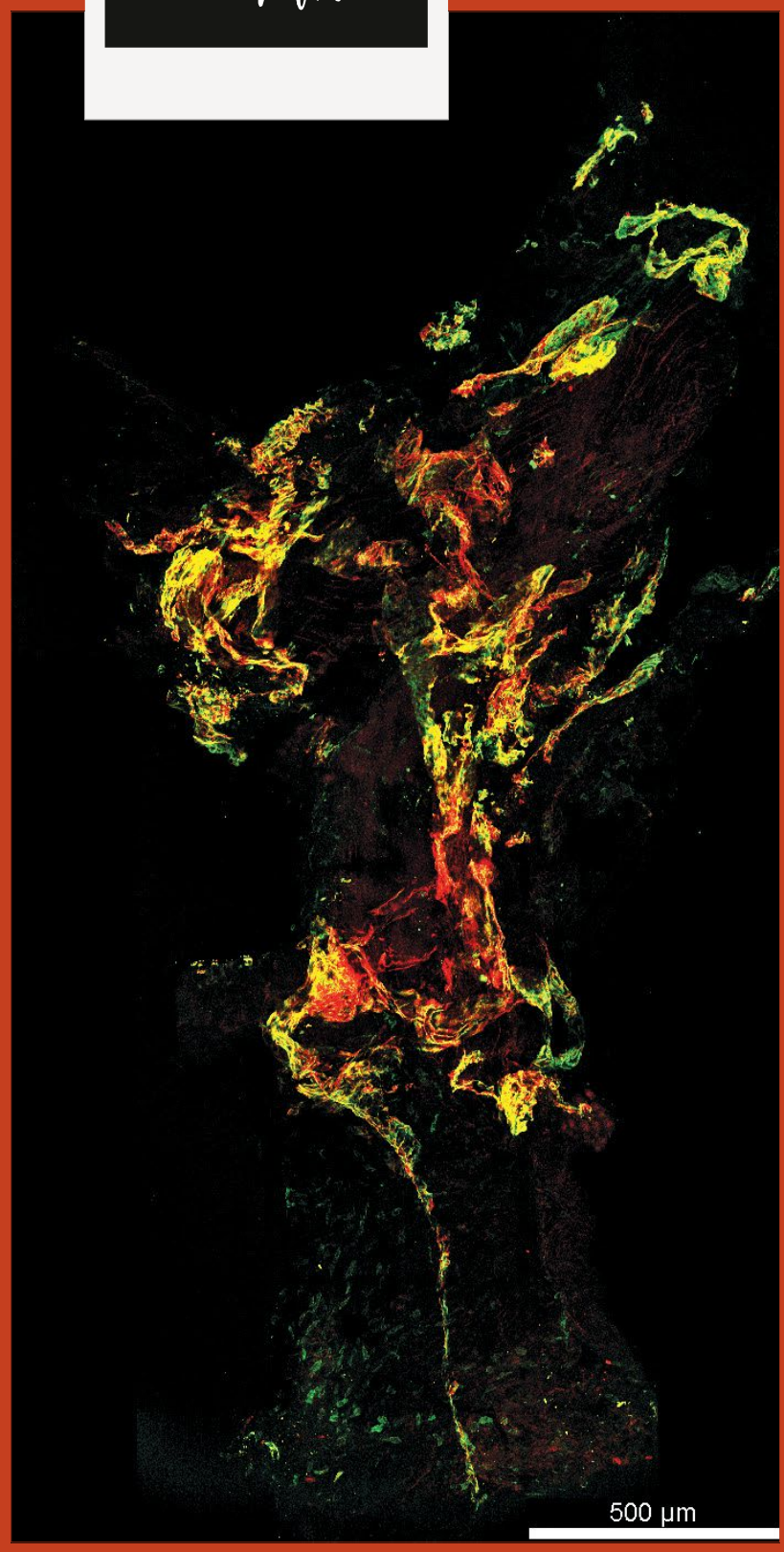
IMAGE of the month



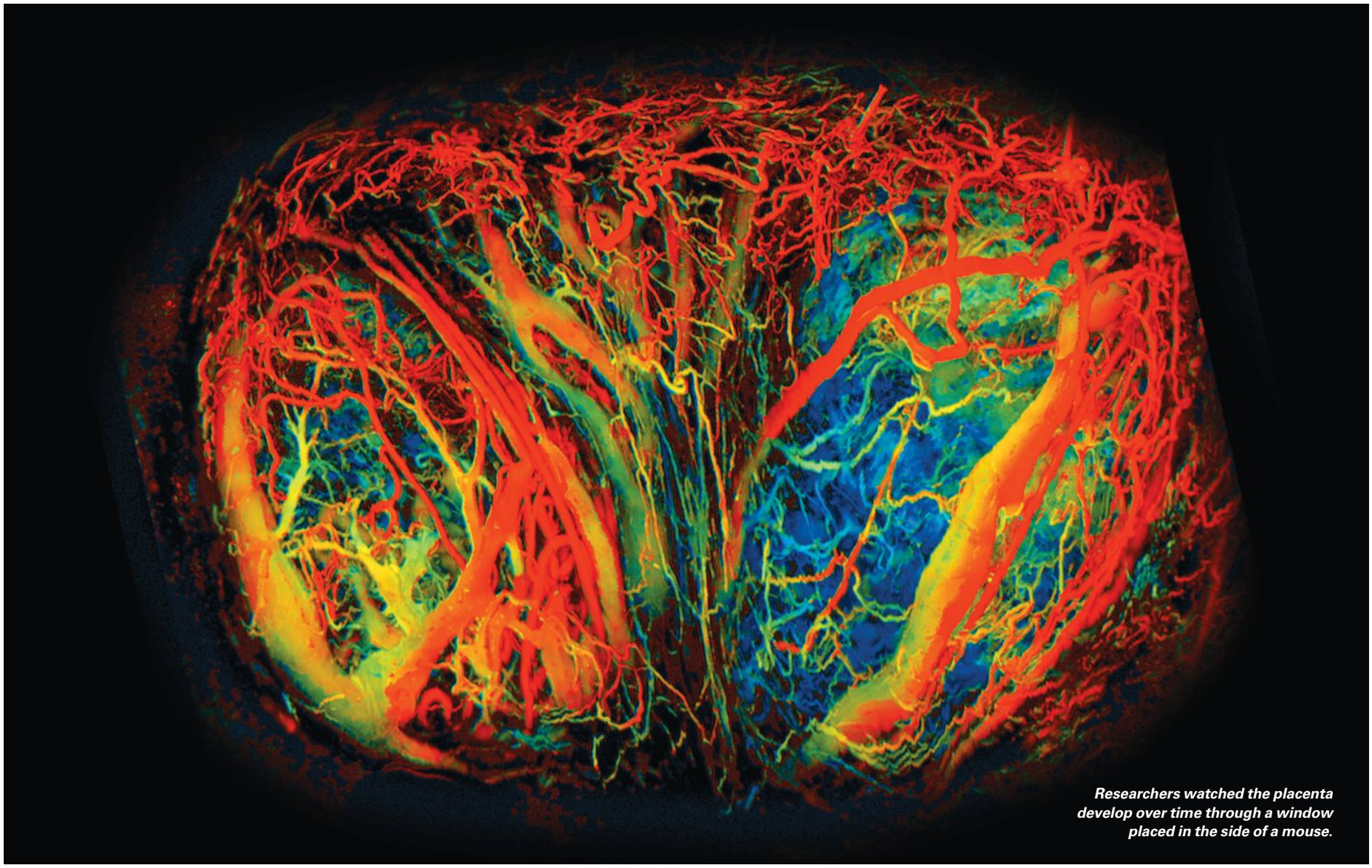
CREDIT: ERIC SONG

Researchers found that the optic nerve sheath that connects the eye and brain also functions as a lymphatic drainage system, shown here in a mouse. Lymphatic cell protein markers that are expressed in lymphatic vessels that lie inside the sheath are stained in green and red.

spatial biology

The Spatial Secrets of the Placenta

While vital for a healthy pregnancy, the placenta is not well understood. Researchers now take advantage of spatial biology approaches to plumb its secrets.



Researchers watched the placenta develop over time through a window placed in the side of a mouse.

CREDIT: JUNJIE YAO

BY STEPHANIE DEMARCO, PHD

FOR AN ORGAN ESSENTIAL TO LIFE, scientists know surprisingly little about the placenta. It may be a transient organ that only appears during pregnancy, but the placenta provides a vital link between the developing fetus and mom.

"Pregnancy is not a disease, but it's a physiologic state," said Yalda Afshar, a maternal-fetal medicine physician-scientist at the University of California, Los Angeles. "Unfortunately, in women's health, we're a little bit behind in understanding some of the basic biology behind one of the most common events."

During a healthy pregnancy, cells from the embryo called trophoblasts implant in the mucosal layer of the uterine wall, which turns into a structure called the decidua (1). Further specialized trophoblasts then invade the decidua, and the fetal cells begin to differentiate and grow to form the placenta.

> "Pregnancy is not a disease, but it's a physiologic state. Unfortunately, in women's health, we're a little bit behind in understanding some of the basic biology behind one of the most common events."
>
> *– Yalda Afshar, University of California, Los Angeles*

If trophoblasts don't infiltrate the decidua properly, life-threatening conditions can arise for both the fetus and the mother. These include preeclampsia, placenta previa, and placenta accreta spectrum disorder, among others. When conditions like these come about, there is very little that clinicians can do.

"The treatment options are really limited. Partially, it's because we don't really understand what's going on," said Junjie Yao, a biomedical engineer at Duke University.

To change that, researchers leverage advances in spatial biology techniques to investigate the placenta in more detail than ever before. Through innovative *in situ* imaging approaches, new computational tools, and single cell and spatial transcriptomics, scientists are determined to let the placenta remain a mystery no longer.

From frogs to placental windows

Before Yao began studying the placenta, he was enthralled with glass frogs. "These [are] very magical frogs from South America. They can become much more transparent when they're sleeping, so they can hide from their predators," Yao said.

How exactly these frogs became transparent was unknown, so Yao and his colleagues developed new imaging systems to figure it out. They discovered that the frogs removed the blood from their circulatory

systems and packed it into their livers while they slept (2).

As the head of a "very hardcore imaging lab," Yao explained that he and his research team develop new imaging technologies to answer unresolved biological questions; the mysterious glass frogs were just one example. One of his team's latest questions focused on how to get high-resolution images of the placenta over the course of development. One way to do this is to study placental development in an animal model using a window that researchers implant into the animal's abdomen.

"People have been developing windows for high resolution imaging, for example, for the brain, for other organs, liver, even the embryo itself," Yao said, but not for the placenta. "When I talk to pediatric surgeons, the answer is always that the placenta is very delicate."

Imaging windows exert pressure on the walls of the biological tissue they are sandwiched into and consequently on the placenta itself, which could affect its development. Windows can also allow too much heat to escape, leaving the placenta too cold. Finally, if not built with placental growth in mind, an imaging window could leave the placenta with too little space to develop completely.

Yao and his team, however, were up for the challenge.

They carefully designed their placental window to sit over one of a pregnant mouse's embryos, and they watched the placenta develop over the course of 12 days, from embryonic day seven to embryonic day 19 (3). All of the mice that were born after being observed in the placental window developed into healthy adult mice, indicating that the window did not adversely affect their long-term development.

With a successful imaging window in place, Yao and his team just needed the perfect imaging tool to study the placenta. They started with photoacoustic microscopy (PAM), which uses both light and sound to image deep inside tissues. In this technique, the researchers shoot a laser pulse at the tissue they want to image, and some photons from the laser get absorbed by the molecules in the tissue. Just like in a car parked outside in the summertime, the photons heat up the tissue. This increase in heat causes the molecules to expand and push against the neighboring molecules in the tissue. The pressure from one molecule on the other propagates as a pressure wave through the tissue, and the researchers detect it as a sound wave via an ultrasound.

"By doing the ultrasound detection, we can achieve a deeper imaging depth and better resolution, and you still keep the functional information of the light," Yao explained. "This whole process is really a physical combination of light and sound, but with their best merits. So, it's a great technology especially suitable for studying functions and molecular information of tissues."

Their next challenge was to find a way to use PAM on a sample that is not still. Because it is located deep within the body, the placenta is always moving. It's subject to motion from the embryo and the mother's breath, so Yao and his team developed a technique called ultrafast functional PAM imaging that could capture an image faster than the placenta moves. With their imaging technology and placental window ready to go, the team could finally see exactly how the mouse placenta changed over the course of a pregnancy.

"I was really amazed by the delicacy of this organ," said Yao. "The information is so rich, especially when you're looking at the early stages of placenta. I'm just so totally taken away by how complex the biology is, even for a transient organ."

Just as the human placenta develops in a hypoxic environment, Yao and his team observed that the mouse placenta does as well (4). This hypoxia signals to the placenta to form blood vessels out of the maternal arteries in the uterus, allowing the fetus access to nutrients and oxygen.

CREDIT: JUNJIE YAO

Junjie Yao and his team develop innovative imaging approaches to answer pressing biological questions, including how the placenta develops.

They then assessed how different factors affected the placenta, starting with alcohol exposure. While alcohol consumption during pregnancy is harmful to the fetus, alcohol's influence on placental development was not as well understood. The researchers injected a small amount of alcohol into the pregnant mouse's abdominal cavity, and over the course of ten minutes, they saw a dramatic increase in the oxygenation level in the placenta.

"That actually gives the placenta a wrong signal. They say, 'Ah, I have too much oxygen. No, I don't have to develop more blood vessels,' with this just a single sip. Imagine if it's a chronic drink," Yao said. "We always knew it was not good drinking alcohol during pregnancy. This is the first time we actually saw it clearly down to the single vessel level."

> "We always knew it was not good drinking alcohol during pregnancy. This is the first time we actually saw it clearly down to the single vessel level."
>
> *– Junjie Yao, Duke University*

Yao and his team also investigated how a model of maternal cardiac arrest and chronic inflammation affected the placenta as well as monitored how an adeno-associated virus, which is often used for gene therapy delivery, moved through the placenta. Moving forward, Yao wants to use this placental imaging system to study how other environmental stressors such as climate change and water pollutants such as PFAS influence the placenta and overall pregnancy.

Yao and his team plan to make their imaging system even better. "You cannot put everybody under the window, so we're developing technologies right now to do this [in a] totally non-invasive [way]," he said.

A placental Google Maps

When Roser Vento-Tormo, now a genomics and bioinformatics scientist at the Wellcome Sanger Institute, was a postdoctoral researcher, she became fascinated with the unique immune interface at the decidua and the placenta where maternal and fetal cells happily mingle without conflict.

"It was very interesting to know how the immune cells really make this happen. Instead of rejecting the tissue, they collaborate with the tissue to ensure the proper implantation and embryo development," she said. "That was the very first thing that got me into going into the placenta."

As a postdoctoral researcher Vento-Tormo used single cell transcriptomics to profile the transcriptomes of approximately 70,000 single cells from the placenta, which included fetal cells from the placenta, maternal immune cells in the decidua, and many other cell types (5). While her findings revealed the gene expression programs of cells present in the placenta and decidua, it lacked an important component: spatial information.

"Their identity depends on the spatial location," Vento-Tormo explained. As trophoblasts, the specialized placental cells, migrate in the decidua, they acquire new identities. "Having the spatial [data] allow us to understand the process and what are the cells doing," she said. She also wanted to know how the migrating trophoblasts interacted and communicated with maternal decidual cells as the placenta developed.

To answer those questions, Vento-Tormo and her colleagues performed both single cell transcriptomics and spatial transcriptomics on placental samples from early

CREDIT: ONUR PINAR/WELLCOME SANGER INSTITUTE

Using spatial and single cell transcriptomics, Roser Vento-Tormo and her team study the interactions between fetal and maternal cells during placental development.

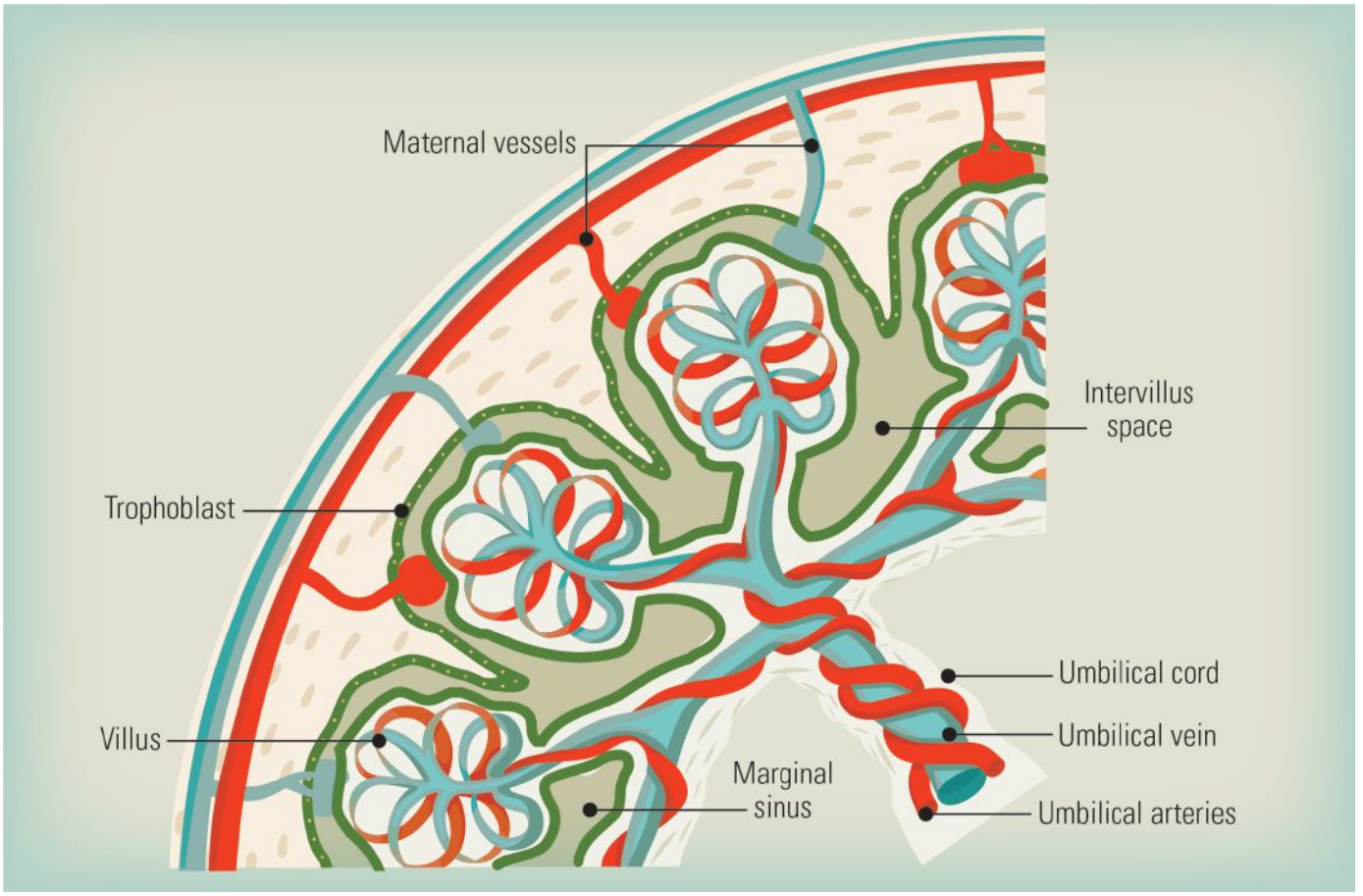
pregnancy that were collected by Ashley Moffett, a reproductive immunologist at Cambridge University (6). They acquired a wealth of spatial information, including how fetal placental and maternal decidual cells interacted with each other. In collaboration with Oliver Stegle's group at the European Molecular Biology Laboratory, Vento-Tormo and her team even developed a new statistical tool to model cell migration to create a complete map of placental cell migration and invasion into the uterus during the first trimester of pregnancy.

"This is like a Google Map," Vento-Tormo said. "We know what we have in the cell, all the details, but then we also have where it sits, who are the neighbors, and how it really forms an organized neighborhood."

They identified potential interactions between the placental cells and maternal immune cells, supporting prior research that showed that maternal immune cells support a healthy pregnancy, not hinder it. She and her team used this new single cell and spatial information to validate their *in vitro* placental models. Their new findings will inform *in vitro* placental models with even more complexity such as incorporating cells from two different individuals — mother and fetus — in the same model.

Now that she and her team have a map of healthy early placental development, Vento-Tormo wants to find out how those instructions go awry in diseases such as preeclampsia or placenta accreta. She is excited about the potential that single cell and spatial biology methods have to answer these kinds of questions.

"This is quite a black box, so we know very little. The good thing about using -omics is that it is unbiased," she said. "The thing that is most exciting [is] that we started uncovering the secret of this environment without really knowing much about what's happening."



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Trophoblast cells invade the decidua to allow the placenta to implant properly.

CREDIT: MARYAM RASSOULI

As a maternal-fetal medicine physician-scientist, Yalda Afshar takes care of pregnant people with placenta accreta and studies ways to diagnose and prevent this condition.

> "This is like a Google Map. We know what we have in the cell, all the details, but then we also have where it sits, who are the neighbors, and how it really forms an organized neighborhood."
>
> *– Roser Vento-Tormo, Wellcome Sanger Institute*

The seed and the soil in placenta accreta

During a normal birth, the mother delivers the child followed by the placenta, but sometimes the placenta invades the decidua so tightly that it doesn't detach when it should. This condition is called placenta accreta. It occurs in one in 272 births, and it can be deadly (7).

"Early detection of accreta is imperative to improve the outcome of that pregnant person because they need to be at a center with blood and expert surgeons," said Afshar. "If it's diagnosed in a center without those, women die from this because of hemorrhage."

The biggest risk factor for placenta accreta is history of a prior Cesarean (C-) section, but placenta previa (when the placenta attaches low in the uterus, sometimes covering the cervix) and *in vitro* fertilization are risk factors as well.

"My interest in placenta accreta is, at the end of the day, really inspired by the women I take care of in the clinic. They come to me scared and appropriately apprehensive of why them? And I don't know why them," Afshar said.

To unpack the molecular mechanisms that underly placenta accreta, Afshar and her colleagues took a single cell and spatial biology approach (8). They collected placentas at the time of birth from six patients with placenta accreta and six healthy individuals as controls. This protocol was no small feat, she added, "This is RNA work that you have to do really fresh and quickly, and it doesn't matter what time the baby's born."

For the placentas with placenta accreta, the researchers cut a two-centimeter cubed sample of the organ from the part that had adhered to the uterine wall and one from the non-adherent site. For the controls, they took the same sized sample from where the placenta had adhered normally to the uterine wall.

"The placenta is a giant organ, and a lot of work is done on just a tiny little histologic block. But there's so much difference in adherence in placenta accreta. There's one area that's adhered, so what's different about that adherent area versus a non-adherent part?" Afshar asked. "The power of spatial is really to be able to decouple that."

They found the most differences in gene expression between the cellular populations in the placenta accreta patients and the healthy controls at the sites of adherence, but there were also substantial differences between the adherent and non-adherent sites in the placenta accreta samples as well. They identified transcriptional differences in extracellular matrix genes, growth factors, and angiogenesis. In particular, none of the genes that they identified as upregulated in placenta accreta samples had ever been identified as contributing to the disease before, highlighting the value of the single cell approach.

"Historically, we always assumed it was the placenta that was this invasive organ that was the culprit, but now there is, I think, enough robust clinical and biological data that it's really not just the placenta," Afshar explained. The maternal decidua plays a significant role too. "It is some kind of lack of a stop signal and a loss of these boundary limits in the implanting pregnancy in that maternal environment that led to this high-risk pregnancy complication."

Afshar likened this phenomenon to a garden. If the placenta is a seed, "of course it's the decidua that is the soil, and the soil is perturbed."

She now plans to study the impaired pathways involved in placenta accreta using *in vitro* models, and she hopes to find surgical ways to prevent placenta accreta from developing in patients who give birth via C-section.

"All of this has implications way broader than accreta. It's scarring. It's fibrosis. How do we translate that to other fibrotic diseases? How do we translate that to better pregnancy outcomes in other normal and non-pathological states?" she asked. "I hope that this work really pushes to understanding why some people develop this, others don't, and approaches to blocking the abnormal placental growth in these regions of scarring." ■

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Bioprinting Tumors to Fight Them

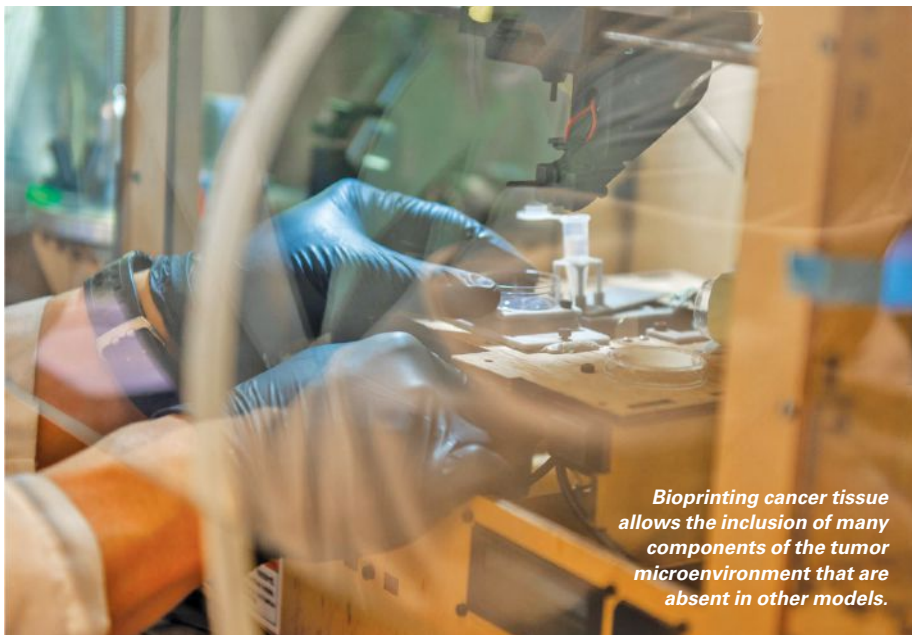
3D bioprinted models of the tumor microenvironment could make the preclinical research process more reliable and accelerate translation to the clinic.

BY ALEJANDRA MANJARREZ, PHD

A TUMOR IS MUCH MORE than a lump of cancer cells. It houses immune cells, blood vessels, and an extracellular matrix, which are key to the disease's development and progression. Yet, traditional flat cultures fail to capture the complexity of the tumor microenvironment and the interactions among its various components. While some 3D models such as organoids are better at mimicking this intricate ecosystem, they still lack some of these vital components and are not highly reproducible. Because of this, some scientists turned their attention to bioprinting technologies.

3D bioprinters operate much like traditional 3D printers, building solid objects by layering material in a predesigned pattern. The twist lies in the "bio" of bioprinting, where bioinks replace traditional inks. Instead of thermoplastic materials, bioinks consist of living cells, active molecules, or biomaterials such as alginate or collagen. Ideally, bioinks mimic the mechanical and biological properties of the target tissues, so to model tumors, researchers need to know the cell types that exist in the cancer microenvironment as well as its extracellular matrix components.

Replicating the heterogeneous architecture of solid cancerous tumors opens a world of possibilities for exploring tumor biology. For example, researchers can investigate the individual or combined roles of each cell type in tumor growth, aggressiveness, or metastatic properties. The approach may help scientists identify the components within this microenvironment that contribute to therapy resistance or that induce blood vessel formation within the tumor. Furthermore, bioprinted models could serve as platforms for screening drugs in a high-throughput manner, assessing their efficacy, and contributing to the development of precision medicine approaches.



Bioprinting cancer tissue allows the inclusion of many components of the tumor microenvironment that are absent in other models.

CREDIT: KATE MYERS, PENN STATE

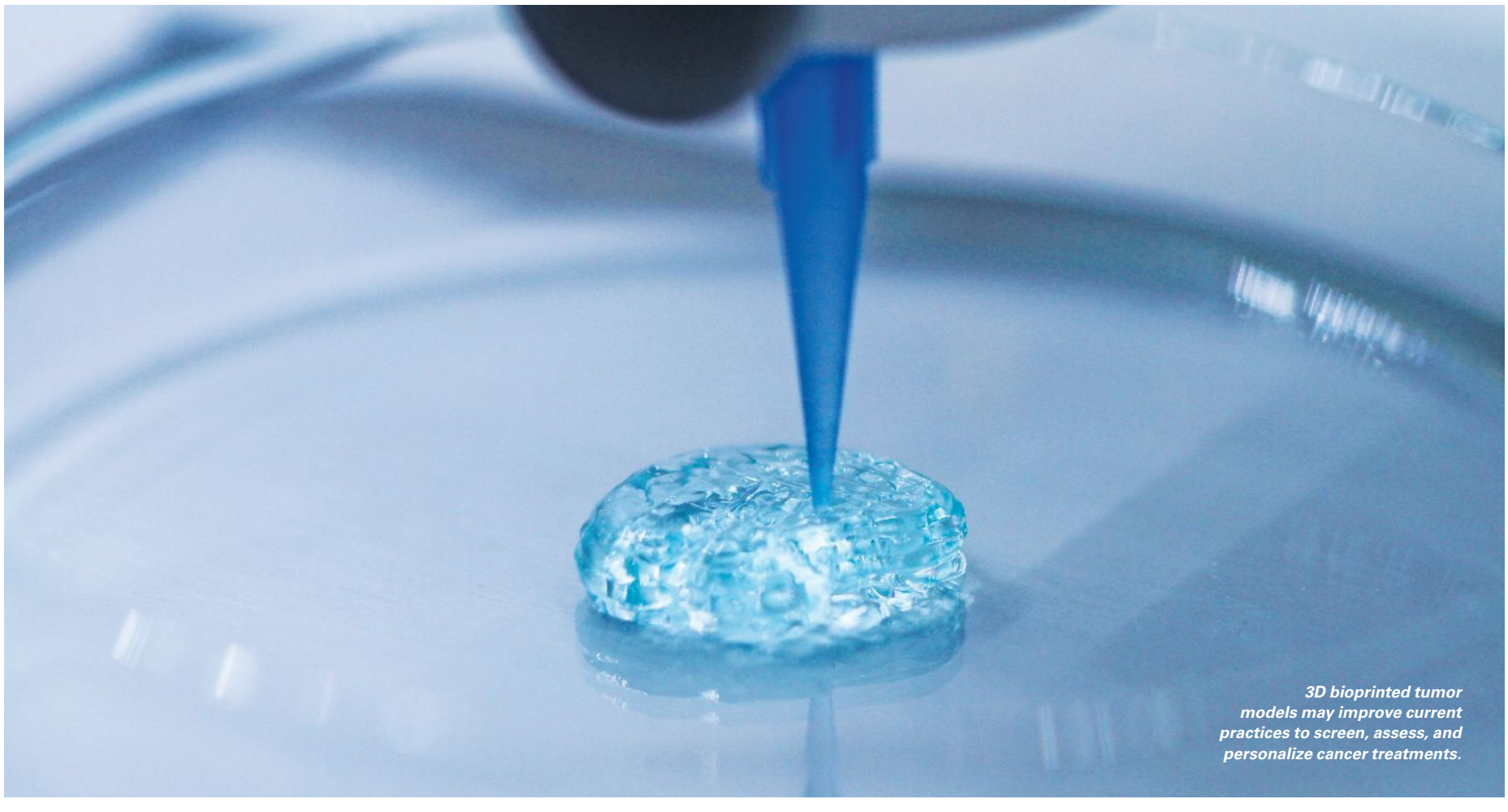
> "When I saw a bioprinter, I thought it was an interesting way of being able to make more tissues in a shorter period of time."
>
> *– Stephanie Willerth, University of Victoria*

Beyond flat and round cultures: the power of ink

While growing human cancer cell lines in a dish is a practical way to study cancer physiology, experiments in these oversimplified models may not always translate successfully to animal models, much less to clinical applications. The advantages of bioprinted models over flat cultures might seem evident, but what about other non-printed 3D models, such as organoids or spheroids?

For many years, University of Victoria's biomedical engineer and bioprinting expert Stephanie Willerth has engineered 3D neural tissue from stem cells to study neurological disorders such as Alzheimer's and Parkinson's diseases. But before moving into bioprinting technologies, her team created these structures manually. For example, they used a pipet to encapsulate spheroids into 3D scaffolds. "When I saw a bioprinter, I thought it was an interesting way of being able to make more tissues in a shorter period of time," she said. "I was also intrigued by the idea of actually placing certain types of cells on certain areas."

Controlling the location of each cell subpopulation is one of the key advantages of bioprinted models compared to other 3D techniques. It allows for a more precise model architecture than that achieved in organoids, biomaterial scaffolds, or cancer-on-chip systems (1). In bioprinted models, "You can control cell-cell interactions by facilitating the right positions of specific cell types," said İbrahim Özbolat, a bioengineer and bioprinting specialist at



3D bioprinted tumor models may improve current practices to screen, assess, and personalize cancer treatments.

CREDIT: AXOLOTL BIOSCIENCES

Pennsylvania State University. This predefined local arrangement also makes bioprinted 3D structures more homogeneous compared to organoids, he said. Willerth added, "[Bioprinted models] tend to be more reproducible than organoids."

Other 3D models also may miss some components of the tumor microenvironment. They might include fewer cell types or lack the extracellular matrix (2). Many of these models also fail to accurately mimic how blood vessels grow and infiltrate the tumor (2,3). In contrast, "You can induce vascularization with the 3D bioprinting," Özbolat noted, which is another key advantage. While it is also possible to manually achieve vascularization in organoids, he acknowledged, the controlled printing approach allows extra capabilities. "You can build some channel structures where you can perfuse and make blood vessel-like organization."

However, 3D bioprinted models also have limitations and fail to represent other tumor properties. The native tumor starts with the gradual growth of cancer stem cells, and throughout that process, it recruits other cell types from the neighborhood. "Everything has some spatial as well as some temporal interactions, versus in the printing process, [where] you have all the cell types and then you put all these together," Özbolat said. The model may recapitulate the spatial arrangement, but not the temporal one. "You still miss that important aspect in your 3D printed model," he noted.

Bioprinting versus animal testing

Animal models are another vital tool in cancer research, but they do not entirely represent human physiology. "One of the main ways people test anti-cancer therapeutics right now is to put a tumor in a mouse that lacks an immune system," said Willerth. "But the immune system plays a huge role, whether it's regulated or dysregulated, on how we respond to cancer. So, it's not a very good system for testing these drugs."

The use of animal models also raises ethical concerns (4). In this sense, some experts view bioprinted structures as a means to reduce and potentially replace animal experimentation (5). In addition to facilitating the inclusion of healthy human tissue, these structures reduce experimentation time and costs and offer greater control.

Özbolat emphasized that, for now, researchers still need to test most cancer therapies in animal models before moving into clinical translation. "There are a lot of different things contributing to cancer that exist in the live body versus in the 3D model," he said. "We're not really 100 percent sure about the 3D model, its capabilities, or its relevance to native biology, so that's why we still have some more confidence if we test on the animal model as well."

Printing tumors for refining cancer therapies

In the landscape of cancer drug development where the probability of success for drugs entering clinical trials is below ten percent, a model with the characteristics of bioprinted tumors is promising (6,7). For instance, high-throughput drug screening in these 3D models would enable a faster transition to the animal testing phase in addition to offering higher confidence for drug efficacy (8).

Since the properties of the extracellular matrix and other components of the tumor microenvironment influence drug diffusion, bioprinted tumors offer superior models for testing therapies compared to flat cell cultures.

Researchers can use cancer cell lines or patient-derived cells to conduct bioprinting experiments.

CREDIT: KATE MYERS, PENN STATE

> "There are a lot of different things contributing to cancer that exist in the live body versus in the 3D model."
>
> *– İbrahim Özbolat, Pennsylvania State University*

Based on several studies on printed tumors, researchers have already questioned findings from 2D experiments, particularly regarding treatment sensitivity and resistance. For example, many chemotherapy drugs such as cisplatin and 5-fluorouracil are highly effective at killing cultured cancer cells in a monolayer, but their efficacy reduces both in 3D models and in mice with patient-derived tumors (9,10).

Willerth and her colleagues have also explored how drugs behave using different modeling structures. Her team has a strong focus on neurodegenerative diseases, but they got into bioprinting cancer models when Chris Lee, who was a talented undergraduate student at the time, approached her to join the lab. "He really wanted to work on cancer, so we decided to work on brain cancer."

The team developed a bioprinted glioblastoma tumor model (11). "We made a printable version of fibrin, which is the protein that clots your blood," explained Willerth, who is also the cofounder and chief executive officer of the bioink developer company Axolotl Biosciences. "We got really huge, lovely tumors," she added.

The team tested the glioblastoma cell responses to a small molecule cocktail that aims to reprogram cancer cells into non-proliferating neurons. While the treatment impaired the cells' proliferation abilities in the 3D tumor, the cancer cells showed higher resistance to the treatment compared to those grown in a dish (12). "It's super easy to kill cancer in 2D; it's much harder to kill it when it's a tumor," Willerth said.

Her team plans to continue comparing cell responses in such different settings for other drug treatments (13). "Those comparisons are really important to make for [3D bioprinted] models really to be adoptable," she argued.

Bioprinted models may also aid in understanding why some therapies are ineffective against certain cancers and in devising ways to improve them. CAR T cell therapies, for example, are great at fighting blood cancers, but they are not as effective against solid tumors. In blood cancers, doctors inject the T cells directly into the patient, and the engineered cells circulate throughout the body to meet, interact with, and kill the cancer cells. For solid tumors, T cells need to find the cancer cells and then infiltrate into a very dense environment. Özbolat compared this search to a person looking for a gas station in different surroundings. "You can always find a gas station next to the highway easier [than] in the middle of downtown," he said.

Özbolat and his colleagues were interested in modeling the crowded environment CAR T cells face when treating solid tumors. They bioprinted a breast tumor that included various representative cell types, such as vein endothelial cells and vasculature (14). They then perfused the 3D model with CAR T cells. They varied the number of CAR T cells they delivered, treatment duration, and target antigens. By modeling these processes, they learned how the CAR T cells attach to the innermost layer of the blood vessels and then migrate to finally invade the tumor and kill it. Since infiltration is one of the main limitations of CAR T cell therapies for treating solid tumors, a better understanding of this process in different treatment conditions may help improve these therapies.

The next question is whether scientists can develop more personalized therapies based on these 3D models, Özbolat said, for example, by printing tumors derived from patient-specific cells. This approach could help predict the dosage and the drug cocktail for each person or identify a cancer's susceptibility or resistance to a given therapy (15). Tumor bioprinting, therefore, holds the promise to potentially guide clinical decisions towards optimal cancer treatments in a timely and more efficient manner than current practices. ■

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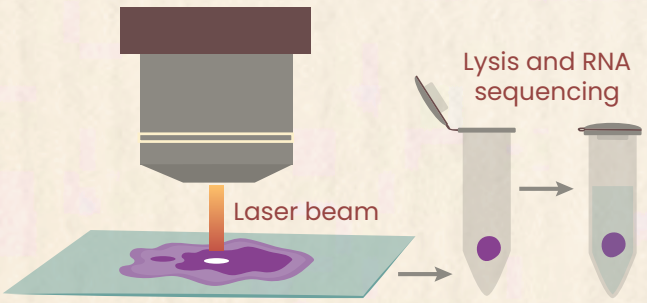
CHARTING A CELLULAR TREASURE MAP WITH spatial transcriptomics

EXPLORING DRUG DISCOVERY AND DEVELOPMENT

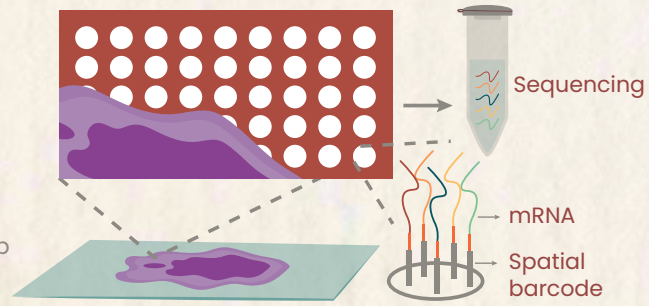
By Yuning Wang, PhD | Illustrated By Alisha Vroom

Just like a treasure map leads explorers to hidden riches, knowing the precise location of gene expression within cells and tissues can guide researchers to uncover many biological mysteries. In the past, scientists could only collect fragmented pieces of this map through traditional RNA sequencing, lacking the vital spatial context. Now, with spatial transcriptomics technologies, researchers are charting detailed landscapes of gene expression and cellular organization across various tissues, unlocking a treasure trove of knowledge about health and disease.



SPATIAL MICROARRAYS

This technique involves mounting the tissue section onto a microarray coated with thousands to millions of spatially barcoded oligonucleotide probes. These probes capture RNA molecules from the tissue onto distinct spots on the array. Scientists then sequence the RNA and map the gene expression patterns back to their original locations in the tissue (2,3).



MICRODISSECTION

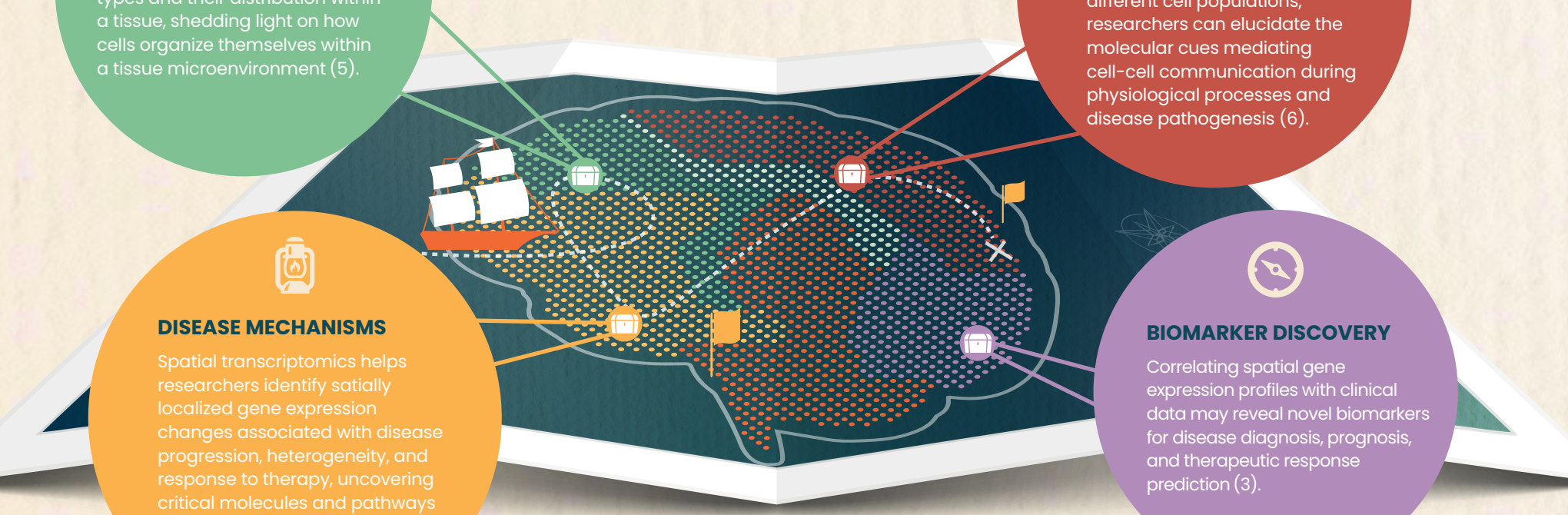
Using an infrared laser, scientists cut out specific regions from the tissue section under the microscope while ensuring that the surrounding tissue remains unaffected. They then extract RNA from these isolated cells and analyze their gene expression using techniques such as high throughput RNA sequencing (1).

CELLULAR HETEROGENEITY AND TISSUE ARCHITECTURE

Researchers can pinpoint cell types and their distribution within a tissue, shedding light on how cells organize themselves within a tissue microenvironment (5).

CELL-CELL INTERACTIONS

By analyzing the expression of signaling molecules, receptors, and cell adhesion proteins across different cell populations, researchers can elucidate the molecular cues mediating cell-cell communication during physiological processes and disease pathogenesis (6).



DISEASE MECHANISMS

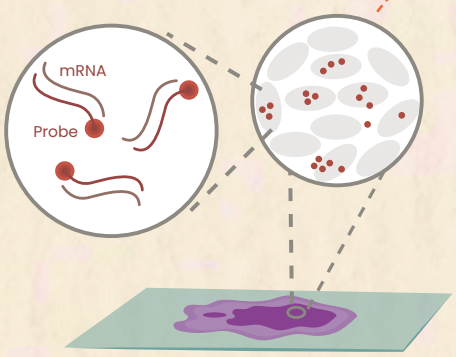
Spatial transcriptomics helps researchers identify satially localized gene expression changes associated with disease progression, heterogeneity, and response to therapy, uncovering critical molecules and pathways driving pathology (3).

BIOMARKER DISCOVERY

Correlating spatial gene expression profiles with clinical data may reveal novel biomarkers for disease diagnosis, prognosis, and therapeutic response prediction (3).

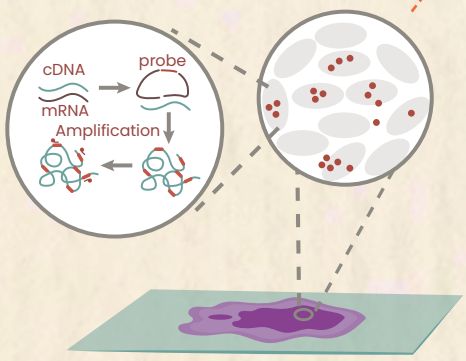
IN SITU HYBRIDIZATION

Scientists design fluorescently labeled oligonucleotide probes that target specific RNA sequences of interest and apply them to the tissue section, where they bind to their target RNA. Using fluorescence microscopy, they visualize the bound probes, mapping gene expression patterns across different cell types and tissue regions (3).



IN SITU SEQUENCING

After reverse transcribing RNA molecules in the tissue into complementary DNA (cDNA) templates, scientists hybridize targeted probes to specific cDNA sequences and amplify and sequence them *in situ*. During sequencing, they use fluorescently labeled nucleotides that bind to specific DNA bases, each emitting a unique color. Analyzing fluorescence signals enables researchers to determine RNA sequences and their spatial locations within the tissue (4).



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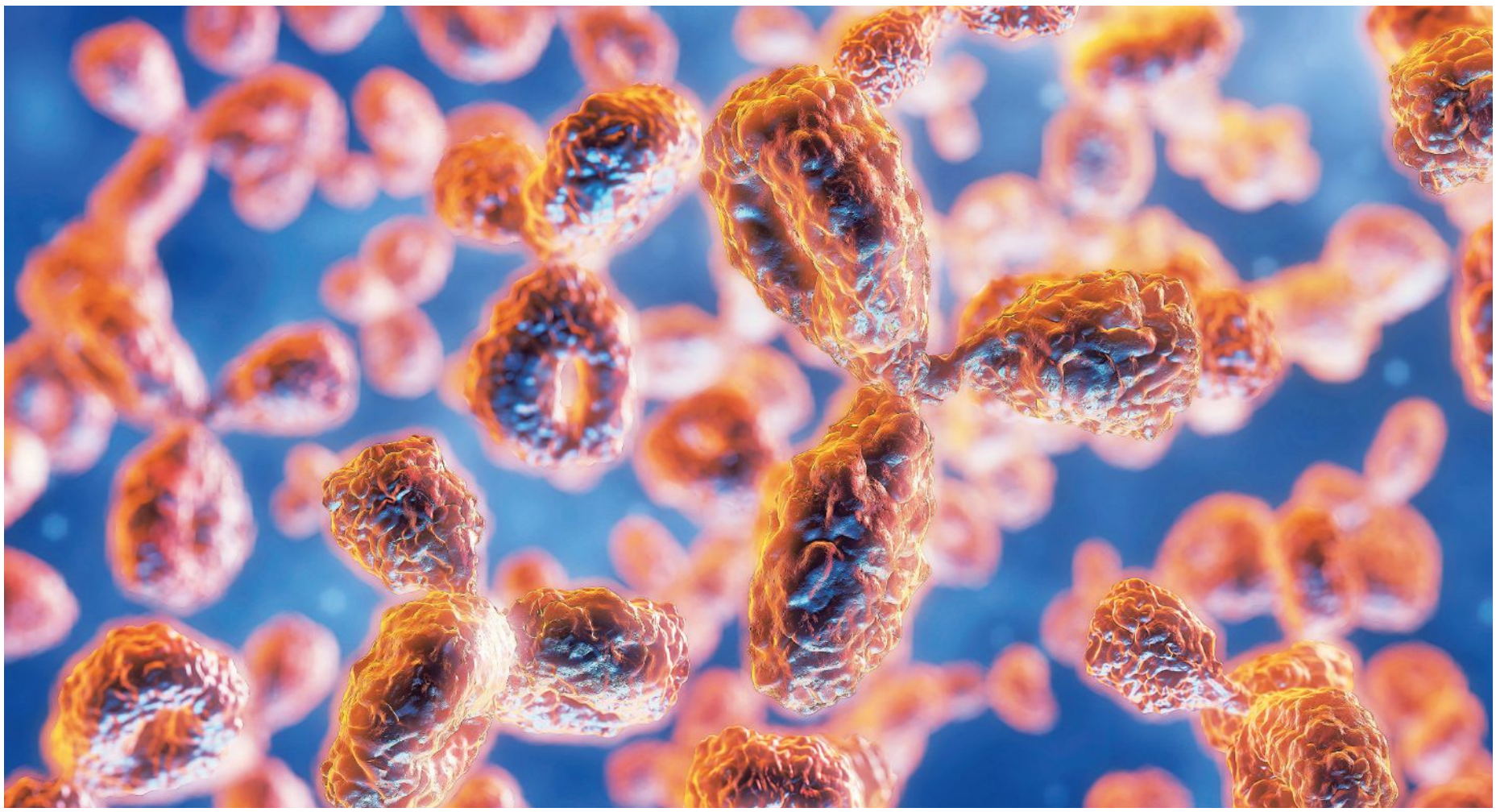
CAR T Cells in a SNAP

A modular CAR T cell could make cancer therapy safer and more effective.



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Lohmueller's team engineered cells to express a SNAP-CAR on their surface so that antibodies added separately could bind to the SNAP-CAR and make it recognize tumor antigens.

BY APARNA NATHAN, PHD

TEN YEARS AGO, CHIMERIC antigen receptors (CAR) promised to change the landscape of cancer therapy forever (1). With the help of genetic engineering, a person's own immune cells could get retrofitted with a surface receptor that recognized cancer antigens. This would muster an immune response against cancer cells, training the body to defend itself from malignancy.

Even with a handful of FDA-approved CAR therapies — largely using a patient's own T cells to go after blood cancers — drastically changing the treatment landscape for some of the most challenging conditions, the field is now reckoning with the limitations of the approach. "One of the caveats of CAR T cell therapy is that this is a living drug," said Daniel Powell Jr., a cancer biologist at the University of Pennsylvania. "Once it's administered to patients, we have no means to control the activity."

Acknowledging that the original architecture may not have been flexible or controllable enough for an effective cancer treatment, researchers are now going back to the drawing board to rethink CAR designs and capabilities. A new paradigm is the "universal CAR," a deconstructed CAR that researchers can customize to go after a nearly unlimited range of antigens.

CREDIT: AIMEE OBIDZINSKI/UNIVERSITY OF PITTSBURGH

Jason Lohmueller developed the SNAP-CAR system to make more potent universal CAR T cells.

"It could be a very personalized therapeutic way down the line. You can screen a patient's tumor, figure out what antigens they have, and figure out what [antibody] adaptors to use, all using the same CAR T cell."

— Jason Lohmueller, University of Pittsburgh

In 2023, Jason Lohmueller, a synthetic biologist at the University of Pittsburgh developed a new way to engineer universal CAR: SNAP-CAR (2). These modular CAR provide a way to make immunotherapy less toxic while keeping pace with rapidly evolving cancers. Alongside Coeptis Therapeutics, a biotechnology company that has licensed the technology, Lohmueller hopes that bringing this approach to the clinic will make CAR therapies a more effective option for more patients.

A universal solution?

In the early 2010s, the first conventional CAR-based cell therapies raced toward the clinic. The CAR T cells that achieved early successes in clinical trials for treating blood cancers were part of the second generation, wherein engineered cells expressed a cancer-antigen-specific receptor attached to a T cell

surface protein, alongside a costimulatory molecule such as CD28 or 4-1BB to make the cells even more potent (1). However, this approach wasn't flawless.

The first FDA-approved CAR T cell therapy, Kymriah, targeted a B cell surface protein called CD19 (3). B cells proliferate wildly in patients with cancers such as acute lymphoblastic leukemia, and these CAR T cells used CD19 to find and kill these cells to control the cancer. But if even a few cancerous B cells don't express CD19, those cells can evade the CAR T cell therapy and drive cancer relapse. This process, called antigen loss or antigen escape, limits the long-term effectiveness of CAR therapies; in CD19 CAR clinical trials, as many as 50 percent of participants ultimately relapsed (4).

CAR therapies can also have nasty side effects. CD19, for example, is on the surface of both cancerous and healthy B cells. When anti-CD19 CAR T cells kill healthy B cells, patients require transfusions of immune molecules to maintain their defenses against infections. For other organs, it isn't as easy to compensate for killing healthy cells.

Powell suspected that these limitations of CAR T cells could be addressed with a relatively simple change: uncoupling the internal molecular pathways mediating the immune response from the external receptors that recognize a cancer antigen. By separating the functional elements of a CAR T cell, physicians could turn it on or off or redirect it toward a new antigen by modifying just its external components.

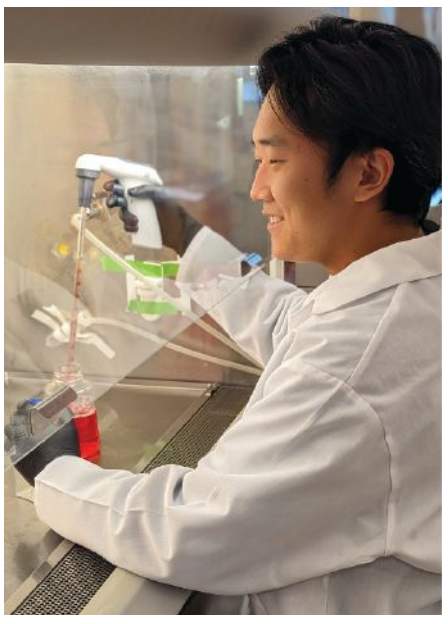
In 2012, Powell's team first proposed the idea of universal CAR in a paper presenting a modular system based on a pair of molecules, biotin and avidin, that bind to each other (5). The researchers engineered the T cells to express avidin on their surfaces, attached to internal proteins that could trigger an immune attack. They also engineered biotin-labeled antibodies that targeted an antigen of interest. By administering the T cells alongside the antibodies, the antibodies' biotin tags would bind to the T cell's avidin molecules to essentially reconstruct a functional CAR T cell with more flexibility to determine its target.

Powell hoped that this would help avoid antigen escape. Rather than just targeting CD19, the researchers could administer antibodies that bind multiple leukemia antigens so that the CAR T cells have multiple ways to identify and kill cancer cells.

"You're responding to the evolution of the cancer by creating additional agents that allow you to target new antigens," he said. "That's probably the single biggest benefit."

CREDIT: JASON LOHMUELLER

Avani Parikh, a graduate student in the Lohmueller group, helped test SNAP-CAR T cells' cancer-killing abilities.



CREDIT: JASON LOHMUELLER

Victor So, a graduate student in Lohmueller's laboratory, helped develop SNAP-CAR T cells.

"The therapy is really waiting for a patient as opposed to a patient waiting for the therapy."

– Colleen Delaney, Coeptis Therapeutics

Better building blocks

Lohmueller heard about Powell's universal CAR when he was a graduate student at Harvard University, and he was intrigued by its potential. "This is much better than the alternative of making two different CAR T cell products that recognize different antigens because it's double the work," he said. "[With this] incredible technology, you can have plug-and-play targeting of CAR T cells."

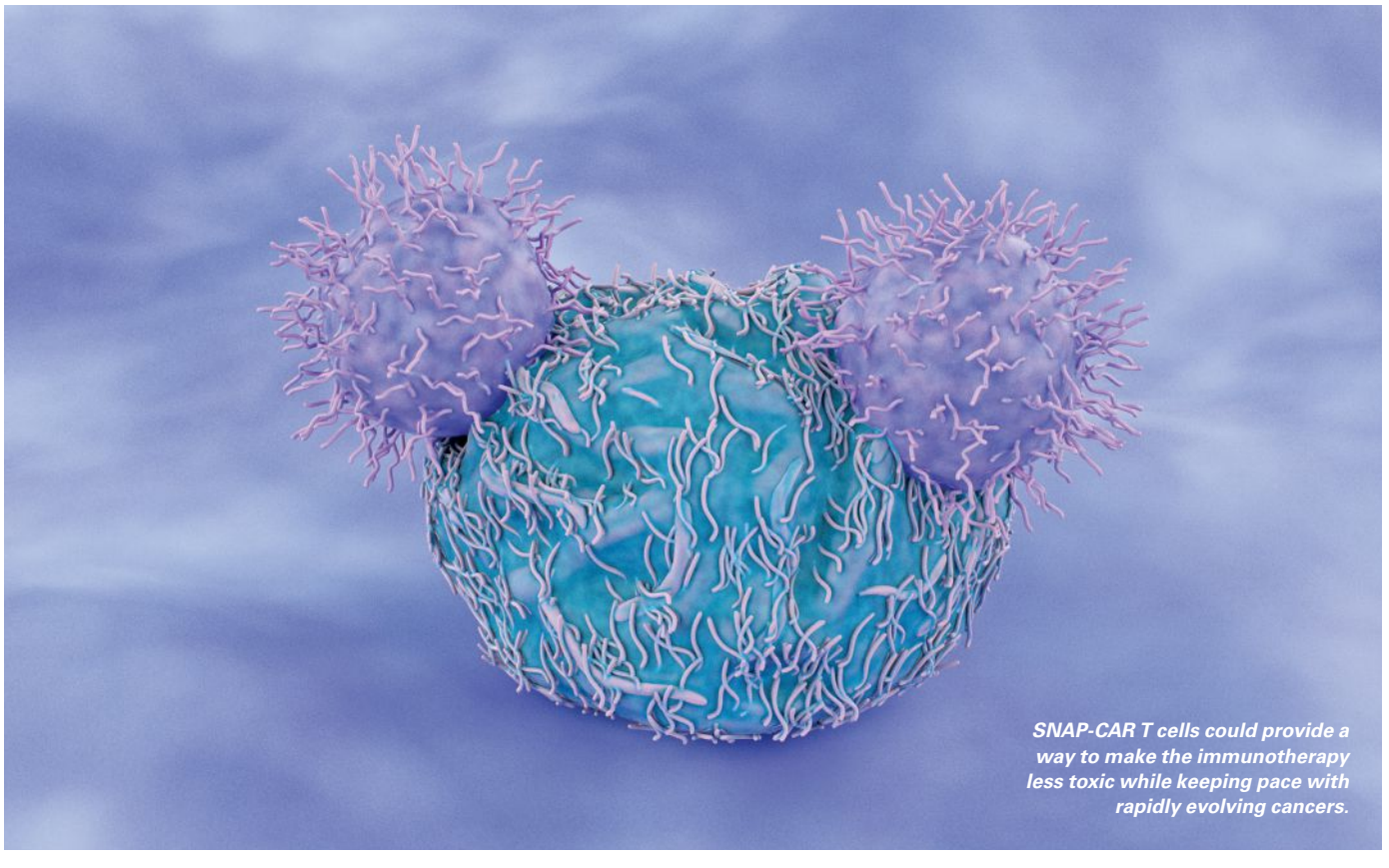
When he tried to use existing universal CAR approaches to develop CAR T cells targeting mucin 1 (MUC1), an antigen found on many types of cancer, it wasn't as effective as he had hoped. He turned his attention to designing more potent universal CAR. This required increasing the affinity between the T cell and the antibody. At first, he just tried tweaking avidin and biotin to make them bind more tightly, but he realized that the CAR activity would always be limited by intermittent binding of avidin and biotin (6).

To maximize activity, he needed to permanently attach the antibody to the T cell with a covalent bond — something that Powell's team had also begun to incorporate into their universal CAR (7). "It mimics the natural traditional CAR [fusion proteins] in being attached," he said. "By using that covalent bond, we can achieve more traditional CAR-like activity with a universal system."

To accomplish this, his team used the SNAP-tag system that had been previously developed to make fusion proteins held together by a covalent bond (8). SNAP is a self-labeling enzyme. When it sees its target molecule, benzylguanine, it attaches itself to the target. SNAP could take the place of avidin in the recipe for universal CAR, and benzylguanine could replace biotin. When benzylguanine-tagged antibodies encountered SNAP on a T cell's surface, they covalently attached to the T cell to form a functional CAR that could recognize a target antigen and trigger the T cell to release cell-killing molecules.

In a 2023 study, Lohmueller's team found that SNAP-CAR T cells could successfully fight a tumor in a mouse model (2). Importantly, however, SNAP-CAR also offered a path to tuning the potency of the therapy by increasing or decreasing the levels of the antigen-targeting antibody. "If we titrate the amount of [antibody] adaptor, we can get different levels of tumor cell killing," Lohmueller said. "It's really nice to have that extra control."

Lohmueller also noted that the SNAP-CAR system accommodates multiple antibodies to target a wider range of antigens. The limit of how many antibodies can be used for one patient is still unknown. However, he proposed that these antibodies could be administered all at once to target multiple antigens simultaneously or in sequence, depending on how the cancer evolves during treatment. After all, the antibody building blocks of these CAR T cells are dynamic. After being infused into the patient, they float around for weeks on average, and even the antibodies that bind to the T cells only remain active there for a few days. This gives physicians the opportunity to continually update the mixture of antibodies available to form CAR T cells in the patient.



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SNAP-CAR T cells could provide a way to make the immunotherapy less toxic while keeping pace with rapidly evolving cancers.

"It could be a very personalized therapeutic way down the line," Lohmueller said. "You can screen a patient's tumor, figure out what antigens they have, and figure out what [antibody] adaptors to use, all using the same CAR T cell."

New frontiers for SNAP

Even before the study was published, Lohmueller's SNAP-CAR technology attracted Coeptis Therapeutics' attention. In 2022, the company, which focuses on developing cell therapies, entered into an agreement with the University of Pittsburgh to license the technology for the development of SNAP-CAR therapeutics. "There's a clear attraction to therapies that could be broadly applicable," said Colleen Delaney, chief scientific and medical officer of Coeptis Therapeutics and an oncologist at the University of Washington.

In particular, Coeptis Therapeutics is interested in making off-the-shelf allogeneic SNAP-CAR therapies that can be used for any patient. Typically, cells are extracted from a patient, engineered to express the CAR, and infused back into the patient to avoid immune rejection of cells from a different donor. But this autologous approach is time-consuming and expensive. An allogeneic therapy could be manufactured in bulk in advance and distributed to hospitals or pharmacies for many different patients to use. "The therapy is really waiting for a patient as opposed to a patient waiting for the therapy," Delaney said.

SNAP-CAR add another dimension to the flexibility of an allogeneic therapy: Now, one therapy can be used for many patients with many diseases, as well, since the CAR wouldn't have a pre-defined target.

There are still many hurdles to making allogeneic T cell therapies because T cells have receptors that can trigger graft-versus-host disease (GVHD) when infused into a different person (9). While Lohmueller's team continues to engineer T cells, Coeptis Therapeutics focuses on a different type of immune cell: natural killer (NK) cells. These cells are easier to transplant from one person into another without triggering GVHD. Phase 1/2 clinical trials have already shown that CAR NK cells can target cancer with minimal side effects, and SNAP-CAR could make these therapies even more versatile, Delaney said (10).

"In the future, you could create master lots of NK cells that are expressing universal CAR and utilize them to treat tens or hundreds of patients," Powell said.

Coeptis Therapeutics is currently optimizing SNAP CAR for NK cells. They start with stem cells that the researchers differentiate into NK cells, a process that is modeled after an allogeneic cell therapy platform that Delaney previously developed at the University of Washington.

The team is testing lentivirus and retrovirus vectors to deliver the DNA encoding the SNAP protein and its internal components into the cells. But viral vectors can be an expensive and scarce resources. To reduce the amount and the cost of virus required, Coeptis Therapeutics is transfecting the stem cells early in their differentiation phase when the number of cells is still low. They've found that the SNAP-CAR proteins persist in the NK cells as they differentiate, allowing them to create batches of allogeneic SNAP-CAR NK cells that can be repeatedly infused into patients alongside antibodies to point the cells at their targets.

Once they optimize the system and test it in animal models of leukemia and solid tumors, the Coeptis Therapeutics team hopes to apply for Investigational New Drug status within the next 24 months. Delaney thinks Coeptis Therapeutics' approach will stand out in a crowded field of CAR therapies. "A lot of people are doing this, so what I hope to get across is that we're going to do this in a more efficacious way," Delaney said.

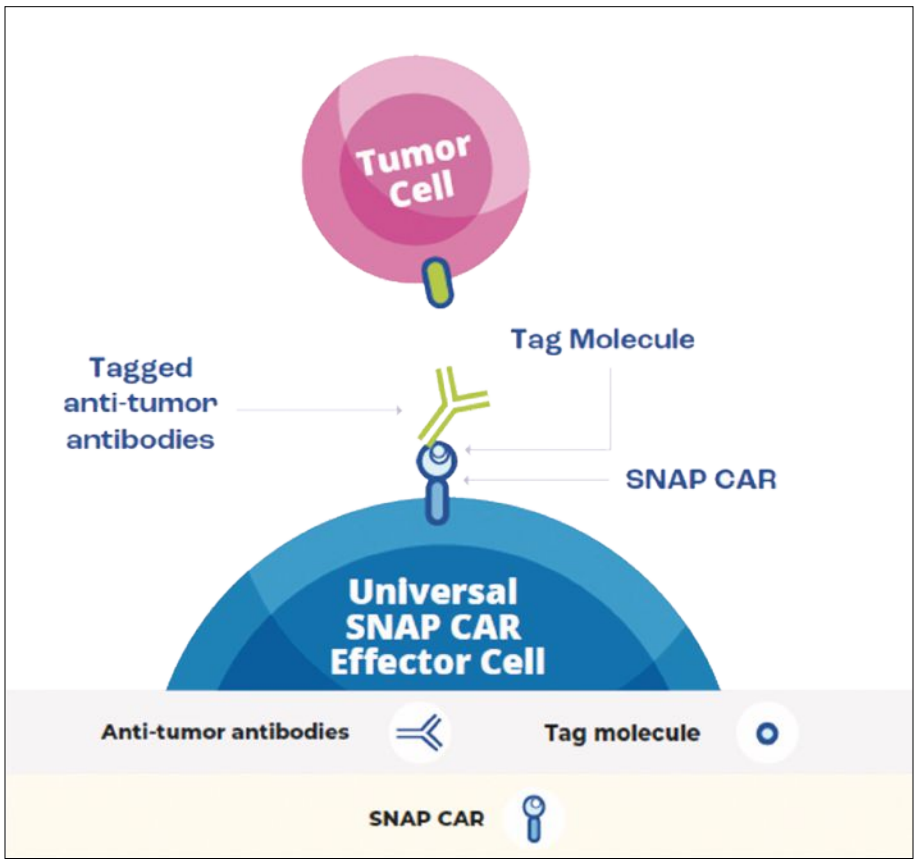
From bench to bedside

For SNAP-CAR cell therapies to make it to the clinic, researchers will have to show that they can work at least as well as conventional CAR. Lohmueller said that the data already show that, and now he hopes to demonstrate that SNAP-CAR can do even more. For example, conventional CAR can have off-target effects that lead to toxicity, but Lohmueller recently showed that it's possible to design the covalent link between the antibody and SNAP protein so that it can be severed by an "off switch," such as UV light or a small molecule (11).

Similarly, the researchers can build in "on-switches," for example, so that the antibody only binds to the SNAP protein in the low-oxygen conditions found in a tumor. "We can do all kinds of cool chemistry to make that chemical tag available for binding," Lohmueller said. Even though allogeneic T cell products are more challenging to develop, he still believes that would be the ideal final product, not only for cancer treatment. "You can have it ready to go to tailor to whatever disease you have," Lohmueller said.

Coeptis Therapeutics licensed the SNAP CAR technology for autoimmune indications as well. For example, in lupus nephritis, where B cells attack the patient's own cells, researchers tested whether CAR that can target CD19 could offer a new treatment avenue. According to Delaney, SNAP CAR could boost these therapies and might even help treat infectious diseases in immunocompromised people.

This is an area where Delaney has seen the need firsthand as a practicing physician, a role she still holds. "It helps me maintain an understanding of what the unmet need is for these patients," she said. "My hope is to make these therapies more clinically accessible." ■



CREDIT: COEPTIS THERAPEUTICS

SNAP-CAR could lead to an off-the-shelf universal CAR T cell therapy.

CREDIT: ISTOCK.COM/WIRESTOCK

The modular nature of SNAP-CAR T cells could tune the power of CAR T cell therapy.

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Bewildering Biologics

Biologics like monoclonal antibodies and mRNA vaccines are complex drugs. Yongchao Su uses biophysical tools and innovative strategies to understand them better.

INTERVIEWED BY ALLISON WHITTEN, PHD

BIOLOGICAL DRUGS TAKE after nature. Often called biologics for short, these therapeutics are made of proteins, sugars, nucleic acids, viruses, and even whole cells or tissues. Biologics treat a wide range of conditions such as autoimmune diseases and cancers, or they act as vaccines that prevent sickness.

Yongchao Su, a senior principal scientist at Merck, studies the molecular intricacies of biologics to create better drug products. Drawing on his training as a structural biophysicist, Su seeks to bridge basic science and pharmaceutical science. He focuses on the formulation of biologics and the analytical functions of peptide and protein drug products.

On Su's first day at Merck over a decade ago, his manager emphasized the influence of their work on human health. To this day, Su carries that sense of purpose with him as he teams up with his colleagues to improve the stability, bioavailability, efficacy, and delivery efficiency of biologics.

CREDIT: ISTOCK.COM/ANDY

Biologics harness the power of nucleic acids, proteins, viruses, and more to treat a vast array of diseases.

CREDIT: MERCK

Yongchao Su applies tools from structural biophysics to advance the understanding of complex biologics.

Why do you study biologic drugs?

My passion for biologics comes from my inherent appreciation of the beauty of proteins. In structural biophysics, we see beautiful 3D structures, and it's amazing that Mother Nature made these proteins to perform valid functions. When we compare naturally occurring proteins and human-made therapeutic compounds that we use as drugs, we see that even though their functions are totally different from each other, they share similar structures. It's very exciting to get to study and appreciate the beauty of proteins and how biological drugs can help human health.

If we look at the current modern medicine market, biologics and small molecule drugs are becoming the primary drugs on the market. Compared to small molecule drugs, biologics have larger molecular weights. That carries opportunities because they better target different sites for therapeutic purposes, and they can be more selective than small molecule drugs, which means less toxicity. But that also brings complexity.

Biologics include monoclonal antibodies, fusion proteins, antibody drug conjugates, mRNA vaccines, and cell and gene therapies. The route of delivery is also diverse, as it includes intravenous, subcutaneous, intramuscular and oral delivery. Working in this space, how can we design all those drugs with different molecular properties and formulation processes?

How have you advanced the molecular understanding of peptide and protein drugs?

We studied glucagon, which is a peptide drug for treating hypoglycemia that's been on the market since it was approved in 1960 (1). To this day, glucagon drug products are provided in a lyophilized powder formulation. They don't have liquid formulations because glucagon can aggregate and fibrilize in solution, especially in acidic solutions. When a drug molecule starts to aggregate, it can lose its therapeutic function and the quality that regulatory agencies require. So, even though glucagon has been such an important drug in the past more than half a century, the community still faces challenges with developing a stable liquid formulation, which is more convenient to administer.

In 2019, our team published a paper in *Nature Structural & Molecular Biology* describing the mechanism that causes glucagon to aggregate (2). We used an advanced technology, solid-state nuclear magnetic resonance (NMR), to determine the mechanism. We found that similar to other peptide and protein drugs, glucagon exhibits intermolecular interactions in solution that lead to the formation of aggregates or fibrils.

Our paper serves as an example that in pharmaceutical science, we need to bring fundamental analytical tools and structural biophysics methodology to understand the molecular mechanisms needed for modifying drugs and overcoming challenges.

> "There's a gap in our mechanistic understanding of biologics, particularly in unraveling the molecular details of how these drugs are stabilized and delivered."
>
> — Yongchao Su, Merck

How will applying new structural biophysics tools further biologic drug development?

We analyze our drug particles to ensure the size of the particles, for example, but that's not enough because having the same particle size doesn't mean the same internal structure. Our team has been advancing NMR as a biophysical tool to understand the structural properties of biologics that modulate their stability and delivery. One example is our recent article reporting a novel NMR method for probing the internal structure of lipid nanoparticles, which is largely inaccessible by routine laboratory tools (3).

This is important to me because there's a gap in our mechanistic understanding of biologics, particularly in unraveling the molecular details of how these drugs are stabilized and delivered. Filling this gap is crucial as it provides the structural basis for the design of peptide and protein drugs. By applying NMR, I'm using my training as a structural biophysicist, and I'm also using my network between the biophysical community and the pharmaceutical community to hopefully bring awareness and eventually bring interest from others to fill that gap.

How have you and your team approached finding new ways to study biologics?

We understand that a protein drug often exists in a combination drug product, which means that there are interactions with different parts of a device. How do we know that they get along with each other? We can put together the formulation and wait for a long time. Then we conduct a shelf life stability study where we test whether the formulation is stable at the end of two years.

A faster way of answering that question is to do a stress condition study, where we put together a formulation and add a stress condition like high temperature. That's one way that we try to understand long-term stability from a short-term study. Yet, we still need innovation and creativity at every step of the formulation design process.

We recently came up with another way to stress the formulation. We connected two syringes through a needle and pushed the syringes back and forth. In that case, the proteins actually experience the interaction because we are mixing them. It's only another way to agitate, but it is a better way because we are going through the delivery device itself. We published that paper in the *Journal of Pharmaceutical Sciences* (4). The goal was to enhance awareness that we need to be creative in the way we do things and also to share the knowledge with the community.

What does the future hold for biologic drugs?

We are in an era of pharmaceutical science where we will keep advancing biologics for unmet medical needs. We're going to see more interdisciplinary collaborations and advancements than we have thought about before.

The complexity will continue to grow as well. We always pick the lower hanging fruit first. In this case, it is picking molecules that are stable and then making the overall process simpler. But once we get to more difficult molecules, things will become even more challenging. ■

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Probiotics to Alleviate Depression

Researchers target the gut to see if they can treat depression with fewer side effects and less stigma than current drugs.

Many people already take probiotics in their daily lives, so unlike antidepressants, there is little to no stigma associated with them.

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BY NORA BRADFORD

DEPRESSION IS MORE COMMON than many might think; it affects 4.4 percent of the US population and 3.8 percent of the global population (1, 2). It also disproportionately hits women, who experience it about 50 percent more frequently than men (2). Despite its prevalence, effective treatment remains elusive for many. Current antidepressant treatments come with a plethora of unwanted side effects, from nausea to insomnia. Even with treatment, about a third of people with depression don't experience improved symptoms (3).

However, a new and promising area of research may offer hope for those seeking alternative treatments. Researchers are delving into the world of probiotics, investigating their potential to serve as a harmless yet effective treatment for depression.

"The link between the gut and the mind has been known forever. We always say 'I feel it in my gut,'" said Roumen Milev, a psychiatrist at Queen's University. "There is this link that we never paid attention to because our way of differentiating portions of science we study did not necessarily cut it in the right way."

Depression is a particularly difficult disorder to define. Each patient has their own somewhat unique constellation of symptoms that can vary widely from those of other patients. Some diagnostic criteria are even opposites, like weight loss and weight gain or loss of sleep and sleeping too much (4).

> "The link between the gut and the mind has been known forever. We always say 'I feel it in my gut.'"
>
> — *Roumen Milev, Queen's University*

This was especially frustrating for Anna-Chiara Schaub, a psychologist at the University of Basel, who recently decided to move away from this research area. "Depression and in general, mental disorders are so heterogeneous that when you just put depressed patients together, they have so many different etiologies and different patterns, so you can't just put them in one group," she said.

Recent advances have shed light on the intricate communication network between the gastrointestinal tract and the central nervous system known as the gut-brain axis. This bidirectional pathway involves the autonomic nervous system, which drives involuntary body functions such as breathing and heart rate; the enteric (gut) nervous system; the neuroendocrine system; and the immune system (5). Over the last decade, many mental health researchers have turned their attention to the gut-brain axis, specifically the microbiome, to uncover the underlying mechanisms and potential new treatments for a variety of disorders (6).

Based on early research into the microbiome's role, researchers suggested that a lack of microbial diversity might be a hallmark of depression. However, scientists recently used more nuanced meta-analyses to point to specific microbial imbalances underlying the disorder rather than overall microbial diversity. Depression and other psychiatric disorders generally go hand-in-hand with fewer anti-inflammatory bacteria and more pro-inflammatory bacteria within the gut compared to controls (7,8). In one study, researchers investigated the effect of fecal transplants from depressed humans to rats and found that symptoms of depression, like the inability to feel pleasure, became more common in the rodents (9).

"The understanding on the gut-brain is still at the early stage," said Hein Tun, a microbiome researcher at the Chinese University of Hong Kong. "The gut-brain axis is always bidirectional … so in the microbiome, there's always this chicken or egg issue."

Probiotics versus antidepressants

Results from meta-analyses and clinical trials have begun to illustrate probiotics' potential as a viable treatment for mild-to-moderate depression, whether as

standalone therapies or adjuncts to traditional antidepressants (10-12). These studies suggest that probiotics may not only improve depressive symptoms but also do so with fewer side effects and greater tolerability than many pharmacological alternatives. Many people already take probiotics in their daily lives, so unlike antidepressants, there is little to no stigma associated with them.

After noticing how many university students showed signs of mild depression, Tun was motivated to find a stigma-free treatment for depression. "We found that more than 45 percent of students experience depression, anxiety, or stress," he said. "I became very interested in understanding why our next generation is suffering from so many mental health problems."

He led a meta-analysis comparing probiotic treatments to prescription medications and found that probiotics were as effective as most common antidepressants (13). "I was very skeptical about it at first," Tun said. Because the studies that he and his team analyzed used different compositions and doses of probiotics, he didn't expect to see an effect. But "all the probiotics were working well," he said. "This was shocking to me."

Probiotics vary widely in their composition, with some targeting certain microbes more than others. They also differ in their concentration of bacteria by up to 50 times (14). However, "now that we know more about the gut-brain axis, hopefully we can develop the next generation microbiome-targeted intervention," said Tun. He hopes to develop specific probiotics called synbiotics to contain the most influential microbes to speed up and maximize their effect. He also plans to conduct longitudinal studies to better understand whether probiotics make lasting changes to mood and microbiome composition.

While investigating probiotics as a depression treatment is one approach, Schaub was curious if probiotics could work in parallel to antidepressants as an add-on therapy. "When you are in the clinics working with patients, it's very obvious that many of the patients have issues with nutrition," said Schaub. "That's one of the main reasons to look deeper into this."

She and her team conducted the first randomized control trial of short-term, high-dose multi-strain probiotics for depression (15). They studied patients who were already being treated for depression with antidepressant medications. Half of the participants took a strong probiotic with eight different strains of bacteria for a month, while the other half took a placebo.

After sequencing participants' gut microbiota from stool samples, the team found that probiotics increased the abundance of the bacteria *Lactobacillus* and maintained microbial diversity. Participants in the treatment group also showed slightly decreased scores on the Hamilton Depression Rating Scale eight weeks after the intervention, suggesting that probiotics may help increase depression remission rates. Participants who received probiotics also showed a decrease in neural responses to neutral faces, which are typically heightened in people with depression (15). A further analysis of this data showed that participants in the probiotic group also performed better on a memory task and had normalized hippocampal function compared to controls (16).

While these findings point to a positive effect of probiotics on depression, researchers are still unsure about the mechanism behind it. "One hypothesis is that if probiotics reduce inflammatory processes, you could also see an effect on general health or more somatic symptoms like bad sleep," said Schaub.

Milev did not seem to mind the ambiguity about probiotics' mechanism. "If we don't show that it works, who cares how it doesn't work? The first step is to really show robust efficacy and good tolerability and safety, and once we show that and there is something in it, then the second step is how it does it," he said.

Milev is working with the Canadian Biomarker Integration Network in Depression, a program aimed at gaining a holistic understanding of depression and its treatments using brain scans, clinical assessments, and blood samples. In a clinical pilot study of people with major depressive disorder who had never taken antidepressants before, his team found that probiotics alleviated symptoms after just four weeks and improved sleep quality after eight weeks (17). However, because the study was done on only 10 participants and had no placebo group, Milev hopes to conduct follow-up research with more people.

Future challenges

The burgeoning research into probiotics as a potential therapy for depression offers a glimmer of hope to researchers, medical professionals, and patients, but a few key challenges lie ahead. One is that many patients with depression are already on antidepressants or have a history of antidepressant treatment. In addition to disentangling the effect of antidepressants and probiotics on mood, researchers also struggle to account for how antidepressants affect the growth of certain microbes, sometimes stunting their growth (16). Tun and others emphasized the importance of testing probiotics on people who show signs of depression but who have never been treated to get a clearer idea of probiotics' effectiveness.

Milev agreed with Tun that testing untreated patients is imperative. His ideal study would assess young adults soon after their depression diagnosis who have significant symptoms that interfere with their daily lives. With a strong dose of probiotics over the course of twelve weeks and follow up assessments, Milev believes that such a dream study "would definitively answer only one question: do they work?" Researchers would need to conduct additional studies to determine why and for whom probiotics have antidepressant effects.

The research community will continue to unravel the complexities of the microbiome and its relationship to depression to develop a new avenue for treatment without the side effects of traditional antidepressants. This new direction will hopefully encourage medical professionals to search for solutions outside of the traditional pharmacological box.

"It's good to keep the gut in mind," said Schaub. "It's nice to see the person more in a holistic way." ■

> "The gut-brain axis is always bidirectional, so in the microbiome, there's always this chicken or egg issue." – *Hein Tun, Chinese University of Hong Kong*

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Current antidepressant treatments come with a plethora of unwanted side effects, from nausea to insomnia. Even with treatment, about a third of people with depression don't experience improved symptoms.

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