DISCOVERY REPORT

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The future of gene editing

CRISPR was just the beginning. Inside, sharpen your knowledge of the companies transforming medicine.

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The future of gene editing

It's easy for people to get the impression that any research that lands a Nobel Prize has reached the pinnacle of perfection. Last fall, scientists celebrated a Nobel Prize in Chemistry for CRISPR genome editing. The technology is transformative. It is simple to use. It is powerful. Yet it is not perfect. It can introduce unwanted mutations, and it remains difficult to deliver the technology where it needs to go in the body.

Those limitations are critical to acknowledge as the world edges closer to applications of CRISPR and other gene-editing tools in medicine. Last year, the US Food and Drug Administration authorized the first CRISPR technology—a COVID-19 diagnostic. Human clinical trials of gene-editing therapies are ongoing. And ethicists continue to discuss the ramifications of technology that tinkers with the instructions for life in the wake of 2018's bombshell news of babies born from embryos with edited genes.

The field's pioneers certainly don't act as though their work is done. Spending a jam-packed day with CRISPR codiscoverer and Nobel laureate Jennifer Doudna will disabuse anyone of that notion. Doudna continues to publish papers and create spin-off companies that promise more-precise gene editing. On the opposite coast of the US, David Liu is doing the same, as are other scientists worldwide. Inside this Discovery Report, you'll meet some of the many entrepreneurs who aim to use gene-editing technology to provide more organs for transplants, combat antibiotic-resistant bacteria, and more.

Contributing editor Carmen Drahl, who has covered organic chemistry and green chemistry for C&EN, edited this report. It includes a reading list of papers and patents curated by our sources, as well as by information scientists at the CAS division of the American Chemical Society.

As an ACS member, you get exclusive access to the Discovery Report, a quarterly publication bringing you cutting-edge research defining the chemical sciences and our industry. Look for the next one in the second quarter of 2021.

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INSIDE

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CHEAT SHEET

5 questions and answers about gene editing for biomedicine

Q. Why are medical researchers excited about gene-editing approaches?

» **Repairing the root causes of genetic illnesses,** including sickle cell anemia or Huntington's disease, may become possible by precisely rewriting the genetic code.

» **Gene editing has the potential to reprogram a person's immune cells** so that they will attack cancers.

» **Systems that target antibiotic resistance or virulence genes** could offer another defense against superbugs while leaving beneficial microbes intact.

» **Diagnostics** that detect a disease's DNA or RNA fingerprint in biological specimens can be designed by harnessing the search function in gene-editing tools.

Q. What does the technology landscape look like now?

» **Gene editors are not new.** Artificial DNA-cleaving enzymes called zinc finger nucleases have been in commercial development for over 2 decades. Transcription activator-like effector nucleases (TALENs) and meganucleases are also established.

» **CRISPR** and CRISPR-associated (Cas) proteins, which can be designed to cut any DNA at a predetermined site, have democratized gene editing.

» **Base editors and prime editors** extend the CRISPR archetype, targeting and editing a single base pair of DNA.

Q. What are the limitations of existing gene editors?

» **It remains challenging to customize** some gene-editing motifs, such as zinc finger nucleases.

» **CRISPR and base-editing systems are not perfectly exact** and can introduce errors or unwanted mutations that become problematic in a medical context (see page 8).

» **Delivering a gene editor** into the cells of the organ where it's needed remains a major hurdle.

Q. How are scientists searching for new options?

» **Some start-ups are mining genomes** to uncover gene-editing proteins that can power specific applications.

» **Other experts are increasing the precision of gene editors** by adjusting particular components, such as the RNA that guides the cutting enzyme.

Q. What's next for gene-editing technology?

» **The first CRISPR technology authorized by the US Food and Drug Administration,** a COVID-19 diagnostic, reached the market in 2020, potentially paving the way for additional products (see page 19).

» **Multiple human clinical trials are underway** for gene-editing treatments that take place either outside or inside the body. Observers will want to see if the treatments edit genes accurately, whether any trigger immune responses, and if their effects are long lasting.

» **Next-generation gene-editing tools, as well as safety switches** that keep gene editors under control, are active areas of research.

8 experts identify the biggest challenges facing gene editing now

Françoise Baylis

» Bioethicist and professor, Dalhousie University

When using CRISPR to treat disease, scientific questions of safety, efficacy, and manufacturing are only one-half of the challenge. Equally important are issues of justice, equity, and broad societal consensus, according to Françoise Baylis. Even if a technology is proved to work scientifically, these questions must be addressed before deploying it broadly. "I worry about the ways in which we only pay attention to the scientific aspects of the technology," Baylis says. "Science is a piece of the answer, but it is not *the* answer."

Baylis has wrestled with the ethics of research on human embryos since the 1980s in the context of in vitro fertilization, preimplantation genetic tests, and research using embryonic stem cells. CRISPR-based approaches to treat disease, whether by making edits that affect only one organism or that can be passed to offspring, raise ethical questions similar to the ones those other techniques did in the past. But Baylis sees one fundamental difference. "The possibility of taking over the human evolutionary story with gene therapy is much closer to reality," she says. "CRISPR exercises the imagination in a different way."

In 2015, when researchers demonstrated the possibility of gene editing in nonviable embryos, Baylis and others organized the first international summit on human gene editing. They agreed that two conditions were essential for editing of the human genome to proceed: safety and efficacy from a scientific standpoint, and broad societal consensus. Baylis continues to work on these issues as a member of the World Health Organization (WHO) advisory committee for the oversight of human genome editing. "I care about scientific validity," she says. "But it's important that a technology also have social value."

> **"CRISPR-based tests are out in the field being used on real patient samples."**

William Blake

» Chief technology officer, Sherlock Biosciences

CRISPR-based kits to detect viruses such as SARS-CoV-2 or other pathogens are a significant improvement over traditional polymerase chain reaction (PCR)- and antibody-based tests in their precision and speed. But as a new technology, CRISPR tools still need to prove they're on par with existing methods, William Blake says.

Detection tools based on new CRISPR-Cas technologies are one part of Sherlock Biosciences' portfolio. Less than a year after its 2019 launch, the company hurried to adapt its technology to develop a COVID-19 test that relies on the nuclease Cas13a, which cuts RNA, not DNA (see page 19).

Sherlock's CRISPR-based test detects two regions in the SARS-CoV-2 genome in a single reaction and works in about an hour. It detected SARS-CoV-2 with accuracy similar to established reverse transcriptase PCR (RT-PCR)-based techniques when applied to standard samples distributed to all test developers by the US Food and Drug Administration (FDA), Blake says. In May, the test received emergency use authorization from the FDA—the first authorization for any CRISPR-based technology. "It's certainly a milestone for us as a company and also a milestone for CRISPR," Blake says.

The firm's eventual goal is real-time detection

CREDIT: DANIEL ABRIEL (BAYLIS); ALL OTHER IMAGES COURTESY OF INSTITUTIONS

and at-home kits that function much like paper strips in pregnancy tests. “One of the most exciting things now is that, for the first time, these CRISPR-based tests are out in the field being used on real patient samples,” Blake says. “That demonstrates the robustness of the technology.”

Janice Chen

» Cofounder and chief technology officer, Mammoth Biosciences

Janice Chen was a doctoral student in Jennifer Doudna’s lab at the University of California, Berkeley, when Feng Zhang’s lab at Broad Institute of MIT and Harvard discovered a new nuclease enzyme named Cas12—one that, unlike Cas9, would snip a single strand of its DNA target. Chen pivoted her research to understand how the protein worked. In 2017, Chen and collaborators at the University of California, San Francisco, confirmed that Cas12’s ability to cut a specific DNA sequence could be used to detect pathogens such as the human papillomavirus, which can cause cancer.

Their discoveries led to the formation of Mammoth Biosciences in 2017. The company’s active programs aim to use Cas12-based technology to detect viral and bacterial pathogens, including drug-resistant strains of bacteria, as well as to spot cancer-linked genetic variants. Last year, the team raced to deploy its detection platform to devise a diagnostic tool for the SARS-CoV-2 virus, which causes COVID-19 (see page 19).

In addition to CRISPR-based detection, the firm aims to expand the utility of CRISPR systems and overcome challenges such as protein delivery and enzyme specificity by seeking out new Cas proteins. Their protein discovery workflow uses algorithms to seek out suspected CRISPR systems in DNA sequences from environmental microbes. Discovering new nucleases helps overcome issues such as off-target effects and reveals ways to improve the accuracy of CRISPR systems and edit more parts of the genome. “There’s not really a one-size-fits-all protein,” Chen says. “Having a diversity of nucleases in our toolkit is really critical to continue to innovate and meet the challenges we face today.”

Alexis Komor

» Chemistry professor, University of California San Diego

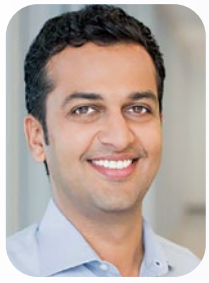
When using CRISPR to make a genomic edit, researchers rely on the cell’s own machinery to repair the cut CRISPR leaves behind. Understanding the DNA repair processes that determine how a cell recovers from an edit is an overlooked challenge in the field, according to Alexis Komor.

Komor was a nucleic acid chemist new to the world of genome editing when she began postdoctoral research. Just a year in, though, she developed the first cytosine base editor by fusing the Cas9 enzyme with two other proteins. Unlike native Cas9, this editor could swap an individual cytosine-guanine base pair in DNA for a uracil-adenine or thymine-adenine duo. Shortly after that breakthrough, Komor and colleague Nicole Gaudelli turned to directed evolution to create an adenine base editor that could similarly swap out an adenine-thymine base pair.

Now, Komor aims to better understand the repair mechanisms that process the reaction intermediates produced by base editors. Identifying these players could help engineer new editors that could perform other kinds of changes, she says.

For example, while much is known about how cells repair the double-stranded DNA breaks made by Cas9 and other nucleases, far less is known about how cells repair single-stranded nicks created by base editors. “There’s lots for me to do,” Komor says.

> **“We’re going from a model where we chronically treat patients to just curing them.”**



Samarth Kulkarni

» CEO, Crispr Therapeutics

CRISPR-based treatments for diseases face different hurdles depending on where they’re being used, Samarth Kulkarni says. When the cells to be edited can be extracted from a patient, treated in a dish, and then put back, he sees the main challenges as involving keeping cultured cells functional and unharmed. But when a therapeutic must be used in a patient’s body, the challenge is to deliver the drug safely and reliably to the target tissues.

Crispr Therapeutics began with “cell therapy” for sickle cell anemia and β-thalassemia, in which blood cells are modified with a relatively simple edit and then administered to patients. The firm built on that capacity to develop engineered CAR T cells with a few different edits for cancer immunotherapies; they are also working on engineering stem cells to create an artificial pancreas. All three applications rely on cells being engineered outside patients. “There’s lower technical risk to enable these,” Kulkarni says, “and it’s one building on another in terms of these three uses.” The next step is therapies

where CRISPR machinery must be packaged and delivered in a patient's body. The company is working on this type of treatment for liver and musculoskeletal diseases.

In June 2020, the company treated its first US patients with CTX001, its therapy for sickle cell anemia and β-thalassemia. Study participants were cured of their genetic diseases—usually lifelong conditions—within months of treatment. "It's a paradigm shift," Kulkarni says. "We're going from a model where we chronically treat patients to just curing them."

Karen Maxwell

» Biochemistry professor, University of Toronto

To Karen Maxwell, controlling CRISPR's cuts is likely to be the field's biggest challenge yet. To avoid off-target edits, "having multiple layers of safety built in is going to be really important," she says.

Some of those layers might stem from nature's own CRISPR regulators—anti-CRISPR proteins found in bacteria-infecting viruses, called phages. Just as microbes evolved CRISPR-Cas systems to protect themselves from the viruses, so phages countered by evolving sophisticated systems to overcome CRISPR-based defenses. Maxwell studies this tiny evolutionary arms race to understand the myriad mechanisms by which anti-CRISPRs help phages bypass bacterial shields.

Some anti-CRISPRs work by blocking guide RNA from binding to DNA; others inhibit the Cas enzyme from binding to its target. Still others act by preventing CRISPR systems from recognizing new viruses, or act like enzymes to prevent CRISPR-Cas action. "Anti-CRISPRs seem to be able to inhibit every aspect of CRISPR biology," Maxwell says. "It shows how strong this evolutionary pressure is on phages to overcome CRISPR-Cas immunity."

Although Maxwell focuses on understanding the evolution and mechanisms of these naturally occurring proteins, she sees immense utility for commercial applications. One area would be controlling gene drives—self-replicating systems that can quickly change a population's genome. Anti-CRISPRs could also help keep CRISPR-based therapeutics switched off in nontarget tissues, so that a treatment meant for the liver, for example, does not act on heart cells. "You don't want to cure one disease and then make some off-target mutation that causes a whole other problem," she says.

Dave Ousterout

» Cofounder and chief scientific officer, Locus Biosciences

Many CRISPR-based therapeutics aim to fix flawed genes. But Locus Biosciences' technology aims to destroy normal DNA—in pathogens, that is. The company develops CRISPR-based antimicrobials that rely on a Pac-Man-like nuclease named Cas3. Unlike other nucleases that make precise cuts, Cas3 dices DNA.

Dave Ousterout sees CRISPR-Cas3 as the ideal platform for precision antibacterial drugs to target the genomes of specific pathogens without killing the microbes that promote health. The search for a delivery system led the company to bacteria-killing viruses known as phages. Locus's platform loads phages up with CRISPR-Cas3 systems designed to eliminate problematic bacteria.

The firm's products focus on diseases for which better treatments are needed urgently and on infections caused primarily by one species of microbe as opposed to several, Ousterout says. The growing threat of drug-resistant infections provides strong rationale for the development of these drugs, Ousterout says. For instance, one product in the works targets recurrent urinary tract infections caused by *Escherichia coli*. The company started human clinical trials to treat urinary tract infections in January 2020 and expects results in the second quarter of 2021. "One of the key issues is that patients are plagued with reinfections," Ousterout says. "We want to cure multidrug-resistant infections but also clear the reservoirs that cause recurrence."

Joshua Rosenthal

» Senior scientist, The Marine Biological Laboratory in Woods Hole

The world of CRISPR-based therapeutics has largely focused on diseases encoded in DNA that require permanent fixes. But RNA editing opens up the possibility of using gene editing to treat something without permanently altering the genome.

Unlike DNA editing, which relies on bacterial enzymes to snip genomes, RNA-editing proteins exist in human cells. Joshua Rosenthal spent years studying RNA editing in squid. Being immersed in that biology was a catalyst for thinking of RNA-editing systems as therapeutics. Eventually, Rosenthal cofounded Korro Bio, a start-up that aims to harness human RNA-editing machinery to treat transient conditions such as pain (see page 16). One approach would be to simply guide human enzymes to the RNA sites that need to be fixed. The therapeutic effects of such changes are likely to last much longer than a standard painkiller today, Rosenthal says. The strategy may also circumvent the risk of addiction to strong painkillers, since RNA editing would act on sensory circuits and avoid the brain's reward centers.

In nature, the guide RNAs used to direct RNA-editing enzymes to their targets are fairly large. To get to a point where RNA editors are viable therapeutics, Rosenthal thinks guide RNAs used to direct enzyme binding will need to shrink. In humans, "the chances of causing adverse immune effects increase as the RNA gets bigger," he says. Although RNA-editing systems are unlikely to trigger an immune response as large as Cas9 DNA-editing systems do, he adds, "the smaller we can keep them the better." ■

BY THE NUMBERS

Discover CRISPR technology trends

Global interest

We looked at 8 diseases commonly addressed in CRISPR company pipelines to see how frequently they are mentioned alongside CRISPR in patents from the US and China.

US

Disease	Number of Patents
Sickle cell disease	17
β-Thalassemia	15
Alzheimer's disease	12
Huntington's disease	10
Retinitis pigmentosa	8
Inflammatory bowel disease	2
Rheumatoid arthritis	1

China

Disease	Number of Patents
β-Thalassemia	22
Alzheimer's disease	6
Huntington's disease	5
Rheumatoid arthritis	5
Sickle cell disease	5
Retinitis pigmentosa	2
Non-Hodgkin's lymphoma	1

Who's who

We found the most active companies and institutions filing patents that mention CRISPR alongside 5 diseases of interest.

β-Thalassemia

Assignee	Number of Patents
Guangdong Trement Medical Science	4
Guangzhou GeneRulor	4
Bioray Laboratories	3
Editas Medicine	3
East China Normal University	2

Sickle cell disease

Assignee	Number of Patents
Editas Medicine	6
Fred Hutchinson Cancer Research Center	2
Guangdong Trement Medical Science	2
Beam Therapeutics	2
Edigene	2

Alzheimer's disease

Assignee	Number of Patents
Laval University	5
Alcyone Lifesciences	2
AVROBIO	2
Neuroproof	2
Xiamen University	2

Retinitis pigmentosa

Assignee	Number of Patents
Editas Medicine	4
Casebia Therapeutics	3
Johns Hopkins University	2
University of Science and Technology of China	2
Cedars-Sinai Medical Center	1

Huntington's disease

Assignee	Number of Patents
Alcyone Lifesciences	3
Southwest University, China	3
Lausanne University Hospital	2
Children's Hospital of Philadelphia	2
Chan Zuckerberg Biohub	1

CRISPR stats

Stay current with our selection of key metrics and milestones in gene editing.

83.7%
Percentage of CRISPR patents held by US- or China-based institutions, 2006–20

57.6%
Percentage of CRISPR publications held by US- or China-based institutions, 2007–21

2020
Scientists test a CRISPR treatment inside a person's body for the first time

$5.3 billion
Projected CRISPR market size by 2025

300,000
Babies born globally each year with severe genetic blood diseases, which are targets of several experimental CRISPR therapies

100
Entries in the latest "Women in CRISPR" list of scientific experts

Sources: Ahead Intelligence; Associated Press; CAS, a division of the American Chemical Society; Google; World Health Organization.

Notes: CAS information scientists searched CRISPR patents from 2006 to 2020. Casebia Therapeutics is now part of Crispr Therapeutics. Patents commonly mention more than one disease.

Beyond Cas9, a world of possibility for new CRISPR technologies

JYOTI MADHUSOODANAN, SPECIAL TO C&EN

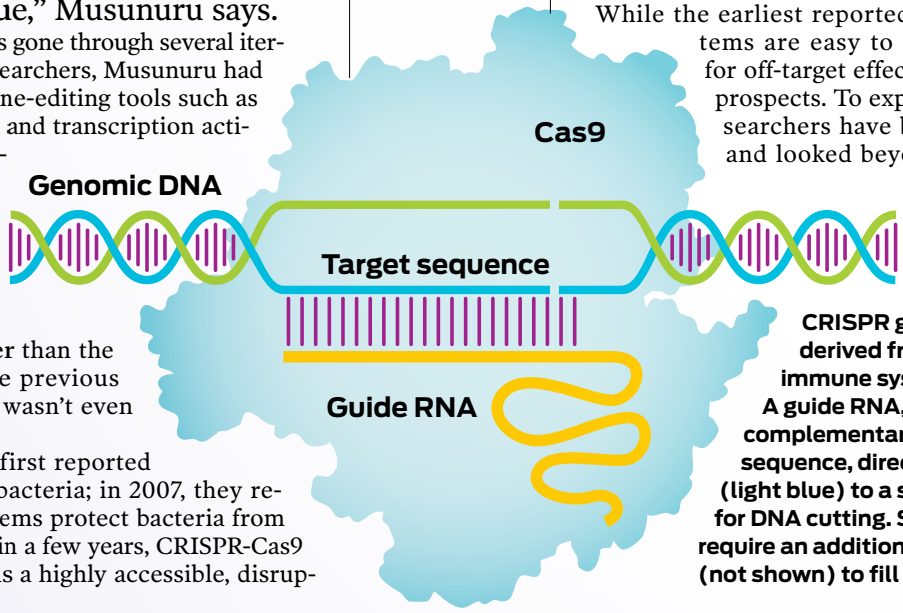
After a person has a heart attack, clinicians often prop the patient's blood vessels open with balloons and stents, then prescribe a large dose of statins, which are to be taken every day for the rest of the person's life. Cardiologist Kiran Musunuru of the University of Pennsylvania knows that a year later, between one-third and over one-half of those people will fail to take their pills daily. "My dream is that when a patient comes in with a heart attack, we give them a once-and-done gene therapy and adherence is no longer an issue," Musunuru says.

It's a dream that has gone through several iterations. Like other researchers, Musunuru had worked with early gene-editing tools such as zinc finger nucleases and transcription activator-like effector nucleases (TALENs). Everything changed when he tried the CRISPR-Cas9 system. "The least efficient CRISPR targeting was way better than the best targeting by the previous tools," he recalls. "It wasn't even close."

In 1987, scientists first reported CRISPR systems in bacteria; in 2007, they revealed that such systems protect bacteria from viral infections. Within a few years, CRISPR-Cas9 was being heralded as a highly accessible, disruptive way to edit genes—upending the view of gene therapy as suitable only as a last-resort treatment for deadly genetic diseases. Musunuru cofounded Verve Therapeutics in 2018 to tackle heart disease using gene therapy, such as by modifying genes that regulate lipid metabolism (see page 14). It is one of dozens of outfits forging ahead with CRISPR-based therapeutics for rare genetic disorders, cancers, bacterial infections, and more.

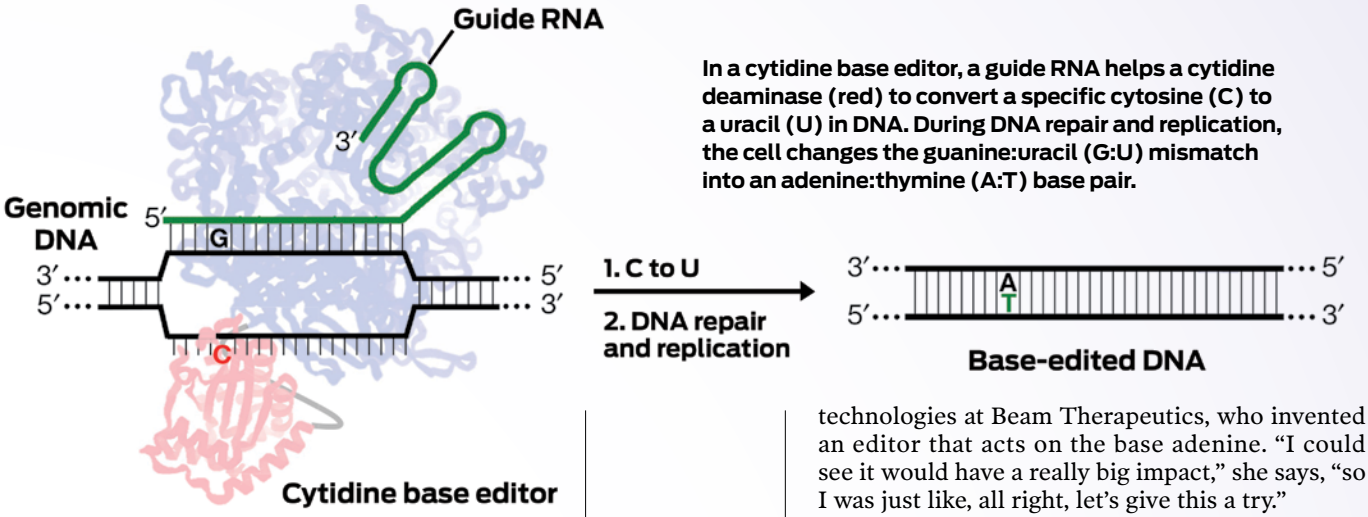
"CRISPR may be the most compelling, promising 8-year-old technology we've had in molecular biology ever since PCR," the now-ubiquitous polymerase chain reaction, says Rodolphe Barrangou of North Carolina State University, who was part of the team that discovered CRISPR's functions in 2007. But at the same time, he adds, "we're only 8 years in," which leaves plenty of room to improve.

CRISPR stands for clusters of regularly interspaced short palindromic repeats, referring to DNA; Cas9 is a DNA-cleaving nuclease enzyme. While the earliest reported CRISPR-Cas9 systems are easy to use, their potential for off-target effects limits therapeutic prospects. To expand possibilities, researchers have both upgraded Cas9 and looked beyond it. They've harnessed other enzymes—some discovered in bacteria and others engi-



CRISPR gene editing is derived from a primordial immune system in bacteria. A guide RNA, which is complementary to a target DNA sequence, directs the Cas9 enzyme (light blue) to a specified location for DNA cutting. Some applications require an additional DNA template (not shown) to fill in the cut.

SOURCE: ADAPTED FROM ORIGENE TECHNOLOGIES.



In a cytidine base editor, a guide RNA helps a cytidine deaminase (red) to convert a specific cytosine (C) to a uracil (U) in DNA. During DNA repair and replication, the cell changes the guanine:uracil (G:U) mismatch into an adenine:thymine (A:T) base pair.

neered in labs, each with different mechanisms of action. This diversity is key to the technology's success, Barrangou says. "To equate it to a toolbox, we have some molecular scalpels in there, and they're great to do precise surgery, but if you want to cut down a tree, you're gonna need a chain saw."

Other groups are addressing the drug delivery challenges of getting CRISPR systems—which tend to be bulky proteins—into target tissues in the human body, as well as safety mechanisms that keep CRISPR in check.

Building the toolbox

In their classic form, CRISPR-Cas9 systems rely on a guide RNA molecule that directs Cas9 to its DNA target. Once bound, the nuclease cuts both strands of the target sequence. This part of the process is very precise, earning CRISPR-Cas9 its moniker of molecular scissors.

Sensing this damage, the host cell activates its repair systems and fixes the rip.

But when faced with a double-stranded cut in a chromosome, "the cell's repair response is to generate a mixture of insertions and deletions" to the DNA sequence, says chemical biologist David Liu of Broad Institute of MIT and Harvard and the Howard Hughes Medical Institute. "Researchers don't know how to control that yet." Although the slice-and-repair system of classic CRISPR-Cas9 silences targeted genetic defects, the haphazard nature of the repair can create other problems.

Liu's lab is among those finding other ways to make specific genome changes, such as by snipping single strands. In 2016, his team published the first report of base editors, which can make single-letter changes to one strand of a DNA sequence without double-stranded cuts. Whereas Cas9 is the equivalent of "editing" a book by ripping out a page to fix a single spelling error, base editors are like pencils with erasers that allow researchers to zoom in on an incorrect letter, erase it, and write in the correct one. "There were a lot of people skeptical that this could work," says Nicole Gaudelli, director and head of gene-editing technologies at Beam Therapeutics, who invented an editor that acts on the base adenine. "I could see it would have a really big impact," she says, "so I was just like, all right, let's give this a try."

> **"When it comes to delivering CRISPR therapies to specific tissues in the body, the challenges are much the same as they were 5 years ago."**

Gaudelli and chemical biologist Alexis Komor, now at the University of California San Diego, were postdoctoral researchers in Liu's lab when they began working on base-editing technologies (see page 5). Komor developed the first base editor by fusing three proteins together: a hobbled Cas9 that can bind to a DNA target but not cleave it, an enzyme that chemically converts a cytosine nucleotide to uracil in one strand of DNA, and an inhibitor that prevents the cell from removing uracil from its genome.

When this three-part fusion acts on DNA, the Cas9 portion opens up double-stranded DNA, creating a little bubble of unwound double helix with one strand paired to the guide RNA. All the cytosines on the other, unpaired strand of this bubble are "edited" into uracil, and the inhibitor prevents the cell from simply deleting this chunk of bases. Other parts of the system then encourage the cell to "repair" its DNA using the new sequence containing uracil, ultimately converting what was once a cytosine-guanine base pair into a thymine-adenine pair.

Designing an editor to turn a thymine-adenine pair into a cytosine-guanine pair was a different challenge, because no naturally occurring enzymes can induce chemical changes to thymine or adenine akin to converting cytosine to uracil. Gaudelli turned to repeated rounds of directed evolution to force a protein to become an adenine editor.

While base editors significantly improve on Cas9's abilities, they're still far from ideal, Komor says. The editors struggle for precision if two cytosines or two adenines are next to each other on the same strand and cause so-called bystander mutations. Gaudelli and others continue to work on improving the efficiency of base editors and reducing these off-target effects.

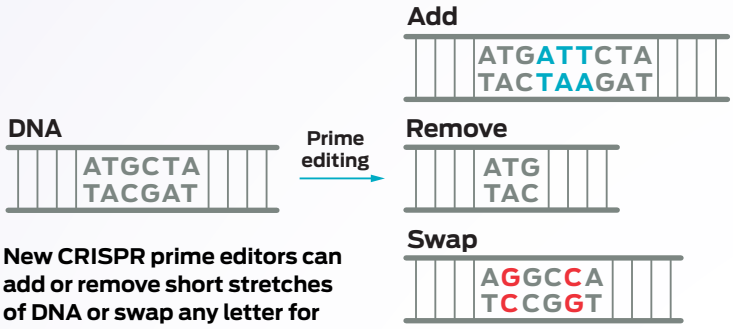
In 2019, Liu's team further adapted CRISPR technologies by developing prime editors, which can directly write new genetic information into DNA at a target site using Cas9 combined with an enzyme that reverse transcribes guide RNA into the DNA to be inserted. Like base editors, prime editors cut only a single strand of DNA. Base editors are more efficient than prime editors, though the latter don't

SOURCE: ADAPTED FROM *NATURE*

run into the bystander mutation problem.

For now, no single technology is better than the others, and each has its own uses, Liu says. "There doesn't need to be a single editing technology to rule them all," he says. "We have the luxury of being able to develop tailor-made editing agents for different cell types or targets. I don't see the need to imagine a future where only one technology is used exclusively."

That includes expanding beyond the specific application of gene-modification therapies. For example, Cas9 relatives called Cas12 indiscriminately cut up DNA once a target is detected. The minced remnants are easily detectable with a fluorescent reporter, making Cas12 enzymes an ideal tool to develop DNA detection kits for pathogens (see page 5). Researchers have adapted yet another enzyme, Cas13, to edit RNA targets. Altered Cas9 and Cas12 enzymes can also make chemical modifications to the genome, including methylating or demethylating DNA and acetylating histones. Such epigenetic changes can alter how genes are expressed.



New CRISPR prime editors can add or remove short stretches of DNA or swap any letter for another. In these examples, prime editing adds a three-base-pair sequence to DNA, removes three base pairs, and swaps two base pairs for alternatives.

Delivering the tools

Expanding CRISPR's repertoire of abilities widens the field of possible therapeutic applications. But challenges with drug delivery remain, Gaudelli says. Most first-generation CRISPR therapies are cell therapies—meaning cells are extracted from a person's body, edited in a lab, and then injected back into the body. "When it comes to delivering CRISPR therapies to specific tissues in the body, the challenges are much the same as they were 5 years ago," Gaudelli says. "We've gotten the cargo side of things much more mature than the delivery of that cargo."

To advance to delivering enzymes and guide RNAs to specific organs, researchers are continuing to develop options such as lipid nanoparticles and virus-based systems, which rely on viruses' natural ability to recognize host cells and put genetic material into them. Liver-targeted treatments may be the easiest to achieve with nanoparticles, Gaudelli says, because the organ is easily accessed—anything we eat or inject usually transits through the liver.

In fact, the most advanced experimental gene-editing medicine at Musunuru's Verve Therapeutics is a base editor licensed from Beam wrapped inside a lipid nanoparticle. The treatment, which is delivered through a vein, is designed to permanently edit a target gene in the liver.

Timing is also critical to delivering CRISPR-based drugs in the body. How long a lipid nanoparticle—and its gene-editing cargo—can survive in the body depends on the specific molecules used to design the nanoparticle, as well as how they're stacked up to create the particle. Typically, nanoparticles are designed to reach a target organ and then to degrade quickly. Viral delivery systems are easier to dispatch to specific tissues, but they're also long lived and will continue to express gene-editing enzymes unless kill switches or other controls are added. In addition, packaging bulky DNA-snipping enzymes into relatively tiny viruses remains a problem. "It's still about finding the right tool for the job," Gaudelli says.

> **"We have the luxury of being able to develop tailor-made editing agents for different cell types or targets."**

Safeguards

Improving the precision and efficiency of CRISPR tools and finding ways to deliver them to cells are vital to realizing the promise of gene therapy. But just as crucial is the ability to stop CRISPR.

Chemist Amit Choudhary at Broad Institute of MIT and Harvard heard about the enzymes and their revolutionary potential from Liu and others. From a chemist's perspective, the risk was obvious: in most enzyme reactions, a few molecules of the protein work in a sea of substrates. But when deploying CRISPR as a therapy, many enzymes flood scarce DNA targets, making it inevitable that some enzymes will cut off-target. Controls that can inhibit or degrade the enzymes therefore become a necessity. "Cas9 is not an enzyme nature designed for genome editing; it's a protein running with scissors inside our cells," Choudhary says.

Some researchers are considering how nature solves the problem: as bacteria developed CRISPR systems to protect themselves against viruses, microbe-killing viruses adapted by evolving anti-CRISPR proteins that block Cas enzymes in myriad ways. Another route to controlling CRISPR lies with small molecules, an option Choudhary is exploring in his research.

In addition to physical controls for CRISPR, regulatory and ethical tools will prove crucial. That takes on new urgency now that the US Food and Drug Administration has permitted the first CRISPR-based technologies—COVID-19 diagnostics—to enter the market on an emergency basis (see page 19) and as more experimental CRISPR therapies like Verve's advance through human clinical trials. "A thoughtful regulatory framework that keeps pace with the development of these new technologies is also important," Liu says. "One long-term challenge is to make sure there's enough education and dialogue among all stakeholders in society, so the benefits of gene editing are not simply relegated to a small fraction of people."

Jyoti Madhusoodanan is a freelance writer based in Portland, Oregon.

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We chose 20 promising companies that are elevating gene editing to the next level

» **Arbor Biotechnologies**
» **arbor.bio**
» **Based:** Cambridge, Massachusetts
» **Launched:** 2016
» **Money raised in start-up funding rounds:** $15.6 million
» **Publicly traded:** No
» **Key partnerships:** Lonza, Vertex Pharmaceuticals, Vor Biopharma
» **Strategy:** Arbor's proprietary platform combs through genetic information from prokaryotes to discover proteins that can power applications in sustainability and human health. The company first wants to expand the tool kit of CRISPR enzymes.
» **Why watch:** Within 18 months after launch, the company's scientists identified new CRISPR-Cas systems with distinctive features that might be applicable to RNA editing and diagnostic detection of disease.

» **ArsenalBio**
» **arsenalbio.com**
» **Based:** South San Francisco
» **Launched:** 2019
» **Money raised in start-up funding rounds:** $85 million
» **Publicly traded:** No
» **Key partnerships:** Bristol Myers Squibb
» **Strategy:** ArsenalBio is a spin-off of the Parker Institute for Cancer Immunotherapy, created by tech entrepreneur and philanthropist Sean Parker. Arsenal integrates CRISPR gene editing with machine learning and other technologies to design immune cell therapies that recognize and attack cancers.
» **Why watch:** In January 2021, ArsenalBio announced a collaboration with Bristol Myers Squibb on cell therapies for solid-tumor cancers, which have proved more challenging to treat than cancers of the blood.

» **Beam Therapeutics**
» **beamtx.com**
» **Based:** Cambridge, Massachusetts
» **Launched:** 2017
» **Money raised in start-up funding rounds:** $222 million
» **Publicly traded:** Yes, IPO 2020
» **Key partnerships:** Bio Palette, Magenta Therapeutics, Verve Therapeutics
» **Strategy:** Beam's base-editing technology can precisely rewrite a single letter of the genome, potentially correcting a disease-causing point mutation permanently. The company's portfolio also includes projects that silence deleterious genes or reactivate dormant genes.
» **Why watch:** The company plans in the second half of 2021 to seek approval from the US Food and Drug Administration to launch human testing of its therapy for sickle cell disease and β-thalassemia.

» **Crispr Therapeutics**
» **crisprtx.com**
» **Based:** Zug, Switzerland
» **Launched:** 2013
» **Money raised in start-up funding rounds:** $123 million
» **Publicly traded:** Yes, IPO 2016
» **Key partnerships:** Bayer, Vertex Pharmaceuticals, ViaCyte
» **Strategy:** One of the original CRISPR companies, Crispr Therapeutics develops disease treatments that use CRISPR-Cas9 gene editing. Investigational treatments in human clinical trials include a therapy for sickle cell disease and βthalassemia, as well as cell therapies designed to attack cancers (see page 5).
» **Why watch:** The most advanced treatments in Crispr Therapeutics' pipeline involve removing cells from a patient's body, editing them in cell culture, and returning them to the person. The company is now moving toward in-body approaches. In December, the company received a grant from the Bill and Melinda Gates Foundation to use this approach against HIV.

» **Caribou Biosciences**
» **cariboubio.com**
» **Based:** Berkeley, California
» **Launched:** 2011

» **Money raised in start-up funding rounds:** $52.5 million
» **Publicly traded:** No
» **Key partnerships:** DuPont, Genus, MaxCyte, Memorial Sloan Kettering Cancer Center
» **Strategy:** One of the original CRISPR companies, Caribou harnesses hybrid RNA-DNA guide sequences that provide more precise genome editing than the RNA guides in canonical CRISPR approaches.
» **Why watch:** In September 2020, the US Food and Drug Administration cleared the company to begin human clinical trials on its gene-edited cell therapy for B-cell non-Hodgkin's lymphoma when people have not responded to treatment or have relapsed.

EDIGENE 博雅辑因

» **EdiGene**
» **edigene.com**
» **Based:** Beijing
» **Launched:** 2015
» **Money raised in start-up funding rounds:** $118 million
» **Publicly traded:** No
» **Key partnerships:** Genetron Health, Immunochina
» **Strategy:** EdiGene's four technologies include a platform for editing blood stem cells outside the body to treat anemias; a platform to edit immune system T cells outside the body to treat cancers; RNA base-editing technology conducted inside the body to treat Hurler syndrome, a disease involving the processing of cellular waste; and high-throughput gene editing to discover new treatments for solid tumors.
» **Why watch:** In January 2021, China's National Medical Products Administration gave EdiGene permission to begin a clinical trial of its gene-edited cell therapy for transfusion-dependent β-thalassemia.

editas MEDICINE

» **Editas Medicine**
» **editasmedicine.com**
» **Based:** Cambridge, Massachusetts
» **Launched:** 2013
» **Money raised in start-up funding rounds:** $210 million
» **Publicly traded:** Yes, IPO 2016
» **Key partnerships:** Allergan, AskBio, BlueRock Therapeutics, Celgene, GenEdit, Juno Therapeutics, Sandhill Therapeutics
» **Strategy:** Another of the original CRISPR companies, Editas developed EDIT-101, the first in-body CRISPR treatment to be administered to a person in a clinical trial. The investigational therapy, which is injected into the eye, is designed to treat Leber congenital amaurosis 10, a rare inherited disease that threatens vision.
» **Why watch:** The company plans to share initial clinical data from its trial of EDIT-101 by the end of 2021.

egenesis ENGINEERING LIFE

» **eGenesis Bio**
» **egenesisbio.com**
» **Based:** Cambridge, Massachusetts
» **Launched:** 2015
» **Money raised in start-up funding rounds:** $140 million
» **Publicly traded:** No
» **Key partnerships:** Duke University School of Medicine, Massachusetts General Hospital, Qihan Biotech
» **Strategy:** eGenesis leverages gene-editing technology to transform organs, tissues, and cells from pigs into human-compatible versions for transplantation.
» **Why watch:** In 2020, eGenesis acquired its partner ICBiotec, which raises gene-edited livestock. The move integrates the company's organ supply chain.

eligo bioscience

» **Eligo Bioscience**
» **eligo.bio**
» **Based:** Paris
» **Launched:** 2014
» **Money raised in start-up funding rounds:** $27.4 million
» **Publicly traded:** No
» **Key partnerships:** Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator (CARB-X), GlaxoSmithKline, European Commission, Institut Pasteur
» **Strategy:** Eligo's antibiotics combine a CRISPR-Cas system with a delivery system derived from bacteriophages, which are viruses that infect bacteria. The technology engineers the composition of the microbiome to address diseases, including drug-resistant infections.
» **Why watch:** In January 2021, Eligo announced a deal with GlaxoSmithKline potentially worth $224 million to develop strategies to remove pro-inflammatory bacterial strains, a root cause of acne, from the skin microbiome.

GENEDIT GENE THERAPY DELIVERED

» **GenEdit**
» **genedit.com**
» **Based:** South San Francisco
» **Launched:** 2016
» **Money raised in start-up funding rounds:** $8.5 million
» **Publicly traded:** No
» **Key partnerships:** Editas Medicine
» **Strategy:** GenEdit focuses on polymer nanoparticle-based delivery technology for CRISPR-based and other gene editors. The company screens its library of nanoparticles to achieve the best delivery of the therapeutic gene editor to the target organ.
» **Why watch:** Most gene-editing companies are focused on genes in blood cells, immune cells, or the liver. But a version of GenEdit's delivery concept fixed in mice the mutation that causes Duchenne muscular dystrophy.

GRAPHITE BIO

» **Graphite Bio**
» **graphitebio.com**
» **Based:** South San Francisco
» **Launched:** 2020
» **Money raised in start-up funding rounds:** $45 million
» **Publicly traded:** No
» **Key partnerships:** Jasper Therapeutics
» **Strategy:** Graphite's approach to gene editing is called targeted DNA integration, and the company's initial focus is correcting the mutation that causes sickle cell disease. Targeted DNA integration involves precisely cutting the mutated DNA encoding the variant of hemoglobin that causes sickle cell and then correcting the mutation by providing a stretch of nonmutated DNA to guide natural repair mechanisms.

» **Why watch:** In December 2020, the firm received clearance to initiate clinical trials of its investigational sickle cell therapy from the US Food and Drug Administration.

» **Intellia Therapeutics**
» **intelliatx.com**
» **Based:** Cambridge, Massachusetts
» **Launched:** 2014
» **Money raised in start-up funding rounds:** $85 million
» **Publicly traded:** Yes, IPO 2016
» **Key partnerships:** GEMoaB, Novartis, Regeneron, TeneoBio
» **Strategy:** One of the original CRISPR therapy companies, Intellia's strengths include an adaptable CRISPR delivery system made with lipid nanoparticles. Its pipeline includes experimental treatments for hemophilia, acute myeloid leukemia, and solid tumors.
» **Why watch:** The company's NTLA-2001 is administered through a vein to edit genes in the liver of the human body, and it is the first such therapy to be given to people in a clinical trial. The treatment is intended for the protein accumulation disorder transthyretin amyloidosis.

» **KSQ Therapeutics**
» **ksqtx.com**
» **Based:** Cambridge, Massachusetts
» **Launched:** 2015
» **Money raised in start-up funding rounds:** $156 million
» **Publicly traded:** No
» **Key partnerships:** Crispr Therapeutics, MaxCyte, Takeda Pharmaceutical
» **Strategy:** Rather than employing CRISPR gene editing as a therapy, KSQ built a drug discovery engine that's based on CRISPR. KSQ has used CRISPR to systematically knock out each gene in the genomes of approximately 600 cell lines in order to understand each gene's function. The results have revealed opportunities to develop new small-molecule treatments and cell therapies for cancer.
» **Why watch:** KSQ's pipeline includes preclinical studies of experimental treatments for ovarian cancer, solid tumors, and a type of breast cancer that is particularly difficult to treat.

» **Locanabio**
» **locanabio.com**
» **Based:** San Diego
» **Launched:** 2016
» **Money raised in start-up funding rounds:** $155.6 million
» **Publicly traded:** No
» **Key partnerships:** Muscular Dystrophy Association
» **Strategy:** Locanabio develops CRISPR systems that edit RNA rather than DNA. Viral vectors deliver the systems to target tissues as a one-time treatment. The company's road map includes programs aimed at Huntington's disease, amyotrophic lateral sclerosis, and myotonic dystrophy type 1.
» **Why watch:** CEO James Burns joined Locanabio from Casebia, a biotech firm that has been absorbed by Crispr Therapeutics. At Casebia, Burns led a team discovering CRISPR therapies for blood disorders, blindness, and heart disease.

» **Locus Biosciences**
» **locus-bio.com**
» **Based:** Morrisville, North Carolina
» **Launched:** 2015
» **Money raised in start-up funding rounds:** $39 million
» **Publicly traded:** No
» **Key partnerships:** Biomedical Advanced Research and Development Authority, Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator (CARB-X), IDbyDNA, Johnson & Johnson Innovation
» **Strategy:** The CRISPR-based antibiotics from Locus involve not the precise DNA-cutting enzyme Cas9 but the indiscriminate enzyme Cas3, which chews up bacterial DNA beyond recognition (see page 6). By combining this CRISPR system with a bacteriophage-based delivery platform, Locus hopes to address the threat of antibiotic-resistant bacteria.
» **Why watch:** The company launched its clinical trial of a treatment for a urinary tract infection caused by *Escherichia coli*. It is the first clinical trial of a CRISPR-enhanced bacteriophage.

» **Metagenomi**
» **metagenomi.co**
» **Based:** Emeryville, California
» **Launched:** 2018
» **Money raised in start-up funding rounds:** $65 million
» **Publicly traded:** No
» **Key partnerships:** Vor Biopharma
» **Strategy:** Metagenomi screens microbial genomes with the help of artificial intelligence to find next-generation gene-editing systems that can help treat genetic diseases or cancer. The company tunes up candidate enzymes with data-driven protein engineering.
» **Why watch:** Metagenomi has discovered over 100 potential CRISPR enzymes, most of them smaller and potentially less error prone than Cas9.

» **Snipr Biome**
» **sniprbiome.com**
» **Based:** Copenhagen, Denmark
» **Launched:** 2017
» **Money raised in start-up funding rounds:** $50 million
» **Publicly traded:** No
» **Key partnerships:** Not disclosed
» **Strategy:** Snipr is developing CRISPR technology to kill bacteria containing a specific DNA fingerprint while leaving beneficial bacteria unharmed. The approach turns disease-causing microbes' own CRISPR machinery against itself.
» **Why watch:** In addition to initiatives to pursue treatments for drug-resistant infections, the company's programs include efforts to remediate microbiome imbalances in cancer and autoimmune diseases.

» **Spotlight Therapeutics**
» **spotlighttx.com**
» **Based**: Hayward, California
» **Launched:** 2018

» **Money raised in start-up funding rounds:** $44.2 million
» **Publicly traded:** No
» **Key partnerships:** Not disclosed
» **Strategy:** Instead of relying on viruses or nanoparticles to deliver CRISPR systems to target cells and tissues inside the body, Spotlight links CRISPR systems to cell-permeable peptides, ligands, or antibodies that can be customized depending on the desired destination.
» **Why watch:** The company's scientific advisory board includes Carolyn Bertozzi, a serial entrepreneur and chemical biology heavy hitter.

SYNTHEGO

» **Synthego**
» **synthego.com**
» **Based:** Redwood City, California
» **Launched:** 2012
» **Money raised in start-up funding rounds:** $259.7 million
» **Publicly traded:** No
» **Key partnerships:** Eurofins Genomics, National Institute of Standards and Technology (NIST) Genome Editing Consortium, Thermo Fisher Scientific
» **Strategy:** Founded by former SpaceX engineers, Synthego is a CRISPR tool company that produces gene-editing kits and engineered cells for researchers. The company's product line of synthetic guide RNAs is intended to lower the cost of CRISPR research, therefore reducing barriers to entry.
» **Why watch:** Synthego makes CRISPR gene-editing experiments something that scientists can plan and purchase with the click of a mouse. During the COVID-19 pandemic researchers have used the firm's gene-edited cells to study factors that contribute to coronavirus infections.

Note: Companies were included because of the novelty and promise of their methods, amount of capital raised, number of partnerships, and number and identity of investors.

Sources: Crunchbase (accessed February 2021), company websites, news reports.

verve THERAPEUTICS

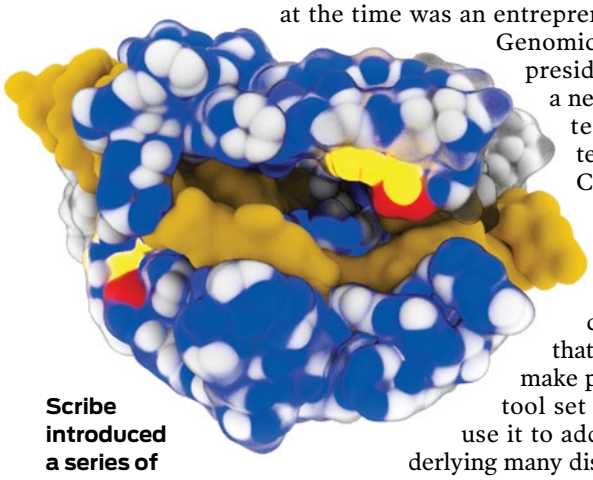
» **Verve Therapeutics**
» **vervetx.com**
» **Based:** Cambridge, Massachusetts
» **Launched:** 2018
» **Money raised in start-up funding rounds:** $215.5 million
» **Publicly traded:** No
» **Key partnerships:** Beam Therapeutics, Broad Institute of MIT and Harvard, Harvard University, Verily
» **Strategy:** Verve develops gene-editing medicines that could protect people from coronary heart disease. The company uses CRISPR enzymes or base editors to re-create naturally occurring gene variants that lower levels of triglycerides and so-called bad cholesterol, which contribute to heart disease.
» **Why watch:** In January 2021, Verve announced that a one-time experimental base-editing treatment safely lowered cholesterol in monkeys for 6 months. The company is laying the groundwork for human clinical trials to begin in 2022.

Scribe Therapeutics promises next-gen CRISPR and neuroscience drug development with Biogen

LISA M. JARVIS, C&EN STAFF

Scribe Therapeutics, a biotech firm focused on developing next-generation gene-editing technology, emerged from stealth on October 6, 2020, having raised $20 million in its first major funding round, backed by Andreessen Horowitz. Separately, the firm unveiled a deal with Biogen to develop CRISPR-based treatments for amyotrophic lateral sclerosis (ALS).

Scribe was cofounded in 2018 by several University of California, Berkeley, scientists, including gene-editing pioneer Jennifer Doudna and protein engineer Benjamin Oakes, who at the time was an entrepreneurial fellow at the Innovative Genomics Institute, of which Doudna is president. Their goal was to engineer a newly discovered class of Cas proteins to make them behave better as therapies than the original CRISPR-Cas9 system.



Scribe introduced a series of mutations to the CasX protein (shown) to arrive at improved gene editors.

That system was found in bacteria, which use it to recognize and chop up DNA from invading pathogens. Scientists, including Doudna, quickly realized that the system could be co-opted to make precise cuts to human DNA. The tool set off a race among companies to use it to address the genetic mutations underlying many diseases.

But even with its promise, the CRISPR-Cas9 system comes with "evolutionary baggage," says Oakes, who is now Scribe's CEO. Those systems "aren't designed to work within the context of the human cell or even the human genome," which complicates efforts to turn the technology into drugs, he says.

Several years ago, a group of Berkeley researchers identified two new classes of Cas proteins, including CasX.

Oakes, working in Doudna's lab, helped characterize the features of CasX, reporting in 2019 several ways the class improves on Cas9. CasX is smaller, so it can be more easily packaged inside adeno-associated viruses for therapeutic delivery and can more efficiently and precisely snip DNA.

Scribe scientists have engineered CasX to arrive at a collection of proteins—the firm calls them X-Editing molecules—that Oakes says perform as well as or better than all the other CRISPR-based gene-editing systems. The Scribe team is now focused on turning those molecules into therapeutics. The company made public its first big pharma partnership, scoring $15 million from Biogen to develop CRISPR-based drugs to treat ALS. ■

Scribe Therapeutics at a glance

- **Launched:** 2018
- **Based:** Alameda, California
- **Strategy:** Compact, high-performing CRISPR systems for treating disease
- **Money raised in start-up funding rounds:** $20 million

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CREDIT: SCRIBE THERAPEUTICS

Watch out, CRISPR. The RNA-editing race is on

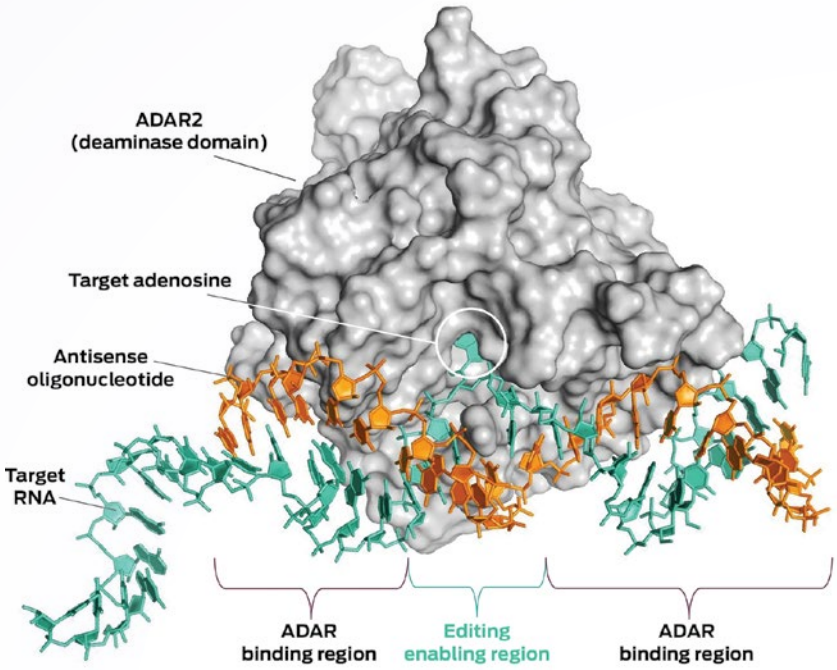
RYAN CROSS, C&EN STAFF

Joshua Rosenthal isn't your typical biotech entrepreneur. The cephalopod scientist at the Marine Biological Laboratory in Woods Hole, Massachusetts, has spent most of his life studying the nervous systems of squid—along with the occasional octopus. But in April 2018, Rosenthal found himself in Boston pitching to investors at Atlas Venture an idea for a new kind of therapy, inspired by a mechanism that squid use to edit their RNA.

RNA, a short-lived cousin to its better-known partner, DNA, is the blueprint for protein production in cells. Rosenthal told the Atlas investors about how squid and octopuses make prolific use of an enzyme called ADAR to catalyze thousands of single-letter changes to their RNA code. Those minor edits alter the structure and activity of proteins that control electrical impulses in the animals' nerves.

Humans have ADAR enzymes in our bodies, too, where they do the same thing, just less prolifically. Rosenthal's studies inspired him to hijack the squid ADAR and program it for making precise edits to human RNA. By attaching a molecule called a guide RNA to ADAR, Rosenthal's lab could direct the enzyme to edit a complementary RNA strand. It's analogous to CRISPR gene editing, which uses a guide RNA to direct an enzyme called Cas9 to a complementary DNA strand.

Unlike DNA editing, which is permanent, the effects of RNA editing are reversible, since cells are constantly churning out new copies of RNA. If Rosenthal's RNA editors work in humans, they could be used to repeatedly treat genetic diseases without confronting the unknown, long-term risks of permanent DNA editing with CRISPR. More importantly, they would offer a new strategy for treating conditions, like pain or inflammation, in which a patient needs just a temporary fix. RNA editing could also be easier to turn into a therapy than CRISPR. Since ADAR already exists in our cells, in theory all that's needed is a guide RNA to lasso the enzyme and tell it where to go.



A model of the RNA-editing enzyme ADAR binding an antisense oligonucleotide and its target RNA

The advantages must have been obvious to Nessan Bermingham, a venture partner at Atlas and the former CEO of Intellia Therapeutics, one of several firms developing CRISPR-based therapies. Atlas founded a new company, called Korro Bio, to develop RNA-editing therapies, with Bermingham as CEO and Rosenthal as a scientific adviser.

Last September, Korro raised $91.5 million in its first major funding round. It's becoming clear that the excitement about RNA editing is mounting. At least four other biotech companies are developing RNA-editing therapies, and more academic labs are trying to design new RNA editors.

The concept of RNA editing isn't new. In 1995, researchers at Ribozyme Pharmaceuticals discovered that synthetic strands of RNA, called antisense oligonucleotides, could recruit ADAR to make edits—albeit sloppily—on matching strands of RNA inside cells (*Proc. Natl. Acad. Sci. U.S.A.* 1995, DOI: 10.1073/pnas.92.18.8298). The team

CREDIT: PROQR THERAPEUTICS

coined the term "therapeutic RNA editing." The paper was cited once and then forgotten.

More than a decade later, two academic researchers—the MBL's Rosenthal and Eberhard Karls University of Tübingen chemist Thorsten Stafforst—independently revived the idea of developing ADAR-based RNA editors. Each devised his own system for connecting a guide RNA to ADAR: Stafforst linked the two components chemically (*Angew. Chem., Int. Ed.* 2012, DOI: 10.1002/anie.201206489), and Rosenthal added a small RNA-binding protein to ADAR, which in turn bound the guide RNA (*Proc. Natl. Acad. Sci. U.S.A.* 2013, DOI: 10.1073/pnas.1306243110).

Both systems rely on ADAR's natural ability to change one letter of the RNA code, an adenosine (A), into a different base, inosine (I). When cells use that edited RNA to make proteins, they interpret the unusual inosine as a normal base, guanosine (G). The result is effectively the change of an A to a G. Although scientists can do a lot with this tool, it doesn't allow them to make any edit they want. Other labs are looking for ways to edit the other bases in RNA.

But by the time Rosenthal's research came out, CRISPR gene editing had been invented; it quickly overshadowed both labs' work. People told Stafforst not to waste his time on RNA. "It was pretty difficult to actually publish this," he says. "Everyone wanted to play around with CRISPR."

While the CRISPR field exploded, Stafforst and Rosenthal continued refining their RNA editors. Early on, it became clear there might still be room for their approach: researchers discovered that CRISPR can sometimes introduce permanent unintended mutations in DNA. In contrast, off-target mutations in RNA are temporary, making RNA editing a potentially safer alternative to gene editing.

In 2017, one of CRISPR's inventors, Feng Zhang from Broad Institute of MIT and Harvard, created his own version of RNA editing that linked up the catalytic portion of the ADAR enzyme to a Cas protein and a guide RNA (*Science* 2017, DOI: 10.1126/science.aaq0180). Zhang's superstar status lent an air of credibility to the RNA-editing field. In fact, two CRISPR companies, Beam Therapeutics—cofounded by Zhang—and Locana, are developing RNA-editing therapies that require Cas enzymes.

> **"An advantage of antisense oligos is that they've been studied for decades already."**

Those complex designs are creating opportunities for others to showcase improved and simpler versions of RNA editors. In 2018, University of California, Davis, chemist Peter Beal revealed a system in which an engineered ADAR enzyme and a chemically modified guide RNA interlock to reduce off-target mutations. "We're really excited about this because it was the first thing we tried," Beal says. "We think there's a lot of room for improvement" (*Cell Chem. Biol.* 2018, DOI: 10.1016/j.chembiol.2018.10.025).

Others are trying to simplify the delivery system to make it easier to turn it into a therapy. Until recently, the various flavors of RNA editors all had to get both ADAR and the guide RNA into cells, creating a drug delivery headache. In fact, drug delivery remains one of the biggest challenges for the CRISPR gene-editing field. To that end, in 2019 Stafforst published a strategy for designing chemically modified guide RNAs with built-in structures that effectively lasso the ADAR proteins found naturally in our own cells (*Nat. Biotechnol.* 2019, DOI: 10.1038/s41587-019-0013-6).

Also in 2019, Prashant Mali, a CRISPR researcher at the University of California San Diego, published a similar study using engineered guide RNAs that bind ADAR and direct it to fix RNA mutations in mouse models of two genetic diseases: muscular dystrophy and ornithine transcarbamylase deficiency (*Nat. Methods* 2019, DOI: 10.1038/s41592-019-0323-0). Mali has also founded a company called Shape Therapeutics to develop RNA-editing therapies.

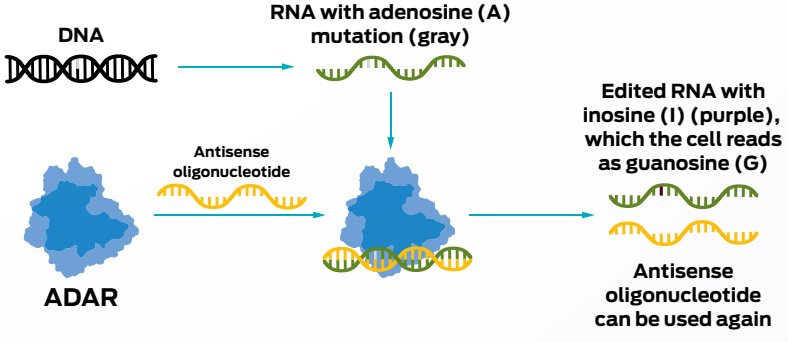
Stafforst's and Mali's approaches demonstrate RNA editing's two big advantages over CRISPR gene editing. First, using only a guide RNA to hijack the body's own ADAR circumvents the problem of introducing a foreign protein into the body—something that could pose problems for CRISPR, whose Cas proteins come from bacteria. Second, chemically synthesized guide RNAs are essentially the same thing as antisense oligonucleotides, a class of drugs well established for treating diseases of the brain, eye, and liver.

"An advantage of antisense oligos is that they've been studied for decades already," says Daniel de Boer, CEO of the Dutch antisense oligonucleotide company ProQR Therapeutics. De Boer says his company has been designing antisense oligonucleotides to recruit the body's own ADAR for RNA editing since 2014. The firm is conducting an early-stage clinical trial of its investigational RNA therapy in people with a form of hearing and vision loss called Usher syndrome or with a form of vision loss called nonsyndromic retinitis pigmentosa.

The RNA-editing field is starting to attract more researchers, too. In 2019, a rare-disease organization called the Rett Syndrome Research Trust (RSRT) awarded more than $5 million to several

A simple way to edit RNA

To correct single point mutations in RNA, researchers are designing antisense oligonucleotides that bind to an enzyme in our cells called ADAR. By also binding a complementary strand of RNA, these oligonucleotides coax ADAR into changing a mutation in an adenosine (A) base of the RNA into an inosine (I), which the cell reads as guanosine (G).



“It will be quite a high burden to make this work decently, but the idea is that once you can do it, there are countless opportunities.”

academic labs to develop RNA-editor therapies for the neurological disease. Rett is driven by a mutation that causes insufficient production of a protein called MeCP2, which is essential for regulating genes in brain cells. RNA editors could fix the blueprints for making the protein.

The nonprofit is also funding gene therapy, which would add a new copy of the gene for MeCP2, and CRISPR gene-editing approaches, which would fix the gene’s DNA mutation. “It is an exciting time for Rett,” says Monica Coenraads, executive director of RSRT. “We don’t want to sit idle.”

For a genetic disease like Rett, RNA-editing therapies would need to be administered repeatedly. Gene-therapy and gene-editing approaches could offer one-and-done cures. But having just the right amount of MeCP2 protein in cells—not too much or too little—is critical, explains Gail Mandel from Oregon Health and Science University, who received funding from RSRT to develop RNA editors with UC Davis’s Beal. The gene therapy approach carries the risk of making too much MeCP2, a liability avoided with RNA and DNA editors, Mandel says.

Other groups are beginning to investigate RNA editing’s potential for temporary treatments. Stafforst, for instance, suggests that RNA editing could be used to boost or weaken inflammation as a cancer or immune-disease treatment. And Rosenthal’s lab is designing RNA editors that can lower the sensitivity of pain receptors by making a single change to their RNA blueprints.

None of the RNA editors are perfect yet. “It will be quite a high burden to make this work decently, but the idea is that once you can do it, there are countless opportunities,” Stafforst says. RNA editing is just getting its start as a small field, and Stafforst thinks that over the next few years, as more researchers turn from DNA to RNA editing, more applications for the technology will become clear. As for his own entrepreneurial ambitions? “It is a bit too early to say,” he says. “But there will definitely be a company.” ■

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Sherlock Biosciences' COVID-19 test becomes the first FDA-authorized CRISPR technology on the market

MEGHA SATYANARAYANA, C&EN STAFF

On May 6, 2020, Sherlock Biosciences received an emergency use authorization (EUA) from the US Food and Drug Administration for its COVID-19 diagnostic assay. Sherlock beat out several other companies and academic groups also trying to use the powerful gene-editing technology to figure out who is infected with the novel coronavirus.

The EUA, which makes Sherlock's test the first FDA-authorized use of CRISPR technology, allows the company to scale up production of its assay for use by laboratories that conduct complex diagnostics.

A new diagnostic test for COVID-19 based on CRISPR technology accepts nasal-swab samples.

CEO Rahul Dhanda says the test is inexpensive and can be done in about an hour. A kit containing 33 tests is priced at $995, for a cost of about $30 per COVID-19 diagnostic test. Dhanda says the EUA represents the maturing of a technology that holds great potential in understanding disease.

"It's just a remarkably exciting moment for industry," he says.

Sherlock's test is a molecular diagnostic intended to identify people with SARS-CoV-2 infection. It capitalizes on a CRISPR–based technology developed in the lab of Feng Zhang, a scientist at Broad Institute of MIT and Harvard and a cofounder of Sherlock.

The test is one of many in a crowded field of SARS-CoV-2 diagnostics. Although Dhanda says Sherlock's test can be run on basic machines that

CREDIT: SHUTTERSTOCK

many hospitals likely have, it remains to be seen who decides to vet this new technology against standard tests.

Like those standard tests, Sherlock's assay detects the presence of SARS-CoV-2 viral RNA. It starts with a respiratory specimen from the mouth, nose, or lungs. To make the viral genome easier to identify, scientists convert it into DNA, which can be copied repeatedly. The method they use—isothermal amplification—is done at a constant temperature, unlike the method used by most conventional diagnostics, which is polymerase chain reaction.

The sample then goes through Sherlock's CRISPR gauntlet. CRISPR-Cas is a bacterial defense system that chops up invasive viral RNA; scientists have turned it into a technique that makes precise cuts in genetic code through various Cas enzymes. Sherlock's system uses Cas13, which William Blake, its chief technology officer, says is a little more flexible than other Cas enzymes in the genetic regions it can target (see page 4).

The CRISPR part of the assay involves converting the amplified DNA back into RNA, which is the type of genetic information the Cas13 enzyme recognizes. The enzyme is led to any viral RNA in the sample based on "guides," short bits of RNA that match the actual code of the virus. Once it finds any viral RNA, the enzyme cuts that RNA.

Blake says the assay targets two distinct parts of the SARS-CoV-2 genome: the recipe for the nucleocapsid—which helps the virus assemble itself—and ORF1ab, a stretch of the genome that leads to the precursor of an enzyme that helps the virus copy itself.

Blake says those targets were chosen over better-known ones like the SARS-CoV-2 spike protein and the protease because viruses are a bit sloppy when they copy their genetic information.

Sometimes they make mistakes, and while those mistakes may not affect the virus's ability to copy itself and infect cells, it might affect the precision of a diagnostic based on CRISPR.

"We wanted to ensure that our tests enabled detection of all the sequences that are out there for SARS-CoV-2," Blake says.

When activated, the Cas13 enzyme cuts other nucleic acids, as well as the viral RNA, Blake says. And that nonspecific cutting is how Sherlock knows the reaction has worked. Within the assay are strands of genetic material that have a fluorescent molecule at one end and a molecule that quenches, or blocks, the fluorescence on the other. As activated Cas13 chomps its way around the genetic material in the sample, it cuts those strands, freeing the fluorescents bits from the quenching bits. Blake says that most fluorescent-plate readers can read the test.

This is different from how the technology is being used as a gene-editing device, Blake says, where it must be very specific and have no extraneous cutting.

Like other SARS-CoV-2 diagnostics to receive EUAs, Sherlock's test was validated through experiments to determine analytical sensitivity, its ability to work on clinical samples, and tests to determine cross-reactivity, Dhanda says. That validation was conducted using samples from people who had tested negative for SARS-CoV-2 or who had a non-COVID-19 respiratory disease.

"We wanted to ensure that our tests enabled detection of all the sequences that are out there for SARS-CoV-2."

The company is working with an experienced manufacturer, Danaher's Integrated DNA Technologies division. Dhanda says its main customers for now will be hospitals, a setting that should provide real-world data about the fidelity of the first CRISPR-based diagnostic.

Mammoth Biosciences, a CRISPR diagnostic company cofounded by Jennifer Doudna of the University of California, Berkeley, received an EUA for its own COVID-19 test in August 2020 (see page 5).

In April 2020, the Mammoth team had published a peer-reviewed study of the system and the effort to validate it, as some molecular diagnostic tests had come under fire for their poor reliability (*Nat. Biotechnol.* 2020 DOI: 10.1038/s41587-020-0513-4).

Like Sherlock's assay, Mammoth's test starts with a constant-temperature amplification step, but its CRISPR-Cas system looks for different viral targets, including the envelope protein of SARS-CoV-2. The test can be read in two ways: with fluorescence, similarly to the Sherlock product, or via lateral flow—adding the final processed sample to a small cassette and looking for a color-change signal that is similar to a pregnancy test. Sherlock is also working on a lateral-flow system.

Caspr Biotech is also working on a CRISPR COVID-19 diagnostic. In March 2020, the firm published proof-of-principle details of its assay to a preprint server—before it was peer reviewed—also analyzing the use of lateral flow as a readout (bioRxiv 2020, DOI: 10.1101/2020.02.29.971127).

In all three cases, the companies were working on CRISPR-based diagnostics for other diseases, and as more information about SARS-CoV-2 became available, they decided to shift large portions of their efforts to the growing pandemic. While these tests may not end up widely used, COVID-19 has presented an opportunity to get CRISPR technology, and the platforms upon which diagnostics can be built, into hospitals much faster than under normal circumstances.

In March 2020, as Mammoth was navigating San Francisco's shelter-in-place order and validating its test using patient samples from the University of California, San Francisco, Chief Technology Officer Janice Chen described to C&EN why the pivot to SARS-CoV-2 was important.

"Based on everything we are living through today, having this tool available would be hugely beneficial to public health efforts," she said. "What we've learned with this public health crisis is that there's actually also a large need for getting point-of-care patient testing available. In some ways, this is kind of an important milestone." ■

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READING LIST

Our picks of the patent and journal literature on CRISPR technology

2020

» Synthego. **"Automated Modular System and Method for Production of Biopolymers."** US Patent 10,814,300, filed Jan. 17, 2020, and issued Oct. 27, 2020.

» Institut Pasteur de Lille and Eligo Bioscience. **"Optimized Vector for Delivery in Microbial Populations."** US Patent 10,808,254, filed July 31, 2019, and issued Oct. 20, 2020.

» Lee, Rose A., Helena De Puig, Peter Q. Nguyen, Nicolaas M. Angenent-Mari, Nina M. Donghia, James P. McGee, Jeffrey D. Dvorin, Catherine Klapperich, Nira R. Pollock, and James J. Collins. **"Ultrasensitive CRISPR-Based Diagnostic for Field-Applicable Detection of Plasmodium Species in Symptomatic and Asymptomatic Malaria."** *Proc. Natl. Acad. Sci. U.S.A.* 117, no. 41 (Sept. 21, 2020): 25722–731. https://doi.org/10.1073/pnas.2010196117.

» Katrekar, Dhruva, Nathan Palmer, Yichen Xiang, Anushka Saha, Dario Meluzzi, and Prashant Mali. **"Comprehensive Interrogation of the ADAR2 Deaminase Domain for Engineering Enhanced RNA Base-Editing Activity, Functionality and Specificity."** bioRxiv 2020.09.08.288233 (Sept. 9, 2020). https://doi.org/10.1101/2020.09.08.288233.

» Snipr Technologies. **"Selectively Altering Microbiota for Immune Modulation."** US Patent 10,765,740, filed Oct. 4, 2019, and issued Sept. 8, 2020.

» Crispr Therapeutics. **"Materials and Methods for Engineering Cells and Uses Thereof in Immuno-Oncology."** US Patent 10,729,725, filed June 7, 2019, and issued Aug. 4, 2020.

» Sinnamon, John R., Susan Y. Kim, Jenna R. Fisk, Zhen Song, Hiroyuki Nakai, Sophia Jeng, Shannon K. McWeeney, and Gail Mandel. **"In Vivo Repair of a Protein Underlying a Neurological Disorder by Programmable RNA Editing."** *Cell Rep.* 32, no. 2 (July 14, 2020): 107878. https://doi.org/10.1016/j.celrep.2020.107878.

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» Stadtmauer, Edward A., Joseph A. Fraietta, Megan M. Davis, Adam D. Cohen, Kristy L. Weber, Eric Lancaster, Patricia A. Mangan, et al. **"CRISPR-Engineered T Cells in Patients with Refractory Cancer."** *Science* 367, no. 6481 (Feb. 6, 2020): eaba7365. https://doi.org/10.1126/science.aba7365.

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» Massachusetts Institute of Technology and Broad Institute. **"CRISPR Enzyme Mutations Reducing Off-Target Effects."** US Patent 10,494,621, filed Oct. 11, 2018, and issued Dec. 3, 2019.

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» Behan, Fiona M., Francesco Iorio, Gabriele Picco, Emanuel Gonçalves, Charlotte M. Beaver, Giorgia Migliardi, Rita Santos, et al. **"Prioritization of Cancer Therapeutic Targets Using CRISPR–Cas9 Screens."** *Nature* 568 (April 10, 2019): 511–16. https://doi.org/10.1038/s41586-019-1103-9.

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Note: This list was chosen by experts who work in the field, CAS information scientists, and C&EN editorial staff.


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